Glycoconj J (2013) 30:281–461
DOI 10.1007/s10719-013-9474-x

GLYCO 22
XXII International Symposium on Glycoconjugates

Abstracts

June 23–28, 2013

Dalian, China

中国 大连

Organising Committee

Co-Chairs

Jianing Zhang (Dalian, China)
Xinmiao Liang (Dalian, China)

Scientific Program Committee

Co-Chairs

Yukui Zhang (Dalian, China)
Jianxin Gu (Shanghai, China)

Committee

Jinku Bao (Chengdu, China)
Kan Ding (Shanghai, China)
Yuguang Du (Dalian, China)
Yuguo Du (Beijing, China)
Xiaodong Gao (Wuxi, China)
Zhongwu Guo (Jinan, China)
Cheng Jin (Beijing, China)
Haojie Lu (Shanghai, China)
Xiaohong Qian (Beijing, China)
Aiguo Shen (Nantong, China)
Lei Wang (Tianjin, China)
Zhongfu Wang (Xi'an, China)
Min Xiao (Jinan, China)
Pengyuan Yang (Shanghai, China)
Qiu Yan (Dalian, China)
Xinshan Ye (Beijing, China)
Biao Yu (Shanghai, China)
Chuyi Yu (Beijing, China)
Guangli Yu (Qingdao, China)
Xiaolian Zhang (Wuhan, China)
Yan Zhang (Shanghai, China)
Yifa Zhou (Changchun, China)

The International Glycoconjugate Organisation

Iain B. H. Wilson, *President*
Jianxin Gu, *President-elect*
Gerald W. Hart, *Immediate Past-president*
Sandro Sonnino, *Secretary*
Thierry Hennet, *Treasurer*

National Representatives

Pedro Bonay (Spain)
Nicolai Bovin, Бовин Николай (Russia)
Inka Brockhausen (Canada)
Jin Won Cho (Korea)
Henrik Clausen (Denmark)
José Luis Daniotti (Argentina)
Anne Dell (UK)
Jukka Finne (Finland)
Paul Gleeson (Australia)
Jianxin Gu 顾建新 (China)
Gerald Hart (USA)
Thierry Hennet (Switzerland)
Jaroslav Katrlík (Slovakia)
Gordan Lauc (Croatia)
Hakon Leffler (Sweden)
Jean-Claude Michalski (France)
Paulo Mourão (Brasil)
Andrei Petrescu (Romania)
Celso Albuquerque Reis (Portugal)
Werner Reutter (Germany)
Sandro Sonnino (Italy)
Avadhesha Surolia (India)
Ken Kitajima 北島健 (Japan)
Maciej Ugorski (Poland)
Johannes F.G. Vliegenthart (The Netherlands)
Iain Wilson (Austria)
Michaela Wimmerová (Czech Republic)
Albert M. Wu 吳明道 (Taiwan)
Lode Wyns (Belgium)
Yehiel Zick (Israel)

Past Presidents
Eugene. A. Davidson (USA)
Alan B. Foster (UK)
Paul Gleeson (Australia)
Mary Catherine Glick (USA)
Gerald W. Hart (USA)
Colin Hughes (UK)
Roger W. Jeanloz (USA)
Akira Kobata 木幡陽 (Japan)
Jerzy Koscielak (Poland)
Bengt Lindberg (Sweden)
Jean Montreuil (France)
Jürgen Roth (Switzerland)
Harry Schachter (Canada)
Roland Schauer (Germany)
Nathan Sharon (Israel)
Avadhesha Surolia (India)
Guido Tettamanti (Italy)
Johannes F.G. Vliegenthart (The Netherlands)
Tamio Yamakawa 山川民夫 (Japan)

International Glycoconjugate Organisation Award 2013

Professor Kurt DRICKAMER

Kurt Drickamer is a household name in the glycobiology field for his seminal discovery of and subsequent work on calcium-dependent or "C-type" lectin domains. The succession of field-leading discoveries that Drickamer has made during the last 30 years, and the insights they afford, has had incomparable influence on the forefront of research in several disciplines, including biochemistry, structural biology, immunology and microbiology.

Beginning with his 1981 JBC publication on the hepatic glycoprotein receptor, which controls serum-glycoprotein turnover by recognising changes with age in their carbohydrate moieties, Drickamer initiated molecular analysis of what turned out to be a huge family of glycan-binding lectins. After this pioneering (single-authored!) study, he went on to demonstrate that the soluble mannose-binding protein (MBP) (alternatively called the mannose-binding lectin or MBL) of blood and lymph also contains CRDs. In this protein he showed the CRDs are linked to collagen-like sequences, as is also the case for pulmonary surfactants. Thereby he also saw the functional parallel between mannose-binding protein and C1q: the former detecting pathogens to initiate innate immunity, the latter initiating adaptive immunity through detection of pathogen-specific antibody bound to the pathogen. This was a major contribution to our understanding of innate immunity in a time (*i.e.* pre-Toll receptors) when innate immunity was not all the rage. From these seminal observations of CRDs in soluble and cell-surface receptors with different functions in blood homeostasis and immunity, Drickamer predicted that CRDs were likely to be a feature shared by many other carbohydrate receptors. To test this hypothesis he initiated systematic investigation and classification of glycan-binding receptors based on the presence of different types of CRDs. From comparison of the CRDs in those C-type lectins that were discovered using traditional assays of biological function, Drickamer had defined the sequence motifs that typify this class of CRDs, which enabled him to identify several CRD-containing proteins that were not known to bind carbohydrates. He pioneered the scanning of whole-genome sequences to identify novel, uncharacterised proteins that contain CRDs and are thus candidates for having glycan-binding function. Complementing this approach for the identification of candidate lectins, Drickamer has been a leader in using glycan array technology to test such candidates for their capacity to bind glycans and determine their sugar specificities.

He is also well known for co-writing, with his colleague and collaborator Maureen Taylor, the text book "Introduction to Glycobiology", now in its third edition. Their book enunciates the established and emerging principles of glycobiology in a manner accessible to students and the broader scientific community and has been translated into Chinese, Japanese, and Korean.

To summarise, Drickamer is a scientist who has both made seminal 'text book' discoveries and followed up on them to become the leading authority on CRDs and their role in cell-cell and cell-microbe recognition. Moreover he unselfishly gives an enormous amount of his time supporting international glycobiology—for example he served as the deputy chair of the Consortium for Functional Glycomics Steering Committee for a decade and personally made substantial contributions to the CFG Paradigm Pages. Since 2005 he is Professor of Biochemistry at Imperial College London and in 2012 he received the Karl Meyer Award from the Society for Glycobiology. In 2013 he has been selected to be recipient of the International Glycoconjugate Organisation Award.

Michael A.J. Ferguson
University of Dundee, Scotland
IGO Award Recipient 1999

Iain B. H. Wilson
Universität für Bodenkultur Wien, Austria
IGO President 2011–2013

The IGO Young Glycoscientist Awards 2013 supported by Genos

Dr. Amanda LEWIS

Dr. Amanda Lewis completed her Ph.D. and postdoctoral studies at the University of California San Diego, under the mentorship of Ajit Varki and Victor Nizet respectively. Training at the intersection of glycobiology and host-microbe interactions led her to an Assistant Professor position at Washington University School of Medicine in St. Louis, USA where she has built a successful research program supported by multiple foundation and government grants. The Lewis lab investigates mechanisms of host sialic acid mimicry and sialoglycan degradation in host-bacterial interactions within the female urogenital tract. Dr. Lewis' work on Group B Streptococcus (GBS), the leading cause of sepsis and meningitis in newborns, has contributed to our understanding of mechanisms by which the bacterium uses its sialic acid-containing capsule to survive in the maternal urogenital tract and the infant bloodstream. Dr. Lewis also led studies showing that the presence of GBS and its ability to precisely mimic host sialic acids influences the severity of urinary tract infection (UTI) caused by E. coli, the most common cause of human UTI. Genomic and biochemical investigations by Dr. Lewis and colleagues suggest that bacterial mimicry of host sialic acids may be a more common theme in bacterial immune evasion than previously recognized.

The Lewis lab has also made recent strides in understanding an enigmatic polymicrobial imbalance of the vaginal microbiota known as bacterial vaginosis (BV)—a condition linked with preterm birth and other pregnancy complications. Lewis' group has demonstrated new biochemical and cellular phenotypes of BV and is building new experimental models of the condition by combining approaches in genomics, microbiology, biochemistry, and infection studies. Recent findings demonstrate that a controversial contributor in BV, Gardnerella vaginalis, is sufficient to cause BV-like phenotypes in an animal model of infection and to engage in degradation, foraging, and depletion of mucus barrier sialoglycans. Ongoing studies in the Lewis lab explore novel contexts and mechanisms of bacterial glycan mimicry, roles of bacterial glycosidases in colonization and infection, and concepts in glyco-ecology within the female reproductive tract.

Dr. Wen YI

Dr. Wen Yi received his bachelor degree from Fudan University in China in 2002. He went to the U.S for graduate study, and in 2008 obtained his Ph.D. degree in Biochemistry from the Ohio State University under the guidance of Prof. Peng G. Wang. His doctoral research mainly focused on chemo-enzymatic synthesis of complex carbohydrates, and *in vitro* characterization of bacterial polysaccharide biosynthesis. From 2008 to 2012, he conducted his postdoctoral training with Prof. Linda C. Hsieh-Wilson in California Institute of Technology, where he carried out studies to understand the role of O-GlcNAcylation in regulating cancer metabolism and cell growth. After finishing his postdoctoral training, he moved back to China and accepted a faculty position at the College of Life Sciences in Zhejiang University. He was awarded multiple prestigious research grants, including the Concept Award from the Department of Defense Breast Cancer Research Program, and the Postdoctoral Fellowship from the Tobacco-Related Disease Research Program. He was recently elected to the Thousand Young Talents Program, a prestigious award given by the Chinese government for recruiting outstanding young talents from overseas.

Plenary Lectures

001: A structural and genomic view of receptors for cell surface glycans

Kurt Drickamer; Department of Life Sciences, Imperial College, London SW7 2AZ, United Kingdom
k.drickamer@imperial.ac.uk

Mammalian receptors that recognise specific oligosaccharides represent an important way in which information in glycan structures can be decoded. We are interested in defining the complement of human glycan-binding receptors and understanding how they function. Our focus is on receptors that contain calcium-dependent carbohydrate-recognition domains. Biochemical, structural and glycan array studies have revealed multiple different mechanisms by which these receptors bind selectively to different types of glycans. In receptors found on macrophages, dendritic cells and endothelial cells in the innate immune system, the binding mechanisms often favour interaction with broad classes of sugar structures, facilitating selective interaction with viral, bacterial and fungal pathogens. In receptors that mediate either glycoprotein trafficking in cells and organisms or cell-cell adhesion, the binding sites are more restrictive, leading to interactions with a much more limited set of oligosaccharides. Knowledge of the structures of carbohydrate-recognition domains has led to identification of novel glycan-binding receptors. Investigation of these receptors and their target ligands has allowed us to proposal novel roles for carbohydrate-mediated communication between cells.

002: Glycomics and glycoproteomics: Windows to glycan function

Anne Dell; Department of Life Sciences, Imperial College London, SW7 2AZ, UK
a.dell@imperial.ac.uk

Ultra-high sensitivity mass spectrometric strategies incorporating MALDI-MS/MS and nano-electrospray(ES)-MS/MS enable very complex mixtures of glycans and glycopeptides from biological extracts of cells and tissues to be studied thereby revealing the types of glycans present and, importantly, providing clues to structures that are likely to be functionally important. Glycomic methodologies seek to define the total N-glycan and/or O-glycan repertoire in a biological sample, whilst glycoproteomic strategies are concerned with the analysis of glycopeptides in order to define heterogeneity at individual glycosylation sites.

Data emerging from our glycomic and glycoproteomic programmes of collaborative research, which are helping to provide new insights into the functions of glycans in health and disease, will be described. Exemplar projects in the fields of human reproduction, pathogen-host interactions, glycoimmunology and congenital disorders of glycosylation will be discussed.

In addition our progress on the development of informatic tools to manage the large volumes of data being acquired will be outlined.

Acknowledgements: My research is primarily supported by the Biotechnology and Biological Sciences Research Council. The work described in this talk is a group effort and I am grateful to my Imperial College colleagues for their contributions. I also thank our numerous collaborators worldwide for giving us the opportunity to exploit our technology in many biological fields.

003: The complex interplay of "yin and yang" in branched N-glycans: from bisecting GlcNAc to core fucose

Naoyuki Taniguchi; Global Research Cluster, Systems Glycobiology Research Group and RIKEN-Max Planck Joint Research Center, Wako, 351-0198, Japan
tani52@wd5.so-net.ne.jp

Glycosyltransferases, which catalyze the biosynthesis of N-glycan branching, have different kinds of functions as our group and other groups have reported. Among them GnT (UDP-N-acetylglucosaminyltransferases)-III, IV, V, IX (Vb), and Fut8 (alpha1,6-fucosyltransferase) play key roles in various physiological and pathological situations. These enzymatic products such as bisecting GlcNAc, beta1,6-GlcNAc branching and core fucose are attached to specific target proteins, including E-cadherin, integrin RPTP (receptor protein tyrosine phosphatase)-beta, TGF (transforming growth factor)-beta receptors, EGF (epidermal growth factor) receptors, glucose transporters, and play key roles in various diseases such as the development of cancer, type II diabetes, COPD (chronic obstructive pulmonary disease) and neurodegenerative diseases such as Alzheimer's disease and schizophrenia *etc.* Serum proteins such as AFP (alpha fetoprotein) are also being used or planned for use as cancer biomarkers. Moreover glycan modifications, such as core fucose, result in an enhancement in the activity of ADCC (antibody dependent cellular cytotoxicity) for antibody therapy against leukemia and several other diseases. On the other hand, the deletion of core fucose in mice is semi-lethal and the survivors develop emphysema. In humans and mice, decreased levels of core fucose result in the development of sensitivity to emphysema and COPD, respectively. Bisecting GlcNAc was found to have a suppressive action against

cancer metastasis and to be involved in the development of a protective effect against Alzheimer's disease. In some cases, the addition of bisecting GlcNAc in the brain results in the opposite behavior. In the case of GnT-V, its activity is high in most cancers with metastatic properties, whereas it is decreased in some.

In this lecture I will present an overview of the complex and yin and yang behavior of branched N-glycans when added to target proteins.

004: Cytoplasmic Glycosylation Targets a Polyubiquitin Ligase to Control Oxygen Sensing in Protists

Christopher M. West[1], M. Osman Sheikh[1], Christopher M. Schafer[1], Hanke Van Der Wel[1], and Ira Blader[2]; [1]Departments of Biochemistry & Molecular Biology, and [2]Microbiology & Immunology, Oklahoma Center for Medical Glycobiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104 USA
Cwest2@ouhsc.edu

Studies on a novel form of cytoplasmic glycosylation in the social amoeba Dictyostelium have led to the discovery of an O2-sensing mechanism related to that of humans, with a novel twist that instead of generating a degron for the polyubiquitination of the transcriptional factor hypoxia inducible factor alpha (HIFα), the ubiquitin ligase itself appears to be regulated. The target of this modification is a single proline on Skp1, an adaptor subunit of the large SCF (Skp1/cullin-1/F-box protein) family of E3 ubiquitin ligases that control physiological and developmental timing *via* targeted protein degradation. Genetic studies show that prolyl hydroxylation and five sequential glycosylation reactions modulate O2-sensing by a mechanism that does not affect Skp1 stability. Cellular and biochemical studies reveal that modifications affect Skp1 conformation and promote binding to F-box proteins, the first step in SCF assembly. The terminal glycosyltransferase is particularly influential, contributing both positive enzymatic and negative non-enzymatic functions to pathway activity and Skp1 function. Key elements of the pathway have conserved O2-sensing functions in other protists including the agent for human toxoplasmosis, Toxoplasma gondii. The findings suggest a model in which developmental progression is tuned by environmental cues *via* a multi-step hydroxylation/glycosylation pathway that controls the rate of assembly of E3SCFUb-ligases which, in turn, influence lifetimes of specific regulatory targets according to the genetic program of F-box protein expression.

005: Complicated N-linked glycans in simple organisms

Iain B. H. Wilson, Shi Yan (闫 石), Alba Hykollari, Birgit Schiller, Dubravko Rendic, Simone Kurz and Katharina Paschinger; Department für Chemie, Universität für Bodenkultur Wien, 1190 Wien, Austria
iain.wilson@boku.ac.at

Although countless genomes have now been sequenced, the glycomes of the vast majority of eukaryotes still present a series of unmapped frontiers. However, strides are being made in a few groups of invertebrate and unicellular organisms as regards their N-glycans and N-glycosylation pathways. Thereby, the traditional classification of glycan structures inevitably approaches its boundaries. Indeed, the glycomes of these organisms are rich in surprises including a multitude of modifications of the core regions of N-glycans and unusual antennae. The trifucosylation of nematode N-glycan cores can now, from our data, be explained enzymologically; the pentosylation of trichomonad and *Acanthamoeba* glycans is based on different $Man_5GlcNAc_2$ or $Man_{8\text{-}9}GlcNAc_2$ based scaffolds; zwitterionic glycans with phosphorylcholine or phosphorylethanolamine are found in nematodes, cestodes and some *Trichomonas* strains; phosphate and sulphate are modifications of glycans from one trichomonad strain, from *Dicytostelium*, from oysters and possibly even insects. From the actually rather limited glycomic information we have, it is nevertheless obvious that the biotechnological, developmental and immunological relevance of these modifications, especially in insect cell lines, model organisms or parasites means that deciphering unusual glycomes is of more than just academic interest.

006: Targeting siglecs for modulation of immune responses

Mathew S. Macauley[1], Fabian Pfrengle[1], Christoph Rademacher[1], Corwin M. Nycholat[1], Cory D. Rillahan, Andrew J. Gale[2], Annette von Drygalski[2,3], and James C. Paulson[1]; [1]Departments of Cell and Molecular Biology, and Chemical Physiology, and [2]Department of Molecular Experimental Medicine, The Scripps Research Institute, La Jolla, CA, 92037 USA; [3]Division of Hematology/Oncology, School of Medicine, University of California, San Diego, La Jolla, CA, 92037, USA
jpaulson@scripps.edu

The sialic acid binding immunoglobulin lectin (siglec) family of cell adhesion molecules are differentially expressed on white blood cells that confer innate and adaptive immune responses. Using rational design and high-throughput

synthesis and screening of sialoside analogs we have developed siglec ligands that selectively recognize a single siglec, suitable for *in vivo* targeting of liposomal nanoparticles loaded with a therapeutic agent or immune modifier to siglec bearing cells. Using this platform, we have investigated the roles of B cell siglecs, CD22 and Siglec-G, in modulation of B cell receptor signaling. CD22 and Siglec-G are well documented as inhibitory co-receptors of the B cell receptor. We hypothesized that the inhibitory activities of CD22 and Siglec-G cooperate to tolerize B cells that recognize a cell surface autoantigen, as a result of their recruitment to the site of the immunological synapse *via* sialic acid containing ligands on the antigen bearing cell. To test this, we generated liposomes displaying an antigen and siglec ligands to mimic a cell expressing an autoantigen. Remarkably, injection of the liposomes into mice results in induction of apoptosis in reactive B cells, and the mice are then incapable of mounting an antibody response to that antigen in a subsequent challenge. Since development of inhibitory antibodies to FVIII is a serious problem in treatment of hemophilia A patients, we investigated the potential of this approach for inducing tolerance to FVIII in a hemophilia mouse model. Our tolerogenic liposomes prevented formation of inhibitory FVIII antibodies, allowing for effective administration of FVIII to hemophilia mice to prevent bleeding. Further studies have documented that the same tolerogenic mechanism is induced by natural siglec ligands present on cells that contain a membrane bound antigen. Thus, through a combination of genetic and biochemical approaches, we have obtained strong evidence for a major function of the B cell siglecs as receptors that recognize sialic acid as 'self' to induce apoptosis in autoreactive B cells for maintenance of peripheral tolerance. Exploiting this mechanism has therapeutic potential in the areas of autoimmunity, allergies, and biotherapeutics (NIH grants AI050143, AI099141, CA013889 and HFSP Fellowship LT001099/2010-L).

007: Large-scale identification of *in vivo* target proteins of glycosyltransferases by Lectin-IGOT-LC/MS, an LC/MS-based glycoproteomic approach using knock-out mouse

Hisashi Narimatsu; Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan

Model organisms containing deletion or mutation in a glycosyltransferase-gene exhibit various physiological abnormalities, suggesting that specific glycan motifs on certain proteins play important roles *in vivo*. Identification of the *in vivo* target proteins of each glycosyltransferase is the key to understand the roles of glycans. Here, we demonstrated the large scale identification of the target proteins specific for several glycosyltransferases by Lectin-IGOT-LC/MS glycoproteomic approach.

Although β4GalT-I is the most characterized glycosyltransferase, its distinctive contribution to β1,4-galactosylation has been hardly described so far. We identified a large number of the target proteins specific for 4GalT-I by comparative analysis of 4GalT-I-deleted and wild-type mice using the LC/MS-based technique with the isotope-coded glycosylation site-specific tagging (IGOT) of lectin-captured N-glycopeptides.

In addition, we are now determining the large number of target proteins of some glycosyltarnsferases, such as b3GnT, b3Gnt5 (polyLacNAc synthases) and two LacdiNAc(LDN) synthases. Our approach to identify the target proteins offers unique information regarding the molecular characteristics common to the target proteins, which facilitate understanding of the mechanism that controls protein glycosylation *in vivo*.

008: Roles of O-glycans in Cellular Interactions and Cancer

Richard D. Cummings, Yingchun Wang, Xiaokun Ding, Sean Stowell, and Tongzhong Ju; Department of Biochemistry, The Emory Glycomics Center, Emory University School of Medicine, Atlanta, GA

Altered expression of glycan structures is seen in many human diseases and disorders, including many types of cancers. A common modification of membrane and secreted glycoproteins is O-GalNAc type O-glycans linked to Ser/Thr residues. These O-GalNAc O-glycans are normally extended, and occur as branched, sialylated, sulfated, and/or fucosylated forms. Our studies show that such O-glycans are essential for normal development and are involved in numerous cellular interactions. In addition, normal O-GalNAc O-glycans are essential to for normal hemostasis, and truncations of O-glycans result in altered blood cell and endothelial cell functions, including abnormal hemostasis from loss of functions in platelets and Von Willebrand factor. In some human diseases in human diseases such O-GalNAc glycans are be truncated. Among the most common tumor-associated carbohydrate antigens are the Tn and Sialyl Tn antigen. Extension of the Tn antigen (GalNAcα1-Ser/Thr), which is a normal precursor for extended O-glycans, is under the control of two key genes, the X-linked gene encoding Cosmc (Xq24) and the autosomal gene encoding the T-

synthase (7p14-p13). Expression of functional T-synthase, a β3-galactosyltransferase that is required for formation of normal extended core 1-derived O-glycans of the structure Galβ1-3GalNAcα1-Ser/Thr, depends on the expression of the unique molecular chaperone Cosmc. Our studies show that Cosmc is a resident protein in the endoplasmic reticulum that prevents oligomerization of the T-synthase into inactive forms that are targeted for degradation, and promotes formation of active T-synthase dimers that leave the ER to function in the Golgi apparatus. Immunohistochemical screening of a wide variety of human tumor specimens in tissue microarrays with specific monoclonal antibody to the Tn antigen show that it is expressed in a majority of human carcinomas. There are multiple mechanisms that cause Tn expression, including acquired loss-of-function of Cosmc. Thus, the expression of normal O-glycans are required in a surprising number of biological systems and leads to normal glycoprotein function and expression. Moreover, expression of the Tn antigen is uniquely associated with neoplastic transformation and Cosmc is implicated as a major contributor to the expression of altered O-glycans in neoplasia.

009: General Tactics and New Glycosylation Methods for the Assembly of Naturally Occurring Glycosides

Biao Yu; State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China
byu@mail.sioc.ac.cn,

The secondary metabolites (or natural products), such as flavonoids, steroids, triterpenes, lipids, and antibiotics, are frequently post-modified with saccharides. These ubiquitous glycosides are extremely diverse in structures and functions. Purification of a homogeneous glycoside from the nature sources, especially in an appreciable amount, is always difficult. Chemical synthesis provides a feasible access to the homogenous glycosides and their congeners. This lecture discusses the general tactics for the synthesis of the diverse glycosides and the newly developed gold(I)-catalyzed glycosylation method for installation of saccharides onto aglycones which are poorly nucleophilic or extremely labile to acid or electrophile.

Reference:
Assembly of naturally occurring glycosides, evolved tactics and glycosylation methods. B. Yu, J. Sun, X. Yang, Acc. Chem. Res. 2012, 45, 1227-1236.

010: Linking Nutrients to Signaling: Many Kinases are Regulated by O-GlcNAcylation

Gerald W. Hart[1]; [1]Dept. of Biol. Chem., Johns Hopkins Univ., Sch. of Medicine, 725 N. Wolfe St., Baltimore, MD 21205
gwhart@jhmi.edu

Dynamic O-GlcNAcylation serves as a major sensor of cellular nutrient status, and has extensive crosstalk with phosphorylation. O-GlcNAc also crosstalks with other abundant post-translational modifications, such as ubiquitination, methylation, and acetylation. This extensive crosstalk regulates transcription, signaling and cellular metabolism in response to nutrient status. O-GlcNAcylation plays a fundamental role in gene transcription at many levels, including regulation of the basal machinery, as well as modifying the interactions and localizations of transcription factors. Prolonged excess O-GlcNAcylation, as occurs in diabetes, underlies fundamental mechanisms of glucose toxicity. Abnormal O-GlcNAcylation is associated with neurodegenerative disease and is elevated many types of cancer. Recent glycomic analyses have found that many kinases are both modified and regulated by O-GlcNAcylation. For example, O-GlcNAcylation either inhibits or activates, depending upon the kinase, and can alter substrate specificity, as exemplified by casein kinase II. Hyper-O-GlcNAcylation of mitochondrial proteins in diabetic tissues contributes directly to production of reactive oxygen species (ROS). Mitochondrial O-GlcNAc transferase (OGT) is strikingly elevated and mis-localized in cardiac mitochondria from diabetic rats. O-GlcNAcylation is very abundant at nerve terminals, is abundant on myriad synaptic vesicle proteins, and appears to play a direct role in learning and memory. OGT is highly enriched in the post-synaptic density. Supported by NIH R01CA42486, R01DK61671; N01-HV-00240; P01HL107153 and the Patrick C. Walsh Prostate Cancer Research Fund. *Dr. Hart receives a share of royalty received by the university on sales of the CTD 110.6 antibody, which are managed by JHU*

IGO Young Glycoscientist Awards

011: Degradation, Foraging and Depletion of Mucus Sialoglycans by the Vagina-Adapted Actinobacterium Gardnerella vaginalis

Warren G. Lewis, Lloyd S. Robinson, Nicole M Gilbert, and Amanda L. Lewis; Washington University in St. Louis, United States
mandylew@gmail.com

Bacterial vaginosis (BV) is a polymicrobial imbalance of the vaginal microbiota associated with reproductive infections, preterm birth, and other adverse health outcomes. Sialidase activity in vaginal fluids is diagnostic of BV and sialic acid-rich components of mucus have protective and immunological roles. However, while mucus degradation is believed to be important in the etiology and complications associated with BV, the role(s) of sialidases and the participation of individual bacterial species in the degradation of mucus barriers in BV have not been investigated. Here we demonstrate that the BV-associated bacterium Gardnerella vaginalis uses sialidase to break down and deplete sialic-acid-containing mucus components in the vagina. Biochemical evidence using purified sialoglycan substrates supports a model in which 1) G. vaginalis extracellular sialidase hydrolyzes mucosal sialoglycans, 2) liberated sialic acid (N-acetylneuraminic acid) is transported into the bacterium, a process inhibited by excess N-glycolylneuraminic acid, and 3) sialic acid catabolism is initiated by an intracellular aldolase/lyase mechanism. G. vaginalis engaged in sialoglycan foraging *in vitro*, in the presence of human vaginal mucus, and *in vivo*, in a murine vaginal model, in each case leading to depletion of sialic acids. Comparison of sialic acid levels in human vaginal specimens also demonstrated significant depletion of mucus sialic acids in women with BV compared to women with a "normal" lactobacilli-dominated microbiota. Taken together, these studies show that G. vaginalis utilizes sialidase to support the degradation, foraging, and depletion of protective host mucus barriers, and that this process of mucus barrier degradation and depletion also occurs in the clinical setting of BV.

012: Protein O-GlcNAcylation Regulates Cancer Metabolism and Cell Growth

Wen Yi[1,2], Peter Clark[2], Daniel Mason[3], Eric Peters[3], Edward Driggers[4], and Linda C. Hsieh-Wilson[2]; [1]College of Life Sciences of Zhejiang University, Hangzhou, Zhejiang 310058, China; [2]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA; [3]Genomics Institute of the Novartis Research Foundation, San Diego, CA 92121, USA; [4]Agios Pharmaceuticals, 38 Sidney Street, Cambridge, MA 02139, USA
wyi@zju.edu.cn

The dynamic post-translational modification of intracellular proteins with O-linked β-*N*-acetylglucosamine (O-GlcNAc) has recently emerged as an important regulator of biological processes, including transcription, translation, stress response, and insulin signaling. More recently, O-GlcNAcylation is also implicated in tumor pathogenesis. For instance, O-GlcNAc levels are increased in multiple tumor types, and O-GlcNAc ablation has caused inhibition of tumor growth and cancer metastasis. However, the functional role of O-GlcNAc in cancer development and progression is largely unknown. Here we demonstrate that O-GlcNAcylation of a key glycolytic enzyme phosphofructokinase 1 (PFK1) plays an important role in regulating cancer cell growth and metabolism. Glycosylation inhibited PFK1 activity and redirected glucose flux through the pentose phosphate pathway, thereby conferring a selective growth advantage to cancer cells. Blocking glycosylation of PFK1 reduced cancer cell proliferation *in vitro* and impaired tumor formation *in vivo*. These studies reveal a previously uncharacterized mechanism for the regulation of metabolic pathways in cancer and a possible target for therapeutic intervention.

Mon-Glycoproteomics

013: Keynote Lecture: Oligosaccharide Microarrays: Past, Present and Future Tools in Glycoscience

Ten Feizi; The Glycosciences Laboratory, Imerial College London, W12 0NN, United Kingdom
t.feizi@Imperial.ac.uk

Oligosaccharide microarrays have revolutionized the molecular dissection of carbohydrate-protein interactions. With the increased awareness that oligosaccharides are involved in diverse molecular interactions in health and, directly or indirectly, in the majority of disease processes, oligosaccharide microarrays have become essential tools in the biomedical sciences.

Dating from work with human *Mycoplasma pneumoniae* infection and the monoclonal antibodies that it elicits to its carbohydrate receptors, our speciality has been the discovery of oligosaccharide ligands for proteins involved in innate and acquired immunity, pathogen-host interactions and in endogenous recognition systems.

The oligosaccharide microarray system we established in 2002 for sequence-defined oligosaccharides is based on the neoglycolipid (NGL) technology, which we introduced in 1980s whereby oligosaccharides chemically linked to a long-chain lipid are immobilized and probed for protein-recognition in conjunction with sequence determination by mass spectrometry. I will present highlights among them discoveries of hitherto unsuspected ligands arising from screening analyses; these include: host-cell binding proteins for infective agents such as Simian Virus 40 (SV40), human JC virus, the pandemic H1N1 2009 influenza virus, parasites of the Apicomplexan family that include *Toxoplasma gondii*, and a monoclonal antibody AE3 directed to a cancer-associated epithelial antigen. I will also discuss designer arrays such as those that enabled the discovery of sulphated ligands for the selectins, assignments of those for Dectin-1 and other glucan-recognition systems of biological and medical importance, including malectin, a newly discovered resident protein in the endoplasmic reticulum of all animals, and potentially therapeutic antibodies elicited following experimental vaccination with fungal polysaccharides. I will proceed to discuss our aspirations for *meta*glycomic arrays in the future.

The Carbohydrate Microarray Facility in the Glycosciences Laboratory is a Wellcome Trust-supported Biomedical Resource to serve the biomedical community.

014: Age- and sex-associated differences in the glycopatterns of human salivary glycoproteins and their roles against influenza A virus

Yannan Qin[1], Yaogang Zhong[1], Minzhi Zhu[1], Liuyi Dang[1], Zhuo Chen[1], Hanjie Yu[1], Xiurong Wang[2], Hua Zhang[3], Zheng Li[1]; [1]Laboratory for Functional Glycomics, College of Life Sciences, Northwest University, Xi'an, 710069, China, [2]National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Science, Harbin, 150001, China, [3]Department of Oncology, Shaanxi Provincial People's Hospital, Xi'an, 710068, China
zhengli@nwu.edu.cn

Recent studies have elucidated that expression of certain glycoproteins in human saliva are increased or decreased according to age, meanwhile, human saliva may inhibit viral infection and prevent viral transmission. However, little is known about the age- and sex-associated differences in the glycopatterns of human salivary glycoproteins and their significant roles against influenza A virus (IVA). Here we investigate the glycopatterns of human salivary glycoproteins with 180 healthy saliva samples divided into six age/sex groups using lectin microarrays and fabricate saliva microarrays to validate the terminal carbohydrate moieties of glycoproteins in individual saliva samples. Furthermore, we assess the binding activity of saliva against two strains of influenza A (H9N2) virus. We find that seven lectins (*e.g.*, MAL-II and SNA) show significant age differences in both females and males, and seven lectins (*e.g.*, WFA and STL) show significant sex differences in children, adults and elderly people. Interestingly, we observe that elderly individuals have the strongest resistance to IVA mainly by presenting more terminal α2-3/6-linked sialic acid residues in their saliva, which bind with the influenza viral hemagglutinations. We conclude that age- and sex- associated differences in the glycopatterns of human salivary glycoproteins may provide pivotal information to help understand some age related diseases and physiological phenomenon.

015: Analysis of glycan and lectin arrays printed on functionalized ITO slides by Mass Spectrometry and Fluorescence

Ana Beloqui[1], Javier Calvo[2], Manuel Martin-Lomas[1,3], Niels-Christian Reichardt[1,3]; [1]Biofunctional Nanomaterials Unit, CICbiomaGUNE, Donostia-San Sebastian, 20009, Spain, [2]Mass Spectrometry Platform, CICbiomaGUNE, Donostia-San Sebastian, 20009, Spain, [3]*CIBER-BBN,* Donostia-San Sebastian, 20009, Spain
nreichardt@cicbiomagune.es

Microarrays are now the preferred format for most high-throughput screening applications in genomics, proteomics and glycomics and the ability to analyze them by more than a single readout method is desirable to broaden their applications.[1] We have prepared micrometer sized glycan and lectin arrays on functionalized indium tin oxide (ITO) slides that can be analyzed by fluorescence spectroscopy, MALDI-Tof-MS and optical microscopy without a change of the format. No prior tagging of analytes is required[2] and ligand libraries can be printed directly onto a non-covalent sandwich composed of activated bi-dentate lipids embedded in a hydrophobic layer conjugated to ITO. These array surfaces are transparent, conductive and very resistant to repeated aqueous washing steps. Moreover, this effective ligand immobilization by hydrophobic interactions provided the signal intensity required for the mass spectrometric readout of

micrometer-sized spots including the structural assignment of enzymatic modifications by MS/MS fragmentation and peptide sequencing by MALDI-Tof MS.

To demonstrate its scope this platform was applied to study the action of seven recombinant glucosyltransferases on multi-antennary N-glycan acceptors, assign the specificity of a fucosyltransferase by on-chip product fragmentation, profile the proteins bound to a lectin array by mass spectrometry and to identfy a lectin bound to a glycan array by on-chip tryptic digestion and *in situ* sequencing of peptide fragments.

[1] a)P. Jonkheijm, D. Weinrich, H. Schröder, C. M. Niemeyer, H. Waldmann, *Angew. Chem. Int. Ed.* **2008**, *47*, 9618–9647; b)A. J. Vegas, J. H. Fuller, A. N. Koehler, *Chem. Soc. Rev.* **2008**, *37*, 1385–1394.
[2] a)F. A. Jaipuri, B. Y. Collet, N. L. Pohl, *Angew. Chem. Int. Ed.* **2008**, *47*, 1707–1710; b)S.-H. Chang, J.-L. Han, S. Y. Tseng, H.-Y. Lee, C.-W. Lin, Y.-C. Lin, W.-Y. Jeng, A. H. J. Wang, C.-Y. Wu, C.-H. Wong, *J. Am. Chem. Soc.* **2010**, *132*, 13371–13380; c)T. R. Northen, J.-C. Lee, L. Hoang, J. Raymond, D.-R. Hwang, S. M. Yannone, C.-H. Wong, G. Siuzdak, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 3678–3683.

016: Identification of metastasis related glycans on breast cancer cells

Shu-Min Zhou[1,2], Li Cheng[1,2], Shu-Juan Guo[1,2], Sheng-Ce Tao[1,2†]; [1]Shanghai Center for Systems Biomedicine, Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200240, China, [2]State Key Laboratory of Oncogenes and Related Genes, Shanghai, 200240, China
taosc@sjtu.edu.cn (S.-C. T.)

Membrane-localized glycans which usually conjugated to proteins and lipids are modifiers of cellular motility processes and thus are of crucial relevance for the onset of cancer metastasis. The aim of this study was to identify altered glycans which take the responsibilities in the metastasis of breast carcinoma cells. To this end, human breast carcinoma cell lines, MDA-MB-231 and its single cell progenies (SCPs) of different metastatic abilities were examined by lectin microarray with 91 lectins. As a result, we found lectins, PTL II, RCA-I and black bean crude express an increasing binding with the rising of metastatic ability of breast cancer cells. In contrary, lectins, VFA, PSA and WGA express a decreasing binding with the rising of metastatic ability of the same cells. And most interestingly, WFA, a lectin binds to glycans terminating in N -acetylgalactosamine linked alpha or beta to the 3 or 6 position of galactose, showed a different binding to the breast cancer cells of close metastatic ability, but different organ-specificities, which indicate that the glycans recognized by WFA plays a role in the tropism of metastatic breast tumor cells. To our knowledge, we are the first one to show the global picture of membrane- localized glycans related to tumor metastasis in breast cancer. We believe the results from this study will help us to pave the way for studying the correlation of aberrant glycans and breast cancer metastasis, and the underlying mechanism.

References:
1. Tao SC, Li Y, Zhou J, Qian J, Schnaar RL, Zhang Y, Goldstein IJ, Zhu H, Schneck JP. Lectin microarrays identify cell-specific and functionally significant cell surface glycan markers. Glycobiology. (2008); 18(10):761–9.
2. Zhou SM, Cheng L, Guo SJ, Zhu H, Tao SC. Lectin microarrays: a powerful tool for glycan-based biomarker discovery. Comb Chem High Throughput Screen.(2011); 14(8):711–9.
3. Yang L, Guo S, Li Y, Zhou S, Tao S. Protein microarrays for systems biology. Acta Biochim Biophys Sin (Shanghai). (2011); 43(3):161–71.
4. Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. Cancer Cell (2011); 19(2):192–205.

017: A Computational Framework for Identification and Quantification of Intact Glycopeptides in Complex Proteome Samples

Haixu Tang[1*], Anoop Mayampurath[1], Chuan-yih Yu[1], Ehwang Song[2] and Yehia Mechref[2,*]; [1]School of Informatics & Computing, Indiana University, Bloomington IN USA 47408, [2]Department of Chemistry, Texas Tech University, Lubbock TX USA 79409
hatang@indiana.edu (Haixu Tang) or Yehia Mechref: yehia.mechref@ttu.edu

Glycosylation is an important protein modification that involves the attachment of sugars to amino acid residues. The functional roles of glycoproteins include cell signaling and immune response. Thus, understanding the structure of sugars and effects of glycosylation are vital for developing indicators of disease. Although computational methods based on mass spectrometry data have proven to be effective in monitoring changes in the glycome, developing such methods for the glycoproteome can be challenging, largely due to the inherent complexity in studying glycan structures in tandem with corresponding glycosylation sites. Here, we present a computational framework for identifying *intact glycopeptides*, *i.e.* glycopeptides with glycans attached to

their glycosylation sites, in complex proteome samples. Scoring algorithms are presented for tandem mass spectra of glycopeptides resulting from Collision-Induced Dissociation (CID), High-energy C-trap Dissociation (HCD) and Electron Transfer Dissociation (ETD) fragmentation methods. An empirical false-discovery rate (FDR) estimation method is derived based on a target-decoy search approach to assign confidence to the identified glycopeptides. The power of our method is further enhanced when multiple datasets are pooled together from technical and biological replicates. Using this framework, a considerable number (~100) of highly confident glycopeptides are identified from complex human serum proteome samples using conventional liquid chromatography mass spectrometry platforms without any further glycopeptide enrichment. Finally, we developed a novel linear statistical model for quantifying and comparing site-specific glycosylation events across multiple samples. We applied the model to an esophagus cancer study based on blood serum samples to discover potential glyco-biomarkers that show significant abundance alteration at glycopeptide level, but not at the corresponding whole glycoprotein levels. A total of five glycoproteins were detected in this study showing significantly different abundances at site-specific glycosylation levels within cancer/control samples, indicating that our method is ready to be used for the discovery of biomarkers on site-specific protein glycosylations for human disease. Our framework was implemented in a new software tool called GlycoFragWork, which analyzes multiple pre-aligned LC-MS/MS datasets, and reports a list (termed as a *glycomap*) of identified intact glycopeptides with their mass, elution time and abundances. The software will be freely available at the conference.

018: Keynote Lecture: Enabling glycoanalysis with informatics: discovery of glycans involved in infection

Arun Everest-Dass, Wai Yuen Cheah, Terry Nguyen-Khuong, Liisa Kautto, Robyn Petersen, Frederique Lisacek, Daniel Kolarich, Matthew P. Campbell, Nicolle H Packer; Macquarie University, Australia
arun.dass@mq.edu.au

As has been the case in proteomics and genomics, an essential requirement for glycomics and glycoproteomics to progress out of our relatively small community into the greater scientific sphere is the development of informatics tools to interpret and store diverse experimental glycan data and enable public accessibility. Ultimately the analytical data must relate to the function of these glycans and their glycoconjugates.

There currently are three main analytical approaches to the analysis of protein glycosylation which are at various levels of technological development: Glycomics (the global structural analysis of N- and O-glycans attached to proteins); Glycoprotein (glycan structure and site analysis of a single protein); and Glycoproteomics (glycan structure and site analysis of complex mixtures of glycoproteins).

Glycomics analysis still gives the most detailed structural information in terms of the branching, linkage and overall topology of the surface of the cell as it presents to the surrounding environment. In fact all the proteins produced in one cell type carry the same glycomic profile, since all are products of the same intracellular glycosylation machinery. In particular, the human epithelial cells and secreted fluids display a dense, heterogeneous array of cell specific glycan structures to invading microorganisms. I will present the analytical strategy and informatics tools we are developing for glycomics analysis, and give some examples of how specific structures affect the adhesion of different microbes.

019: The use of Glycoproteomics for the discovery of biomarkers of HCC-From GP73 to the development of biomarkers of AFP negative cancer

Anand Mehta[1], Mary Ann Comunale[1], Mengjun Wang[1], Lucy Betesh[1], Timothy Block[1]; [1]Drexel University College of Medicine, Doylestown, PA 18902, United States of America.
anand.mehta@drexelmed.edu

Hepatocellular carcinoma (HCC), caused by infection with hepatitis B virus (HBV) (and/or hepatitis C virus), is one of the most common solid malignancies worldwide. Despite advances in medical technology, the 5-year survival rate is 8–13 %, likely due to the fact that the majority of patients with HCC are diagnosed at advanced stage. Currently, alpha-feto protein (AFP) is used in combination with several imaging methodologies to help identify HCC. Unfortunately, AFP is elevated only in 40–60 % of patient with HCC, limiting its usefulness. In this setting of a significant increase in the number of patients with HCC, early detection and treatment are vital to improve outcome. The association of carbohydrates with cancer has long been known and we were one of the first groups to combine glycomic strategies with proteomic methods to identify modified glycoproteins in cancer. One such protein was Golgi protein 73 (GP73), which has shown great promise as a biomarker of HCC and is now commercially available in China.

However, GP73 has limitations and is better in the detection of AFP positive cancers than in the detection of AFP negative cancers. Thus in this study, we have performed biomarker discovery in AFP and GP73 negative, HCC positive

individuals. Our discovery effort involved the examination of the N-linked glycans on serum proteins following resolution *via* two dimensional electrophoresis (2DE). This approach has led to the discovery of specific change in glycosylation that were associated with AFP negative HCC. The changes that were observed included increased levels of alpha 2,6 linked sialic acid and increased level of branching on several proteins. These specific glycoforms were subsequently analyzed in the serum of a large patient cohort. Together, with AFP, these new biomarkers could be used to identify close to 100 % of those with HCC.

020: LC-MS/MS Quantification of Neu5Ac, Neu5Gc and KDN Levels in Urine and Potential Health & Disease in 3~5 Years Old Children

Yue Chen[1], Lili Pan[1], Ni Liu[1], Frederic A. Troy II[1,2], Bing Wang[1,3]; [1]School of Medicine, Xiamen University, Xiamen City 361102, China; [2]Department of Biochemistry and Molecular Medicine, University of California School of Medicine, Davis, CA 95616, USA; [3]School of Molecular Biosciences, The University of Sydney, NSW 2006, Australia
bing.wang@sydney.edu.au/bwang@xmu.edu.cn

Sialic acids (Sias) are a diverse family of sugars based on the parent compound, neuraminic acid, a nine-carbon sugar acid. *N*-acetylneuraminic acid (Neu5Ac) and its hydroxylated form, *N*-glycolylneuraminic acids (Neu5Gc), are the two major forms of Sia in mammal. A less common Sia is ketodeoxynonulosonic acid (KDN) that occurs frequently in lower vertebrates. Previous studies show that Sia is an essential nutrient for neural structure and function. Red meat and dairy products contain relatively higher Sias, including non-human Sias Neu5Gc, which correlate with human inflammatory diseases including atherosclerosis and cancer, but the metabolic fate and health impact in children remains unknown. Our aim is to quantify the levels of urinary Sias, Neu5Ac, Neu5Gc, KDN, and to discuss health & disease risk of preschool children in China.

Spot urine was collected from 386 healthy children at the ages of 3 (n=108), 4 (n=144) and 5 (n=134) at 6:30~7:00, 11:30~12:00, and 16:30~17:00. Children were provided breakfast, lunch and afternoon dessert from 7:30 to 17:30 and food intake was recorded on the day of urine collection. Sia levels were quantified using LC-MS/MS. This study was approved by the Human Ethics Committee of Xiamen University, with written informed consent from all subjects.

Our results showed that total urinary Sia levels in healthy preschool children ranged from 40~79 mmol Sia/mol creatinine. The predominated form of urinary Sia levels were conjugated Neu5Ac (~70.8 %), followed by free Neu5Ac (~21.3 %), conjugated KDN (~4.2 %) and free KDN (~3.7 %). Neu5Gc was detected in the urine of only one 4 year-old girl. Total urinary Sia levels were highest in the morning and declined over time in the 4 and 5 year-old children (P<0.05), but not 3 year-old children. We also found 3 year-old children had a relatively higher Sia intake at breakfast, lunch and dessert than other age-groups, their urinary Sia concentration was lower than that of 5 year-old children. The age difference of urinary Sia suggests that metabolism and utilization rates of dietary Sia are age dependent.

This study was supported by a research grant from the School of Medicine of Xiamen University and Nestle Research Center, Beijing.

021: Quantitative Glycomics Reveals specific N-glycans change of cell-secreted proteomes in Metastasis of Epithelial Ovarian Cancer

Xingwang Zhang[1], Yisheng Wang[2], Yifan Qian[1], Shifang Ren[1], Congjian Xu[2], Jianxin Gu[1]; [1]Key Laboratory of Glycoconjugate Research Ministry of Public Health; Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai, P.R. China, 200032, [2]Obstetrics and Gynecology Hospital, Department of Obstetrics and Gynecology of Shanghai Medical School, Fudan University, Shanghai, P.R. China, 200032
renshifang@fudan.edu.cn

It has been known that glycans which conjugated to proteins of secretome play a prominent role in cell signaling, communication and migration. Quantitative and/or qualitative variations in glycans may result in aberrant glycosylation patterns, which are linked to cancer metastasis. In this study, we attempted to identify alterations of N-glycans involved in tumor metastasis in SKOV3(human serous ovarian cancer cell line) and its high metastatic derivative cell line. Using quantitative glycomics technology, we determined the detailed N-glycan profile of secreted proteins from this two ovarian cancer cell lines. Interestingly, the N-linked glycomic profiles revealed that the levels of some specific glycoforms like tri-antennary glycans that were identified altered in SKOV3ip compared to high metastastic derivative cell line. Real time PCR, western blotting analysis, transwell experiments and immunohistochemical study were further employed to validated these specific changes. All the results were in accordance with the change of the N-glycans revealed by quantitative glycomics methods. With the methods, we revealed specific N-glycans changes of cell-secreted proteomes associated with metastasis of epithelial ovarian cancer. Our data suggest alterations of these

glycoforms in human ovarian cancer cells correlate with cancer metastasis, and their glycan subclass could poteintially be used as a biomarker for metastatic prognosis. Also, this study are helpful to understand the mechanism of metastasis of epithelial ovarian cancer.

022: Monoclonal IgM cryoglobulins show patient specific glycosylation patterns that differ from average polyclonal human IgM

[1,2]Daniel Kolarich, [2,3]Pia H. Jensen, [4]Mariel Bartley, [4]Vicky Vallas, [4]William Farrugia, [2]Nicolle H. Packer, [4]Paul A. Ramsland; [1]Max Planck Institute of Colloids and Interfaces, Department of Biomolecular Systems, 14424 Potsdam, Germany, [2]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW 2109, Australia, [3]Institute of Molecular Medicine, University of Southern Denmark, 5230 Odense, Denmark, [4]Centre for Immunology, Burnet Institute, Melbourne, VIC 3004, Australia
daniel.kolarich@mpikg.mpg.de

Cryoglobulinaemia occurs in many clinical settings and is characterised by the presence of serum cryoglobulins (IgM and/or IgG) which form insoluble aggregates in the cold [1]. The clinical severity varies widely and correlates with the type of cryoglobulin (monoclonal or mixed Igs) and the physical nature of the aggregates (precipitate, gel or crystal). We present a comprehensive glycoproteomic study of two monoclonal IgM cryoglobulins (Pot [precipitating] and Yvo [gelling]) isolated from plasma of patients with Waldenström's macroglobulinemia [2], and compare this data with commercially available polyclonal IgM isolated from pooled normal human plasma.

Porous Graphitized Carbon (PGC) LC-ESI-IT-MS/MSn glyco-profiling of the glycan structures [3] revealed a significant difference between the two cryoglobulin IgMs and normal IgM. The majority of *N*-glycans in all three samples contained bisecting GlcNAc and core α1,6 fucosylation. Both the Pot and Yvo IgM cryoglobulins showed a higher amount of neutral N-linked oligosaccharides, with considerable amounts of oligomannosidic and hybrid type structures, which were found to just constitute only a minor fraction of the normal IgM *N*-glycans. There was no indication of cryoglobulin O-glycosylation.

Analysis of IgM heavy chain glycopeptides was performed after HILIC enrichment using LC-ESI-Q-TOF-MS and LC-ESI-IT-MS/MS^n as described previously [4]. Combining data derived from Collision Induced Dissociation (CID) and Electron Transfer Dissociation (ETD) experiments enabled simultaneous identification and characterisation of peptide sequences and the glycan(s) structures at specific attachment sites. In addition, the *N*-glycan distribution at specific sites on the peptide was determined from the MS-scan spectra using the software tool GlycoSpectrumScan (www.glycospectrumscan.org) [5]. The three N-glycosylation sites closer to the N-terminus of the protein were found to carry mostly complex type *N*-glycans, whereas the more C-terminally positioned sites preferentially carried high mannose type structures. Despite overall structural *N*-glycan differences this glycan type distribution pattern was similar in all three IgM preparations analysed. This study presents the first comprehensive glycoproteomic characterisation of IgM heavy chains clearly showing that glycosylation of this important immunoglobulin is affected in disease.

References:
[1] Ramsland PA, Terzyan SS, Cloud G, Bourne CR, Farrugia W, Tribbick G, Geysen HM, Moomaw CR, Slaughter CA, Edmundson AB., Biochem J. **2006**; 395(3):473–81
[2] Vallas V, Farrugia W, Raison RL, Edmundson AB, Ramsland PA. J Mol Recognit. **2007**; 20(2):90–6.
[3] Jensen PH, Karlsson NG, Kolarich D, Packer NH., Nat Protoc. **2012**; 7(7):1299–310.
[4] Kolarich D, Jensen PH, Altmann F, Packer NH.; Nat Protoc. **2012**; 7(7):1285–98
[5] Deshpande N, Jensen PH, Packer NH, Kolarich D.; J Proteome Res. **2010**; 9(2):1063–75.

023: Keynote Lecture: Mass spectrometry-based strategies for qualitative and quantitative glycoproteomics

Haojie Lu and Pengyuan Yang; Department of Chemistry and Institutes of Biomedical Sciences, Shanghai, 200032, China
luhaojie@fudan.edu.cn

As one of the most ubiquitous post-translation modifications, glycosylation is involved in a variety of physiological and pathological processes. Qualitative and quantitative of the glycoproteome, which involves the identification and quantitation of protein components in various biological systems, therefore becomes an important area for biomarker research.

However, the huge dynamic range of proteins expressed in the complex biological always masks the detection of the inherent low abundance glycoproteins, and the glycan microheterogeneity further reduces the relative amount of glycopeptides and decreases the detection sensitivity. To

gain thorough insights into glycosylation and elucidate the functional relationship among proteins, efficient separation of glycoproteins for their analysis is absolutely necessary. Therefore, one of our research interests is the development of novel methods for highly specific and efficient capture of glycoproteins. Different kinds of boronic acid-functionalized nanomaterials have been developed and successfully applied to glyco-specific enrichment in large-scale glycosylation identification. Sensitivity for glycoproteins was improved by 1~2 orders using these newly synthesized nanocomposites. Especially, 165 new N-glycosylation sites were identified by our new methods. The other research interests of our group focuses on developing mass-spectrometry based strategies for the relative quantification of glycoproteins as well as the changes of occupancy of N-glycosylation at specific sites between healthy and diseased individuals. 18O atom incorporated into the N-glycosylation site of asparagines-linked sugar chains specifically *via* a N-glycosidase F (PNGase F)-mediated hydrolysis served as one isotope-code. Other labeling methods, for example 18O or iTRAQ, introduced an additional isotope-code. Thus, the relative quantities of N-glycosylated and its parent protein levels were obtained simultaneous by measuring the intensity ratios of 18O/16O for glycosylated peptides and 18O/16O or 114/115/116/117 for nonglycosylated peptides, respectively. A comparison of these two ratios was utilized to evaluate the changes of occupancy of N-glycosylation at specific sites. For example, N-glycosylation site ratios on serum haptoglobin (Hp) beta-chain in healthy individuals as well as patients with hepatitis B virus (HBV), liver cirrhosis (LC) and hepatocellular carcinoma (HCC) were quantified and glycosite ratios of VVLHPN#YSQVDIGLIK were observed to change significantly in HCC patients compared with LC and HBV patients.

024: Characterization of the Yeast Glycoprotoeme by Using Hydrophilic Affinity Enrichment Coupled to Mass Spectrometry

Long Yu[1], Xinmiao Liang[1]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China
souron@126.com

Glycosylation, which is a common and important post-translational modification of proteins in eukaryotic origanisms, involves in many cellular activities. It is essential to acquire the structure information of glycosylation, *i.e.*, the glycosylation site and the attached glycan structure, prior to understand the biological functions of glycosylation. We developed a bottom-up glycoproteomic strategy based on the hydrophilic affinity enrichment, where a customized hydrophilic material named Click TE-Cys was used for the selective enrichment of glycopeptides. We first evaluated the selectivity and the glycosylation site coverage of Click TE-Cys by using the immunoglobulin G (IgG) and horseradish peroxidase (HRP) as model glycoproteins, respectively. We have identified 21 IgG glycopeptides in the glycopeptide fraction enriched by Click TE-Cys from the IgG digest mixed with human serum albumin digest (w/w, 1:10). We also identified 11 glycopeptides with different peptide backbone from the enriched HRP glycopeptide fraction. Thereafter, we applied the enrichment method to the lysate of the baker's yeast *Saccharomyces cerevisiae*, where 174 glycosylation sites from 102 glycoproteins were identified. By comparison, only 48 glycosylation sites from 31 glycoproteins were identified from the yeast glycopeptide fraction enriched by concanavalin A-immobilized lectin affinity chromatography. These results indicated the superior selectivity and glycosylation site coverage of the Click TE-Cys material. We hope the Click TE-Cys material and the corresponding enrichment method could be a powerful tool for the glycoproteomics analysis.

025: The GlycoFilter: A Simple and Comprehensive Sample Preparation Platform for Proteomics, N-Glycomics and Glycosylation Site Assignment

Hui Zhou[1], John W. Froehlich, Andrew C. Briscoe, Richard S. Lee; [1]Department of Urology and The Proteomics Center, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115 USA
Hui.Zhou@childrens.harvard.edu

N-glycosylation, one of the most abundant protein post-translational modifications, is highly involved in many biological and pathological processes. The amount of glycoproteins, glycosylation site occupancy, and the glycoforms are well known to alter between normal and disease. Unfortunately, current strategies to study N-glycoproteins in complex samples are often distinct, focusing on either N-glycans, or N-glycosites enriched by sugar-based techniques such as the hydrazide beads or lectins. In this study we report a simple and rapid sample preparation platform, the GlycoFilter, which allows a comprehensive characterization of N-glycans, N-glycosites, and proteins in a single workflow. Both PNGase F catalyzed de-N-glycosylation and trypsin digestions are accelerated by microwave irradiation and performed sequentially in a single spin filter. Both N-glycans and peptides (including de-N-glycosylated peptides) are separately collected by filtration. The condition to effectively collect complex and heterogeneous N-glycans, including those with multiple sialic acid residues, was established on model glycoproteins, bovine ribonuclease B, bovine fetuin, and human serum IgG. With this platform, the N-glycome, and proteome of human urine and plasma were characterized. Furthermore, a total of

912 and 318 N-glycosites were identified from three pairs of urine and plasma samples, respectively. Many sites were defined unambiguously as partially occupied by the detection of both their sugar-modified and non-sugar-modified peptides (149 from urine and 75 from plasma), demonstrating that partial occupancy of N-glycosylation occurs frequently, even in healthy adults. Given the likely high prevalence and variability of partial occupancy, an unbiased approach that captures both the sugar-occupied and non-sugar occupied peptide will lead to improved glycoprotein quantifications.

026: Selective Enrichment of N-linked Glycopeptides Using Hydrophilic Interaction Chromatography-based Materials

Xiuling Li*, Bingcheng Yang, Yanyan Zhao, Xinmiao Liang; Key Lab of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Chinese Academy of Science,457 Zhongshan Road, Dalian 116023, China
lixiuling@dicp.ac.cn

N-linked protein glycosylation is the most common post-translational modification (PTM). However, characterizing this PTM is difficult for mass spectrometry (MS) because of low concentration of glycopeptides and suppression effect of non-glycosylated peptides. Therefore it is imperative to enrich glycopeptides prior to MS analysis. Hydrophilic interaction chromatography (HILIC) in solid phase extraction (SPE) mode has been increasingly employed to enrich glycopeptides in the last few years. Various types of N-linked glycopeptides can be selectively separated from non-glycosylated peptides and the glycosylation heterogeneity coverage is improved. However, the enrichment selectivity for glycopeptides is moderate because of co-elution of non-glycosylated peptides containing multiple serine/threonine residues or larger molecular weight. In order to improve the enrichment selectivity of glycopeptides, two types of novel HILIC-based materials including Click aspartic acid (Click Asp) and Click OEG-CD were synthesized in our group and applied to enrich N-linked glycopeptides. For the case of Click OEG-CD, N-linked glycopeptides could be selectively enriched through reversed phase depletion coupled with hydrophilic affinity enrichment. Compared to single HILIC mode, the method exhibited remarkably higher selectivity for glycopeptides. Concerning Click Asp, It demonstrated good hydrophilic property for separation of highly polar compounds. Click-Asp based HILIC SPE was observed to efficiently bind both neutral and sialylated glycopeptides with short to long peptide chain. The application of Click-Asp to simulated proteomic samples proved its specialty toward glycopeptides. The high hydrophilicity of these Click-based HILIC materials result from their unique structure constructed *via* simple click chemistry. We believe these HILIC materials will become promising tools in glycoproteomic studies.

027: Glycoproteomics in the diagnosis and understanding the pathogenesis of Rheumatoid Arthritis

Ashish Saroha[1,3], Sagarika Biswas[1], Sarvanan Kumar[1], Hasi R Das[1], Bishnu P Chatterjee[2*]; [1]Glycomics and Molecular Medicine Division, CSIR-Institute of Genomics and Integrative Biology, Delhi-110007, India, [2]Department of Natural Science, West Bengal University of Technology, Salt Lake, Kolkata-700064, India, [3]Department of Biological Chemistry, Weizmann Institute of Science, Rehovot-76100, Israel.
cbishnup@gmail.com

Glycosylation studies of plasma proteins can provide information about the onset and progression of disease. Rheumatoid Arthritis (RA) is an autoimmune, chronic systemic inflammatory disease characterized by swelling of synovial joint leading to there deformation and destruction. The most notable changes in the development of RA are alteration of glycosylation in plasma proteins and there level of expression. The increased level of agalctosylated IgG (IgG0) correlates with the disease severity of RA and thus showing its role in disease pathogenesis. Our study demonstrates plasma glycoproteins enrichment by *wheat germ* agglutinin (WGA) followed by 2DE. The protein spots were identified by MALDI-TOF-MS. Database search of MS-MS spectra identified alpha 1-acid glycoprotein (AGP), haptoglobin alpha 2 (Hp-α2) and haptoglobin beta 2 (Hp-β) chain respectively. High performance anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD) revealed the presence of significantly higher levels of GlcN ($p=0.02$), Gal ($p=0.01$) and Man ($p=0.0$) in plasma of AGP in RA patients. Similarly by HPAEC-PAD analysis showed the decreased amount of Man ($p<0.5$) in Hp-β chain of RA patients. These results were further validated by ELISA using ConA lectin which showed higher binding of ConA with AGP in RA plasma indicating possible increased expression of biantenary glycan. In another study *O*-linked glycoproteins in plasma of RA patients enriched by jacalin affinity chromatography followed by 2DE revealed a number of differentially expressed protein spots as compared to healthy control. Eighteen protein spots were found to have statistically significant ($p<0.5$) difference in their expression level from four sets of gel and were identified by MALD-TOF-MS. Most of the proteins were identified to be *O* glycosylated by Net-O-Gly 3.1 algorithm. Among these the alpha 2-HS glycoprotein (A2HSG) were found to be down regulated whereas inter alpha trypsin inhibitor 4 (ITIH4) was up regulated and this

was validated by western blotting. Thus glycoproteomics is a promising approach to discover novel glyco-markers in diagnosis of RA and understanding the disease pathogenesis.

Biosynthesis & Metabolism of Glycoconjugates

028: Keynote Lecture: Development of Glyco-enzyme Inhibitors

Chun-Hung Lin[1]; [1]Institute of Biological Chemistry, Academia Sinica, Taipei 115 Taiwan
chunhung@gate.sinica.edu.tw

Fucosyltransferases (FucTs) usually catalyze the final step of glycosylation and are critical to many biological processes. High levels of specific FucT activities are often associated with various cancers. Here I will discuss the development of a chemoenzymatic method for synthesizing a library of guanosine diphosphate β-L-fucose (GDP-Fuc) derivatives, followed by *in situ* screening for inhibitory activity against bacterial and human α-1,3-FucTs. Several compounds incorporating appropriate hydrophobic moieties were identified from the initial screening in a recent publication of *Advanced Synthesis & Catalysis* in 2012 (volume 354, pages 1750–1758). These compounds were then individually synthesized, purified and characterized in detail for their inhibition kinetics. Out of these products, one had a K_i of 29 nM for human FucT-VI, and is 269 and 11 times more selective than for *Helicobacter pylori* FucT (K_i=7.8 μM) and for human FucT-V (K_i=0.31 μM).

029: Sequencing pond life in the search for CAZymes

Ellis O'Neill,[a] Martin Trick,[a] Bernard Henrissat,[b] Robert A. Field [a*]; [a] Depts of Biological Chemistry and Computational and Systems Biology, John Innes Centre, Norwich, NR4 7UH, UK, [b] Lab Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, CNRS, UMR 7257, 13288 Marseille cedex 9, France
rob.field@jic.ac.uk

Euglena gracilis is a metabolically flexible protozoan alga with a complicated evolutionary history. Genetically, it is more closely related to the African trypanosome, *Trypanosoma brucei*, than it is to many other algae. Euglena is capable of growing to very high cell density and is capable of both photosynthesis and absorbing exogenous nutrients. It contains high levels of vitamins and essential amino acids and it produces polyunsaturated fatty acids. In contrast to plants, the storage polysaccharide in *Euglena* is paramylon, an insoluble β-1,3-glucan with interesting biomedical properties. Unexpectedly, the cell surface of Euglena is proposed to contain few carbohydrates. In order to gain greater understanding about the metabolic potential of Euglena, we have obtained *de novo* transcriptome sequencing data as a surrogate for full genome sequencing. The transcriptome sequences contain a wide variety of carbohydrate active enzymes, including many involved in β-glucan metabolism. An equally wide range of metabolic pathways are also encoded, including several natural product biosynthesis pathways that are unprecedented in these organisms.

030: 1,3/6-Linked Galactotrioses for the Identification of Arabinogalactan Biosynthetic Enzymes

Matilde Aguilar-Moncayo, Stephan Goetz, Robert A. Field.*; Department of Biological Chemistry, John Innes Centre, Norwich, NR4 7UH, United Kingdom
matilde.aguilar-moncayo@jic.ac.uk

Arabinogalactan-proteins (AGPs) are an abundant class of plant cell surface proteoglycans, which have been implicated in many processes involved in plant growth and development. The structure of AGPs contains highly complex and diverse polysaccharide units (arabinogalactan) with a β-(1→3)-D-galactan backbone and β-(1→6)-D-galactan side chains. In order to identify and characterize glycosyltransferases (GTs) involved in the biosynthesis of arabinogalactans, a methodology recently developed in our research group was applied. In this methodology, APTS-labelled oligosaccharides were used as suitable glycosyl acceptors in biochemical assays catalysed by GTs of plant cell membrane extracts, allowing the high sensitivity analysis of the resulting products by capillary electrophoresis with laser-induced fluorescence detection (CE-LIF).

In combination with the chemical synthesis of different galactotrioses as standards, previously unreported β-(1→3)-galactosyltransferases in the model plant *Arabidopsis thaliana* could be identified with the use of this methodology. Here we describe the preparation of three β-(1→3)/(1→6)-linked galactotrioses, their APTS-labelling and analysis by CE. The comparison of the elution times obtained for the synthetic standards and the products formed in biochemical reactions catalysed by over-expressed candidate galactosyltransferases confirmed the identity of the new glycosidic linkages.

031: Synthesis of sialyllactose derivatives by bio-conversion

Jae Kyung Sohng; Institute of Biomolecule Reconstruction (iBR), Department of Pharmaceutical Engineering, Sun Moon University, #100, Kalsan-ri, Tangjeong-myeon, Asansi, Chungnam 336-708, Korea
sohng@sunmoon.ac.kr

Sialylated sugar chains are present at the cell surface of various animal species. Due to their position, they are thought to serve important roles in a large variety of biological functions such as cell–cell and cell–substrate interactions, bacterial and virus adhesion, and protein targeting. We present a bio-conversion process for the conversion of N-acetylglucosamine and CMP into CMP-neuraminic acid with five enzymes. Key enzyme is *N*-acyl-D-glucosamine 2-epimerase from *Baceroides fragilis*. 2,3-Sialyllactose and 2,6-sialyllactose are synthesized by one-pot reaction from lactose, N-acetylglucosamine and CMP with CMP recycling. Vancomycin sialoside, biotin-sialoside, flavonoid sialoside and multiligand sialosides were synthesized by glycosylation, galactosylation and sialydation with CMP-neuraminic acid.
Acknowledgement: This research was supported by the Converging Research Center Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20090082333).

032: Keynote Lecture: *C. elegans* DPY-19 is a *C*-mannosyltransferase

Hans Bakker; Cellular Chemistry, Hannover Medical School, Hannover, 30625, Germany
Bakker.Hans@mh-hannover.de

C-mannosylation of tryptophan residues of the consensus sequence WXXW forms a unique type of glycosylation next to the more known *N*- and *O*-glycosylation. It is an abundant type of glycosylation typically, but not exclusively, occurring on proteins with thrombospondin type I repeats and on cytokine type I receptors. Mutagenesis of the WSXWS motif in cytokine receptors already established an important role of this motif for receptor function. The genetic base of the enzyme catalyzing *C*-mannosylation was so far elusive.

We identified *C. elegans* DPY-19 as the *C*-mannosyltransferase. DPY-19 is homologous to the catalytic subunit of oligosaccharyltransferase responsible for N-glycosylation. The overall membrane topology is identical and amino acids involved in the binding of the common dolichol-phosphate moiety of the donor are conserved. Overall identity is, however, very limited.

DPY-19 was already known from a *C. elegans* mutant defective in neuroblast migration. *In vitro* assays showed that the *dpy-19* mutant is devoid of *C*-mannosyltransferase activity. Moreover, by recombinant expression of DPY-19 and mass spectrometric analysis, we could show *C*-mannosylation of *C. elegans* proteins UNC-5 and MIG-21, which both contain two thrombospondin type I repeats with a WXXWXXW sequence. Lack of mannosylation of MIG-21 in the *dpy-19* mutant is most likely responsible for the neuroblast migration phenotype. *Dpy-19* and *mig-21* mutants have the same neuroblast migration defect.

033: *In vitro* catalytic properties of ER α-mannosidase I

Jun-ichi Aikawa[1], Yoichi Takeda[2], Ichiro Matsuo[3], Yukishige Ito[1, 2]; [1]Synthetic Cellular Chemistry Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama, 351-0198 JAPAN, [2]ITO Glycotrilogy Project, ERATO, JST, 2-1 Hirosawa, Wako, Saitama, 351-0198 JAPAN, [3]Graduate School of Engineering, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma, 376-8515 JAPAN
aikawa@riken.jp

Backgrounds: Processing of asparagine-linked sugar chains of newly synthesized glycoproteins is essential for those glycoproteins to be delivered to the final destination. In this process, trimming reaction of α-1,2 linked mannose moieties is catalyzed by members of glycosylhydrolase family 47, which include ER α-1,2 mannosidase I (ERManI), Golgi α-1,2 mannosidase IA-C, and EDEM1-3.

We had synthesized various high-mannose type oligosaccharides by our convergent methods and utilized them to analyze biochemical characteristics of proteins involved in ER quality control[1]. As a part of the studies, we had revealed that a recombinant form of human ERManI had an activity to generate smoothly $Man_{6\text{-}5}GlcNAc_2$ from $Man_9GlcNAc_2$[2]. In addition, hERManI removed more mannose moieties from some glycoproteins, when they had been exposed to denaturing conditions[2, 3]. In this study, we have examined to reveal influencial factors and reaction conditions which could accelerate trimming of mannoses from the oligosaccharides by ERManI in living organisms.

Methods: Pyridylaminated $GlcMan_9GlcNAc_2$ was prepared from oligosaccharides in chicken immunoglobulin Y[3]. Mannotrioses of Man(α-1,2)Man(α-1,2)Man-dansyl[2], Man(α-1,2)Man(α-1,3)Man-dansyl, and Man(α-1,2)Man(α-1,6)Man-dansyl were synthesized. A solution of polyethyleneglycol (PEG) was included in each de-mannosylation reaction[2].

Results: First, chemicals to induce macromolecular crowding effects were included in the reaction. As a result, removal of mannoses from $GlcMan_9GlcNAc_2$-PA was markedly accelerated in the presence of 50 % PEG 400. The result may suggest that de-mannosylation by hERManI could be enhanced under macromolecular crowding circumstances.

Second, we have revealed that all three mannotrioses were susceptible to hERManI. The reactivity of hERManI to Man(α-1,2)Man(α-1,6)Man was, however, much lower than the two other compounds, suggesting that the efficiency of de-mannosylation by hERManI was highly dependent on the structure at the non-reducing end. When reactions were performed at pH 5–7, maximum processing of those compounds was observed around pH 5.0. In addition, hERManI exhibited highest efficiency at pH 5.5 of de-mannosylation from heat-treated immunoglobulin Y. These results indicated that de-mannosylation by hERManI was accelerated under acidic conditions.

[1]Takeda, Y. *et al. Curr. Opin. Chem. Biol.* **13**, 582–591 (2009)
[2]Aikawa, J. *et al. Glycoconj. J,* **29**, 35–45 (2012)
[3]Aikawa, J. *et al. 26th International Carbohydrate Symposium* (2012)

034: Expression regulation of glycosyltransferase gene β3Gn-T8 by transcription factor c-jun in gastric cancer cell line SGC-7901

Zhi Jiang[1], Lan Xu[1], Ying-hui Zhou[1], Shi-liang Wu*[1]; Department of Biochemistry and Molecular Biology, School of Medicine,Soochow University, Suzhou, China 215123
wushiliang@suda.edu.cn

Objective: To clarify the expression regulation of glycosyltransferase gene β3Gn-T8 by transcription factor c-jun in gastric cancer cell line SGC-7901. Methods: Online prediction of the transcription factor c-jun binding sites in the β3Gn-T8 promoter region was performed with a transcription factor database. Eight β3Gn-T8 promoter segments and one mutated segment were cloned by PCR from the gastric cancer cell line SGC-7901 genomic DNA. Then, they were used to construct recombinant plasmids with luciferase reporter gene respectively. The above β3Gn-T8 promoter segment reporter gene expression vectors, as well as an internal control plasmid pRL-SV40 and transcription factor expression vector pCI-neo-jun were co-transfected into the SGC-7901 cells. A Dual-Luciferase Reporter Assay System was used to detect the activation of the β3Gn-T8 promoter by the transcription factor c-jun. Whether the activation relied on the c-jun binding site (-561/+8) of β3Gn-T8, and whether it was dose-dependent on c-jun was also observed. Furthermore, the binding of c-jun protein to β3Gn-T8 DNA in gastric cancer cells was detected using chromatin immunoprecipitation (ChIP). Results: The transcription factor c-jun binding sites resided within the β3Gn-T8 promoter region and could activate the transcriptional of β3Gn-T8. pGL3-luc (-561/+8) showed the highest luciferase activity, which was dose-dependent on c-jun. The ChIP results verified the binding of c-jun to β3Gn-T8 DNA fragments. Conclusion: The transcription factor c-jun is involved in the expression regulation of β3Gn-T8 in the gastric cancer cell line SGC-7901.

035: Epigenetic regulation of glycosyltransferase gene expression

Yasuhiko Kizuka[1,2], Shinobu Kitazume[1,2], Naoyuki Taniguchi[1, 2, 3]; [1]Disease Glycomics Team, [2]RIKEN-Max Planck Joint Research Center, RIKEN, Wako, 351-0198, Japan;[3]Alliance Laboratory of ISIR, Osaka Univ. and RIKEN, Ibaraki, 567-0047, Japan.
y.kizuka@riken.jp

Glycan expression is regulated by several factors such as nucleotide-sugars, acceptor substrate molecules, glycosidase *etc.* Expression level of glycosyltransferase is a key factor to control glycan expression, and the tissue-specific regulation of glycosyltransferase expression is indispensable for distinct glycan biosynthesis in tissues. However, it is poorly understood how each glycosyltransferase is expressed in a tissue specific manner, suggesting that unidentified mechanisms exist to regulate glycosyltransferase gene expression.

Recently, we have reported that the expression of one of brain-specific glycosyltransferases, GnT-IX (also designated as GnT-Vb), is epigenetically regulated at chromatin level (Kizuka *et al.* J. Biol. Chem. 2011). Particularly, active and repressive histone modification marks are well-correlated with the GnT-IX mRNA expression level in a tissue or cell-type specific manner, indicating that brain specific GnT-IX expression is regulated by an epigenetic mechanism. So far, however, little is known about detailed mechanisms of how glyco-genes including GnT-IX are regulated epigenetically.

In this study, we investigated epigenetic mechanisms of how chromatin of GnT-IX gene is activated. In addition, epigenetic regulation mechanisms of other glycosyltransferase genes are also analyzed including GnT-III, -V and Fut8. As target epigenetic factors, we focused on 1) HDAC (histone deacetylase) that downregulates histone acetylation, 2) histone methylation particularly at histone H3 lysine 27 that is involved in gene repression, 3) TET (ten-eleven translocation) that is required for hydroxylation of methylated DNA. As a result, we found that histone acetylation selectively activates GnT-IX gene among the glyco-genes tested while histone methylation down-regulates several glycosyltransferase genes in a cell type specific manner. In addition, it was revealed that TET enzyme is involved in

activation of the specific GnT genes. These results indicate that glycosyltransferase genes are systematically regulated by multiple epigenetic mechanisms and that each gene is distinctly regulated by different epigenetic factors.

036: Keynote Lecture: Plasma membrane glycohydrolases and new strategies for medical dignosis and therapeutic treatments

Sandro Sonnino, Nicoletta Loberto, Rosaria Bassi, Vanna Chigorno, Alessandro Prinetti and Massimo Aureli; Department of Medical Biotechnology and Translational Medicine, University of Milan, Italy

The following enzymes of the sphingolipid metabolism have been found associated to the external leaflet of the plasma membranes: sialidase, sialyl transferase, -hexosaminidase, -galactosaminyl transferase, β-galactosidase, β-glucosidase, sphingomyelinase, sphingomyelin synthase.

The association of glycosphingolipid metabolic enzymes with the plasma membranes, the information on their activity on natural substrates in living cells, the availability of couples of enzymes capable to catalyze opposite reactions, suggest that changes of glycosphingolipid structures at the plasma membrane could be the way to change rapidly the GSL plasma membrane concentration, to modify the glycosphingolipid pattern and to modulate cell functions.

All the enzymatic activities found associated to the plasma membranes result strictly related to the extracellular pH, being in general optimal under mild acidic conditions. Mild acidic conditions are found in specific membrane domains known as lipid rafts, where glycosphingolipids are highly enriched together with cholesterol, receptor proteins, proteins involved in cell signaling, and antiports or general ion exchange proteins. Several proton pumps are present on the cell surface and inserted into lipid rafts together with the glycohydrolases. Thus their activity and the following catabolic process at the cell surface, that under particular conditions can lead to apoptosis, can be increased by activating the proton pump and decreasing the local pH of their extracellular environment. In addition to this, it has been demonstrated that a cross talk among the glyoshingolipid hydrolases exists. This introduces the possibility to pharmacologically activate a single glycohydrolase to activate a series of enzymes working in sequence.

Changes of the cell surface glycosphingolipid hydrolase activities in sphingolipidosis and during radiotherapy will be presented and discussed.

037: Exploring the "life" of *N*-glycans on glycoproteins

Tadashi Suzuki; Glycometabolome Team, Systems Glycobiology Research Group, RIKEN Advanced Science Institute (ASI), Wako, Saitama 351-0198, JAPAN
tsuzuki_gm@riken.jp

Cell surface glycans can be regarded as a "face" of cells, and their structures are known to change depending on developmental stages or environment. Therefore cell surface glycans are utilized for identification of stem cells such as iPS/ES cells, or as valuable biomarkers in diagnosis/detection of cancer. To understand the molecular mechanism of "change of face", biosynthetic pathway for glycan chains on glycoproteins/glycolipids has been, in most part, revealed, while there are still many unresolved issues on their transport/degradation aspect.

We have been studying enzymes involved in the formation and degradation of free oligosaccharides derived from the *N*-glycans or their precursor (dolichol-linked oligosaccharides) in the cytosol, *i.e.* peptide:*N*-glycanase (PNGase) (1), endo-β-N-acetylglucosaminidase (ENGase)(2), and α-mannosidase (Man2C1) (3). These enzymes constitute a major pathway for the non-lysosomal catabolic processing of free oligosaccharides in mammalian cells. We have shown that free oligosaccharides in yeast can serve as a read-out of *N*-glycan processing for misfolded glycoproteins during a process called ER-associated degradation (ERAD) (4, 5).

In this symposium, current knowledge for catabolic pathway of glycans in *S. cerevisiae* will be presented. We will also present our new approach to visualize "glycoforms" of proteins of interest using transmembrane FRET (fluorescence resonance energy transfer) technique. This technique will help us understand how glycan structures can affect the stability and/or intra/intercellular trafficking of carrier proteins. We will also present our new approach to visualize "glycoforms" of proteins of interest using transmembrane FRET (fluorescence resonance energy transfer) technique (6). This technique will help us understand how glycan structures can affect the stability and/or intra/intercellular trafficking of carrier proteins (6, 7).

References

(1) Suzuki, T., *et al.* ***J. Cell Biol.*** 149, 1039 (2000); (2) Suzuki, T., *et al.* ***Proc. Natl. Acad. Sci. USA*** 99, 9691 (2002); (3) Suzuki, T., *et al.* ***Biochem. J.*** 400, 33 (2006); (4) Hirayama, H., *et al.* ***J. Biol. Chem.*** 285, 12390 (2010); (5) Hosomi, A., *et al.* ***J. Biol. Chem.*** 285, 24324 (2010); (6) Haga, Y., *et al.* ***Nature Commun.*** 3, 907 (2012); (7) Haga, Y., *et al.* ***J. Biol. Chem.*** 286, 31320 (2011).

038: Inhibition of nucleotide sugar transport in *Trypanosoma brucei* alters surface glycosylation

Li Liu,[1] Yu-Xin Xu,[1] [1] Kacey L. Caradonna,[2] Emilia K. Kruzel [3], Barbara A. Burleigh[2], James D. Bangs [3], and Carlos B. Hirschberg[1];** [1]Dept. of Molecular and Cell Biology, Boston University Goldman School of Dental Medicine, Boston, MA 02118, USA, [2]Dept.of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115, USA, [3]Dept. of Microbiology and Immunology, School of Medicine and Biomedical Sciences, University at Buffalo, SUNY, Buffalo, NY 14214, USA, [1**]Present address: Center for Human Genetic Research and Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA 02114, USA
liul@bu.edu

Nucleotide sugar transporters (NSTs) are indispensible for the biosynthesis of glycoproteins by providing the nucleotide sugars needed for glycosylation in the lumen of the Golgi apparatus. Mutations in NST genes cause human and cattle diseases and impaired cell walls of yeast and fungi. Information regarding their function in the protozoan parasite, *Trypanosoma brucei*, a causative agent of African trypanosomiasis, is unknown. Here, we characterized the substrate specificities of four NSTs, TbNST1-4, which are expressed in both the insect procyclic form (PCF) and the mammalian bloodstream form (BSF) stages. TbNST1/2 transport UDP-Gal/UDP-GlcNAc, TbNST3 transports GDP-Man, and TbNST4 transports UDP-GlcNAc, UDP-GalNAc and GDP-Man. TbNST4 is the first NST shown to transport both pyrimidine and purine nucleotide sugars and is demonstrated here to be localized at the Golgi apparatus. RNAi-mediated silencing of TbNST4 in PCF caused underglycosylated surface glycoprotein EP-procyclin. Similarly, defective glycosylation of the variant surface glycoprotein (VSG221) as well as the lysosomal membrane protein, p67 was observed in Δ*TbNST4* BSF *T. brucei*. Relative infectivity analysis showed that defects in glycosylation of the surface coat resulting from TbNST4 deletion were insufficient to impact the ability of this parasite to infect mice. Notably, the fact that inactivation of a single NST gene results in measurable defects in surface glycoproteins in different life cycle stages of the parasite, highlights the essential role of NST(s) in glycosylation of *T. brucei*. Thus, results presented in this study provide a framework for conducting functional analyses of other NSTs identified in *T. brucei*.

039: POFUT1/2 Promotes Embryonic Cell Adhesion and Migration at Fetal-Maternal Interface

Shuai Liu[1], Xuesong Yang[1], Jiao Wang[1], Xiaoqi Wang[2], Qiu Yan[1]; [1]Department of Biochemistry and Molecular Biology, Dalian Medical University, Liaoning Provincial Core Lab of Glycobiology and Glycoengineering, Dalian 116044, China; [2]Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
yanqiu63@126.com

Implantation is a complex developmental event that is initiated by the recognition and adhesion of the embryo to the endometrial epithelium. The fucosylation of glycoproteins both on the embryonic and uterine epithelial cell surface plays critical roles in the molecular interactions at fetal-maternal interface. Our previous study shows that the occurrence of N-linked fucosylated sugar chain, such as sialyl LeX (sLeX), is closely related to the adhesive potential of embryo to the endometrium. However the function of O-fucosylation in this process is not still clearly stated till now. In this study, the expression of protein O-fucosyltransferases 1 and 2 (POFUT1/2), which are the two subtypes of synthetic enzymes transferring GDP-Fuc to the Ser/Thr residues of the specific substrate protein molecules, was detected in human embryonic cells (Jar) and human uterine epithelial cells (RL95-2 and HEC), respectively. The results showed that both embryonic cells and uterine epithelial cells expressed POFUT1/2 by RT-PCR, Western blot and immunofluorescent assays. The POFUT1/2 expression level was dose-dependently regulated by leukemia inhibitory factor (LIF) in embryonic cells, and was correlated to the adhesive potential detected by adhesive percentage *in vitro* implantation model constituting by Jar and RL95-2. Furthermore, the elevated POFUT1/2 increased the invasive capability of embryonic cells by migration and matrix-degradation assays. The primarily study in mouse also showed that the level of POFUT1/2 in murine embryos changed dynamically with the different stages of embryo development. **Conclusion**: POFUT1/2 is expressed at fetal-maternal interface, and the elevated POFUT1/2 is related to the adhesion, invasion as well as developmental processes of embryo. They may be the important markers of reproductive functions and further study is needed.

Tue-Development & Differentiation

040: Keynote Lecture: Transcriptome and biochemical analysis reveals that suppression of GPI-anchor synthesis leads to autophagy and necroptosis in Aspergillus fumigatus

Jianghong Yan, Ting Du, Wan Zhao, Cheng Jin*; State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
jinc@im.ac.cn

Previously, it has been shown that GPI proteins are required for cell wall synthesis and organization in Aspergillus fumigatus, a human opportunistic pathogen causing life-threatening invasive aspergillosis (IA) in immunocompromised population. Blocking GPI anchor synthesis leads to severe phenotypes such as cell wall defects, increased cell death, and attenuated virulence. However, the mechanism by which the phenotypes are induced is unclear. To gain insight into global effects of GPI anchoring in A. fumigatus, in this study a conditional expression mutant was constructed and a genome wide transcriptome analysis was carried out. Our results suggested that suppression of GPI anchor synthesis mainly led to activation of the phophatidylinositol (PtdIns) signaling and ER stress. Biochemical and morphological evidences showed that autophagy and increased necroptosis were induced in response to suppression of the GPI anchor synthesis. Based on our results, we proposed that activation of PtdIns3K and increased cytosolic Ca2+, which was induced by both ER stress and PtdIns signaling, acted as the main effectors to induce autophagy and necroptosis.

This work was funded by the National Natural Science Foundation of China (31030025) to C. Jin.

041: Differential Targeting of the Dolichol Pathway Develops Uneven Cellular Responses

Zhenbo Zhang[1], Aditi Banerjee[1], Elena A. Carasquillo[1], Krishna Baksi[2] and Dipak K. Banerjee[1]; [1]Department of Biochemistry, School of Medicine, University of Puerto Rico, Medical Sciences Campus, San Juan, PR 00936-5067, USA; and [2]Department of Anatomy and Cell Biology, School of Medicine, Universidad Central del Caribe, Bayamon, PR 00960-3001, USA
dipak.banerjee@upr.edu

Assembly of $Glc_3Man_9GlcNAc_2$ on dolichol is the hallmark of asparagine-linked (N-linked) protein glycosylation as the angiogenesis for tumor progression and metastasis. The process is evolutionary conserved and contributes to the structural and functional integrity of the N-linked glycoproteins. Dynamics of the N-glycosylation process indicates the presence of a cross-talk between mannosyphospho dolichol synthase (DPMS) and N-acetylglucosaminyl 1-phosphate transferase (GPT). DPMS has a conserved phosphorylation motif and has been found to in all Dpm1 gene sequenced from 35 different species, *viz*., protozoan parasite to human. Phosphorylation activation of DPMS accelerates the $Glc_3Man_9GlcNAc_2$-PP-Dol (LLO) biosynthesis and turnover, and consequently the protein N-glycosylation and capillary endothelial cell proliferation, *i.e.*, angiogenesis. GPT activity is also increased. Cells transfected with overexpressing DPMS plasmid enhances the expression of GlcNAc-β-(1,3)-GlcNAc)1-4-β-GlcNAc-NeuAc containing glycans and increased angiogenesis. Removal of the phosphorylation motif by site-directed mutagenesis prevents DPMS activation and reduction of *S. cerevisiae* proliferation. Similarly, silencing of DPMS with shRNA down regulates glycan expression, angiogenesis as well as Matrigel™ invasion. Reduced PCNA, Bcl-2 and caspase-3 expression supports that DPMS silencing affects the cell cycle progression without inducing apoptosis. Dol-P-Man is an activator of GPT, and tunicamycin is a competitive inhibitor of GPT. When treated, tunicamycin inhibits DPMS activity in capillary endothelial cells without affecting the protein or the mRNA. The results are G1 arrest and the induction of apoptosis. Silencing of the transcription factor E2F1 has no additive or synergistic effect. Mechanistic details support development of "ER stress" and the cell deaths is due to *unfolded protein response* (upr). Raman Spectroscopy of the proteome at C-O stretching supports protein denaturation. cDNA microarray analysis indicates that among the affected genes, nearly 20 % are transcription factors. The developmentally regulated genes such as c-fos, c-myc and c-jun are all upregulated but not their down-stream regulators. *In vivo*, tunicamycin inhibits angiogenesis in Matrigel™ implants in athymic Balb/c (nu/nu) mice and prevents breast tumor progression. There is ER stress in tumor blood vessels, and reduced N-glycosylation correlates narrowing of the vessel diameter. Supported by grants from the NIH U54-CA096297 (DKB), Susan G. Komen for the Cure BCTR0600582) (DKB) and NIH/NIMHD 8G12MD007583 (KB).

042: GPI-anchor synthesis is indispensable for the germline development of the nematode Caenorhabditis elegans

Daisuke Murata[1,2,3], Kazuko H. Nomura[1,2], Ayako Matsuda[1], Sayaka Akiyoshi[1], Katsufumi Dejima[1,2,4],Lance Wells[5], Karen L. Abbott[6], Nana Kawasaki[2,7], Keiko Gengyo-Ando[2,8,9],Eriko Kage-Nakadai[8], Shohei Mitani[2,8], Kazuya Nomura[1,2]; [1] Department of Biological Sciences, Kyushu University Graduate School, Fukuoka 812-8582, Japan; [2] Core Research for Evolutional Science and Technology, Kawaguchi 332-0012, Japan; [3] Department of Pharmacology, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan; [4] JSPS Postdoctoral Fellow for Research Abroad, Division of Biological Sciences, University of California, San Diego, La Jolla California 92093-0368, USA; [5] Department of Chemistry, University of Georgia, Athens, Georgia 30605, USA; [6] Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30605; [7] Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Tokyo 158-8501, Japan; [8] Department of Physiology, Tokyo Women's Medical University School of Medicine, Tokyo 162-8666, Japan; [9] Saitama University Brain Science Institute, Saitama 338-8570, Japan
knomuscb@kyushu-u.org

GPI (glycosylphosphatidylinositol)-anchor attachment is one of the most common post-translational protein modifications, widely used in organisms from archaebacteria to humans. Pathogens such as *Trypanosoma cruzi* use GPI-anchor variants to escape from host immune surveillance, and the enzymes involved in their synthesis are potential drug targets for the treatment and prevention of disease. GPI-anchored proteins are concentrated in lipid rafts on the cell surface or membrane nanodomains and play essential roles in signal transduction in multicellular organisms. Using *C. elegans*, we determined that GPI-anchored proteins are present in germline cells as well as distal tip cells (DTCs), which are essential for maintenance of the germline stem cell niche. We identified 24 *C. elegans* orthologs of human genes involved in GPI-anchor synthesis by PSI-BLAST search and used RNAi and gene knockout strategies to inhibit their functions. Inhibition of genes involved in various steps of GPI-anchor synthesis resulted in abnormal development of oocytes and early embryos, and both lethal and sterile phenotypes were observed. The *PIG-A* gene codes for the major catalytic subunit (subunit A) of the phosphatidylinositol N-acetylglucosaminyltransferase complex (EC 2.4.1.198), which catalyses the first step of GPI-anchor synthesis. Mutations in this gene would thus inhibit all GPI-anchor synthesis. By isolating a deletion mutant allele of *piga-1* gene (*C. elegans PIG-A* ortholog), we showed that GPI-anchor synthesis is indispensable for the normal development of germline cells. Lack of GPI-anchor synthesis resulted in 100 % lethality with decreased mitotic germline cell number, abnormal oocyte formation and death of cleaving eggs. Using cell specific rescue of the null allele, we showed that expression of PIGA-1 in DTCs or in gonadal sheath cells is sufficient for germline cells to develop normally. These results clearly demonstrate that GPI anchor synthesis is indispensable for the normal development of oocytes and embryos in *C. elegans*. To elucidate the molecular mechanism of GPI-anchored proteins in germline development, we concentrated GPI-anchored proteins from wild type and DTC-rescued worms with alpha toxin column and PI-PLC treatment followed by nanoLC/MS/MS. Results of RNAi of these identified protein genes will be discussed in the paper.

043: Novel Marker Antibodies for Human iPS/ES Cells and their Application

Kawasaki, T.[1] Nakao, H.[1] Matsumoto, S.[1] Toyoda, H.[2] Kusuda Furue, M.[3] and Kawasaki, N.[1]; [1]Research Center for Glycobiotechnology, Ritsumeikan University, Shiga 525-8577, Japan; [2]Laboratory of Bio-analytical Chemistry, Faculty of Pharmaceutical Sciences, Ritsumeikan University, Shiga 525-8577, Japan; [3]Laboratory of Cell Cultures, Department of Disease Bioresources, National Institute of Biomedical Innovation, Ibaraki, Osaka 567-0085, Japan
tkawasak@fc.ritsumei.ac.jp

Carbohydrate recognizing antibodies are very useful tools for monitoring the specific glycans on a specific cell with high sensitivity and sharp specificity. Among the conventional human iPS (hiPS)/ES marker antibodies, SSEA-3 and SSEA-4 recognize specifically globosides, and TRA-1-60 and TRA-1-81 recognize keratan sulfate. However, most of these antibodies were generated against human embryonal carcinoma (hEC) cells. Consequently, these antibodies recognize those glycans, which are common to hiPS/ES and hEC cells. In order to obtain antibodies specific to hiPS cells, we immunized mice with a hiPS cell line, Tic, and selected hiPS-cell positive hybridomas. These hybridomas were then subjected to screening with hEC cells and hEC positive hybridomas were excluded. Upon Western blotting with antibody R-10G, one of these hiPS/ES specific antibodies, following SDS-PAGE, the Tic cell lysate indicated one major but broad immunoreactive band at the position corresponding to over 250 kDa. The antigen protein was isolated from the Tic cell lysate by using an affinity column of R-10G. The epitope was identified as keratan sulfate with little over-sulfation, since digestion of the R-10G antigen with keratanase, keratanase II and endo-beta-galactosidase

reduced completely the immunoractivity of the antigen protein. The carrier protein of the R-10G epitope on hiPS cells was identified as podocalyxin by liquid chromatography/mass spectrometry (LC/MS/MS) analysis of the R-10G positive-protein band material obtained on SDS-PAGE. The R-10G epitopes differ from those recognized by other human pluripotent-cell marker antibodies such as TRA-1-60 and TRA-1-81 and from high-sulfated keratan sulfate-recognizing antibodies such as 5D4 and BCD4, indicating that the R-10G should be a new useful tool for studying the roles of glycans on the cell surfaces. The R-10G epitope is distributed heterogeneously on hiPS cells, suggesting that a single colony of undifferentiated hiPS cells consists of different cell subtypes. Survey of a human tissue array indicated that the R-10G epitope is not confined to hiPS/ES cells but is expressed in the adult brain and cerebellum.

044: The role of multiple exopolysaccharides in the communities of *Pseudomonas aeruginosa* and their coordinate regulation

Luyan Ma; State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
luyanma27@im.ac.cn

Biofilms are surface-associated communities of microorganisms encased in an extracellular substances matrix. Exopolysaccharide is a critical biofilm matrix component. *Pseudomonas aeruginosa* can produce several biofilm matrix exopolysaccharides that include alginate, Psl, and Pel, which each has contributions in biofilm. The overproduction of exopolysaccharide alginate leads to the mucoid phenotype of *P. aeruginosa,* which is believed to play a role in the bacteria's resistance to antibiotics and to evade host defense mechanisms. Our data shows that alginate occupy a lot of space in biofilm, resulting in the muciod biofilm with high biofilm thickness, but little biomass. Psl is an essential matrix component for nonmucoid and mucoid *P. aeruginosa* to initiate and maintain biofilms. Pel is a glucose-rich exopolysaccharide, which is required to form air-liquid interface biofilms (pellicles). We demonstrated that AlgC, a key enzyme that provides sugar precursors for the synthesis of alginate and lipopolysaccharides (LPS) is also required for both Psl and Pel production. We showed that forced-synthesis of Psl in alginate-producing mucoid bacteria reduced alginate production but this was not due to transcription of the alginate biosynthesis-operon. Likewise, when either alginate or Psl were overproduced, levels of B-band LPS decreased. Induction of Pel resulted in a reduction of Psl levels. Since the effects of reduced exopolysaccharide synthesis when another is overproduced didn't appear to be regulated at the transcriptional level, this suggests that the biosynthesis pathways of Psl, Pel, alginate, and LPS compete for common sugar precursors. As AlgC is the only enzyme that provides precursors for each of these exopolysaccharides, we propose that AlgC is a key checkpoint enzyme that coordinates the total amount of exopolysaccharide biosynthesis by controlling sugar precursor pool. Our data also provide a plausible strategy that *P. aeruginosa* utilizes to modulate its biofilm matrix exopolysaccharides.

045: Keynote Lecture: Roles of Heparan Sulfate in Normal and Cancer Stem Cells in intestine

Minoru Fukuda; Glycobiology Unit, Tumor Microenvironment Program, Cancer Center, Sanford-Burnham Medical Research Institute, La Jolla California 92037, USA
minoru@sanfordburnham.org

Differentiation system of intestinal epithelia cells develops from normal stem cells residing crypt of the intestinal cells. From these, enterocytes, mucus secreting cells, and endocrine cells will be developed during the journey to the top of the villi. It has been shown that stem cells to normal differentiated cells develop into these cell types while stem cells maintain self-renewal. To determine the roles of heparan sulfate in intestinal cell development, we utilized Lgr5-driven inducible gene knockout, which was generated by Hans Clevers (Nature; 459: 262–265, 2009). After treatment on mouse with tamoxifen, heparan sulfate was significantly abrogated. As a result, the height of villus was shorter than $EXT1^{wt/wt}$ control mice, and resulted in fewer enterocyte and endocrine cells. Surprisingly, Ki-67 staining level was diminished in crypt cells of EXT1 knockout mice, suggesting that intestinal stem cells require heparan sulfate in stem cell proliferation, but heparin sulfate requirement was not obvious in enterocytes and endocrine cells in the villus domain.

In EXT1 knockout mouse, azoxymethane (AOM) and dextran sodium sulfate induced only adenoma while adenocarcinomas were apparently produced in wild-type mouse counterparts. The results strongly suggest that cancer stem cells also require heparan sulfate to produce progenies. Further work is necessary to determine the role of heparan sulfate in colon cancer formation, possibly by utilizing organoid culture. The work is support by NCI grant, P01CA71932

046: 3-*O*-sulfated heparan sulfate structure contributes to the differentiation of mouse embryonic stem cells by activating Fas signaling

Shoko Nishihara[1], Kazumi Hirano[1], Norihiko Sasaki[1], Toin H. Van Kuppevelt [2], Taichi Miura[1], Tomomi Ichimiya[1]; [1]Laboratory of Cell Biology, Department of Bioinformatics, Faculty of Engineering, Soka University, 1-236 Tangi-cho, Hachioji, Tokyo 192-8577, Japan; [2]Department of Biochemistry, Nijmegen Center for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
shoko@soka.ac.jp

Embryonic stem cells (ES cells) are derived from the inner cell mass of pre-implantation blastocysts, have the characteristic features of self-renewal and pluripotency, and are able to differentiate into all cell types deriving from the different germ layers. Maintenance of self-renewal and pluripotency in mouse ES cells is regulated by the balance between several extrinsic signaling pathways. Recently, we demonstrated that heparan sulfate (HS) chains play important roles in the maintenance and differentiation of mouse ES cells by regulating extrinsic signaling. Sulfated HS structures are modified by various sulfotransferases during differentiation of mouse ES cells. However, the significance of specific HS structures during their differentiation remains unclear. Therefore, we performed a screen by overexpression of various sulfotransferases in mouse ES cells and found that HS 3-*O*-sulfotransferases (3OSTs) contribute to the differentiation of mouse ES cells.

The 3-*O*-sulfated HS structure synthesized by 3OSTs and recognized by the antibody HS4C3 increased during differentiation of mouse ES cells. Expression of Fas on the cell surface of the differentiated cells also increased. Overexpression of the 3-*O*-sulfated HS structure in mouse ES cells induced apoptosis and spontaneous differentiation even in the presence of LIF and serum. Furthermore, knockdown of *3OST* and inhibition of Fas signaling reduced the potential for differentiation into the three germ layers during embryoid body formation. These data showed that the 3-*O*-sulfated HS structure was required for differentiation of mouse ES cells. Up-regulation of the 3-*O*-sulfated HS structure resulted in the recruitment of Fas from the cytoplasm to lipid rafts on the cell surface followed by activation of Fas signaling. The 3-*O*-sulfated HS structure interacted with a region that included the heparin-binding domain of Fas. Reduced self-renewal capability in cells overexpressing *3OST* resulted from the degradation of Nanog by activated caspase-3, which is downstream of Fas signaling, and was rescued by the inhibition of Fas signaling.

This is the first demonstration that activation of Fas signaling is mediated by an increase in 3-*O*-sulfated HS structure and indicates a novel signaling pathway for differentiation in mouse ES cells.

047: The expression of chondroitin sulphate glycosaminoglycans in the development of human foetal articular cartilage

Siyuan Li[1], Rongliang Xue[1], Antony J Hayes[2], Caterson Bruce[2], Clare E. Hughes[2] & Junling Cao[3]; [1]The Department of Anaesthesia, The Second Hospital of Xi'an Jiaotong University, Xi'an, 710004, China; [2]The Department of Pathophysiology and Repair, School of Biosciences, Cardiff University, CF10 3AX, UK; [3]The Institute of Endemic Diseases, Medical School of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061,China
foxlsy@163.com

Introduction: Our previous studies have illustrated that novel chondroitin sulphate sulphation motifs in glycosaminoglycans (GAGs) on proteoglycans can be used to identify early stages of stem/progenitor cell differentiation in the articular cartilage. However, the spatial-temporal expression profiles of these novel chondroitin sulphate sulphation motifs in GAGs during the development of human articular cartilage are largely unknown.

Materials & Methods: Articular cartilage from figure joints was obtained from aborted human fetuses aged 25 and 32 weeks, respectively. Articular cartilage from normal human figure joints aged 2-year-old was used as a postnatal control. The expression of novel chondroitin sulphated sulphation motifs including 3B3, 4C3, 6C3 and 7D4 were investigated immunohistochemically.

Results: 7D4 and 6C3 positive staining was observed in the articular cartilage from both foetal (25-week-old & 32-week-old) and juvenile figure joints (2-year-old). In 25-week-old foetal figure joints, 7D4 positive staining was found in the inter-territorial matrix in the surface layer of articular cartilage, and the cartilage near synovium. Differently, intensive 7D4 positive staining was observed in the superficial, upper middle zone, and deep zone of articular cartilage near subchondrol bone in the figure joints from 32-week-old foetus. Interestingly, 7D4 positive staining was only localised in the territorial but not inter-territorial matrix around some cell clusters in the deep zone of articular cartilage from the juvenile figure joints. 6C3 positive staining mainly localised in the territorial matrix of surface & middle zone of articular cartilage from 25-week-old foetal figure joints. However, its positive staining expanded to the inter-territorial matrix in the surface and deep zone of articular cartilage from the 32-week-old figure joints. In the

juvenile figure joints, 6C3 positive staining can be observed in both territorial and inter-territorial matrix across the whole depth of articular cartilage. There was no or very weak 3B3 or 4C3 positive staining in the articular cartilage from these figure joints.

Conclusion: The different expression profiles of these novel chondroitin sulphated sulphation motifs indicated their dynamics of distributions during articular cartilage development, which may be associated with distinct chondroitin sulphated proteoglycans in the extracellular matrix in the articular cartilage, and tissue mature as well as homeostasis.

048: Direct interaction of FGF2 with polysialic acid regulates the cell growth, but not cell survival

Sayaka Ono[1], Masaya Hane[1,2], Ken Kitajima[1,2], and Chihiro Sato[1,2]; [1]Bioscience and Biotechnology Center, Nagoya University; [2]Program for Leading Graduate Schools IGER, Nagoya University, Nagoya 464-8601, Japan
chi@agr.nagoya-u.ac.jp

Polysialic acid (polySia) is a unique polymer that spaciotemporally modifies the neural cell adhesion molecule (NCAM) in vertebrates. Recently, we demonstrated that polySia functions not only as an anti-cell adhesion molecule, but also as a reservoir for neurologically important molecules such as neurotrophic factors and neurotransmitters. In this study, to gain further insight into the reservoir function of polySia, we focused on FGF2 as a critical molecule in neurogenesis and neural functions. We first demonstrated the direct interaction between polySia and FGF2 by native-PAGEs, gel-filtration, and surface plasmon resonance (SPR) methods. Actually, polySia formed a large complex with FGF2, and the minimum chain length of polySia for the binding was 17 sialic acid residues. Heparan sulfate (HS), which is a well-known FGF2-binding glycosaminoglycan, also formed a large complex with FGF2; however, the size and structural feature of the FGF2 complexes were completely different between polySia and HS. To understand the effects of the expression of cell surface polySia on FGF2-dependent physiological effects, the cells expressing non-polysialylated NCAM were transfected with plasmids encoding either polysialyltransferase ST8SiaII/STX or ST8SiaIV/PST genes and analyzed for cell survival and cell growth under FGF2 treatment. Interestingly, polySia-NCAM-expressing cells inhibited the FGF2-dependent cell growth without affecting the cell survival. These results suggest that the FGF2-polySia complex stimulates a new signal pathway *via* FGF receptor (FGFR) different than the FGF2-HS complex that triggers conventional FGFR signaling to enhance the cell proliferation.

049: Novel regulation of the intracellular Ca^{2+} by microdomain-localized GPI-anchored glycans in animal sperm

Ken Kitajima[1,2,3], Takeru Kanazawa[1,2,3], Estelle Garénaux[1], Waraporn Kasekarn[1], Kazuki Hori[2], Andrés D. Maturana[2], Hiroshi Yasue[4], Chihiro Sato[1,2,3]; [1]Bioscience & Biotechnology Center, Nagoya University; [2]Graduate School of Bioagricultural Sciences, Nagoya University; [3]Program for Leading Graduate Schools IGER, Nagoya University, Nagoya 464-8601, Japan; [4]Transgenic Animal Research Center, NIAS, Tsukuba 305-8602, Japan
kitajima@agr.nagoya-u.ac.jp.

Although membrane microdomains (or lipid rafts) are well recognized as glycosphingolipid-enriched membrane areas involved in interactions and the subsequent signal transductions, very few studies have focused on biological roles of glycan chains of proteins on the microdomains. In 1999, we reported the first biochemical characterization of sperm microdomains from sea urchin [Ohta *et al.* 1999; 2000]. Many reports have since been made on gamete microdomains in various species. Of glycoproteins of sea urchin sperm microdomains, a heavily glycosylated protein localized in sperm flagella, named flagellasialin, is involved in sperm motility by regulating an intracellular Ca^{2+}, $[Ca^{2+}]i$. Flagellasialin is a GPI-anchored protein that contains a high content of glycans (80–90 % by weight) on a short polypeptide (7.2 kDa). We have recently reported that flagellasialin is involved in Ca^{2+}-dependent mechanosensory behavior of sea urchin sperm [Kambara *et al.* 2011]. Regulation of the $[Ca^{2+}]i$ is an important physiological event in animal sperm. However, no report has been made on how heavily glycosylated proteins like flagellasialin are involved in Ca^{2+} regulation through the microdomain. Therefore, we asked if this type of glycoprotein is also present in sperm of other animals. Here we report the presence of such a glycoprotein in pig sperm. The glycoprotein is named WGA-gp due to its high binding property with wheat germ agglutinin (WGA) [Kasekarn *et al.* 2012]. WGA-gp is a highly glycosylated GPI-anchored protein and shares the unique features in localization, structure, and potential function with flagellasialin. Several lines of evidence demonstrated that WGA-gp is identified as CD52, which is known to be a mammals-specific protein. Interestingly, the structure of mature protein and glycans is extensively different among CD52 molecules. Furthermore, the $[Ca^{2+}]i$ started oscillating at sperm population level, when sperm were treated with a monoclonal anti-WGA-gp antibody (4D1). The transient increase of $[Ca^{2+}]i$ was observed at a single cell level. This is a mAb.4D1-specific event, because no other antibody recognizing sperm glycan epitopes induced the $[Ca^{2+}]i$ change. Therefore, the presence of carbohydrate-enriched flagellar proteins involved in $[Ca^{2+}]i$ regulation may be a common feature among animal sperm.

Infection & Immunity

050: Keynote Lecture: Genes affecting glycosylation of human IgG show pleiotropy with autoimmune diseases and haematological cancers

Gordan Lauc; University of Zagreb, Zagreb 10000, Croatia glauc@pharma.hr

Glycosylation of immunoglobulin G (IgG) influences IgG effector function by modulating binding to different Fc receptors. To identify genetic loci associated with IgG glycosylation, we quantitated N-linked IgG glycans in plasma of 2,247 individuals from four European populations. After isolating IgG from human plasma, N-glycans were released and analyzed by ultra performance liquid chromatography (UPLC). Genome-wide association study (GWAS) identified 9 genome-wide significant loci ($P<2.27\times10-9$). Four loci contained genes encoding glycosyltransferases (ST6GAL1, B4GALT1, FUT8 and MGAT3), while the remaining 5 contained genes that have not been previously implicated in protein glycosylation (IKZF1, IL6ST-ANKRD55, ABCF2-SMARCD3, SUV420H1, and SMARCB1-DERL3). However, most of them have been strongly associated with autoimmune and inflammatory conditions (*e.g.*, systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, diabetes type 1, multiple sclerosis, Graves' disease, celiac disease, nodular sclerosis) and/or haematological cancers (acute lymphoblastic leukaemia, Hodgkin lymphoma, and multiple myeloma). Follow-up functional experiments in haplodeficient Ikzf1 knock-out mice showed the same general pattern of changes in IgG glycosylation as identified in the meta-analysis. As IKZF1 was associated with multiple IgG N-glycan traits, we explored biomarker potential of affected N-glycans in 101 cases with SLE and 183 matched controls and demonstrated substantial discriminative power in a ROC-curve analysis (area under the curve = 0.842). Our study indicates that IgG glycosylation is regulated by a complex network of genes through still unknown mechanisms. The observed pleiotropy with autoimmune diseases and haematological cancer indicates that individual variations in IgG glycosylation affect IgG function and contribute to disease development and progression.

051: Glycobiology aspects of the periodontal pathogen *Tannerella forsythia*

Gerald Posch[1], Andrea Koerdt[1], Laura Neumann[2], Friedrich Altmann[2], Paul Messner[1], Christina Schäffer[1]; [1]Department of NanoBiotechnology, NanoGlycobiology Unit, Universität für Bodenkultur Wien, Muthgasse 11, A-1190 Vienna, Austria; [2]Department of Chemistry, Universität für Bodenkultur Wien, Muthgasse 18, A-1190 Vienna, Austria
christina.schaeffer@boku.ac.at

Glycosylation of proteins in bacteria is becoming increasingly documented, including both *O*- and *N*-linked glycosylation, with bacterial glycosylation profiles exceeding by far those known from eukaryotes.

For the recently investigated periodontal pathogen *Tannerella forsythia* glycobiology plays a pivotal role, affecting the cellular integrity of the bacterium, its life-style, and its virulence potential. The bacterium possesses a unique Gram-negative cell envelope with a glycosylated surface (S-) layer as outermost decoration. This S-layer is formed by self-assembly oft two S-layer glycoproteins into a square 2D lattice with a spacing of ~10 nm which is proposed to be anchored to the cell envelope *via* a rough lipopolysaccharide.

The *T. forsythia* S-layer glycan was elucidated by a combination of electrospray ionization-tandem mass spectrometry and nuclear magnetic resonance spectroscopy as an oligosaccharide with the structure 4-Me*O*-β-Man*p*NAcCONH$_2$-(1→3)-[Pse5Am7Gc-(2→4)-]-β-Man*p*NAcA-(1→4)-[4-Me*O*-α-Gal*p*-(1→2)-]-α-Fuc*p*-(1→4)-[α-Xyl*p*-(1→3)-]-β-Glc*p*A-(1→3)-[β-Dig*p*-(1→2)-]-α-Gal*p* and is linked to distinct serine and threonine residues within the D(S/T)(A/I/L/M/T/V) amino acid motif. This S-layer glycan impacts the life style of *T. forsythia* because increased biofilm formation of an UDP-N-acetylmannosaminuronic acid dehydrogenase mutant can be correlated with the presence of truncated S-layer glycans devoid of the charged glycan branch. In addition, several other proteins of *T. forsythia* are modified with that specific oligosaccharide. Proteomics identified two of them as being among previously classified antigenic outer membrane proteins that are up-regulated under biofilm conditions, in addition to two predicted antigenic lipoproteins. Theoretical analysis of the S-layer *O*-glycosylation of *T. forsythia* indicated the involvement of a 6.8-kb gene locus that is conserved among different bacteria from the *Bacteroidetes* phylum. Together, these findings reveal the presence of a protein *O*-glycosylation system in *T. forsythia* that is essential

for creating a rich glycoproteome pinpointing a possible relevance for the virulence of this bacterium.

While the S-layer has also been shown to be a virulence factor and to delay the bacterium's recognition by the innate immune system of the host, the contribution of glycosylation to modulating host immunity is currently unraveling.

Financial support came form by the Austrian Science Fund FWF projects P20605-B12 and P21954-B20 (to C.S.) and the PhD programme "BioToP - Biomolecular Technology of Proteins" (Austrian Science Fund, FWF project W1224).

052: The Impact of Carbohydrate Microarrays in Studies of the Molecular Basis of Virus-host Interactions

Yan Liu[1], Angelina S. Palma[1,2], Wengang Chai[1], Kerstin Reiss[3], Ursula Neu[3], Luisa Stroeh[3], Thilo Stehle[3], Feizi Ten[1]; [1] Glycosciences Laboratory, Department of Medicine, Imperial College London, London, W12 0NN, UK, [2]REQUIMTE, Departamento de Química, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal, [3]Interfaculty Institute of Biochemistry, University of Tuebingen, Hoppe-Seyler-Str. 4, D-72076 Tuebingen, Germany
yan.liu2@imperial.ac.uk

Carbohydrate microarrays are important tools in elucidation of carbohydrate receptors involved in virus–host interactions. The neoglycolipid (NGL)-based oligosaccharide microarray system is among the relatively few in the world that are beyond proof-of-concept. The ability to readily broaden glycan libraries by incorporating both natural and chemically synthesized oligosaccharides, and the clustered and flexible presentation of non-covalently immobilized lipid-linked probes with an element of mobility, are key advantages that render the NGL system powerful in providing information on the molecular basis of the virus-host interactions. The NGL system has the versatility not only in that it lends itself well to analyses of the carbohydrate-mediated interactions of whole viruses, virus-like particles and recombinantly-expressed viral attachment proteins, but also in that the NGLs can be incorporated into live cells for infectivity assays to corroborate the biological significance of *in vitro* binding data. There have been contributions to a number of receptor-binding studies of human pathogenic viruses, such as the pandemic 2009 H1N1 and H5N1 'avian' influenza viruses isolated from infected humans, and the human polyomavirus JCV which, in immunocompromised individuals, causes a fatal demyelinating disease, progressive multifocal leukoencephalopathy.

This communication will be focused on recent applications of the NGL-based microarray system in pinpointing the oligosaccharide receptors for the capsid proteins of viruses that belong to the reoviridae and the polyoma families. In contrast to the haemagglutinin of influenza virus that can bind to a broad spectrum of sialyl-glycans, these viral proteins exhibit narrow specificities for sialylated oligosaccharide sequences, among around 120 in the microarrays. I will highlight the discovery of the ganglioside GM2 glycan as a receptor for the attachment protein Sigma 1 of reovirus strain type 1 Lang (T1L). New findings with B-lymphotropic polyomavirus (LPV) and several recently discovered polyomaviruses will also be described. These findings using microarrays, together with protein structural data, are providing information crucial to our understanding the viral tropisms, and also opportunities for the design of anti-viral agents.

053: Interaction of lectins from pathogens with bacterial surfaces visualised by fluorescence microscopy

Lenka Malinovská[1,2], Lenka Adamová[2], Michaela Wimmerová[1,2,3]; [1]CEITEC – Central-European Institute of Technology, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic, [2]National Centre for Biomolecular Research, Fac of Science, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic, [3]Department of Biochemistry, Fac of Science, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
michaw@chemi.muni.cz

Lectins are of non-immune origin occurring in all organisms and executing various functions. Microbial carbohydrate-binding proteins, lectins, can recognize saccharides on host cells (glycoproteins or glycolipides) and mediate adhesion to the host cells or mucosal surfaces.

Burkholderia cenocepacia and Pseudomonas aeruginosa are opportunistic human pathogens that infect immunocompromised people and are dangerous for example for cystic fibrosis (CF) patients. Both B. cenocepacia and P. aeruginosa produce several soluble lectins located in cytoplasm but significant part of lectins is exported and attached to bacterial surface by an unknown mechanisms. Lectins PA-IL and PA-IIL from P. aeruginosa are proved virulence factors. Soluble lectins from B. cenocepacia are homologues of PA-IIL and are also considered as virulence factors. Lectins differ in fine carbohydrate specificity but all of them could bind heptose and heptose derivatives. Thus, lipopolysaccharides (LPS) from B. cenocepacia could be suitable targets for lectins and anchor them directly on the bacterial surface.

We labelled lectins with fluorescent labels to follow their ability to bind to surfaces of B. cenocepacia

(single cells and biofilm) using fluorescence microscopy. After incubation with lectins-FITC conjugates, both cells and biofilm provided visible fluorescence signal however differently for individual lectins. Binding to LPS could therefore serve as direct attachment of lectins on microbial surfaces which is necessary for adhesion functions.

This work was supported by the European Community's Seventh Framework Programme under the European Regional Development Fund (CZ.1.05/1.1.00/02.0068) and Capacities specific programme (286154).

054: Ficolin-2 inhibits HCV infection, while ApoE3 mediates viral immune escape

Yinglan Zhao[1], Yushan Ren[1], Xuping Zhang[1], Ping Zhao[2], Xiao-Lian Zhang[1*]; [1] State Key Laboratory of Virology, Department of Immunology, Hubei Province Key Laboratory of Allergy and Immunology, Wuhan University School of Medicine, Wuhan 430071, China, [2] Department of Microbiology, Second Military Medical University, Shanghai 200433 China
zhangxiaolian@whu.edu.cn

Ficolin-2 is a human serum complement lectin with a similar structure to Mannan-binding lectin (MBL) and has been implicated in anti-bacterial innate immunity. However, the role of ficolin-2 in viral infection remains elusive. In this study, we found that ficolin-2 inhibited and neutralized HCV cell culture (HCVcc) infection to Huh7.5.1 cells, blocking HCVcc attachment to the receptors of low-density lipoprotein receptor (LDLR) and the scavenger receptor (SR-B1), but weakly interfered with attachment to CD81, and did not interfere with attachment to the claudin-1 and occludin receptors. C-terminal fibrinogen domain (113-313aa) of ficolin-2 was the critical binding region of HCV E1E2 N-glycans and played a critical role in anti-HCV activity. In addition, we found ApoE3 could block the interaction between ficolin-2 and HCV through competitively binding to HCV-E1E2 heterodimers but not by directly interacting with ficolin-2. Extrogenous ApoE3 blocked ficolin-2's effect, while knockdown of endogenous ApoE3 decreased HCV infection and increased L-ficolin-mediated anti-viral activity. This is the first report demonstrating the beneficial therapeutic effects of ficolin-2 against HCV infection. Our findings provide new insights into the important role of ApoE3 in blocking the anti-viral activities of the lectin ficolin-2 and the immune escape mechanism mediated by ApoE3 during HCV chronic infection.

055: Keynote Lecture: Role of Glycolipids in Innate Immune Response

Kazuhisa Iwabuchi[1,2]; [1]Infection Control Nursing, Graduate School of Health Care and Nursing, Juntendo University, Urayasu 279-0023, Japan, [2] Institute for Environmental and Gender-Specific Medicine, Graduate School of Medicine, Juntendo University, Urayasu 279-0021, Japan.
iwabuchi@juntendo.ac.jp

The innate immune system is the first line of defense against pathogenic microorganisms. Professional phagocytes, such as neutrophils and macrophages, are essential for this system. It used to be thought that the innate immune system comprised mechanisms to defend the host from infection by microorganisms in a non-specific manner. However, recent studies indicate this to be untrue. The mechanism of the innate immune system involves interactions of specific molecules. Host cells express several kinds of glycolipids on their surfaces. Because of their physicochemical characteristics, glycolipids tend to form clusters with cholesterol as membrane microdomains on plasma membranes. Membrane microdomains have been shown to be involved in several biological functions. Despite the role of the microdomains in activation of these biological functions, host membrane microdomains, especially glycolipids, are targeted by intracellular pathogens at different points in the infective process as gateways for entry into the cell in order to create environments in which to replicate avoiding host immune responses. In this light, glycolipids are the main players among the microdomain components in host-microorganism interactions.

Here I would like to introduce the role of glycolipids in innate immuno responses of neutrophils. Microorganisms are engulfed by neutrophils *via* PRRs under non-opsonized conditions. Engulfed microorganisms are internalized within intracellular vacuolar compartments called phagosomes; thereafter, these phagosomes sequentially fuse with lysosomes, which contain proteolytic enzymes. Pathogenic mycobacteria, such as *Mycobacterium tuberculosis*, have been shown to avoid fusion with lysosomes, resulting in their escape from killing by phagocytes. A neutral glycolipid, lactosylceramide (LacCer) has been shown to bind specifically to several types of pathogenic microorganisms, including *Escherichia coli, Bacillus dysenteriae,* and *Candida albicans*. LacCer is highly expressed on human phagocytes, and forms membrane microdomains coupled with Src family kinase Lyn on plasma membranes of those cells as pattern recognition receptors (PRRs). LacCer has been demonstrated to mediate neutropihl chemotaxis, phagocytosis and superoxide generation. Recently, we found that neutrophils phagocytosed mycobacteria *via* the carbohydrate-carbohydrate interactions between LacCer-enriched microdomains and

lipoarabinomannan (LAM) expressing on mycobacteria under non-opsonized conditions. Importantly, pathogenic mycobacteria utilized LacCer-enriched microdomains to arrest phagolysosome formation.

056: Reveal the molecular basis of N-glycan core chitobiose modifications

Shi Yan[1], Sonia Serna[2], Niels-Christian Reichardt[2], Katharina Paschinger[1] and Iain B. H. Wilson[1]; [1]Department für Chemie, Universität für Bodenkultur, A-1190 Wien, Austria; [2]Biofunctional Nanomaterials Unit, CICbiomaGUNE, 20009 San Sebastian, Spain
shi.yan@boku.ac.at

Fucosylation is one of the well-recognized N-glycan modifications in eukaryotes. Whereas in vertebrates, α1,6-linked fucose is the sole core modification of N-glycan, studies have shown that the "lower organisms" tend to produce even complicated core structures with multiple fucose and galactosylated fucose residues. The core chitobiose of sheep parasite *Haemonchus contortus* displays up to three fucoses, including an usual α1,3 fucose moiety linked to the distal GlcNAc; the same modification is present in the free-living model organism *Caenorhabditis elegans* as well as the pig parasites *Ascaris suum* and *Oesophagostomum dentatum*. To date, *C. elegans* core α1,3 fucosyltransferase-1 (FUT-1) and core α1,6 fucosyltransferase-8 (FUT-8) are proven to strictly modify the proximal GlcNAc, and no FUT has been found responsible for the distal GlcNAc fucosylation. Since the core α1,3 fucose is immunogenic to mammals, α1,3 fucosylation on the distal GlcNAc might enhance the immunogenicity of any parasite glycoproteins. Therefore, it is of interest to identify the "third" core FUT. We employed MALDI-TOF MS and a glycoarray platform with 22 immobilised glycan structures to investigate the enzymatic activity of recombinant FUTs from *C. elegans*. A homologue of core α1,3 fucosyltransferase was identified, which is capable to fucosylate the distal GlcNAc of the trimannosyl N-glycan core. In combination with other FUTs and hexosaminidase, sequential modifications on a chemically synthesised N-glycan yielded a tri-fucosylated structure; NMR data showed this structure is modified with α1,3 fucose and α1,6 fucose on proximal GlcNAc, and α1,3 fucose on the distal GlcNAc. We also proved that certain biosynthesis pathways have to be followed to achieve a tri-fucosylated N-glycan core.

057: A Trick of Galectins Interaction with Influenza Virus

Evgeny S. Chernyy[1], Hans-J. Gabius[2], Nicolai V. Bovin[1]; [1]Shemyakin Instutute of Bioorganic Chemistry RAS, Moscow, 117997, Russia; [2]Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Munich, D-80539, Germany
bovin@carb.ibch.ru

Influenza virus is known to dock onto sialoglycans at the surface of host cells; recent data suggest the involvement of additional targets in starting viral infection. Of note, the viral neuraminidase generates an enormously high density of terminal galactose residues on the surface of virions. Thus, an interaction of the influenza virus with mammalian gelatins (gals) is a possibility. Six virus strains, three cell lines (as host cells), and panels of human and chicken gals were studied. Gals indeed promote virus binding in a dose-dependent manner, but they do not influence the internalization stage. Gals are able to restore the ability of influenza virus to infect desalinated cells up to the level of completely sialylated surfaces. The extent of adhesion increase depends both on glycan structure and gal nature. The most intriguing yet is the strong inhibition by gals on natural anti-influenza virus antibodies: functional activity of viral haemagglutinin and neuraminidase was not blocked by loaded gals, whereas antigenic determinants were masked.

058: The galactic CvGal1 from the eastern oyster (*Crassostrea virginica*) binds to blood group A oligosaccharides on the hemocyte surface

Chiguang Feng[‡], Anita Ghosh[§], Mario A. Bianchet[§], Barbara Giomarelli[‡], Surekha Sridhar[‡], Aditi Banerjee[‡], Mohammed Amin[¶], Lai-Xi Wang[¶], Iain B. H. Wilson[†], and Gerardo R. Vasta[‡]; [‡]Department of Microbiology and Immunology, University of Maryland School of Medicine, and Institute of Marine and Environmental Technology, Columbus Center, 701 East Pratt Street, Baltimore, MD 21202, USA, [§] Department of Neurology, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA, [¶] Institute of Human Virology and Department of Biochemistry & Molecular Biology, University of Maryland School of Medicine, 725 West Lombard Street, Baltimore, MD 21201, USA, [†]Department für Chemie, Universität für Bodenkultur, A-1190 Wien, Austria
GVasta@som.umaryland.edu

The protozoan parasite *Perkinsus marinus* causes "Dermo" disease in the eastern oyster (*Crassostrea virginica)*, and is responsible for catastrophic damage to shellfisheries and the estuarine environment in North America. The parasite's mechanism(s) for entry into the host's blood cells

(hemocytes) have been largely unknown. We identified and characterized in oyster hemocytes a galactic (CvGal1) of unique carbohydrate-recognition domain organization that, unlike most mammalian gelatins, recognizes both endogenous and exogenous carbohydrate ligands. CvGal1 displays four CRDs in which conserve seven of the nine amino acid residues that bind ligand in bovine galectin-1. The carbohydrate specificity of CvGal1, previously analyzed by hemagglutination-inhibition studies, was further characterized by a glycan array (Consortium for Functional Glycomics) and confirmed that CvGal1 binds preferentially to glycans containing non-reducing terminal N-acetylgalactosamine, fucosylated (α1-2) galactose, and particularly blood group A oligosaccharides. Antibodies specific for CvGal1 showed that attached and spread hemocytes released soluble CvGal1, some of which specifically binds to the hemocyte surface. Glycomic analysis of the hemocyte surface revealed the presence of blood group A oligosaccharides. CvGal1 binds to a variety of microorganisms and phytoplankton components, but preferentially to *Perkinsus* spp trophozoites. Pre-treatment of oyster hemocytes with anti-CvGal antibodies reduced trophozoite uptake, revealing that CvGal1 is a phagocytic receptor for *P. marinus*. We propose that *P. marinus* has evolved to adapt its glycocalyx to be selectively recognized by CvGal1 over algal food or bacterial pathogens, thereby subverting the oyster's innate immune/feeding recognition mechanism to gain entry into the host cells [Supported by grants IOS 0822257 and IOS 1063729 from the National Science Foundation to G.R.V.].

059: Natural anti-glycan antibodies of mammals and birds

Nadezhda V. Shilova[1], Nailya R. Khasbiullina[1] Nicolai V. Bovin[1]; [1]M.M. Shemyakin and Y.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia.
shilova_nv@carb.ibch.ru

Natural anti-glycan antibodies (nAbs) are important part of innate immune system, but at the same time they are least of all studied. Their role and origin are still poor understood. Blood-group related antigens, tumor-associated and xeno-antigens are well-known counter-part of human nAbs. Anti-glycan nAbs pattern of healthy donors have been investigated with help of PGA. It was shown that about half of typical mammalian glycans bound human serum IgG and IgM, most of the specificities are not described yet. Discrete PGA data about anti-glycan nAbs of other mammals as well as birds were summarized (most of them are available from http://www.functionalglycomics.org), and detailed analysis was performed. Baseline and control data from mice, rats, primates and hens were taken into account and compared with humans.

Primates' (baboons) pattern of nAbs is mostly cognate to human: number of detected specificities and scope of repertoire were close to humans. Anti-glycan nAbs repertoire of mice, rats and hens was dramatically shrunk, top-binding glycans are different. Common epitopes recognized by nAbs such as chitooligosaccharides, L-rhamnose, L-fucose, and nAbs to core structures can be mentioned. It's argued for allied origin of nAbs. Note that all examined species have own features in nAbs repertoires which reflect their individual immunological "history" as humans have. The information about the natural anti-glycan antibodies repertoire is necessary because laboratory mammals and birds are widely used as immunological models.

Wed-Glyco(bio)technology

060: Keynote Lecture: Synthetic and biological studies of GPIs and GPI-anchored proteins

Zhongwu Guo[1, 2]; [1]National Glycoengineering Research Center, Shandong University, Ji'nan 250100, China; [2]Department of Chemistry, Wayne State University, Detroit, Michigan 48202, USA
zwguo@chem.wayne.edu, zwguo@sdu.edu.cn

Many surface proteins and glycoproteins are anchored to the cell membrane through glycosylphospha-tidylinositols (GPIs), a group of complex glycolipids sharing a conserved core structure: H_2N-CH_2CH_2-OPO_3-6-α-Man-(1→2)-α-Man-(1→6)-α-Man-(1→4)-α-GlcNH2(1→6)-inositol-1-OPO_3-glycerolipid. All GPI-anchored proteins and glycoproteins have their polypeptide C-termini linked to the conserved phosphoethanolamine moiety. GPI-anchored proteins and glycoproteins play a critical role in various biological and pathological processes. To study these events, it is essential to have access to GPIs and GPI-anchored proteins and glycoproteins in homogeneous and structurally well-defined forms, which is a significant challenge. Our research aims at developing methodologies for the synthesis of natural and functionalized GPI anchors and GPI-peptide, glycopeptide, protein and glycoprotein conjugates, as well as studying GPI biology using synthetic GPIs and GPI conjugates.

We have developed a highly convergent strategy for GPI anchor synthesis utilizing a phospholipidated pseudodisaccharide as the common key intermediate, and applied it to the synthesis of many GPIs and GPI analogs. With *p*-methoxybenzyl group as a permanent protection of hydroxyl groups, the strategy was also applied to preparing GPIs with unsaturated lipids and other functionalities that enabled their further modifications and visualization. We have also developed chemical and enzymatic methods for the synthesis of structurally defined GPI-linked

peptides, glycopeptides, and proteins. For the chemical synthesis, extensively protected GPIs with free conserved phosphoethanolamine moiety and peptides or glycopeptides having free *C*-termini were regiospecifically coupled by a conventional condensation reaction. For the enzymatic synthesis, free GPI anchors and peptides, glycopeptides and proteins were effectively ligated by sortase A, a transpeptidase used by bacteria to anchor surface proteins to the cell wall. We have demonstrated that sortase A could accept GPIs having one or multiple glycine residues attached to the conserved phosphoethanolamine moiety as substrates and couple them with peptides, glycopeptides or proteins. Sortase A could also cyclize peptides and glycopeptides to form macrocyclic peptides and glycopeptides or attach proteins to liposomes. The synthetic GPIs, functionalized GPIs, and various GPI conjugates have been used to study how bacterial toxins interact with GPIs and how GPIs distribute in membranes, and to study the structure-activity relationships of GPIs and cell surface GPIomics as well.

061: Engineering of Cyclodextrin Glucanotransferase as an *O*-Glycoligase

Chao Li[1], Hee-Jeong Ahn[1], Jin-Hyo Kim[2], Young-Wan Kim[1]; [1]Department of Food and Biotechnology, Korea University, Sejong, 339-700, Korea; [2]Chemical Safety Division, National Academy of Agricultural Sciences, Rural Development Administration, Suwon, 441-701, Korea
ywankim@korea.ac.kr

O-Glycoligase approach has been reported as a new category for the enzymatic synthesis of *O*-glycosidic linkages using a retaining glycoside hydrolase mutant whose hydrolysis activity is markedly reduced by the mutation of its general acid/base catalytic residue but its transglycosylation activity still maintained. In this study, a cyclodextrin glucanotransferase mutant with an alanine residue at its general acid/base catalyst position (CGT-E284A) was constructed, and thus no obvious hydrolytic function but significant transfer activity was detected using α-maltosyl fluoride (αG2F) and 4-nitrophenyl β-D-maltoside (4-NPβG2) as the substrates. In order to improve the transglycosylation activity, an E284X library was created by site-directed saturation mutagenesis at the position of its acid/base catalyst. Through thin-layer chromatography analysis of the reaction mixtures, two mutants were screened; One is a mutant with a glycine residue at Glu284 (CGT-E284G) showing better activity than the original alanine mutant, and the other is a mutant with a serine residue (CGT-E284S) with severe hydrolytic activity. Kinetic analysis revealed that the transglycosylation activities of CGT-E284A and CGT-E284G were inhibited at high substrate concentrations of acceptor sugars above 0.8 mM. By contrast, the typical saturation kinetics mode was observed upon varying the donor (αG2F) concentration at a fixed acceptor concentration (0.8 mM). The catalytic efficiencies (k_{cat}/K_M) of CGT-E284G were improved by 3 and 5 folds compared to those of CGT-E284A using 4-NPβG2 and 4-nitrophenyl β-D-glucopyranoside as the sugar acceptors, respectively. Transglycosylation yield of the reaction using αG2F and 4-NPβG2 at 1 mM each was about 80 %, and NMR analysis confirmed that the transfer product was 4-nitrophenyl β-D-maltotetraoside.

062: Biotransformation of Ginseng Pectin and Its Application in Structure-activity Relationship Study

Yifa Zhou, Di Wu, Lin Sun; School of Life Sciences, Northeast Normal University, Changchun 130024, PR China
zhouyf383@nenu.edu.cn

Pectins are the active components of Ginseng, including HG, RG-I, RG-II and AG types. Pectins have very complex structures and diverse activities. It is difficult to study the structures and the activities of their structure domains. In the present work, we tried to transform pectin using micros and enzymes to produce some different domains of pectin molecules, in order to further study their structures and activities relationship.

Ginseng pectin was purified and hydrolyzed by endo-polygalacturonase to produce several HG, RG-I and AG fragments which have been purified and analyzed. The results showed that HG fragments were highly esterified and RG-I domains were good inhibitors of galactin-3. Ginseng pectin was first de-esterified by alkali, and then hydrolyzed by endo-polygalacturonase. The hydrolysates were fractionated by a combination of ion-exchange and size exclusion chromatography, and four de-esterified RG-I domains were obtained. They were all composed of GalA, Rha, Ara and Gal as the main components, and the ratios of Rha/GalA were between 0.28 and 0.94. The molecular weights of these fractions were between $7.7\times10^3-1.2\times10^5$ Da. IR and NMR spectra indicated that they were nearly no methyl-ester and acetyl groups in these fractions. NMR results indicated that the four fractions were RG-I type pectins, and two of them contained arabinogalactan II side chains while the others contained arabinogalactan I side chains.

The numbers of commercial available pectinases are limited. Therefore, producing pectinase fungi were used to transform pectin. Ginseng pectin was transformed by *Aspergillus Niger* and *Penicillium oxalicum* which could ferment pectinases, to produce a series of pectic fragments with molecular weight from 1 kDa to 10 kDa, containing Rha, GalA, Gal, Ara and Glc. Comparatively, sunflower pectin was also biotransformed

to produce different fragments of pectin molecules. The combining results indicated that ginseng pectins were degraded by different fungi to produce different pectin molecule domains. And different pectins were digested by the same fungus to produce different fragments of pectin. Thus, using biotransformation may constitute a new way for studying structure-activity relationship of pectin.

This work was supported by the Chinese New Drug Creation and Manufacturing Program(2012ZX09502001-001), the National Natural Science Foundation of China(No: 31170770).

063: Characterization of The Anti-HIV-1 Mechanism of a Pseudoproteoglycan Produced by Conjugating Unsulfated Dextran with Poly-L-lysine

Haruko Ogawa[1],Fumie Kano[1], Takahiro Otsuki[2], Hiroo Hoshino[2], Kosuke Nakamura[1], Haruyo Mori[3], Hiromi Sakagami[1]; [1]Ochanomizu University, Tokyo, 112-8610, Japan, [2]Gunma University, Gunma 371-8511, Japan, [3]Osaka Prefectural Institute of Public Health, Osaka 537-0025, Japan.
ogawa.haruko@ocha.ac.jp

We reported that pseudoproteoglycan (pseudoPG), Dex-PLL, which had been synthesized by conjugating unsulfated dextran (Dex) with poly-L-lysine (PLL). Dex-PLL was found to have remarkable anti-HIV-1 activity against both the macrophage-tropic R5 virus Ba-L and T-cell line tropic X4 virus IIIB strains, although neither PLL nor dextran has such activity[1)]. Conjugation of PLL with dextran was found to produce an antiviral effect showing the chain-lengths dependency in three kinds of assay systems including a human $CD4^+$ T-cell line. The pseudoPG synthesized using 10 kDa PLL and 10 kDa dextran showed EC_{50} 4–40 times lower than that of sulfated dextran or heparin against Ba-L and EC_{50} equal to that against IIIB virus, indicating that Dex-PLL was more effective against R5 virus than sulfated polysaccharides. Dex-PLL suppressed a clinically isolated R5 virus from primary peripheral blood mononuclear cells.

Dex-PLL significantly inhibited virus adsorption to cells and subsequent virus entry into the cells. On the smear preparations of uninfected or IIIB-infected MOLT-4 T-lymphocytes, Dex-PLL inhibited the reactivities of the IgGs in AIDS patient's serum to the IIIB viruses budding on infected cells but not to uninfected cells, indicating that Dex-PLL bound to the IIIB viruses. Flow cytometry using budding MOLT-4-IIIB cells supported that Dex-PLL bound to the virus at a site different from that of heparin.

Confocal laser-scanning microscopy demonstrated that Alexa488-labelled Dex-PLL bound to the surface of human glioma NP-2 cells at 4 °C and 37 °C, and entered into a cytoplasmic matrix to localize near nuclear region at 37 °C, while *N*-acetylation of Dex-PLL abolished the endocytosis and the HIV-1-suppressing activity. On membrane analysis using separated HIV-1 proteins, Dex-PLL was found to bind to viral core proteins but not to envelope glycoproteins including GP160. These results suggest that Dex-PLL may have unique multiple preventive mechanisms against HIV-1 at not only adsorption and entry to the cells, but at the intracellular steps after denucleation of the virus. Elucidating the action mechanisms of Dex-PLL will provide new insights into the molecular processes of the viral infection to develop a novel anti-HIV-1 treatment.

[1)]Nakamura K., *et al.*, *Antiviral Res*.,**94** (2012)89–97.

064: A novel approach to studying the protein–carbohydrate interactions employing cancer cells grown on a QCM biosensor surface

Xueming Li[1], Yuxin Pei[1], Teodor Aastrup[2], Zhichao Pei[1,2,*]; [1]First author's affiliation, City, Zip code, Country; [2]Second author's affiliation, City, Zip code, Country; [1]College of Science, Northwest A&F University, Yangling, Shaanxi, 712100, China, [2]Attana, SE-11419, Stockholm, Sweden
peizc@nwsuaf.edu.cn

A novel approach to studying the protein–carbohydrate interactions on the surface of cancer cells using a QCM (quartz crystal microbalance) biosensor was developed, where a PANC-1 cell line and a SKOV-3 cell line were grown onto polystyrene-coated quartz crystals. To evaluate lectin–cancer cell interactions, the kinetics of binding of a diverse range of lectins were evaluated. The binding and dissociation between the lectins and the cells as well as the inhibition of the binding by monosaccharides were monitored in real time; the kinetic parameters of the interaction of ConA with SKOV-3 cells were studied. The real-time lectin screening of a range of lectins enabled the accurate study of the glycosylation changes between cells, such as the changes associated with cancer progression and development. This methodology provides a novel tool to study cell surface glycosylation which binding events take place at the cell surface, more closely mimicking an *in vivo* system.

References

1. Sharon N., Lis H. **2004**, *Glycobiology. 14 (11)*, 53R-62R.
2. Elovsson K, Pei Z, AastrupT. *Am. Lab.* **2011**, *6*: 13.
3. Dahlbäck M, Jørgensen LM, Nielsen MA, Clausen TM, Ditlev SB, Resende M, Pinto VV, Arnot DE, Theander TG, Salanti A. *J. Biol. Chem.* **2011**, *286:18*, 15908–15917
4. Pei Z, Saint-Guirons J, Käck C, Ingemarsson B, Aastrup T. *Biosens. Bioelectron.* **2012**, *35*, 200–205

065: Structure-Function Studies on Glycosyltransferases: Design of Novel Glycosyltransferases for the Site-Specific Conjugation of Biomolecules

Pradman Qasba, Boopathy Ramakrishnan and Natalia Mercer; National Cancer Institute, National Institutes of Health, United States
qasbapr@helix.nih.gov

Outer surface of a cell is decorated with information-rich complex oligosaccharides (glycans) of glycoproteins, glycolipids, and proteoglycans which participate in several biological processes. Cell's glycophenotype changes with the cellular growth, development and differentiation as well as in the pathological state such as inflammation and cancer. In the growing glycomics field, there is a need for the development of new strategies for detecting and identifying the glycan moieties associated with the cellular status. The glycan chains are synthesized by a large family of enzymes called Glycosyltransferases (GT) that transfer a sugar moiety from a sugar nucleotide donor to an acceptor molecule. The structural information on GTs from our laboratory has shown that there are flexible loops that undergo marked conformational changes upon binding of metal ion and sugar-nucleotides and simultaneously create at the C-terminal region of the flexible loop an oligosaccharide acceptor binding site that did not exist before. The loop acts as a lid covering the bound donor substrate. Furthermore, there are few residues in the catalytic pocket of GTs that determine their sugar donor specificity. Mutation of these residues broadens their donor sugar specificities, including towards the sugars carrying a chemical handle such as keto or azido group at C-2. Furthermore, the N-acetyl group of the donor sugars, e. g GalNAc and GlcNAc, binds in a cavity of their respective glycosyltransferases that can also accommodate a sugar analogue with a chemical handle. The transfer of a modified sugar residue that has a chemical handle by the mutant or wild type GTs to a specific sugar residue on a glycoconjugate has made it possible to develop a highly sensitive chemoenzymatic method for detecting and isolating glycan biomarkers. The mutant and wild type glycosyltransferases, currently being produced in our laboratory, are turning out as powerful tools for investigating the cell's glycophenotype with high specificity.

066: High-throughput screening for discovering glycosidase from metagenomic libraries using droplet-based microfluidics

Xiaohui Wang[1, 2], Runtao Zhong[1, 3], Bingcheng Lin[1, 3], Yuguang Du[*1, 3]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China; [2]University of Chinese Academy of Sciences, Beijing, 100049, China; [3]Research Center of Lab on a Chip, Dalian, China
duyg@dicp.ac.cn

Glycoside hydrolases (also called glycosidases) catalyze the hydrolysis of the glycosidic linkage to release smaller sugars. They are extremely common enzymes with roles in nature including degradation of biomass, in anti-bacterial defense strategies, in pathogenesis mechanisms and in normal cellular function. Together with glycosyltransferases, glycosidases form the major catalytic machinery for the synthesis and breakage of glycosidic bonds. Metagenomics has emerged as an alternative approach to conventional microbial screening that allows exhaustive screening of microbial genomes in their natural environments. It has been proven to be a powerful tool to isolate new enzymes and drugs of industrial applications. However, searching for desired traits from metagenomes is often problematic because of the lack of ultrahigh-throughput and cost-effective screening approaches.

This work aims to establish a general ultrahigh-throughput screening platform using droplet-based microfluidics that overcomes the limitations mentioned above. To demonstrate its performance, we have been identifying new types of chitinases exhibiting more efficient and specific enzymatic activity of breaking down glycosidic bonds in chitin. Transformed *E.coli* cells in a metagenomic library obtained from deep sea were encapsulated individually into aqueous picoliter-volume droplets, dispersed in inert carrier oil and then cultured off chip. After expression of the encoded enzymes, fluorogenic substrates were added into each droplet and incubated with the contents in the droplet. Finally, the droplets were sorted based on their own fluorescent intensities, which represent the activities of the expressed enzymes, and the sequences of the functional genes can be recovered from the *E.coli* cells contained in collected droplets. The droplet-screening frequency is in the range of hundreds to thousands of droplets per second. Compared to screening using microtiter plate-based systems, the volume and cost of the reagents are reduced by almost 10^4~10^5-fold, allowing the screening of 10^6~10^7 genes using only microliter-scale reagents.

067: Keynote Lecture: N-GlcNAc and O-GlcNAc Modification of Plant Proteins: Origin and Function

Neil E. Olszewski[1]; [1]University of Minnesota, St. Paul, MN, 55108, USA
neil@umn.edu

Plant proteins can be post-translationally modified with monosaccharides consisting of N- or O-linked β-D-*N*-acetylglucosamine (GlcNAc). In *Arabidopsis thaliana*, two enzymes, SPINDLY (SPY) and SECRET AGENT (SEC) modify serine and threonine residues of nuclear and cytosolic proteins with O-linked GlcNAc. *spy* mutants have defects in hormone signaling, light signaling, response to stress and the circadian clock, indicating a role for O-GlcNAc modifications in many plant processes. While *sec* mutants have only subtle phenotypes, *sec spy* double mutant embryos die before completing seed development suggesting that SEC and SPY have overlapping functions and indicating that O-GlcNAc modification is required for seed development. SEC modifies the capsid protein of *Plum pox virus* (PPV) and *sec* mutants are weakly resistant to PPV suggesting that PPV co-opts the host O-GlcNAc modification machinery to enhance its infectivity. Recently, a number of arabidopsis proteins have been found to be N-GlcNAc modified at the Asn of the Asn-X-Ser/Thr sequon. These proteins transit through the secretory system suggesting the modification may be produced when N-linked glycans are removed. Consistent with this hypothesis but also suggesting that N-GlcNAc modifications arise by multiple mechanisms, N-GlcNAc modification of some, but not all, proteins is blocked in endo-β-N-acetylglucosaminidase mutants. Recent results on regulation of plant processes by N- and O-GlcNAc modification will be presented.

068: Marine oligosaccharides production and application in plant protection

Heng Yin, Xiaoming Zhao, Yuguang Du; Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China
duyg@dicp.ac.cn

There are lots of polysaccharides resources in the sea. Many papers reported that these polysaccharides have several functions. But some of these polysaccharides are unsoluble in water and inconvenient for application. So we identified some glycosidases to hydrolysis the polysaccharides (chitin, chitosan, alginate, carrageenan and *etc.*,) to soluble oligosaccharides.

Furthermore, we developed a rapid and novel method to separate and purify the different DP (degree of polymerzation) oligosaccharides by using hydrophilic interaction liquid chromatography. Now, more than 1,000 oligosaccharides mixtures and more than 200 specific oligosaccharides with clear structure were identified in our lab.

As functional materials with excellent absorption and high bio-activity, marine oligosaccharides are widely used in pharmaceutical, food materials, cosmetic and agriculture. As plant elicitors or called plant disease vaccines, marine oligosaccharides have the ability to induce plant immunity system to resist plant diseases and stresses. Based on our experiments and other literatures, we conclude that there are five steps in the process of marine oligosaccharides activated plant defense: signal reception by receptor, secondary signal messengers activation, plant hormones accumulation, responded genes regulation and proteins expression, defense-related secondary metabolites production.

Some oligosaccharides based bio-pesticides and plant growth regulators were developed by our lab. These bioreagents are widely used in some provinces of China. They have good effect on food crops, economic crops, vegetables, fruits and horticultural plants.

069: The mitogenic and antioxidant properties of Andrias davidianus glycopeptides

Li Wei[1], Min Qu[2], Changqing Tong[3], Qiao Jin[1], Wenli Wang[1], Xinying Yu[1], and Lukyanov Pavel[4]; [1]College of Food Engineering, Dalian Ocean University, Dalian, Liaoning, 116023 China, [2]College of Food Science, Shenyang Agriculture University, Dalian, Liaoning, 116023 China, [3]Alkali Soil Natural Environmental Science Center, Northeast Forestry University, Harbin, Heilongjiang, 150040 China, [4]G.B.Elyakov Pacific Institute of Bioorganic Chemistry, Far East Branch of the Russian Academy of Science, Vladivostok, 690022 Russia
paluk@mail.ru

The *Andrias davidianus* glycopeptides (ADGPs) was prepared from skin secretion of Chinese giant salamander. MALDI-TOF mass spectrometry showed that molecular weights of hydrolysates were below 3.5 kDa. ADGPs are stabile at 0.1 M HCl, trypsin and pepsin hydrolysis. The glycopeptides–FITC binding with the surface of human epithelial cells was investigated by fluorescence microscope. Mitogenic activity of ADGPs on human epithelial cells was studied by the MTT. ADGPs show potent mitogenic effect on human epithelial cells, with the highest cell survival increase of 29 % and 48 % for a ADGPs concentration of 6 μg/mL and 98 μg/mL, respectively. Glycopeptides have strong antioxidant properties, and normalize NO-

synthase activity in human peripheral white blood cells stimulated with lipopolysaccharide, inhibit hyperproduction of reactive oxygen species (ROS) in them.

The work was supported by FEB RAS (09-I-P24-05, Russia) and NSFC (31071612 and 21075012, China).

070: Chitosan Oligosaccharides: A New Feed Additive

Qingsong Xu[1], Yuguang Du[1*]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, PR China.
articles1805@gmail.com

There is increasing urgency in the livestock production business worldwide to develop good alternatives to the in-feed antibiotics that have been a significant component of intensive livestock production for many years. Although the antibiotics are powerful agents to control enteric animal disease, the same antibiotics are also the bottleneck restricting the development of Chinese livestock. Chitosan oligosaccharide (COS) prepared from chitosan is a oligosaccharide with free amino group derived through deacetylation from chitin. It has been used as immunity regulator, antimicrobial agent, health food, feed additive, *etc.* This paper summarized recent progress made on chitosan oligosaccharide feed additive, including: preparation of chitosan oligosaccharide; application in the livestock; application in the poultry; application in the fisheries; mechanism of action of chitosan oligosaccharide. Based on our former results, we deduce that the mode of chitosan oligosaccharide act on animal health, So we conclude that chitosan oligosaccharide is a potential feed additive to substitute the in-feed antibiotics.

071: Probing Glucan Oligosaccharide Sequences by Negative-Ion Electrospray Tandem Mass Spectrometry and their Recognition by Glucan-Binding Proteins using a Designer Microarray

Angelina S. Palma[1,2], Yan Liu[1], Hongtao Zhang[1], Yibing Zhang[1], Barbara Mulloy[1], Ten Feizi[1], Wengang Chai[1]; [1]Glycosciences Laboratory, Department of Medicine, Imperial College London, London W12 0NN, UK, [2]REQUIMTE, Departamento de Química, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
w.chai@imperial.ac.uk

Glucans are homopolymers of D-glucose and are constituents of fungal, bacterial, and plant cell walls. Their specific recognition by glucan-binding proteins is important in mediating diverse biological processes in animals, plants and microbes. These include innate and acquired immunity to pathogens, modulation of immune responses, pathogen virulence, and plant cell wall biodegradation.

The molecular features of the glucan-protein interactions include specific glucose linkages, sequence and chain length. In this communication we demonstrate a strategy of negative-ion electrospray tandem mass spectrometry with collision-induced dissociation for high-sensitivity determination of linkage (1,2-, 1,3-, 1,4- and 1,6-) and sequence (linear and branched) in major types of gluco-oligosaccharide chains, and the combined use of a designer gluco-oligosaccharide microarray for binding analyses of the specificities of glucan recognition.

We have established a comprehensive gluco-oligosaccharide library, prepared from glucan polysaccharides of various fungal, plant and bacterial origins and from chemical synthesis, and constructed a gluco-oligosaccharide microarray, based on the neoglycolipid-technologyfor generating lipid-linked probes. The microarray comprises ~150 probes: linear sequences with 1,2-, 1,3-, 1,4- and 1,6-linkages of α- and β-configurations ranging from 2 up to 16mers, with mixed β1,3/β1,4- and α1,4/α1,6-linkages, and also branched β1,3/β1,6-sequences.

The prototype gluco-oligosaccharide microarray was instrumental in assigning oligosaccharides recognized by the major receptor of the innate immune system for fungal pathogens, Dectin-1, and by murine monoclonal antibodies elicited using glucan-based vaccines. We now extend our study by using the more diverse microarray to investigate a panel of 16 glucan-binding proteins, including mammalian lectins, monoclonal anti-glucan antibodies and microbial carbohydrate-binding modules of glycoside hydrolases. The microarray analyses reveal different patterns of glucan recognition among these proteins.

For proteins and gluco-poly- and oligosaccharides, our grateful acknowledgements are due to the following collaborators: Alisdair Boraston (Victoria); Gordon Brown (Aberdeen); Antonio Cassone & Antonella Torosantucci (Rome); Andres Ciocchini (Buenos Aires); Harry Ensley (Tulane); Harry Gilbert (New Castle); Jianxin Gu (Shanghai); Yvette van Kooyk (Amsterdam); Eckhard Loos (Regensburg); Ana Luís, Ana-Luísa Carvalho & Carlos Fontes (Lisbon); Barry McCleary (Wicklow); Takashi Tonozuka (Tokyo); Denong Wang (Stanford); David Williams (Johnson City); Guangli Yu (Qingdao); and Lina Zhang (Wuhan).

072: Exploring sugar code *via* macromolecular self-assembly

Guosong Chen; State Key Laboratory of Molecular Engineering of Polymers and Department of Macromolecular Science, Fudan University, 220 Handan Rd., Shanghai, China.
guosong@fudan.edu.cn

Carbohydrates are the most abundant organic species in the world and also the third most important biological macromolecules after nucleic acids and proteins. The self-assembly of DNA and proteins make a significant contribution to our lives and they have been employed to make functional self-assembled materials recently. Compared to the development of DNA and proteins, our knowledge and manipulation to the self-assembly of carbohydrates as well as their functionality is quite limited. The major obstacle for our understanding of carbohydrates is their complicated chemical structure of oligosaccharides and perplexing glycoforms and microhetrogeneity on proteins, which currently is a problematic and long-term task. Under this circumstance, macromolecular self-assembly might provide an alternate insight to this problem. Many of the biological functions of carbohydrates are related to their binding ability to proteins, especially for the sugars on the cell surface, which are called glycocalyx. By using our macromolecular self-assembly method, vesicles with a heavy layer of glycopolymers on their outer surface and inner surface were obtained, which could mimic the structure and function of glycocalyx. Besides, the molecular recognition of lectin and sugars were employed to construct self-assembled nanoparticles as well.

073: Two-step Hydrolysis for Preparing Low- and High-molecular Weight β-1,3-glucan Oligosaccharides from Curdlan

Jing Li [1], Li Zhu [2], Zhi-Yong Zheng [1], Xiao-Bei Zhan [1, 2, *], Chi-Chung Lin [1], Hong-Tao Zhang[1]; [1] Key Laboratory of Carbohydrate Chemistry and Biotechnology of Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, Jiangsu 214122, China; 2 Jiangsu Rayguang Biotech Co. Ltd., Wuxi, Jiangsu 214125, China
xbzhan@yahoo.com (X.-B. Zhan)

Bioactive β-1,3-glucan oligosaccharides (GOSs) are increasingly important for many biomedical applications. The simple and reproducible processes for the preparation of high and low molecular weight GOSs with degree of polymerization (DP) from 2 to 10 (identified by ESI-MS and TLC) by 1 mol/L sulfuric acid hydrolysis of curdlan aqueous suspension at various temperature and time were developed. A putative structure of the curdlan insoluble particles in acid suspension is proposed. It is hypothesized that the curdlan particle consists of exposed and relaxing exterior surface susceptible to acid hydrolysis and compact and water-insoluble core hindering hydrolysis. Consequently, two-step acid hydrolysis processes were developed for the GOSs. Combination of hydrolysis processes at 70 °C, 6 h then 80 °C, 3 h is effective for obtaining low molecular weight GOSs (DP 2 to 6). For high molecular weight GOSs (DP 7 to 10), the optimal hydrolyses condition is 70 °C, 4 h and then 80 °C, 1 h.

Thu-Carbohydrate & Disease

074: Keynote Lecture: Alterations of glycosylation in gastric carcinogenesis

Celso A. Reis[1,2,3]; [1]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Portugal, [2]Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Portugal; and [3]Medical Faculty, University of Porto, Portugal.
celsor@ipatimup.pt

Altered glycosylation of cell surface membrane and secreted glycoconjugates is a common modification in cancer.

In the gastric mucosa major alterations are observed during the process of gastric carcinogenesis. *Helicobacter pylori* attachment to human gastric mucosa is mediated by bacterial adhesins, such as BabA, that recognizes neutral histo-blood group antigens expressed by gastric epithelial cells. Persistent *H. pylori* colonization promotes gastric mucosa expression of sialylated glycan structures. These include receptors for the bacterial sialic acid binding adhesin (SabA). We have demonstrated in human gastric tissues and in cell models that *H. pylori* infection induces the expression of glycosyltranferases, namely *B3GNT5,* involved in the biosynthesis of sialylated Lewis antigens leading to an increased *H. pylori* adhesion.

Furthermore, major glycosylation alterations are observed in pre-cancerous and cancerous lesions of the gastric mucosa, such as the aberrant expression of simple mucin-type carbohydrate antigen Sialyl-Tn due to the overexpression of ST6GalNAc-I.

The increased expression of sialylated glycan structures in gastric cancer is associated with more aggressive phenotypes. Gastric cancer cells showing increased expression of ST3Gal-IV and sialyl-Lewis X antigen display an increased activation of signaling pathways leading to more aggressive cancer cell behavior. Furthermore, alterations on N-glycosylation of E-

cadherin have been shown to be crucial in gastric cancer progression. Altogether these alterations constitute a source of cancer biomarkers with clinical application.

These findings show that activation and modulation of the glycosylation pathways in gastric cancer cells play key roles in the process of carcinogenesis and in the biology of the cancer cell.

075: The modification of aggrecan sulphation in articular cartilage from KBD children

Mingxiu Luo[1], Jinghong Chen[1], Siyuan Li[1], Clare E Hughes[2], Bruce Caterson[2] & Junling Cao[1*]; [1]Institute of Endemic Diseases, Xi'an Jiaotong University College of Medicine; Key Laboratory of Environment and Genes related to Diseases (Xi'an Jiaotong University), Ministry of Education, Xi'an, 710061, Shaanxi, P.R. China; [2]Connective Tissue Biology Laboratories, Division of Pathophysiology and Repair, School of Biosciences, Cardiff University, Cardiff, Wales, UK CF10 3AX
caojl@mail.xjtu.edu.cn

Objectives: The sulfate of the proteoglycan is a necessary process in cartilage matrix formation. In this study, we tried to investigate the potential role of the sulfate modification of cartilage proteoglycan on the pathogenesis of Kaschin–Beck disease (KBD) children.

Materials and Methods: Samples of articular cartilage were divided into 2 groups: control children (5 samples from 5 cases), and children with KBD (5 samples from 5 cases). The morphology and pathology of hand joint cartilage of KBD children were examined by light microscopy and the expression of proteoglycans was determined by histochemical staining. Aggrecan, PAPSS2 (3′-phosphoadenosine-5′-phosphosulfate synthetase 2), PAPST1 (3′-phosphoadenosine-5′-phosphosulfate transporter 1), CHST15 (Carbohydrate (N-acetylgalactosamine 4-sulfate 6-O) sulfotransferases 15), ARSB (Arylsulfatase B) and GALNS (N-acetylgalactosamine-6-sulfate sulfatase) localization were performed by inmmunohistochemitry. Analysis of proteoglycan degradation products was performed by immunohistochemistry using antibodies 1B5, 2B6 and 3B3(+), which are specific for the sulphation patterns of CS (chondroitin sulfate) substituted on the aggrecan core protein. Articular cartilage was classified in 3 zones, and positive findings were counted by light microscopy for cytoplasmic staining by antibodies of PAPSS2, PAPST1, CHST15, ARSB and GALNS.

Results: There were lesser amounts of aggrecan in the ECM (extracellular matrix) of the articular cartilage of KBD children and the sulphation patterns of CS substituted on the aggrecan core protein in KBD articular cartilage proteoglycans were altered. The percentages of chondrocytes staining for PAPSS2, PAPST1 and CHST15 in the upper(18.38 %±3.22 %, 16.79 %±3.42 % and 13.14 %± 1.17 % respectively) and middle (12.47 %±1.47 %, 7.35 %± 1.42 % and 6.75 %±0.61 % respectively) ($p<0.05$) zone of KBD children were significantly lower than in controls, while the percentage of chondrocytes staining for the ARSB and GALNS was significantly higher than in controls in the upper(43.79 %±11.05 %, 35.53 %±4.24 % respectively) and middle zone (27.12 %±3.45 %, 14.45 %±1.53 % respectively) of articular cartilage.

Conclusion: The decreased sulfation of aggrecan maybe related to several enzymes about the sulfated modification in KBD patients. Our results suggest that the altered sulfate modification of cartilage proteoglycan has an important effect on the pathogenesis of KBD. Our study may provide strong evidence for further studying the etiology and pathogenesis of KBD.

This research was supported by the National Natural Science Foundation of China (Nos.31070725,30872187 and 30471499).

076: Developmental process and tumor progression are mediated by carbohydrate to carbohydrate interaction

Sen-itiroh Hakomori[1,2] and Kazuko Handa[2]; [1]Depts of Pathobiology and Global Heath, Univ. of Washington, Seattle, WA, USA; [2]Pacific Northwest Research Institute, Seattle, WA 98122, USA,
hakomori@u.washington.edu

Various types of cell-cell interactions and signal transductions were found to be mediated by protein to protein interaction (PPI), or protein to carbohydrate interaction (CPI), and these processes were well established by many previous studies. In addition to the interactions, carbohydrate to carbohydrate interaction (CCI) has been found as a novel molecular interaction, which is involved in cell-cell interactions, signal transductions and cell phenotype changes: (i) Compaction process at the "morula stage" of embryos is mediated by Le^x to Le^x interaction leading to development into "blastocyst". In this process, Le^x binding proteins were not involved. (ii) Interaction between GM3 and Gg3 or GM3 and LacCer has been suggested to be involved in adhesion of certain tumor cells to endothelial cells, which is considered a crucial step in metastasis of the tumor cells; and the metastasis was inhibited by mimetics of Gg3 or LacCer. (iii) In contrast to CCI mediating cell-cell

interaction, CCI between molecules expressed within the same cell surface membrane microdomain was termed *cis*-CCI. The interaction of ganglioside GM3 to multi (>3) GlcNAc termini of N-linked glycans of epidermal growth factor receptor (EGFR) has been indicated as the molecular mechanism for the inhibitory effect of GM3 on EGFR activation. Also, the complex of GM3 and GM2 has been shown to inhibit the activation of hepatocyte growth factor receptor, cMet, through its association with tetraspanin CD82. Studies on CCI, especially using quantitative biophysical methodologies, have been accumulating.

077: Gene Regulations during Apoptotic Induction in Metastatic Breast Cancer and in Embryonic Brain Cells

Subhash Basu, Manju Basu and Rui Ma; Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556
sbasu@nd.edu

New apoptotic agents identified during our recent studies can be employed as a new generation of anti-cancer drugs after being properly delivered to the patients. In order to study glyco-gene regulation, we used four clonal metastatic cancer cells of colon and breast cancer tissue origin (Colo-205, SKBR-3, MDA-468, and MCF-3). The glyco-genes for synthesis of SA-Le^x and SA-Le^a (which contain N-acetylglucosamine, sialic acid, and fucose) in these cells were modulated differently at different phases induced by D-PDMP (inhibitor of glucosylceramide biosynthesis), Betulinic Acid (a triterpinoid isolated from the bark of certain trees and used for cancer treatment in China), Tamoxifen (a drug in use in the west for treatment of early stages of the disease in breast cancer patients in China), and *cis*-platin (an inhibitor of DNA biosynthesis used for testicular cancer patients) when used for induction of apoptosis in the above mentioned cell lines. Within 2 to 6 h, transcriptional modulation of a number of glyco-genes was observed by DNA micro-array (containing over 300 glyco genes attached to the glass cover slips) studies. Under a long incubation time (24 to 48 h) almost all of the glyco-genes were downregulated. The cause of these glyco-gene regulations during apoptotic induction in metastatic carcinoma cells is unknown and needs future investigations for further explanations. At least six GSL-glycosyltransferase activities (GLTs) of Basu-Roseman pathway catalyzing the biosynthesis of GD1a gangliosides have been characterized in developing chicken brains. Most of these glyco-genes are expressed in the early stages (7 to 17 days) of brain development and lowered in the adult stage, but the cause of reduction of enzymatic activities of these GLTs in the adult stages is also not known. Our recent attempt of *in vitro* Glyco-gene regulation in metastatic cells may lead us to explain the *in vivo Glyco- gene regulation* in normal or diseased animal organs.

078: Galectin-binding fine specificities, from small saccharides to glycoproteins—mechanisms, evolution, functions and medical use

Michael Carlsson[1], Ulf, Ulf Ryde[2], Derek Logan[3], Mikael Akke[4], Ulf J Nilsson[5], Hakon Leffler[1]; [1]Laboratory Medicine; [2]Theoretical Chemistry; [3]Biochemistry and Structural Biology; [4]Biophysical Chemistry; [5]Organic Chemistry[5], Lund University, SE 22362 Lund, Sweden
hakon.leffler@med.lu.se

Methods. Galectin-ligand interactions have been analyzed by fluorescence anisotropy, affinity chromatography and other binding-assays, and interactions have been mapped using galactic mutants, galactic orthologues from different species, and an array of different natural and synthetic ligands. For galectin-3, the structure and dynamics of complexes with small ligands have been analyzed by NMR-spectroscopy, ultra high resolution (~0.85 Å) X-ray-crystallography and neutron crystallography.

Mechanism. The conserved galactic-defining amino acid motif provides a preorganized binding site for interaction with galactosides, but an increase in conformational entropy of the protein upon ligand binding is also a newly discovered contributor to the affinity, as large as the enthalpy contributed by the previously well-known hydrogen bonds and van der Waals interaction. Interactions in neighboring sites may enhance or decrease the affinity in different ways for different gelatins, and it is possible to alter fine specificity by mutagenesis. Selectivity and affinity of gelatins for natural glycoproteins, such as found in human serum, is much higher than expected from the structure of their glycans. This suggests that the interaction also involves the specific context of the glycan in the protein and possible interaction with protein parts.

Functions and relationship to physiological regulation and disease. Different aspects of galactic fine specificity appear to be important in different contexts, for example, the tolerance of galectin-3 for 2-3 sialylated galactosides is not required for its activation of neutrophils but it is for its binding to serum glycoproteins. Galectin fine specificity determines intracellular traffic of the galactic itself and of bound glycoproteins, as shown for transferrin and haptoglobin-hemoglobin complexes, after their uptake into their respective target tissue cells. Increase or decrease of specific galactic-binding glycoforms in serum correlates in different ways with diseases.

Galectin inhibitors for medical use. The understanding of galactic fine specificity has made it possible to design highly potent (K_d in low nM range) and selective small molecule galactic inhibitors. Modified citrus pectin and other plant polysaccharides were found to have low or no affinity for the canonical galactic carbohydrate-binding site of a range of gelatins, which puts into question their proposed mechanism of action as specific galactic inhibitors.

079: Caveolin-1 up-regulates ST6Gal-I expression and integrin α2,6-sialylation to promote integrin α5β1-dependent hepatocarcinoma cell adhesion and FAK signaling

Shujing Wang [1]*,Shengjin Yu [1], Jianhui Fan [1], Linhua Liu [1], Lijun Zhang [1], Nanyang Li [1], Jianing Zhang [1]*; [1] Department of Biochemistry, Institute of Glycobiology, Dalian Medical University, Dalian 116044, Liaoning Province, China
**Correspondence to: Jianing Zhang; Shujing Wang.*
jnzhang@dlmedu.edu.cn; wangshujing82101@sina.com.

The alternations of cell surface sialylation play an important role in tumorigenesis and development. Although an increase in α2,6-sialylation is observed in hepatocellular carcinogenesis (HCC), the regulation mechanisms and roles involved remain unclear. Caveolin-1 is a major structural protein of caveolae and involved in many biological processes including protein glycosylation. Hca-F and Hepa1-6 are mouse hepatocarcinoma cell lines with high and low malignant potential, respectively. This study showed that caveolin-1 overexpression in Hepa1-6 cells up-regulated sialyltransferase ST6Gal-I expression and activated FAK-mediated adhesion signaling, and down-regulation of ST6Gal-I attenuated caveolin-1-induced increase in the cell adhesive ability to fibronectin. Conversely, caveolin-1 knockdown in Hca-F cells inhibited ST6Gal-I expression and FAK signaling-mediated cell adhesion to fibronectin. Re-expression of wild-type caveolin-1 or ST6Gal-I rescued the decreased ST6Gal-I expression and adhesion of Hca-F cells caused by caveolin-1 silencing. Further studies demonstrated caveolin-1 up-regulated cell surface α2,6-linked sialic acid *via* stimulating ST6Gal-I transcription. Cell surface α2,6-sialylation was required for integrin α5β1-dependent cell adhesion to fibronectin, and an increase in α2,6-linked sialic acid on α5-subunit facilitated fibronectin-mediated FAK phosphorylations. Taken together, these results indicate for the first time that caveolin-1 can up-regulate ST6Gal-I expression and integrin α2,6-sialylation, and further contribute to promoting mouse hepatocarcinoma cell adhesion to fibronectin. This study provides new insights into the biological functions of caveolin-1 and the significance of sialylation modification for integrin α5β1 in HCC metastasis. This work was supported by grants from the Major State Basic Research Development program of China (2012CB822103), and National Natural Science Foundation of China (31000372, 31170774 and 31000618).

080: Roles of sialidase in cancer initiation and progression

Taeko Miyagi [1], Kohta Takahashi[1], Masahiro Hosono [2], Koji Yamamoto[1], Kazuo Nitta[2]; Divisions of [1]Cancer Glycosylation Research and [2] Cell Recognition Study, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, Japan
tmiyagi@tohoku-pharm.ac.j

Plasma membrane-associated sialidase (NEU3), a key enzyme for ganglioside degradation, is remarkably up-regulated in several human cancers, and its up-regulation leads to increased cell invasion, motility and survival of cancer cells (1). Up-regulation of NEU3 is also of importance for the promotion stage of colorectal carcinogenesis *in vivo,* from experiments using NEU3 transgenic (2) and *Neu3*-deficient mice (3), possibly *via* activation of EGF signaling. These results suggest that alteration in the sialidase expression thus may be a crucial defining factor in malignancy. We then analyzed whether and how NEU3 contributes to cancer promotion as well as to progression. When NEU3 was transfected into NIH3T3-cells, NEU3 exhibited significant increase in colonogenic growth, clonogenicity on soft agar and *in vivo* tumor growth in the presence of EGF, as compared with vector control. Although endogenous level of EGFR in the cells is extremely low, NEU3 enhances markedly its phosphorylation, and probably mostly determines EGFR activation. In the cells, NEU3 also stimulated ERK and Akt, whereas the NEU3-mediated activation was largely abrogated by PP2 (a src inhibitor) or AG1478 (an EGFR inhibitor), suggesting that NEU3 is indeed involved in tumorigenesis through EGFR/src signaling pathway. Furthermore, NEU3-silencing in colon cancer cells resulted in significant decrease in clonogenic growth, chemosensitivity to oxaliplatin, clonogenicity on soft agar and *in vivo* tumor growth, along with down-regulation of stemness genes and Wnt–related genes. Further analyses revealed that NEU3 enhanced phosphorylation of the Wnt receptor LRP6 and consequently β-catenin activation by accelerating complex formation with LRP6 and recruitment of GSK3β and Axin, whereas its silencing exerted the opposite effects. These results suggest that NEU3 contributes to tumorigenic potential of cancer cells by regulating Wnt/β-catenin signaling and/or EGFR signaling. The detailed relationship between these signaling pathways remains to be solved.

(1) Kawamura, S. *et al. Cell Death Differ.* **19**, 170, 2012 (2) Shiozaki, K. *et al. Cancer Sci.* 100, 588–594, 2009 (3) Yamaguchi, K., *et al. PLoS ONE* 7, e41132, 2012

081: Keynote Lecture: Protein *O*-mannosylation and its pathological role

Tamao Endo; Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, JAPAN endo@tmig.or.jp

Glycosylation is the most common post-translational modification of proteins. The past decade of research on glycan function has revealed the etiology of a growing number of human genetic diseases with aberrant glycan formation.

Protein *O*-mannosylation is important in muscle and brain development. α-Dystroglycan (α-DG) is a highly glycosylated surface membrane protein. The main glycan of α-DG was found to be *O*-mannosylglycan. We identified and characterized glycosyltransferases, protein *O*-mannose β1,2-*N*-acetylglucosaminyltransferase (POMGnT1) and protein *O*-mannosyltransferase 1 (POMT1) and its homolog, POMT2 are involved in *O*-mannosyl glycan synthesis. Then *POMGnT1* is found to be responsible for muscle-eye-brain disease (MEB) and *POMT1/2* are for Walker-Warburg syndrome (WWS). MEB and WWS are congenital muscular dystrophies with brain malformation and structural eye abnormalities. In addition, defects in glycosylation of α-DG are also the cause of other four muscular dystrophies, *e.g.*, Fukuyama-type congenital muscular dystrophy (FCMD caused by fukutin), congenital muscular dystrophy type 1C (MDC1C caused by FKRP), limb-girdle muscular dystrophy 2I (LGMD2I caused by FKRP), and congenital muscular dystrophy type 1D (MDC1D caused by LARGE) since highly glycosylated α-DG was also found to be selectively deficient in the skeletal muscle of these patients. These all are named dystroglycanopathies. Elucidation of the intrinsic characters of these gene products improves out understanding of the pathomechanisms of these diseases. Recently, a phosphodiester-linked modification on an *O*-mannose was critical for laminin-binding and LARGE was found to have xylosyl- and glucuronyltransferase activity. However, the functions of fukutin and FKRP are largely unknown. Additionally, new genes for dystroglycanopathies, *ISPD* and *DPM3*, were reported. Interestingly, both affected *O*-mannosylation.

Collectively, details of molecular pathology of dystroglycanopathies are still unclear and structures and processing of *O*-mannosylglycans are highly complicate. I will focus on the relation between aberrant glycosylation of α-DG and congenital muscular dystrophies. Possible regulatory mechanism of protein *O*-mannosylation will also be discussed.

082: A Rationally Designed Glycomimetic Antagonist of E, P, and L-selectins Inhibits Functional Biomarkers in Sickle Cell Disease Patients in a Phase 1 Clinical Trial

John T. Patton[1], Daniel D. Myers[2], Scott I. Simon[3], Ted Wun[4], Helen Thackray[1] and John L. Magnani[1]; [1]GlycoMimetics Inc., Gaithersburg MD 20879 U.S.A., [2]Univ. Michigan, Ann Arbor MI, USA, [3]Univ California Davis, Davis, CA USA, [4]Univ California Davis School of Medicine, Sacramento, CA, USA. jmagnani@glycomimetics.com

The selectins are a family of carbohydrate binding proteins that have traditionally been studied as adhesion molecules involved inflammatory diseases and cancer. More recently they have been shown to directly activate cells resulting in downstream effects functioning in a variety of diseases thereby making them novel targets for drug development. We rationally designed a small molecule glycomimetic antagonist based on the bioactive conformation of the native ligand (sialyl Le^x) in the E-selectin carbohydrate recognition domain combined with a sulfated domain required for optimal binding to L- and P-selectins. The resulting pan selectin antagonist (GMI-1070) showed efficacy in many animal models including vaso-occlusive crisis in sickle cell disease where the selectins are known to play a critical role. GMI-1070 strongly blocks E-selectin-mediated activation of neutrophils as determined by inhibition of expression of high affinity CD18 with an IC_{50} of 0.5 μM, a value about 10 fold lower than the IC_{50} (5.5 μM) required to inhibit cell adhesion in a flow chamber. E-selectin also functions in thrombus formation and GMI-1070 dosed at 20 mg/kg BID potently inhibits the weight of thrombus formed at day 2 (90.6 %, $p<0.01$) and day 6 (83.6 %, $p<0.01$) post injury in a venous thrombosis mouse model.

Patients with sickle cell disease (SCD) exhibit elevated biomarkers of adhesion, neutrophil activation (Mac-1, LFA-1), and thrombosis (TF, TAT). These E-selectin-mediated mechanisms play roles in the life-threatening vaso-occlussive crises (VOC) periodically experienced by SCD patients. GMI-1070 was administered in a Phase 1 study to SCD patients ($n=15$) not in VOC with an IV loading dose of 20 mg/kg followed by a second dose 10 h later at 10 mg/kg. Blood sample were taken from patients prior to dosing and at 4,8,24 and 48 h after the loading dose. GMI-1070 significantly reduced cell adhesion markers at 8 h (sE-selectin, $p=0.004$; sP-selectn, $p=0.028$; sICAM-1, $p=0.004$), activation markers on monocytes at 4,8,24 and

48 h (Mac-1 p=0.002, 0.001, 0.008, 0.004; LFA-1 p= 0.004, 0.004, 0.012, 0.008) and markers of thrombosis at 4 and 8 h (TF p=0.009, 0.05) and at 4,8,24 and 48 h (TAT p=<0.001, <0.001, <0.001, 0.002). These results suggest that GMI-1070 is affecting its target in patients and supports further clinical evaluations.

083: Hepatitis B virus sensitizes IL-6 stimulation and confers sorafenib resistance *via* N-glycosylation-mediated gp130 membrane retention in hepatocellular carcinoma

Jiejie Xu, Haiou Liu, Jianxin Gu; Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University, Shanghai, China.
jiejiexu@fudan.edu.cn

Chronic active hepatitis (CAH) induced by persistent hepatitis B virus (HBV) infection remains the major etiologic risk factor for hepatocellular carcinoma (HCC) in China. Cytokine bath with interleukin 6 (IL-6) secreted mainly by activated Kuffer cells (KCs) in local liver microenvironment dominates inflammatory response of hepatocytes during chronic hepatitis. Previous studies have indicated the crucial role of IL-6 upregulation in hepatocarcinogenesis due to HBV infection. However, alterations in stimulatory response to IL-6 of hepatocytes undergoing chronic HBV infection remain obscure. In this study, we found that HBV infection sensitized hepatoma cells to IL-6 stimulation, which was dependent on membrane retention of glycoprotein 130 (gp130) on hepatoma cells due to HBV X protein (HBx) expression. Moreover, HBV infection and HBx expression were found to increase gp130 membrane stability due to MGAT5-mediated N-glycosylation. HBV infection and HBx expression could also upregulate MGAT5 transcription through ERK/Ets-1 signal activation. More importantly, HBV infection could confer hepatoma cells with tumor stemness and sorafenib resistance *via* N-glycosylation-mediated gp130 activation. Genetic ablation and pharmacological inhibition of MGAT5/gp130/STAT3 pathway decreased tumorigenicity and sorafenib resistance of hepatoma cells with HBV infection and HBx expression. Immunohistochemial staining in clinical hepatoma samples showed that MGAT5/gp130/STAT3 activation interlinks HBV infection with hepatoma stemness and poor overall survival in HCC patients. Our results reveal that HBV infection sensitizes IL-6 stimulation and confers sorafenib resistance *via* N-glycosylation-mediated gp130 membrane retention in hepatocellular carcinoma, which identifies MGAT5/gp130/STAT3 signal activation as potential therapeutic targets for personal intervention with HBV-associated HCC patients.

084: Serum glycome profiling - a biomarker for diagnosis of ovarian cancer

Karina Biskup[1], Elena I. Braicu[2], Jalid Sehouli[2], Christina Fotopoulou[2], Rudolf Tauber[1], Markus Berger[1] and Véronique Blanchard[1];[1]Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, Charité Medical University, Charité Platz 1, 10117 Berlin, Germany; [2]Department of Gynecology, Charité Medical University, Augustenburger Platz 1, 13353 Berlin, Germany

It has been known that protein glycosylation plays an important role in many biological and biochemical processes. Glycosylation has been shown to change during cancer initiation and progression. Ovarian cancer is mostly diagnosed after the development of metastases. As a consequence, prognoses and overall survival are very poor. In the case of Epithelial Ovarian Cancer (EOC), the common clinically used tumor markers such as CA-125 are known to have poor sensitivity as they often fail to detect the disease at its early stages. Therefore there is a high need for more suited biomarkers. The aim of the work was to analyse the serum N-glycome of EOC patients in order to identify a new potential serum biomarker.

N-glycans were released from serum glycoproteins by PNGase F digestion, purified in a solid phase extraction, permethylated und subsequently analyzed by MALDI-TOF mass spectrometry. 87 samples from preoperative EOC patients and 33 samples from age-matched healthy women were enrolled in this study. Statistical analyses were carried out using the SPSS 18.0 software. A GLYCOV value was calculated from the structures that were over- and underexpressed.

We were able to identify statistical N-glycome differences between primary ovarian cancer and control sera, giving better results than those of the established tumor marker CA-125, and for this reason could potentially be used as a biomarker.

085: Solubule smyloid precrsor protein 770 is secreted in a O-glycosylated form from inflamed endothelial cells

Shinobu Kitazume[1], Akiomi Yoshihisa[2], Takayoshi Yamaki[2], Masayoshi Oikawa[2], Yuriko Tachida[1], Kazuko Ogawa[1], Rie Imamaki[1], Yoshiaki Hagiwara[3], Noriaki Kinoshita[3], Yasuchika Takeishi[2], Nobuhisa Iwata[5], Takaomi Saido[5], Naomasa Yamamoto[6] and Naoyuki Taniguchi[1]; [1]Disease Glycomics Team, RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan; [2]Department of Advanced Cardiac Therapeutics, Cardiology and Hematology, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan; [3]Department of Biological Sciences, Immuno-Biological Laboratories Co. Ltd., 1091-1 Naka, Fujioka, Gunma 375-0005, Japan; [4]Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan; and [5]Department of Biochemistry, School of Pharmaceutical Sciences, Ohu University, 31-1 Sankakudo, Tomita, Koriyama, Fukushima 963-8611, Japan.
shinobuk@riken.jp

Deposition of amyloid β (Aβ) in the brain is closely associated with Alzheimer's disease (AD). Aβ is generated from amyloid precursor protein (APP) by the actions of β- and γ-secretases. In addition to Aβ deposition in the brain parenchyma, deposition of Aβ in cerebral vessel walls, termed cerebral amyloid angiopathy (CAA), is observed in more than 80 % of AD individuals. The mechanism for how Aβ accumulates in blood vessels remains largely unknown. In the present study, we show that brain endothelial cells expressed APP770, a differently spliced *APP* mRNA isoform from neuronal APP695, and produced Aβ40 and Aβ42. Furthermore, we found that the endothelial APP770 had sialylated core 1 type O-glycans. Interestingly, O-glycosylated APP770 was preferentially processed by both α- and β-cleavage and secreted into the media, suggesting that the O-glycosylation and APP processing involved related pathways. By immunostaining of human brain sections with an anti-APP770 antibody, we found that APP770 was expressed in vascular endothelial cells. We then established sandwich ELISA system to detect sAPP770 in human serum samples. We observed that inflammatory cytokines significantly enhanced sAPP770 secretion by endothelial cells. Furthermore, we unexpectedly found that sAPP770 was rapidly released from activated platelets. Our finding showing that plasma sAPP770 is significantly higher in acute coronary syndrome (ACS) patiens raise the possibility that sAPP770 can be a useful biomarker for ACS.

References

1. **S. Kitazume**, A. Yoshihisa, T. Yamaki, M. Oikawa, Y. Tachida, K. Ogawa, R. Imamaki, Y. Hagiwara, N. Kinoshita, Y. Takeishi, K. Furukawa, N. Tomita, H. Arai, N. Iwata, T. C. Saido, N. Yamamoto, and N. Taniguchi (2012) Soluble amyloid precursor protein 770 is released from inflamed endothelial cells and activated platelets: a novel biomarker for acute coronary syndrome. ***J. Biol. Chem.*** (2012) 287, 40817–40825.
2. **S. Kitazume,** Y. Tachida, M. Kato, Y. Yamaguchi, T. Honda, Y. Hashimoto, Y. Wada, T. Saito, N. Iwata, T. Saido and N, Taniguchi (2010) Brain endothelial cells produce amyloid β from amyloid precursor protein 770 and preferentially secrete the O-glycosylated form ***J. Biol. Chem.*** 285, 40097–40103.

086: Sialin as a Therapeutic Target for Solid Tumors

Roger A Laine[1], Ioan Horia Negulescu[2]; [1]Department of Biological Sciences, Louisiana State University and A&M College, and [1,2]TumorEnd, LLC Baton Rouge, Louisiana, USA 70803
rogerlaine@gmail.com

The HP59 lectin gene contains, entirely within its coding region, the Sialin Gene SLC17A5, chromosome 6, solute carrier family 17 (anion/sugar transporter), member 5, which codes for Sialin. Sialin is a lysosomal membrane sialic acid/acidic amino acid transporter, also important in CNS myelination. HP59 has a transcription initiation site 300 bp upstream of the Sialin Gene SLC17A5, and only in humans encodes 41 additional amino acids at the Amino-terminus of Sialin. In humans, there is a functional upstream transcription initiation site and an alternate start codon for HP59, incorporating the entire Sialin gene product. An uniquely human capillary endothelial lumen-expressed form of Sialin was termed HP59/SP55 by Hellerqvist, *et al.*, discovered independently as the receptor for Group B streptococcal 270 kDa polysaccharide toxin, (GBS Toxin, CM101). CM101 binds HP59/SP55 on the luminal face of anoxic driven capillary membranes as a lectin receptor of unknown specificity and function. HP59, having 7 or 12 transmembrane domains, is expressed in the neonate lung in the first 5 days after birth. GBS Toxin (CM101), secreted during sepsis, is the etiologic agent for "Early Onset Disease, to which the neonate is sensitive 5 days post partum during rapid anoxic driven lung vascularization. The CM101-HP59 lectin complex activates complement C3b, initiating an inflammatory cytokine cascade which recruits CD69+, activated granulocytes to destroy HP59 capillaries and surrounding lung tissue causing a 50 % mortality. Post partum in humans, rodents, canines, equines and primates later than 5 days, HP59/SP55 (Sialin) is expressed in wound healing and tumor angiogenesis. Sialin is also expressed on Salivary Acinar Cells plasma membranes, transporting

nitrate, and in Aspartergenic Synaptic Vesicles transporting aspartate. The homologous Sialin form SP55 is in the genomes of all mammals. Ungulates and felines express HP55-sialin in lung capillaries, susceptible to GBS toxin as adult animals. CM101 has been shown in a published Phase I, FDA-IND-approved clinical trial to have clinical safety and 33 % effectivity on select stage IV cancer patients, specifically targeting tumor vasculature. Endothelial involvement of CM101 binding to HP59 is indicated by levels of Soluble E Selectin. References can be found under HP59 in Wikipedia or at www.tumorend.com.

087: O-glycome of *Ascaris suum* and *Acanthocheilonema viteae*

Chunsheng Jin, Iain H Wilson, William Harnett and Niclas Karlsson; Department of Medical Biochemistry, Institute of Biomedicine, Göteborg University
chunsheng.jin@medkem.gu.se

O-glycan structures from free-living nematode Caenorhabditis elegans, porcine parasitic nematode (roundworms) Ascaris suum, and rodent filarial nematode Acanthocheilonema viteae, were analyzed by LC-MS. As for C. elegans, several new fucosylated O-glycans were found together with glycans modified with hexuronic acid (HexA). However, phosphocholine (PC)-linked O-glycan was detected in low amount. In contrast to C. elegans, the predominant O-glycans isolated from A. suum and A. viteae were modified by PC.

088: Another method of controlling Glc assimilation at high α-amylase concentrations in intestine through *N*-glycan-specific interactions

K. Date[1], N. Kawasaki[2], N. Hashii[2], H Sakagami[1], H. Ogawa[1]; [1]Ochanomizu University, Tokyo, 112-8610, Japan; [2]National Institute of Health Sciences, Tokyo, 158-8501, Japan
date.kimie@ocha.ac.jp

Pancreatic α-amylase plays dual modulatory roles in digestion and absorption of D-glucose (Glc) to achieve blood sugar homeostasis[1)]. We previously reported that porcine pancreatic α-amylase (PPA) binds to the *N*-glycans of glycoproteins2). In this study, we discovered a new system that controls Glc assimilation by interaction between pancreatic α-amylase and the N-glycans of glycoproteins in the luminal brush-border membrane (BBM) of enterocytes.

Recombinant human pancreatic α-amylase (recHPA) was expressed in yeast and purified by single-step affinity chromatography on amylose-Sepharose. RecHPA exhibited almost the same carbohydrate-binding specificity as PPA, indicating that mammalian pancreatic α-amylases share a common carbohydrate-binding activity. Immunostaining revealed that both α-amylases are located at the BBM in the porcine duodenum. The binding was inhibited by mannan but not galactan, indicating that α-amylases bind carbohydrate-specifically to BBM. The ligands for α-amylase were identified by combining ligands purified by a PPA-Sepharose column, PAGE, and LC/MS/MS. The ligands identified for PPA in BBM included sucrase-isomaltase (SI) and Na+/glucose-cotransporter 1 (SGLT1), both of which take part in assimilating glucose. Bindings of SI and SGLT1 in BBM to PPA were dose-dependent and inhibited by mannan. Assays using freshly prepared BBM vesicles revealed functional changes in PPA and its ligands: the starch-degrading activity of PPA and maltose-degrading activity of SI were enhanced 240% and 175%, respectively, while Glc uptake by SGLT1 was markedly reduced by PPA at high, but physiologically possible, concentrations after eating food. The effect of PPA on [^{14}C]-Glc uptake by BBM vesicles was attenuated by the addition of mannose-specific lectins, especially *Galanthus nivalis* lectin, supporting the involvement of a mannose-specific interaction.

The interaction of α-amylase with *N*-glycans in the BBM activated starch degradation to produce much more Glc on one hand, while suppressing a sharp increase in Glc absorption on the other[1)]. Therefore, α-amylase plays a key role in regulating Glc assimilation to maintain blood homeostasis through carbohydrate-specific interaction in the intestine.

[1)] Asanuma-Date K. et al., *J. Biol. Chem.*, **287**, 23104–23118 (2012)
[2)] Matsushita, H. et al., *J. Biol. Chem.*, **277**, 4680–4686 (2002)

Structural & Chemical Glycobiology and Glycomics

089: Keynote Lecture: Glycomics technologies identify stem cell and cancer biomarkers

Michael Pierce, Samuel Dolezal, Hua-bei Guo, Jin-Kyu Lee, Michael Tiemeyer, Lance Wells, and Karen Abbott; University of Georgia Cancer Center and Complex Carbohydrate Research Center, Athens, GA 30605, USA
hawkeye@uga.edu

Cell surface glycan expression is highly regulated during embryonic stem cell and iPS cell differentiation, as well as during oncogenesis. Our team has developed novel Glycomics technologies that facilitate the discovery of

glycoproteins and glycans that can serve as stage-specific or disease-specific biomarkers, which can then be further investigated to determine possible functional significance. We have identified a glycan that is expressed on cells of various lineages after human or mouse embryonic stem cells or iPS cells are induced to differentiate. Blocking expression of this glycan inhibits or blocks differentiation. In addition, we have developed methodologies for targeted glycoproteomics that have identified markers for several types of cancers, including invasive breast, pancreatic, and ovarian carcinomas. In the case of pancreatic cancer, we have identified a unique glycan epitope with high sensitivity and specificity for pancreatic adenocarcinoma. In preliminary experiments we can detect this epitope in blood from patients with this cancer. For invasive ductal breast carcinoma, we have identified a novel glycoform of periostin that is expressed during the EMT. During breast ductal carcinoma oncogenesis, periostin is secreted and functions in the matrix as a niche substituent for cancer stem cells. Moreover, stromal cells surrounding the breast tumor cells are induced to produce periostin, providing an amplification step. Initial experiments suggest that serum levels of this specific glycoform of periostin may serve as an accurate biomarker of invasive breast carcinoma and can distinguish serum from cancer patients and those that are non-diseased or have fibrocystic breast disease.

090: Mapping the glycome of Erythropoietin

Sureyya Ozcan[1], Myung Jin Oh[1], Rudolf Grimm[1,2], and Hyun Joo An[1]; [1]Graduate School of Analytical Science and Technology (GRAST), Chungnam National University, Daejeon, Republic of Korea [2]Agilent Technologies, Inc., Santa Clara, California 95052, USA, sureyya.ozcan@gmail.com Tel: 82-42-821-8547, Fax: 82-42-821-8551

Erythropoietin is heavily glycosylated therapeutic glycoprotein that stimulates red blood cell production. Increasing role of complex therapeutic proteins in immune based therapies requires further characterization in many aspects. The quality, safety, and potency of recombinant erythropoietins are determined largely by their glycosylation. Small variations in cell culture conditions can significantly affect the glycosylation, and therefore the efficacy, of recombinant erythropoietins. We have determined the glycosylation sites and glycan heterogeneity with isomer-specific and quantitative glycomic analysis of recombinant erythropoietins isolated from Chinese hamster ovary (CHO) cells and mammalian cells. The platform was used to profile native N and O-glycans from three production batches of darbepoetin alfa (aka NESP), a common form of recombinant erythropoietin and three production batches of epoetin alfa. Glycosylation sites of corresponding glycoproteins are also profiled to show extensive glycan heterogeneity. Results were verified by independent orthogonal analysis with both MALDI-TOF and nano-LC/Q-TOF MS. We found multi-antennary N-glycans with high levels of sialylation, O-acetylation, and dehydration in CHO cell. The detailed structures of these oligosaccharides were successfully characterized by tandem MS. This platform may be applied to quality control and batch analysis of not only recombinant erythropoietin but also other complex, glycosylated biotherapeutics and biosimilars.

091: Glycomics analysis using solid-phase glycan extraction on chip and chipLC-MS

Shuang Yang[1], Yan Li[2], Hanching Chiu[1], Shadi Toghi Eshghi[1], Don L. DeVoe[3], and Hui Zhang[1C]; [1]Department of Pathology, Johns Hopkins University, Baltimore, MD 21231; [2]Institute of Biophyscis, Chinese Academy of Sciences, Beijing, China 100101; [3]Department of Mechanical Engineering, University of Maryland, College Park, MD 20742 hzhang32@jhmi.edu

Glycosylation is one of the most common protein modifications and plays significant roles in many biological processes, including regulation of transcription and translation, signal transduction, cell proliferation, cell interactions and protein degradation. Aberrant glycosylation is reported to associate with many diseases such as cancers and immune disorders. Unlike the protein synthesis, the glycans attached to glycoproteins are enormously complex due to the non-template dependent synthesis of glycans. As a result, the comprehensive analysis of glycans from biological or clinical samples is an unmet technical challenge. Development of the high-throughput method will facilitate the glycomic analysis.

In this study, we developed a novel method for the high-throughput analysis of glycans from glycoproteins using Solid-Phase Glycan Extraction (SPGE) on chip, coupling with chipLC-mass spectrometry (MS) in an integrated microfluidic system. Proteins were immobilized on a solid support and other non-conjugated molecules were removed. Sialylated glycans were acetylated on solid support to enhance their stabilization and hydrophobicity. N-Glycans were then enzymatically released by PNGase F and then separated in a microfluidics device that was packed with porous graphitized carbon particles; O-glycans were then chemically cleaved by β-elimination and separated by chipLC. The glycans were analyzed by mass spectrometry (MS) and verified by tandem

mass spectrometry (MS/MS). We applied this technology on human serum and showed high glycan coverage, specificity, and sensitivity.

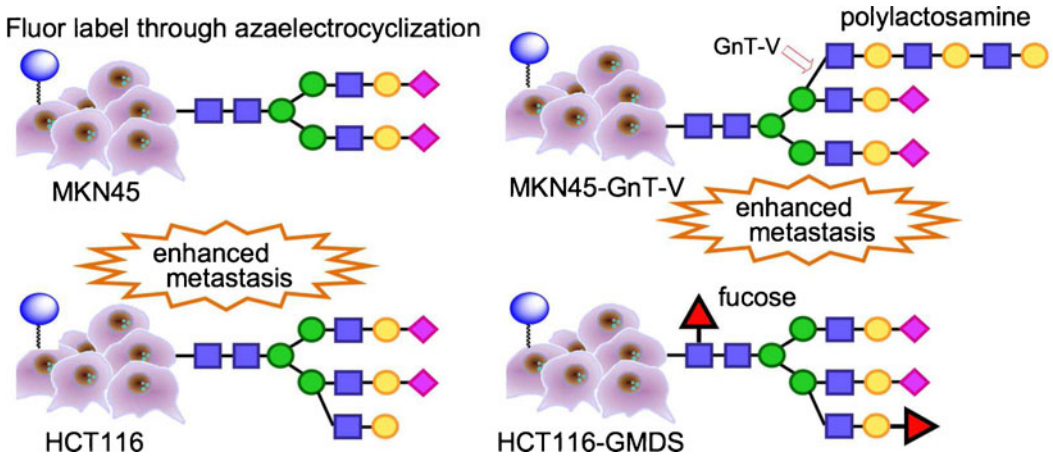
This method was used to analyze glycans from mouse heart and serum. Comparative analysis of extracted glycans from heart tissues, proximal serum, and distal serum showed that unique glycan structures were identified in heart but not in the proximal serum or blood serum.

092: Whole-Body Imaging of Glycan-Dependence on Tumor Metastasis

Katsunori Tanaka[1,2], Kenta Moriwaki[3], Eiji Miyoshi[3], and Koichi Fukase[1]; [1]Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka-shi, Osaka, 560-0043, Japan, [2]RIKEN, Wako-shi, Saitama, 351-0198, Japan, [3]Department of Molecular Biochemistry and Clinical Investigation, Osaka University, Suita-shi, Osaka, 565-0871, Japan
kotzenori@riken.jp

We have recently developed the rapid 6π-azaelectrocyclization to efficiently and conveniently introduce the ^{68}Ga-DOTA or the fluorescent groups to the amino groups of the proteins and on the live cell surface *via* a reaction with unsaturated aldehyde probes under the quite mild conditions (*Angew. Chem. Int. Ed.* **2008**, *47*, 102–105). The efficient labeling procedure led to the successful imaging of the *in vivo* dynamics of glycoproteins (*Angew. Chem. Int. Ed*. **2008**, *47*, 102–105), glycoclusters (*Angew. Chem. Int. Ed*. **2010**, *49*, 8195–8200), and lymphocytes (*J. Carbohydr. Chem*. **2010**, *29*, 118–132), which could precisely be controlled by the surface *N*-glycan structures, e.g, non-reducing sialic acids.

In further applying our azaelectrocyclization chemistry to visualize the glycan dependence on cell trafficking of biological importance, we investigated a whole-body fluorescence imaging of tumor metastasis (*Bioorg. Med. Chem*. **2013**, *21*, 1074–1077). Two kinds of cancer cell lines, *i.e.*, human gastric cancer MKN45 and human colon cancer HCT116, and their transfected versions with the surface glycan-related genes, *i.e.*, MKN45 transfected with GnT-V (β(1-6)*N*-acetylglucosaminyltransferase) and HCT116 transfected with GMDS (GDP-mannose-4,6-dehydrase), were fluorescently labeled by the azaelectrocyclization without affecting the cell viability, and their trafficking and metastasis in BALB/c nude mice were imaged. The metastasis properties depending on glycan structures on their cell surfaces, which were investigated previously in details through *in vitro* and invasive vivo experiments (*J. Biol. Chem*. **2002**, *277*, 16960; *J. Biol. Chem*. **2011**, *286*, 43123), was clearly visualized over a month; namely, the increased polylactosamine structure by overexpression of GnT-V in MKN45 cells and the deficiency of fucosylation in the mock HCT116 cells, enhances the metastatic potential.



093: Human serum N-glycome: milestone to the Human Glycome Atlas

Chanyoung Han[1], Hyun-Joo An[1], Carlito Lebrilla[2], Jeahan Kim[3]*; [1] Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon, Korea, [2] Department of Chemistry, University of California, Davis, USA, [3] Department of Food and Nutrition, Chungnam National University, Daejeon, Korea
jaykim@cnu.ac.kr

Glycan moieties on biological molecules play crucial roles engaging in their stability, functionality and regulation. The changes of glycosylation on proteins or lipids, thus, caused or occurred by the abnormal conditions of biological system such as disease. Genomics, transcriptomics and proteomics enable to draw a big picture of human biological network, yet a huge part of their dynamic interactions or regulations are not completely understood. Despite the necessities to elucidate the structural and positional information of glycosylation, the progress on high throughput analysis of glycans has been hindered by the heterogeneity and complexity of glycosylation site or isomeric structure. Nonetheless, recent advancement in mass spectrometry can routinely provide various information of glycan such that a compositional glycan profile in a single analysis or a quantitative isomeric separations in combination with porous graphite carbon column. Aiming to link the mass spectrometric data of glycosylation in human specimen to their systemic figures within the biological context, we start build up the database called Human Glycom Atlas (HUG-A). N-glycan in human serum was chosen for the first target since the quantitative and qualitative changes of glycosylation has been reported to reflect the host medical conditions.

Total N-glycan was released from the serum of 100 controls (non-cancer) and 100 lung cancer patient followed by the fractionation using PGC cartridge. Three fractions (release by 10 %, 20 %, and 40 % acetonitrile each) were then analyzed by MALDI-TOF/TOF for overall profile and nanoLC/Chip Q-TOF for isomeric separation. Overall, 108 glycans were identified based on their accurate mass and isomeric separations and the glycan synthetic correlation enabled drawing a serum N-glycan map that similar to the metabolic network. Among them, 17, 9 and 11 glycans were major glycan that comprise 90 % of total peak intensity of 10 % 20 % and 40 %, respectively. Several major glycans were showed more than one fraction, however, the nanoLC Chip Q-TOF confirmed that they have same chemical structures. Interestingly, 33 glycans, mostly major glycans, were present regardless of medical condition of samples such as cancer, age or sex albeit the peak intensities varied.

094: Endowing Metabolic Glycan Labeling with Specificity

Wei Lin, Ran Xie, Senlian Hong, Jie Rong, and Xing Chen; Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, China
xingchen@pku.edu.cn

Glycosylation plays a key role in mediating molecular recognition, development, and cell signaling. The metabolic glycan labeling technique has recently emerged as an appealing approach for detecting and imaging glycans on live cells and within living animals. However, this technique lacks cell-type selectivity and protein specificity. Here, we present the development of a cell-specific metabolic glycan labeling strategy using azidosugars encapsulated in ligand-targeted liposomes. The ligands are designed to bind specific cell-surface receptors that are only expressed or up-regulated in target cells, which mediates the intracellular delivery of azidosugars. The delivered azidosugars are metabolically incorporated into cell-surface glycans, which are then imaged *via* a bioorthogonal reaction. We will also present a FRET-based strategy for imaging glycans attached to a specific protein on live cell surface.

Reference:
01. Xie, R.; Hong, S.; Feng, L.; Rong, J.; Chen, X. "Cell-Selective Metabolic Glycan Labeling Based on Ligand-Targeted Liposomes" *J. Am. Chem. Soc.* **2012**, 134, 9914–9917.
02. Rouhanifard, S. H.; Xie, R.; Zhang, G.; Sun, X.; Chen, X.; Wu, P. "Detection and Isolation of Dendritic Cells Using Lewis X-Functionalized Magnetic Nanoparticles" *Biomacromolecules* **2012**, 13, 3039–3045.
03. Hao, Z.; Hong, S.; Chen, X.; Chen, R. P. "Introducing Bioorthogonal Functionalities into Proteins in Living Cells" *Acc. Chem. Res.* **2011**, 44, 742–751.

095: A Novel Membrane Electrophoresis Compatible with Glycoconjugate Analysis

Weijie Dong, Yu-ki Matsuno, Akihiko Kameyama; Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, 305–8568, Japan
aki-kameyama@aist.go.jp

A number of electrophoresis techniques are available to researchers in the life sciences. These include gel electrophoresis, capillary electrophoresis (CE), or its miniaturized technology, chip electrophoresis. Membrane electrophoresis

such as paper electrophoresis is now largely obsolete. However, cellulose acetate membrane electrophoresis is widely used for a clinical laboratory test even now as a simple and robust method. Unlike modern electrophoresis such as 2D-electrophoresis and CE, the membrane electrophoresis does not require skilled techniques and any expensive instruments. That might be one of the reasons why this method has been widely used in clinical labs even though the resolving power is low.

Recently, we have developed a novel membrane electrophoresis, termed supported molecular matrix electrophoresis (SMME), in which hydrophilic polymer such as polyvinyl alcohol (PVA) soaking into porous poly(vinylidene difluoride) (PVDF) membrane is used as separation medium. Although SMME is as easy and cheap as cellulose acetate membrane electrophoresis, it has several advantages arising from the use of the PVDF membrane as follows: compatible with glycan analysis following β-elimination, direct immunostaining the membrane just after electrophoresis, resistant to various chemical treatments, low electro-osmotic flow, and lectins are attachable through hydrophobic interaction.
As application of SMME, we here report human serum fractionation and compare the results with that obtained by cellulose acetate membrane electrophoresis. Moreover, analysis of glycans of each fraction is demonstrated. Additionally, we have reported that SMME could also be used for analysis of mucins and mucopolysaccharides. Alcian blue can sensitively stain these acidic molecules but not mucins with low acidic glycan content, such as porcine gastric mucin (PGM). To overcome this problem, we developed a novel staining method, succinylation-Alcian blue staining. In this method, the glycans of the mucin molecules are modified with succinic anhydride, thereby enabling them to be stained with Alcian blue. Furthermore, we demonstrate the compatibility of this novel staining procedure with glycan analysis using PGM as a model mucin.

096: Expression and purification of recombinant single-subunit oligosaccharyltransferase from *Leishmania braziliensis*

Toshihiko Kitajima, Markus Aebi; ETH Zürich, Zürich, 8093, Switzerland
kitajima@micro.biol.ethz.ch

Oligosaccharyltransferases (OSTs) mediate the *en bloc* transfer of N-glycan intermediates onto asparagine residue of nascent protein in glycosylation sequons (Asn-Xaa-Thr/Ser, Xaa≠Pro). The enzymes are typically heteromeric complexes composed of several membrane-associated subunits, in which Stt3 is highly conserved as catalytic core. The difficulties in preparation of the enzyme have hampered *in vitro* characterization and its ternary structure determination. Recent progress of genome-sequencing analysis revealed that the species within the Kinetoplastida, such as *Leishmania* and *Trypanosoma*, lack the genes encoding non-catalytic subunits of OST, but they code for several different Stt3 proteins [1]. Recent studies demonstrated that protozoan OSTs can complement yeast *stt3* mutant without formation of heteromeric complex with yeast OST [2]. In this study, we show the expression and purification of single-subunit OST from *Leishmania braziliensis* (LbStt3_1). Recombinant genes encoding full length LbStt3_1 proteins were expressed in *Leishmania tarentola*e under the control of a constitutive promoter. Solubilization and purification conditions were optimized. The purified protein is active with fluorescent-labeled peptide as acceptor and lipid-linked chitobiose as donor. We will report our recent progress on the characterization of recombinant LbStt3_1 protein.

References: [1] Kelleher, D. J., *et al.*, (2007) J. Cell Biol. 177, 29–37. [2] Nasab, F. P., *et al.*, (2008) Mol. Biol. Cell 19, 3758–3768.

097: Donor substrate specificity provides insights into sugar donor-OGT recognition and discriminatory proteomic substrate analysis of sOGT and ncOGT

Hui Yan, Xiaofeng Ma, Xiaoyan Liu, Feng Zhou, Yuqiu Wang, Lianwen Zhang*; College of pharmacy, Nankai University, 300071, Tianjin, China
lianwen@nankai.edu.cn

O-linked β-N-acetylglucosamine transferase (OGT) plays an important role in the glycosylation of proteins involving in various cellular events. Three isoforms of human OGT (sOGT, mOGT and ncOGT) are distinct in their N-terminal TPR domain, but share the same C-termianl catalytic domain. For all these isoforms, UDP-GlcNAc is a popular sugar donor though other sugar donors such as UDP-GlcNAz can also be used ncOGT.

We have synthesized a set of donor substrate derivatives and tested them with two isoforms of OGT: sOGT and ncOGT. When CKII3K, a peptide from casein kinase, was used as acceptor substrate, five donor substrate derivatives (UDP-GlcNAz, UDP-GlcNPr, UDP-4Deo-GlcNAc, UDP-6Deo-GlcNAc, UDP-6Deo-GalNAc) could be accepted in sOGT-mediated glycosylation reaction. While using an FITC labeled octapeptide, including the

above five derivatives, other three derivatives (UDP-GlcNPh, UDP-GlcNBu, UDP-GlcNGc) could also be accepted by sOGT, suggesting that (1) sOGT is ambiguous in recognizing sugar donor derivatives, and (2) the sugar donor-sOGT recognition could be affected by different acceptor substrates. Moreover, ncOGT and sOGT could recognize different panel of sugar donor derivatives. UDP-GlcNPh and UDP-GlcNTFA could be accepted by sOGT and ncOGT, respectively. Some derivatives (UDP-GlcNGc, UDP-GlcNAz, UDP-GlcNBu, UDP-GlcNPr) could be accepted by both the isoforms, but their binding capacity to sOGT or ncOGT are significant different. These results provides further insights into sugar donor recognition mechanism of OGT, it should be also used in proteomic substrate analysis of sOGT or ncOGT in various cells.

098: Keynote Lecture: The Structural Epitopes of Glycomic Function

Vernon Reinhold, David Ashline, Andrew Hanneman and Hailong Zhang; Glycomics Center, University of New Hampshire Durham, NH 03824, United States
vnr@unh.edu

Although glycans manifest their activity in a multitude of ways, including modulation of a protein's conformation and solubility, or serving as conjugate receptors, activators, or blocking agents. It is abundantly clear, that a large portion of function resides within properly positioned valence epitopes on the exposed surfaces of lipids and proteins. Protocols to isolate and confirm such structures are lacking, and contemporary analytical applications rarely match that needed for a comprehensive understanding. Accurate mass compositions, chromatographic separations and profiles annotated with undefined cartoons fail to meet the qualitative needs of bioengineers aspiring to design blocking analogs or vaccines to control a disease process. Additionally, an absence of these critical features misses the mark when trying to follow disease progression and metabolic outcome. Such conjugates possess a plethora of isomers, both structural and stereo, details which embody the major obstacles to an understanding of sequence. The literature abounds with chromatographic and MS strategies to unravel carbohydrate structural details and the approaches are largely variations in chromatography, MS ionization and activation. Operationally, the pursuit and expectation that all components of structure can be assigned in a one or two dimensional MS approach may be short-sighted, especially when considering the diverse bond energies and the need to unravel stereo and isomeric complexity. Somewhat ironically, a mass measuring instrument, operating in a sequential manner, can be exploited to resolve structural details better than any current chromatographic system, and thereby provides a different type of resolution, based on ion fragments and their relationships. With these tools we now focus directly of the valance epitopes that drive glycomic function. Given the awareness that these are responsible for most biological interactions, it seems that strategies focused on finding and assigning these substructures would provide a more direct focus on the actual components defining progression in numerous diseases and in developmental processes. Fortunately, these substructures are pendant through relatively labile, N-acetyl-hexosaminyl linkages, a lability and subsequent rupture that allows intact substructure (composition) to be observed as a B-type fragment at earlier stages of disassembly. These tactics open new doors to epitope structural detail.

099: Experimental and theoretical approaches to understand the conformational differences in isomeric glycan pairs

Yoshiki Yamaguchi[1], Kana Matsumoto[1], Kenji Hirose[2], Wataru Nishima[2], Suyong Re[2], and Yuji Sugita[2]; [1]Structural Glycobiology Team and [3]Theoretical Molecular Science Laboratory, RIKEN, Wako-shi, Saitama, 351-0198, Japan; [2]Nihon Waters K.K., Osaka, Japan
yyoshiki@riken.jp

The oligosaccharide moieties have many important biological roles, including protein quality control, cell-cell recognition and protection from proteolysis. Recent evidence suggests that a protein with a particular glycan structure possesses a specific biological function. Further, glycoprotein therapeutics (*e.g.* use of antibodies), is now one of the most promising approaches in the fight against disease. Analysis of glycan structure is therefore critical not only for basic science but also for pharmaceutical purposes.

One of the central issues in the mass analysis of glycans is the ambiguity of structural assignments mainly due to the heterogeneity and complexity of glycan structures. The many possible isomeric glycan structures of the same mass hamper accurate annotation of each peak. Although detailed tandem mass analysis does yield the glycan structure, the process is often time-consuming and is not suited for handling many sets of glycan mixtures such as are required in glycomics studies. In most cases, therefore, the separation of

isomeric glycans has been accomplished by the use of liquid chromatography.

In developing methods for the accurate and rapid identification of glycan structures, we have investigated in detail the ion mobility spectrometry (IMS) separation of a set of fluorescently biantennary *N*-glycans including isomeric structures. The cross section of each glycan derives from the IMS data and the structural characteristics are discussed in the light of molecular dynamics (MD) simulations. To enhance conformational sampling of the *N*-glycans in vacuum, we employ the replica-exchange molecular dynamics (REMD) method. Further, we demonstrate the separation of pyridylaminated *N*-glycans using an ultra-performance liquid chromatography (UPLC) system with hydrophilic interaction liquid chromatography (HILIC), thereby establishing a system that combines ion mobility mass spectrometry and HILIC.

100: Structure of Gal/GalNAc-specific lectin from marine mussel *Crenomytilus grayanus*

Oleg V. Chernikov, Irina V. Chikalovets, Valentina I. Molchanova, Pavel A. Lukyanov; G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, 690022, Russian Federation
chernikov@piboc.dvo.ru

Lectins are sugar-binding proteins which recognize specifically carbohydrate structures. Lectins are found in all taxa. Based on the structural similarity of carbohydrate recognition domain lectins are classified into a number of structurally distinct families. In recent years, many lectins from marine invertebrates have been identified.

1Previously, the Ca^{2+}-independent Gal/GalNAc-specific lectin (CGL) with a molecular mass of 17 kDa was isolated by us from the mussel *Crenomytilus grayanus*. In the present study, the sequence of cDNA encoding CGL was determined for the first time. The obtained cDNA sequence of 750 bp contained an open reading frame of 450 bp encoding a polypeptide of 150 amino acid residues (GenBank ID:JQ314213). The predicted CGL amino acid sequence comprised peptides determined by Edman degradation and ESI-MS/MS earlier. The CGL calculated molecular weight of 17025.3 Da was in agreement with those estimated by MALDI.

Results of NCBI-BLAST and WU-BLAST search revealed that CGL amino acid sequence had not similarity with lectins of known families. NCBI Conserved Domain Search program have not identified any conserved domain in CGL amino acid sequence. SMART server also could not predict in CGL any known domains, but revealed that CGL contained three internal repeats. Multiple alignments of the tandem-repeat amino acid sequences (which we designed as subdomains α, β and γ) have shown their high similarity. CD spectra of CGL and PSIPRED data showed that a characteristic feature of the structural organization of CGL is the predominance of β-structure. 3D-model of CGL built by PHYRE2 server was used for prediction of GalNAc binding site. It was found that CGL contained three putative GalNAc binding sites.

Thus, analysis of the amino acid sequence of CGL revealed that this protein is a member of a novel lectin family which adopts a ß-trefoil fold. However, the elucidation of the three-dimensional structure of CGL and site-directed mutagenesis studies are necessary to verify prediction of CGL fold and sugar-binding sites.

This research was supported by the grant from the Russian Foundation for Basic Research (no. 12-04-00862), a grant from the RAS Presidium (no. № 12-I-П6-10) under the project “Molecular and Cellular Biology”.

101: Bacterial cell wall peptidoglycan fragment library/array for investigation of their protein recognition

Yukari Fujimoto[1], Ning Wang[1], Akiyoshi Hirata[2], Kiyoshi Nokihara[2], Koichi Fukase[1]; [1]Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, 560-0043, Japan; [2]HiPep Laboratories, Kyoto, 602-8158, Japan.
yukarif@chem.sci.osaka-u.ac.jp

Peptidoglycan is a component of bacterial cell wall consisting of glycans (with alternating GlcNAc-β(1→4)-MurNAc) and peptide chains forming a three-dimensional mesh-like structure outside the plasma membrane. There are many proteins that can recognize PGN. For example, multicellular organisms including mammals have innate immune receptor proteins such as nucleotide-binding oligomerization domain protein 1 (Nod1) and 2 (Nod2), peptidoglycan recognition proteins (PGRP), and lectins. However, the comprehensive analyses of their substrate structures have not been really conducted because of the lack of pure chemically synthesized PGN fragments. To analyze the various ligand/substrate-protein interactions, the chemical synthesis of the PGN fragment library as well as the new analysis method are essential, leading to

understand the protecting system against infection of bacteria.

Here, we report the construction of a comprehensive library of PGN with linkers and its application on microarray for recognition protein detection, based on our recent establishment of the PGN fragment synthesis. In order to introduce the PGN fragments to the array, a linker with terminal amine was successfully attached to the PGN fragments. PGN array was then built up by attaching synthesized PGN fragments on the carboxyl covered solid surface of a carbon platform using amide formation. The array could be used to detect the PGN's receptors and recognition proteins and measure the binding properties of these proteins. For evaluation of the binding property of the array, two series of proteins were used, which are wheat germ agglutinin (WGA) with fluorescence label and human PGRP-S with combination of fluorescence labeled antibody. PGRP-S showed stronger affinity with tetrasaccharide tripeptide (GMGM3P), which is identical with our previous report using SPR study. Other proteins which have unknown binding properties are also tested.

References:
1) Reviews: a) Fujimoto, Y., Pradipta, A. R., Inohara, N., Fukase, K., *Nat. Prod. Rep.* **2012**, *29*, 568; b) Fujimoto, Y., Inamura, S., Kawasaki, A., Shiokawa, Z., Shimoyama, A., Hashimoto, T., Kusumoto, S., Fukase, K., *J. Endotoxin Research* **2007**, *13*, 189. (Synthesized PGN fragments for receptor search.)
2) a)Wang, N., Huang, C.-y., Hasegawa, M., Inohara, N., Fujimoto, Y., Fukase, K. *ChemBioChem*, **2013**, *in press*; b) Inamura, S., Fujimoto, Y., Kawasaki, A., Shiokawa, Z., Woelk, E., Heine, H., Lindner, B., Inohara, N., Kusumoto, S., Fukase, K., *Org. Biomol. Chem*. **2006**, *4*, 232.

102: Structures of Pectin Related to Galectin-3 Inhibition

Guihua Tai, Xiaoge Gao, Huiting Xue, Yifa Zhou*; School of Life Sciences, Northeast Normal University, Changchun, 130024, PR China
taigh477@nenu.edu.cn and zhouyf383@nenu.edu.cn

Pectin has been shown to inhibit the functions of galectin-3, a β-galactoside-binding protein associated with cancer progression and metastasis. The structural components of pectin that regulate its activity are unclear. We prepared various pectins, fragments and oligosaccharides from ginseng pectin, modified citrus pectin (MCP) and potato galactan, and studied their inhibitory activities on galectin-3 using several galectin-3-mediated cell assays. A rhamnogalacturonan I-rich ginseng pectin fragment, RG-I-4, was identified as potent inhibitor of galectin-3. Comparative studies showed that RG-I-4 had better activity than MCP and potato galactan, which were two well-established galectin-3 ligands. RG-I-4, MCP and potato galactan had dissociation constants of 22.2 nM, 143 nM and 2,590 nM, respectively, consistent with their relative activities determined by surface plasmon resonance analysis. The structure-activity of RG-I-4, MCP and potato galactan was further investigated by modifying the structure through various enzymatic and chemical methods followed by activity tests. The results showed that β1,4-galactan side chains were pivotal to the activity. The activity of a linear β1,4-galactan chain was proportional to its length up to 4 Gal residues. All long chains with ≥4 Gal residues had similar activities that were slightly better than lactose. Thus, a chain of four Gal residues meets the basic need for maximum activity. Galectin-3 appeared to recognize terminal tetrasaccharides. RG-I-4 contained mainly short β1,4-galactan side chains, consisting of less 4 Gal residues. However, it had 40- to 1000-fold higher activity than a single chain. Extensive structure-activity correlation studies indicated that the high activity of RG-I-4 resulted from the cooperative action of multiple short side chains. However, the existence of multiple side chains did not guarantee cooperative action. Potato galactan, containing multiple side chains, had similar activity to a single long side chain. The inhibitory activity of pectin should be related to the backbone, side chains and even whole molecule conformation.

103: Analysis of Glycosphingolipids by LC-MS

Akemi Suzuki[1], Hideshi Fujiwaki[2], and Yoshikatsu Umemura[2]; [1]Institute of Glycoscience, Tokai University, Hiratsuka, 259-1292, and [2]Shimadzu Co., Kyoto, 604-8511, Japan
akmszk@tokai-u.jp

Recent advances in mass spectrometry make our view of glycosphingolipids (GSLs) completely different from the previous one. LC-MS is applicable to GSL mixtures in a small amount, and provides structural information of carbohydrate chains and ceramides and possibly quantitative results in the presence of appropriate internal standards. However, several subjects remaining to be carefully clarified are the establishment of analytical conditions for obtaining fragmentations useful for structural

characterization, the preparation of standard or isotope-labeled GSLs for quantitation, and the establishment of required informatics including data base construction, collection of MS spectra, and search engine creation. We report here our resent progress in our attempt for solving above requirements.

We have tested several matrices for neutral GSLs and acidic GSLs (gangliosides) in the analysis of negative ion mode. Negative ion mode provides more fragment ions derived from carbohydrate chains and ceramides. Matrices used for LC analysis with a C30 reversed phase column of neutral GSLs, such as ammonium formate, formic acid, ammonium acetate, and acetic acid, give adduct ions and the ratios of molecular ions $[M - H]^-$ and adduct ions are different among molecular species of GlcCer as the simplest neutral GSL. Ammonium bicarbonate containing solvents give lesser amount of adducts and can be used for comparing changes of molecular species of neutral GSLs produced by the different conditions of cultured cells or comparing neutral GSLs of different subsets of cells isolated from living organisms. In the analysis of gangliosides, GM1(NeuGc), GD1a and b, GT1b, and GQ1b were detected as double-charged ions. We detected GM1(NeuAc) as single-charged ions $[M - H]^-$, and GM1(NeuAc) exhibits the highest m/z value among these gangliosides, resulting in that detection efficiency of GM1(NeuAc) becomes very low. We found that this result depends on pH of elution solvents which also effect electrospray ionization. This finding helps to detect GM1(NeuAc) in a better sensitivity. We are now compiling MS, MS/MS, MS/MS/MS spectra of gangliosides.

104: Interactions of carbohydrates with biomolecules Role of the CH/π interactions

Michaela Wimmerová[1,2,3], Stanislav Kozmon[1,2], Josef Houser[1,2], Radek Matuška[2], Jaroslav Koča[1,2]; [1]CEITEC – Central-European Institute of Technology, Masaryk University (MU), Kamenice 5, 625 00, Brno, Czech Republic, [2]National Centre for Biomolecular Research, Fac of Science, MU, Kamenice 5, 625 00, Brno, Czech Republic, [3]Department of Biochemistry, Fac of Science, MU, Kamenice 5, 625 00, Brno, Czech Republic
jkoca@ceitec.cz

Molecular recognition plays crucial role in many biological processes, such as bacteria-host identification. Some of these recognition processes are performed by proteins called lectins, which are able to bind saccharides in a very specific way. In our study we have focused on RSL lectin from world-wide distributed bacteria *Ralstonia solanacearum* causing lethal wilt in many agricultural crops and the AAL lectin from *Aleuria aurantia*. Both above mentioned bacteria interact with hosts through their lectins, binding predominantly L-fucose. In this paper, we are interested in the role of the CH/π interaction in binding abilities of these two lectins.

In the RSL lectin, we have attempted for the first time to quantify how the CH/π interaction contributes to a overall carbohydrate—protein interaction. We have used an experimental approach, creating single and double point mutants, combined with high level computational methods. The structure contains three monomer units of the lectin with six almost identical binding sites, where three of them are intramonomeric and the other three are intermonomeric. Experimentally measured binding affinities were compared with computed carbohydrate-aromatic acid residue interaction energies. Experimental binding affinities for the RSL wild type, phenylalanine and alanine mutants were −8.5, −7.1 and −4.1 kcal.mol−1, respectively. These affinities agree with the computed dispersion interaction energy between the carbohydrate and aromatic amino acid residues for RSL the wild type and the phenylalanine mutant, with respective values of −8.8 and −7.9 kcal.mol−1, excluding the alanine mutant where the interaction energy was −0.9 kcal.mol−1. Molecular dynamics simulations show that discrepancy can be caused by creation of a new hydrogen bond between the α-L-Me-fucoside and RSL. Observed results suggest that in this and similar cases the carbohydrate-receptor interaction can be driven mainly by a dispersion interaction. The AAL lectin contains five different binding sites for L-fucose. However, the main motifs of the binding sites are similar to the RSL ones. Therefore, we have attempted to analyze the impact of dispersion interaction to total binding potency also for AAL.

This work was funded by the European Community's Seventh Framework Programme under the European Regional Development Fund (CZ.1.05/1.1.00/02.0068) and Capacities specific programme (286154). The project is supported within the SoMoPro programme (project No. 2SGA2747, co-funded by FP7/2007-2013 under grant agreement No. 229603). The research is also co-funded by the South Moravian region. The authors thank the Czech National Supercomputing Centre, METACENTRUM, for providing computational resources (research intent LM2010005).

105: Evidence for a species-wide general *O*-glycosylation system for extracellular proteins in *Lactobacillus buchneri*

Julia Anzengruber[1], Martin Pabst[2], Laura Neumann[2], Friedrich Altmann[2], Christina Schäffer[1], and Paul Messner[1]; [1]Department of NanoBiotechnology, NanoGlycobiology and [2]Department of Chemistry, Universität für Bodenkultur Wien Vienna, A-1190, Austria
paul.messner@boku.ac.at

While having been long overlooked, nowadays glycosylation of prokaryotic proteins is becoming increasingly accepted. In the past decade, general protein glycosylation systems have been demonstrated mainly in Gram-negative pathogenic bacteria. Based on health promoting features and general advantages of Gram-positive lactobacilli with GRAS status for applied research, protein glycosylation in this bacterial genus was investigated in detail in this study.

The cell surface of the model organism *Lactobacillus buchneri* CD034 was shown to be completely covered by an oblique 2D crystalline array formed by self-assembly of the glycosylated S-layer protein SlpB. Biochemical and mass spectrometric analyses revealed that SlpB is decorated with an *O*-glycan, consisting of α1,6-linked D-glucose residues, attached to specific serine residues of the S-layer protein within the sequence S-A-S-S-A-S. Strikingly, a second glycoprotein of *L. buchneri* CD034, a glycosyl hydrolyzing enzyme (*Lb*GH25B) contains this characteristic glycosylation sequence and carries the same glycan. Subcellular fractions of strain CD034 showed that either glycoprotein is cell-wall associated and absence of the glycan on cytosolic proteins revealed a link between protein glycosylation and secretion. Protein glycosylation analysis was extended to strain *L. buchneri* NRRL B-30929 where a similar glycosylation scenario could be detected, with the S-layer protein SlpN and the glycosyl hydrolase *Lb*GH25N as major glycoproteins. All identified lactobacillar glycoproteins are extracellular, share the same *O*-glycan and the same glycosylation sequence. These findings corroborate previous data on the protein glycosylation of *L. buchneri* 41021/251 and let us propose the general *L. buchneri O*-glycosylation motif S-A-S-S-A-S and the first example of a general *O*-glycosylation system in Gram-positive and beneficial microbes.
This work was supported by the Austrian Science Fund FWF projects P21954-B20 (to C.S.) and P24305-B20 (to P.M.).

106: Sequence analysis and domain motifs in the decorin glycosaminoglycan chain

Xue Zhao,[1,2] Bo Yang,[2] Robert J. Linhardt,[2,3,4,5] Lingyun Li[2]; [1]College of Food Science and Technology, Ocean University of China, Qingdao P. R. China 266003, [2]Department of Chemistry and Chemical Biology, [3]Department of Chemical and Biological Engineering, [4]Department of Biology, [5]Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA.
zhaoxue@ouc.edu.cn; lil12@rpi.edu

Decorin proteoglycan is comprised of a core protein containing a single *O*-linked dermatan sulfate/chondroitin sulfate glycosaminoglycan (GAG) chain. While the sequence of the decorin core protein is determined by the gene encoding its structure, the structure of its GAG chain is determined in the Golgi. The recent application of modern mass spectrometry (MS) to bikunin, a far simpler chondroitin sulfate proteoglycans, suggests that it has a single or small number of defined sequences. On this basis, a similar approach to sequence decorin's much larger and more structurally complex dermatan sulfate/chondroitin sulfate GAG chain was undertaken. This approach resulted in information on the consistency/variability of its linkage region at the GAG chain's reducing end, its iduronic acid-rich domain, glucuronic acid-rich domain, and non-reducing end. A general motif for the porcine skin decorin GAG chain was established. A single small decorin GAG chain was sequenced using MS/MS analysis. The data obtained in the study suggests that the decorin GAG chain has a small or a limited number of sequences.

Fri-Cell Biology

107: Keynote Lecture: *O*-GlcNAcylation on Transcription Factor Mef2c Regulates Terminal Differentiation of Skeletal Myogenesis

Jin Won Cho, Hanbyeol Kim, Jürgen Roth; Department of Integrated OMICS for Biomedical Science, Yonsei University, Seoul, 120-749, Korea
chojw311@yonsei.ac.kr

O-GlcNAc modification is one of the post-translational modifications found in nucleus and cytosol. This modification which can compete with phosphorylation is dynamically regulated by O-GlcNAc transferase(OGT) and O-GlcNAcase (OGA). UDP-GlcNAc, a final product of the hexosamine biosynthesis pathway is a substrate for OGT. More than 1,800 proteins have been identified as O-GlcNAcylated proteins. O-GlcNAcylation is reported to

associate with the development of skeletal muscle atrophy and with muscle metabolism. Particularly, terminal differentiation of skeletal muscle is repressed by increased O-GlcNAcylation. We observed that the expression of myogenin, a key regulator for skeletal muscle terminal differentiation, decreased after treatment of NButGT, an inhibitor of OGA. In addition, we confirmed that the promoter activity of myogenin decreased after NButGT treatment. To find the promoter region affected by O-GlcNAc, we used shorter length promoter gene and found that at least 169 base pair upstream region of myogenin is affected by O-GlcNAc. We also found that Mef2c protein can be an important transcription factor regulated by O-GlcNAc *via* avidin-biotin complex DNA binding assay on 169 base pair upstream region. We performed immunoprecipitation and mass spectrometry to confirm that Mef2c is O-GlcNAcylated and to find O-GlcNAcylated sites on Mef2c. As we expected, mutagenesis of these serine or threonine to alanine resulted in a decrease in O-GlcNAc modification of Mef2c.

108: O-GlcNAc of p120 inhibits the interaction between p120 and E-cadherin

Haiyang Liu, Cuifang Han, Xinling Zhang, Chuanlin Bi, Leina Ma, Wengong Yu, Yuchao Gu*; Key Laboratory of Marine Drugs, Chinese Ministry of Education; Department of Molecular Biology, School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao, China.
guych@126.com

Protein O-GlcNAcylation is an O-linked glycosylation involving attachment of beta-*N*-acetylglucosamine (GlcNAc) to Ser/Thr residues of intracellular proteins catalyzed by O-GlcNAc transferase (OGT), whose removal is catalyzed by O-GlcNAcase (OGA). Previously, we demonstrated that O-GlcNAc promotes the formation and metastasis of some types of cancer (including breast cancer, lung cancer and colon cancer), which was caused mainly by the decrease of cell surface E-cadherin. However, the molecular mechanism underlying O-GlcNAc induced the disruption of E-cadherin complexes is still unclear. Recently, we found that p120-catenin (p120), which could bind and stabilize E-cadherin by its armadillo repeat domain and thus regulates cell-to-cell adhesion, could be O-GlcNAcylated by OGT *in vivo* and *in vitro*. By using CoIP and pull-down assays, we demonstrated that O-GlcNAcylation of the armadillo repeat domain inhibits the binding of p120 and E-cadherin. We mapped two O-GlcNAc sites in the armadillo repeat domain by MS, which might interpret the role of O-GlcNAc in the regulation of the interaction between p120 and E-cadherin. Additionally, we also found that OGT could directly bind to the armadillo repeat domain of p120, and the presence of OGT could interfere the binding of p120 to E-cadherin *in vitro*. Altogether, these data demonstrated that O-GlcNAc and OGT play important roles in the regulation of the interaction between p120 and E-cadherin and thus E-cadherin-mediated cell-cell adhesion.

109: Ribosomal RACK1 is stabilized by O-GlcNAc modification under hypoxic conditions and promotes IRES-mediated translation in hepatocellular carcinoma

Yuanyuan Ruan and Jianxin Gu*; Key Laboratory of Glycoconjugate Research Ministry of Public Health, Fudan University
yuanyuanruan@fudan.edu.cn

Hepatocellular carcinoma (HCC) is among the most common and aggressive cancers worldwide. RACK1 is a classical scaffold protein present in both ribosome- and nonribosome-bound form, and our previous report also demonstrates that ribosome-associated RACK1 promotes the chemoresistance and growth of HCC. However, what modulates the association between ribosome and RACK1 is little understood. Herein, we show that ribosomal RACK1 is modified and stabilized by O-GlcNAc glycosylation in HCC. Moreover, hypoxia promotes the O-GlcNAc modification of RACK1, leading to the enhanced association between ribosome and RACK1 and IRES-mediated translation under hypoxic conditions. Our data suggest that the O-GlcNAc modification of RACK1 provides the basis for translational regulation in HCC under hypoxic conditions.

110: Modification of Transforming growth factor β-activated kinase 1 (TAK1)-binding protein 2(TAB2) by O-linked-N-cetylglucosamine(O-GlcNAc) in pro-inflammatory signaling

Zengxia Li[1], Guanghui Han[2], Junfeng Ma[2], Gerald W. Hart[2]; [1]Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai, 200032,China; [2] Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore,21205,USA
gwhart@jhmi.edu

O-linked β-N-acetylglucosamine (O-GlcNAc) is a highly dynamic post-translational modification of serine and threonine residues regulating physiological and stress processes. TGF-β activated kinase 1 (TAK1)- binding protein2 (TAB2), binding partner of TAK1, plays important roles in the regulation of various pro-inflammatory signaling pathways including TNF-α and IL-1β-mediated activation of AP-1 and NF-κB. Here, we report that the global changes

of O-GlcNAc affected the IL-1β and TNF-α induced IκB degradation and NF-κB activation signaling. TAB2 is identified as a highly O-GlcNAc modified protein. TAB2 reacted with a O-GlcNAc specific antibody CTD110.6 and the antibody reactivity was diminished by treatment of CPNag J. TAB2 was found to be susceptible to labeling with beta-1,4-galactosyltransferase (GalT). We demonstrate that TAB2 can form a complex with OGT using co-immunoprecipitation approach in both directions. OGT *in vitro* labeling assay showed that TAB2 is a direct substrate of OGT. Using mass spectrometric methods, we validated the O-GlcNAcylation of TAB2 and localized O-GlcNAc sites on 3 separate sequences (GTSSLSQQTPR, TSSTSSSVNSQTLNR, and VVVTQPNTK). Site-directed mutagenesis of identified sites (T164, S165, S166, S168, T348, S349, S350, T351, S352, S353, S354, T456) confirmed these sites to be modified. We also found that the O-GlcNAc modification of TAB2 is a dynamic process and undergoing a rapid cycling. Currently, we are aimed to find the unrecognized role of O-GlcNAc modification of TAB2 in the regulation of pro-inflammatory signaling pathways.

111: Regulation of integrin αV expression by sulphated cerebroside scaffolding in hepatocellular carcinoma cells

Wei Wu, Yi Wei Dong, Peng Cheng Shi, Xing Zhong Wu; Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Key Lab of Glycoconjugate Research, Ministry of Public Health, 138 Yi Xue Yuan Road, Shanghai 200032, P.R. China;
xz-wu@shmu.edu.cn

Integrin expression is very important in cell adhesion and tumour progression. To study the impact of cerebroside sulfation on integrin levels, we investigated the sulphated cerebroside regulation of the integrin αV subunit expression in comparison with cerebroside. Exogenous sulphated cerebroside, rather than cerebroside, elicited a dose- and time-dependent stimulation of integrin αV subunit mRNA and protein expression in hepatocellular carcinoma cells (HCC). Through a comparison of treatments with cerebroside, ManN propanyl perac, and cyclo-ManN propanyl perac, only the cells treated with sulphated cerebroside produced a high expression level of the integrin αV subunit. Furthermore, the stable transfection of cerebroside sulfotransferase (*CST*) into the cells led to high expression of integrin αV, and a knockdown of *CST m*RNA showed down-regulation of integrin αV. This effect occurred with a corresponding phosphorylation of the transcription factors Sp1 and Stat3. In the cells stimulated with sulphated cerebroside, phosphorylation of Erk1/2 as well as c-Src was noted, and inhibition of Erk1/2 activation with either U0126 or PD98059 significantly suppressed Sp1 phosphorylation. Sulphated cerebroside was positively correlated with integrin αV expression in HCC tissues from 125 patients and their expression levels were associated with the patient's disease-free survival. Herein, we demonstrated that sulfation of cerebroside regulated integrin αV expression and cell adhesion *via* Erk activation, and was important in HCC.

112: N-glycan structure and function of cancer stem cell marker CD133

Liu Ying[1], Wei Yuanyan[1], Jiang Jianhai[1$]; [1] Key Laboratory of Glycoconjuates Research, Ministry of Public Health and Gene Research Center, Shanghai Medical College of Fudan University, China, 200032
jianhaijiang@fudan.edu.cn.

CD133/Prominin-1, a pentaspan membrane glycoprotein, has been widely used as a marker of normal and cancer stem cells. Increasing studies indicate that CD133 performs a variety of functions in cell proliferation, differentiation, metabolism and migration. Increasing evidences indicate that the glycosylation status of CD133, rather than the expression of CD133 protein itself, can act as an the marker of stem cells. And, the dynamic glycosylation of CD133 might play a critical role in its biological functions. Thus, identification of CD133 glycosylation status, which might be different in cells at different stages of differentiation and in different tissues, means a crucial step in defining the potential role of CD133 in normal and cancer stem cells. We analyzed the N-linked oligosaccharides of CD133 by biotin acylation lectin PHA-L, PHA-E, SNA and ConA and identified N-glycosylation site by MS. Furthermore, we showed that N-glycosylation regulated the positive effect of CD133 on hepatoma cell growth. We revealed CD133 could be sialylated in neural stem cells and glioma-initiating cells, and the sialyl residues attach to CD133 N-glycan terminal *via* a 2, 3-linkage. These findings explored the fundamental biological aspect of CD133 glycosylation.

113: The role of beta 1,4-galactosyltransferase I in glia activation during neuroinflammation

Aiguo Shen*; The Jiangsu Province Key Laboratory of Neuroregeneration, Nantong University, Nantong 226001, P.R. China
shag@ntu.edu.cn

Protein glycosylation modification exerts great importance on the migration, recognition and inflammatory signal transduction of inflammatory cells,but there isn't much indepth study i this field. We found that β4GalT1 (beta 1,4-

galactosyltransferase I) played an important role in the CNS inflammatory response,but its role in regulating glycosylation modification remains unclear.We have found during the activation of glial cells,β4GalT1 regulates glial cell migration, adhesion, and the release of Inflammatory factors by modulating the glycosylation of E-selectin, intergrin α5 and BMP2 respectively; then we found regulation of SSeCKS and UBE2Q on β4GalT1 location and the modulation of CDK11p58 and LRRK1 on β4GalT1 enzymatic activity, respectively. Our research findings can provide the foundation to thoroughly study the role of protein glycosylation modification in inflammatory response.

114: Core fucosylation of mu heavy chains regulates the assembly of precursor B cell receptors and its intracellular signaling

Wenzhe Li[1], Jinhua Jin[1], Akihiro Kondo[2], Jianguo Gu[3], and Naoyuki Taniguchi[4]; [1] Dalian University, Liaoning 116622, China; [2] Osaka University Graduate School of Medicine, Suita 565-0871, Japan; [3] Tohoku Pharmaceutical University, Sendai, Miyagi 981-8558, Japan; [4] RIKEN, Saitama 351-0198, Japan
liwenzhe46@hotmail.com

GDP-L-Fuc:N-acetyl-b-D-glucosaminide a1,6-fucosyltransferase (Fut8) catalyzes the transfer of a fucose residue from GDP-fucose to the innermost *N*-acetylglucosamine (GlcNAc) residue of hybrid and complex *N*-glycans *via* an a1,6-linkage (core fucosylation) in the Golgi apparatus in mammals. The presence of core fucose in *N*-linked glycoprotein has been shown to be important in glycoprotein processing and recognition. Fut8 knockout (*Fut8*$^{-/-}$) mice showed an abnormality in pre-B cell generation. Membrane assembly of pre-BCR is a crucial checkpoint for pre-B cell differentiation and proliferation both in humans and in mice. The assembly of pre-BCR on the cell surface was substantially blocked in the *Fut8*-knockdown pre-B cell line, 70Z/3-KD cells, and then completely restored by re-introduction of the *Fut8* gene to 70Z/3-KD (70Z/3-KD-re) cells. Moreover, loss of a1,6 fucosylation (also called core fucosylation) of μHC was associated with the suppression of the interaction between μHC and λ5. In contrast to *Fut8*$^{+/+}$CD19^{+}CD43^{-} cells, the subpopulation expressing the μHC/λ5 complex in the *Fut8*$^{-/-}$CD19^{+}CD43^{-} cell fraction was decreased. The pre-BCR mediated tyrosine-phosphorylation of CD79a and activation of Btk were attenuated in *Fut8*-KD cells, and restored in 70Z/3-KD-re cells. The frequency of CD19^{low}CD43^{-} cells (pre-B cell enriched fraction) was also reduced in *Fut8*$^{-/-}$ bone marrow cells, and then the levels of IgM, IgG and IgA of 12-week-old *Fut8*$^{-/-}$ mice sera were significantly lower than those of *Fut8*$^{+/+}$ mice. Our results suggest that the core fucosylation of μHC mediates the assembly of pre-BCR so as to regulate pre-BCR intracellular signaling and pre-B cell proliferation. This work was supported by National Nature Science Fundation of China (No. 30972675 and No. 31270864).

115: O-GlcNAc Transferase Mediates the Regulation of 26S Proteasome Functionality by Endothelial Nitric Oxide Synthase Derived Nitric Oxide

Hongtao Liu[1], Shujie Yu[1], Hua Zhang[1], Yu Li[1,2], Yu-Guang Du[2], Jian Xu[1]; [1]Department of Medicine, Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA; [2]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
jian-xu@ouhsc.edu

Loss of vascular homeostasis leads to morbidity and mortality in diabetics, attributable to early loss of endothelial nitric oxide (NO) bioavailability. In contrast, early diabetes is found to increase 26S proteasome functionality in vasculature resulting in enhanced degradation of certain proteins key to the vascular homeostasis. We wonder whether endothelial NO synthase (eNOS)-derived NO functions as a physiological inhibitor of the 26S proteasome in vasculature. To test this, we exposed proteasome reporter (Ub^{G76V}-GFP)-expressing vascular endothelial cells to NO and monitored proteasome functionality by detecting the polyubiquitinated-GFP reporter proteins. We also generated Ub^{G76V}-GFP-expressing eNOS-knockout mice to determine the role of eNOS-derived NO in whole animal. Like the selective NO donors, NO derived from the activated eNOS (either by pharmacological or genetic approach) induced (poly)-Ub^{G76V}-GFP accumulation in cultured endothelial cells, which was associated with increased O-GlcNAc modification of the proteasome regulatory complex (PA700/Rpt2) and reduced proteasome chymotrypsin-like activity ($p<0.05$, $n=5$/group). Conversely, siRNA knockdown of O-GlcNAc transferase (OGT), the key enzyme for O-GlcNAcylation, abolished the NO-elicited effects. Consistently, adenoviral overexpression of O-GlcNAcase, the enzyme responsible for O-GlcNAc removal, mimicked the effects of OGT knockdown. Finally, compared to the control Ub^{G76V}-GFP mice, eNOS-deleted mice exhibited accelerated Ub^{G76V}-GFP degradation in isolated aortas, in parallel with elevated chymotrypsin-like activity and reduced PA700/Rpt2 O-GlcNAcylation, without changing PA700/Rpt2 protein levels. Importantly, the changes were enhanced in eNOS-lacking mice when rendered diabetic with streptozotocin ($p<0.05$, $n=10$/group). In summary, the eNOS-derived NO functions as an OGT-mediated

suppressive regulator of the 26S proteasome, likely through PA700/Rpt2 O-GlcNAcylation. The lack of eNOS-derived NO may contribute to elevated vascular 26S proteasome functionality in early diabetes.

116: Tumorigenic potential of MEF cells transformed with polyoma virus oncogene correlates with β-1,4-galactosyltransferase V gene dosage

Chiemi Tareyanagi, Takeshi Sato, Yukiha Ishikawa and Kiyoshi Furukawa; Laboratory of Glycobiology, Nagaoka University of Technology, Nagaoka, Japan
furukawa@vos.nagaokaut.ac.jp

Our previous studies showed that β-1,4-galactosyltransferase (β4GalT) V is a lactosylceramide synthase and that its gene expression increases upon malignant transformation of cells. To date, there is another lactosylceramide synthase, β4GalT VI, whose gene expression in cells appears unchanged by malignant transformation. To investigate the biological significance of the increased gene expression of β4GalT V in tumor cells, we introduced the anti-sense β4GalT V cDNA into B16-F10 mouse melanoma cells, and examined the tumorigenic and metastatic potentials of the cells. The results showed that the B16-F10 cells with decreased β4GalT V gene expression reduce remarkably their malignant properties when compared with the control cells. This suggests that the expression level of the β4GalT V gene which finally regulates the amounts of lactosylceramide and its derivatives is quite important for malignant potentials of tumor cells.

In order to investigate the above study further, we first isolated fibroblast cells from wild-type, heterozygous and homozygous β4GalT V-knockout (B4galT5−/−) mouse embryos and transformed them with polyoma virus middle T antigen gene. RT-PCR analysis revealed that three types of mouse embryonic fibroblast (MEF) clones contain almost similar numbers of the gene copies. When they were placed into soft agarose gels, all the clones grew in them. However, the average numbers of colonies formed were high and the average sizes of colonies formed were large both in order of B4galT5+/+, B4galT5+/−, and B4galT5−/− derived MEF clones. Furthermore, the transformed MEF clones were transplanted subcutaneously into athymic mice, and tumors were allowed to develop for 3 weeks. The average volumes of the tumors formed were large also in order of B4galT5+/+, B4galT5+/−, and B4galT5−/− derived MEF clones. These results indicate that the tumorigenic potentials of transformed MEF clones correlate with the gene dosage of β4GalT V, namely with the amounts of lactosylceramide synthesized in the cells. Since lactosylceramide is the precursor for a series of glycolipids, how lactosylceramide and/or its derivatives are important for the malignat potentials in tumor cells remains to be elucidated.

117: Endoplasmic Reticulum Located ppGalNAc-T18 (GALNTL4) is Involved in Cell Survival and Apoptosis

Yan Zhang[1], AiDong Shan[1,2], Xing Li[1], Wei Li[1], AiGuo Shen[2]; [1]Ministry of Education Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, 200240, China; [2]Department of Biochemistry, Medical College of Nantong University, Nantong 226001, Nantong 226001, China
yanzhang2006@sjtu.edu.cn

Mucin-type *O*-glycosylation is initialized by members of the UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase (ppGalNAc-T, EC 2.4.1.41) family. Recently, we have identified a new member, ppGalNAc-T18, also known as polypeptide GalNAc transferase-like protein 4 (GALNTL4), which consists the vertebrate-specific Y subfamily together with ppGalNAc-T8, -T9 and -T17. ppGalNAc-T18 localizes in the endoplasmic reticulum (ER) and lacks classical GalNAc-transferase activity. However, our results have revealed that it can selectively modulate the *in vitro* GalNAc-transferase activity of ppGalNAc-T2 and -T10, and it can interact with ppGalNAc-T2 or mucin substrate. Our further study has found that T18 was up-regulated both in apoptotic neuronal cells of mouse brain after intraperitoneal (i.p.) injection of lipopolysaccharide (LPS) and in apoptotic neuron-like PC12 cells after treatment with inflammatory cytokines. Knockdown of T18 induced cells apoptosis through activating ER stress response, and overexpression of T18 protected cells against apoptosis induced by pathological stimuli. Our results indicated that T18 was an essential protein for maintaining ER functions and cells survival. These results demonstrate that T18, an ER localized ppGalNAc-T like protein, not only could be involved in regulating the *O*-glycosylation, but also participated in maintaining ER homeostasis, it has important roles in cells survival and apoptosis.

Physiology & Signalling

118: Functional crosstalk between cell-cell adhesion and cell-ECM adhesion *via* different expression of N-glycans

Jianguo Gu; Division of Regulatory Glycobiology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai Miyagi, 981-8558, Japan
jgu@tohoku-pharm.ac.jp

Changes in oligosaccharide structures are associated with numerous physiological and pathological events. The effects of cell-cell interactions on *N*-glycans were investigated in epithelial cells. The population of the bisecting GlcNAc-containing *N*-glycans, which formation are catalyzed by *N*-acetylglucosaminyltransferase III (GnT-III), was substantially increased in cells cultured under dense condition in an E-cadherin-dependent manner. In contrast, perturbation of E-cadherin-mediated adhesion abolished the up-regulation of expression of GnT-III. Consistently, the induction of GnT-III was not observed in embryonic fibroblasts or E-cadherin-deficient cells, which was rescued by restoration of the expression of E-cadherin. The induction was also not observed in alpha-catenin-deficient cells, but in the restored cells with the alpha-catenin gene. Taken together, these results strongly suggest that GnT-III expression is tightly regulated by cell-cell adhesion *via* the E-cadherin-catenin complex. However, unexpectedly, GnT-III was up-regulated by knockdown of beta-catenin or inhibition of Wnt/beta-catenin signaling, resulting in an inhibition of integrin-mediated cell adhesion. In fact, integrins undergo glycosylation by GnT-III inhibits cancer metastasis *in vitro* and *in vivo*. Beta-catenin is an essential molecule both in cadherin-mediated cell adhesion and in canonical Wnt signaling, the loss of cadherin-mediated cell adhesion can promote beta-catenin release and Wnt signaling, and then Wnt signaling in turn inhibits E-cadherin-catenin-mediated cell adhesion. Therefore, we postulate that there are positive and negative regulation pathways for GnT-III, *i.e.*, E-cadherin-catenin mediated cell adhesion signaling and Wnt/beta-catenin signaling, and the intersection point is at beta-catenin. The GnT-III expression was down-regulated in the epithelial-to-mesenchymal transition (EMT) induced by TGF-beta. Conversely, *N*-acetylglucosaminyltransferase V (GnT-V), which is closely related with cell migration and cancer metastasis, was up-regulated in the EMT. Furthermore, overexpression of GnT-III inhibited EMT-like changes. Taken together, these results provide new insights into the molecular mechanism of crosstalk among cell-cell adhesion and cell-ECM adhesion during normal development, EMT and cancer metastasis.

119: Hydrophobically modified polysaccharides as effective sorbents of alimental cholesterol

Andriy Synytsya[1], Jan Tůma[1], Tomáš Taubner[1], Milan Marounek[2], Dagmar Dušková[2], Jana Čopíková[1]; [1]Department of Carbohydrate Chemistry and Technology, ICT Prague, Technická 5, 166 28 Praha 6 Dejvice, Czech Republic; [2]Institute of Animal Science, Přátelství 815, 104 00 Praha 22 Uhříněves, Czech Republic
sinicaa@vscht.cz

Lowering of LDL cholesterol level in plasma of patients with hypercholesterolemia may improve the risk of cardiovascular diseases. Cholestyramine, a strongly basic anion-exchange synthetic resin, is an example of effective cholesterol lowering agent. It reduces cholesterol absorption by sequestering bile acid conjugates and, therefore, interrupts the enterohepatic circulation of bile acids, increases their fecal excretion and supports compensatory oxidation of cholesterol in the liver. However, cholestyramine also binds anionic drugs, vitamins, and salts, and the competition for its binding sites in the digestive tract decreases the binding capacity for bile acids. As a result, large doses are required for the effective sorption that may cause serious side effects for some patients.

Another way of cholesterol level lowering is the consumption of dietary fibers that can also remove bile acids from the digestive tract. The combination of cholestyramine with such well-tolerated polysaccharides is effective in the treatment of patients with familial hypercholesterolemia. Hydrophobic modifications may increase cholesterol lowering effect of polysaccharides. Alkyl- or acyl-substituted polysaccharides are amphiphilic polymers with a polar sugar backbone and non-polar hydrocarbon side chains. Hydrophobically modified polysaccharides have a potential value as drug carriers and sorbents for removing non-polar compounds from aqueous media and as potential agent for intestinal sorption of cholesterol, fats and bile acids.

N-octadecylamides of highly methylated (HM) citrus pectin and methylesters of cellulose derivatives (monocarboxy oxidized cellulose and carboxymethyl cellulose) were obtained by amino-de-alkoxylation with *n*-octadecylamine. *N,O*-palmitoylchitosan was prepared by reaction of chitosan with palmitoyl chloride. This chitosan derivative was than *N*-methylated (partially quarternized) with methyl iodide. Organic elemental, spectroscopic (FTIR, Raman and CP-MAS NMR) and thermal (DSC, TG) analyses confirmed high substitution degree of these derivatives. *In vitro* sorption of sodium cholate and cholesterol by these hydrophobically modified polysaccharides was studied in comparison with the initial polysaccharides and cholestyramine. The Langmuir–Freundlich (Sips) model parameters were calculated based on the experimental data. Sorption experiments with modified polysaccharides showed that long alkyl/acyl substitution of studied polysaccharides improved their affinity to cholesterol, while an introduction of quarternized nitrogen groups may improve the binding of cholate. It was found that the long alkyl/acyl modified polysaccharides, but not initial ones are able to bind bile acids and cholesterol effectively and thus can be used as cholestyramine replacers. However, by contrast to cholestyramine, these polysaccharide derivatives preferably adsorb cholesterol than cholate that could be explained by their significant hydrophobicity.

In vivo feeding experiments on rats with high cholesterol level diet confirmed that hydrophobically modified HM pectin significantly altered cholesterol homeostasis in these animals and might be considered as a clinically effective hypocholesterolemic agent; preliminary experiments on the alkylated cellulose derivatives achieved similar results.

This work was supported by the Czech Science Foundation (project No 503/11/2479)

120: The mechanism underlying uptake of polysaccharides *via* Clathrin/Dynamin/Eps15 and Rab5 dependent pathway

Wenfeng Liao, Dianxiu Cao, Ying Wang, Kan Ding*; Glycochemistry & Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 ZuChong Zhi Road, Shanghai 201203, China
kding@mail.shcnc.ac.cn

Polysaccharides play multitudinous and important role in many biological processes such as in the immune system, fertilization, pathogenesis prevention, blood clotting, and system development. However, most glycobiologists believe that polysaccharide can not be absorbed by cells due to its large molecular size and the hydrophily. Here, we firstly employed the Caco-2 model to test the *in vitro* permeability of several polysaccharides extracted from plants and fungus. The results show that the apparent permeability coefficient (P_{app}) values of all the polysaccharides are more than 10×10^{-6} cm/s. Interestingly, molecular size of polysaccharides is not changed significantly before and after they across the Caco-2 monolayer. Subsequently, the rat intubation surgery was employed to study the pharmacokinetics of the polysaccharides. The results indicates that the bioavailability of GFPBW1 (1,3-linked glucan), WGE (1,4-linked glucan) and Lentinan is 69.47 %, 73.91 % and 63.89 %, respectively. In addition, the polysaccharides even do not degrade significantly in plasma within at least 10 h. Using FITC-labeled polysaccharides, we found that various linked polysaccharides can internalized into Human Intestine Mucosa Epithelial Cell (HIMC cell) and co-localize with Clathrin, Dynamin, Rab5 and Eps15. Blocking Clathrin-mediated endocytosis either by the selective inhibitor Pitstop-1 or using small interfering RNA can reduce the internalization of the polysaccharides. Furthermore, silence of Dynamin-1, Dynamin-2 and Rab5 in HIMC cells by small interfering RNA or mutant Eps15 can also abolish polysaccharides internalization. These data suggest that polysaccharides may enter intestine cells by a Clathrin/Dynamin/Eps15 and Rab5-dependent pathway. This work provides novel evidences on the absorption of polysaccharides.

121: Involvement of Ganglioside GD3-raft Signaling in the Growth Cone Behavior of Cerebellar Granule Neurons

Kohji Kasahara; Laboratory of Biomembrane, Tokyo Metropolitan Institute of Medical Science,Tokyo 156-8506, Japan
kasahara-kj@igakuken.or.jp

Gangliosides are known to exist in clusters and form microdomains containing cholesterol at the neuronal membrane called rafts and are thought to play functional roles in neuronal differentiation. Previously we isolated ganglioside GD3 synthase cDNA and investigated expression of GD3 synthase. To clarify GD3 raft-mediated neuronal function, we have been investigating the association of ganglioside GD3 with specific proteins in the nervous system.

Anti-ganglioside GD3 monoclonal antibody (R24) co-immunoprecipitated GPI-anchored neuronal cell adhesion molecule TAG-1, src-family tyrosine kinase Lyn and Csk-binding protein Cbp from cerebellar granule neurons. TAG-1 has neurite extension activity and is transiently expressed on premigratory granule neurons in the external granule cell layer. Antibody-mediated cross-linking of TAG-1 or GD3 induced Lyn activation and tyrosine phosphorylation of Cbp in primary cerebellar granule neurons. R24 also co-immunoprecipitated α subunit of heterotrimeric G protein, Go (Goα). Treatment with SDF-1, a ligand for the G protein-coupled receptor, stimulated GTPγS binding to Go and caused Goα translocation to the rafts, leading to the growth cone collapse of cerebellar granule neurons. Migration of cerebellar granule neurons is known to be impaired in TAG-1 or SDF-1-deficient mice.

Immunoblotting analysis showed that GD3, TAG-1, active form of Lyn, tyrosine phosphorylated form of Cbp and Goα were highly accumulated in the detergent-resistant membrane raft fraction prepared from the developing cerebellum. In addition, GD3, TAG-1, Lyn, phosphorylated Cbp and Goα were also concentrated in the growth cone fraction. These data suggest that GD3 rafts are signaling platforms of growth cone behavior in migrating cerebellar granule neurons.

1) Nara et al. (1994) Proc.Natl.Acad.Sci. 91, 7952–7956
2) Kasahara et al. (1997) J.Biol.Chem. 272, 29947–29953
3) Kasahara et al. (2000) J.Biol.Chem. 275, 34701–34709
4) Kasahara et al. (2000) Glycoconj.J. 17, 153–162
5) Kasahara et al. (2002) Neurochem.Res. 27, 823–829
6) Yuyama et al. (2007) J.Biol.Chem. 282. 26392–26400
7) Yuyama et al. (2011) J.Neurochem. 116, 845–850
8) Sekino-Suzuki et al. (2013) J.Neurochem. 124, 514–522

122: Network-based identification of microRNA-modulated programmed cell death pathways in plant lectin-induced cancer death

Leilei Fu, Jingchun Zhang, Yongxi Liu, Jinku Bao*;
School of Life Sciences, Sichuan University, Chengdu, 610064, China
baojinku@scu.edu.cn

Plant lectins, carbohydrate-binding proteins of non-immune origin, have recently been reported to induce programmed cell death (PCD, referring to autophagy and apoptosis) in a variety types of cancer cells. MicroRNAs (miRNAs), small, non-coding endogenous RNAs, ~22 nucleotides (nt) in length, have been well characterized to play essential roles in regulation of the PCD in cancer; Identification of miRNA-regulated apoptotic or autophagic pathways was carried out through a serious of elegant bioinformatics approaches, such as the human apoptotic and autophagic protein-protein interaction (PPI) network construction, the modification of this network into a plant lectin-induced network, hub protein identification, targeted miRNA prediction, microarray analyses and molecular docking, and finally experimental validation. Together, these results demonstrated that network-based identification of miRNA-modulated apoptotic or autophagic pathways in plant lectin-induced cancer death may shed new light on the discovery of plant lectins as potent apoptotic or autophagic inducers, for future cancer drug discovery.

Society for Glycobiology: Model Systems in Glycobiology

123: Pathways Regulating the Expression of N- and O-linked Glycans in Drosophila

Michael Tiemeyer[1,2], Varshika Kotu[1,2], Marion Marshall[1,2], Nickita Mehta[1,2], Mindy Porterfield[1,3], Tadahiro Kumagai[1], Toshihiko Katoh[1], Kazuhiro Aoki[1];
[1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, 30602, USA; [2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, 30602, USA; [3]Department of Chemistry, University of Georgia, Athens, GA, 30602, USA

Glycan expression is regulated in all multicellular organisms, producing patterns of protein and sphingolipid glycosylation that are specific for developmental stage or characteristic of tissue type. Mechanisms that regulate this universal feature of animal development remain largely unknown. With increasing attention focused on describing cellular, tissue, and organismal glycomes, there is hope that patterns may become recognizable within glycan diversity, potentially revealing nodes of regulation. Although its small size might be thought to preclude detailed glycomic analysis, as a model system the embryonic, larval, and adult stages of Drosophila offer unique opportunities to investigate glycan expression. Advances in the sensitivity and robustness of mass spectrometry over the last few decades have made it possible to harvest large amounts of information from small amounts of tissue, allowing a comprehensive analysis of the Drosophila glycome. Dominated by high mannose structures, the N-linked glycome of the Drosophila embryo is also populated with minor glycans of significant complexity, even glycans bearing sialic acid. The O-linked glycome includes structures seen in vertebrate species but also glycans containing novel composition and linkage. Therefore, both the N- and O-linked glycome of the Drosophila embryo are characterized by some similarity to vertebrate glycomes as well as some novelty, providing a fertile domain in which to investigate the regulation of glycan expression. To that end, we have undertaken genetic screens and reverse genetic manipulations in order to identify key genes that control the expression of neural-specific N-linked glycans and of O-linked glycans relevant for developmental signaling pathways. Our combined genetic and glycomic analyses have demonstrated an important role for intercellular signaling, mediated by toll-like receptors and facilitated by specific adaptor proteins and by specific post-translational modifications, in regulating the organization and function of the Golgi apparatus. Furthermore, altered expression of a glycosyltransferase that caps O-linked glycans leads to catastrophic loss of female fertility, but only in a specific genetic background—one that has otherwise been considered wild-type for most of the past century. Thus, Drosophila glycobiology is revealing the importance of signaling networks, subcellular organization, and the complex interplay of genetic background in regulating glycan expression in animals.

124: Sialoglycoconjugates in Organ Development: A Study in Mouse and Zebrafish

Anja-Katharina Muenster-Kuehnel, Wiebke Schaper, Birgit Weinhold, Rita Gerardy-Schahn; Institute for Cellular Chemistry, Hannover Medical School, 30625 Hannover, Germany
Gerardy-Schahn.Rita@mh-hannover.de

The acidic nine-carbon sugar sialic acid (Sia) terminates the bulk of oligosaccharide chains present on cell surface components and circulating glycoproteins. For a long time Sia was regarded as a mere carrier of negative charge. However, the last decades disclosed that Sia is involved in virtually all areas of vertebrate life. The activation of Sia to CMP-Sia by CMP-Sia synthetase (CMAS) is a prerequisite for the biosynthesis

of sialoglycoconjugates. Only the activated sugar can be transferred into the Golgi apparatus and used by sialyltransferases to build specific sialo-glycotopes. In order to study the role of sialo-glycotopes in organogenesis, mouse models with constitutive or tissue specific defects in the cellular sialylation process have been generated. Importantly, these models demonstrate that sialo-glycotopes provide indispensable information not only for proper cellular functions but also for the shaping of anatomical structures.

Recently, we identified two *Danio rerio* genes with high sequence homology to known *CMAS*. The isolated zebrafish cDNAs encode proteins with high resemblance in primary sequence and quaternary structure. However, the zebrafish enzymes (zfCMAS1 and 2) differ in terms of substrate specificity and subcellular localisation. Initial results that establish zebrafish as a suited model organism to study the spectrum of CMAS functions will be discussed.

125: Heparan sulfate Modifications in Development and Disease

Hannes E. Bülow; Albert Einstein College of Medicine, Department of Genetics, Bronx, NY 10461, USA

Heparan sulfates (HS) are un-branched extracellular glycosaminoglycans exhibiting substantial molecular diversity due to multiple, non-uniformly introduced modifications including sulfations, epimerization and acetylation. HS modifications serve specific and instructive roles in neuronal development leading to the hypothesis of a HS code that regulates nervous system patterning by modulating ligand/receptor interactions. By visualizing specific HS modification patterns in the nematode *Caenorhabiditis elegans* we provide direct evidence for the existence of neuron-specific HS modifications in living animals. Our genetic studies establish that distinct combinations of HS modification patterns are important for development of the nervous system. HS 3-*O*-sulfation is the most enigmatic of all HS modifications due its rarity and a largely expanded family of enzymes predicted to introduce this modification. We show that animals lacking all HS 3-*O*-sulfation are superficially normal. Moreover, the general structure of the nervous system is unaffected. Intriguingly, we find highly specific processes during neural development affected by lack of different HS 3-*O*-sulfatransferase genes suggesting that HS 3-*O*-sulfation may play a combinatorial role in establishing the correct neural circuitry of metazoans. Lastly, we show that mutations in human genes encoding HS sulfotransferase result in neurological disorders which also affect specific aspects of neural development.

126: O-glycoprotein podoplanin initiates and maintains separated blood and lymphatic vascular systems

Lijun Xia; Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, Oklahoma 73104, USA. Phone: (405) 271–7892
lijun-xia@omrf.org

Mucin-type O-glycans (O-glycans) are highly expressed in vascular endothelial cells. However, it is not known whether endothelial O-glycans are important for vascular development. To investigate the roles of endothelial O-glycans, we generated mice lacking T-synthase (T-syn), a critical glycosyltransferase for biosynthesis of core 1-derived O-glycans, in endothelial cells (*EHC T-syn*$^{-/-}$). *EHC T-syn*$^{-/-}$ mice exhibited disorganized and blood-filled lymphatic vessels. We found that the O-glycoprotein podoplanin was significantly reduced in *EHC T-syn*$^{-/-}$ lymphatics, and podoplanin-deficient mice developed blood-filled lymphatics resembling *EHC T-syn*$^{-/-}$ defects. In addition, we and others have identified that interaction between podoplanin on lymphatic endothelial cells and platelet CLEC-2 activates SYK/SLP-76 signaling in platelets, which induces platelet aggregation that seals initial embryonic blood-lymphatic vascular connections during embryonic development. Recently, we show that this interaction is also important for preventing blood from entering the lymphatic system at the lympho-venous junction of the thoracic duct and subclavian vein. These results reveal an essential role of podoplanin for the development and maintenance of separated blood and lymphatic systems.

WorkshopI: Glyco-bioinformatics

127: Recent Efforts of Global Collaborations for Interconnecting Glycan-related Databases on the Semantic Web

Hiromichi Sawaki[1], Kiyoko F. Aoki-Kinoshita[2], Issaku Yamada[3], Shin Kawano[4], Shujiro Okuda[5], Masaki Kato[6], Hisashi Narimatsu[1]; [1]Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan,[2]Department of Bioinformatics, Faculty of Engineering, Soka University, Hachioji, Tokyo, 192-0003 Japan, [3]The Noguchi Institute, Tokyo 173-0003, Japan, [4]Database Center for Life Science (DBCLS), Tokyo, 113-0032, Japan, [5]RitsumeikanUniversity, Kusatsu, Shiga, 525-8577, Japan, [6]RIKEN Advanced Science Institute, Wako, Saitama, 351-0106, Japan
h.narimatsu@aist.go.jp

Recent high-throughput technologies for life-science research has been generating large amount of data and enlarging the size and numbers of storing databases. Technologies for building the present databases cannot keep up with the large amounts of data. Thus, bioinformatics researchers have turned to the Semantic Web as the next-generation of the World Wide Web to reconstruct life science databases such that Big Data can be handled effectively.

Also, in Japan, the Integrated Database Project has conducted to developing infrastructures and reconstructing life-science databases from 2010. JCGGDB project is the glycan part of the Integrated Database Project in Japan. We have surveyed available resources for glycol-science fields in the world for adopting glycoscience data to the Semantic Web technologies in 2011, and felt that international collaboration is necessary for this purpose. Then, we have call up a meeting to glycoscience researchers who hold databases by themselves at 2012. ACGG-DB3 meeting has been successfully held in Okinawa, Japan. 25 attendees from 17 research institutes in 7 countries or areas have discussed actively glyco-informatics and the need for collaborations in a global framework (PMID: 23271684). Based on consensus at the Okinawa meeting, concrete development was started and the first collaboration was organization of glycan-team at the DBCLS BioHackathon 2012 in Toyama, Japan, which consisted of 9 researchers representing for 5 databases; CSDB, GlycomeDB, GLYCOSCIENCES.de, JCGGDB and UniCarbKB.
In the presentation, discussion and progress at these meetings will be reported.

This work was supported by a grant from Japan Science and Technology Agency (JST).

128: WURCS: Unique Representation of Carbohydrate Structures for Semantic Web

Issaku Yamada[1, 2]; [1]Laboratory of Glyco-organic Chemistry, The Noguchi Institute, Tokyo, 173-0003, JAPAN, [2]JST, National Bioscience Database Center (NBDC), Tokyo, 102-0081, JAPAN
issaku@noguchi.or.jp

The Web3 Unique Representation of Carbohydrate Structures (WURCS) is a new linear notation for representing carbohydrates for Semantic Web. In the next-generation of the Web, it is expected that a new technology will be able to utilize various data on the Web as a huge database. In order to use the data effectively, it is necessary to develop a method to represent the carbohydrate structure with the same meaning. This new linear notation can represent ambiguous and incomplete carbohydrate structures as unique character strings. Furthermore, anyone can generate WURCS with the same character strings related to the equivalent carbohydrate structure in glycobiology. For example, lactose is represented by the character strings of "[12112h|1,5]1:1,4:2[X2122h|1,5]". By applying WURCS in a Web, a user will be able to use a virtual huge database.

129: UniCarbKB: Building a Glycomics Platform in the Cloud

Matthew P Campbell[1], Jingyu Zang[1], Julien Mariethoz[2], Elisabeth Gasteiger[2], Catherine A Hayes[3], Niclas G Karlsson[3], Frederique Lisacek[2], Nicolle H. Packer[1]; [1]Biomolecular Frontiers Research Centre, Macquarie University, Sydney, Australia; [2]Swiss Institute of Bioinformatics, Geneva, Switzerland; [3]Department of Biomedicine, Gothenburg University, Sweden
matthew.campbell@mq.edu.au

The UniCarb Knowledgebase (UniCarbKB) is an effort to develop and provide an open access bioinformatic framework for the storage of high-quality data collections on glycoconjugates, including informative meta-data and MIRAGE compliant experimental datasets. Initial efforts have focused on the reengineering of GlycoSuiteDB and EUROCarbDB to provide a unified view of structural, experimental and functional information that acts as a central hub of glycoproteomics knowledge.

This corpus of data is rich in information, but there is a distinct growing gap in our knowledge of glycoproteins, in terms of functional information and the extent of glycosylation heterogeneity and site occupancy. The annotation of individual sites provides more detailed insights between sequence and function, as well as context for the interpretation of experimental data. In general, site annotation has to be extracted from the published literature, and can often be transferred to closely related sequence neighbours.

Here, we describe our approach to reorganise and curate existing data collections associated with proteins in a site-specific manner both from the attachment and the recognition perspective. Furthermore, we showcase our efforts to control high-quality data depositions, notable the inclusion of the glycosylated structures characterised from over 100 glycoproteins localised in different tissues and body fluids and its integration with the UniProt and NextProt platforms. These entries are published in a Semantic Web compatible format (GlycoRDF), which is now facilitating integration with other international resources by making connections

between datasets. Ultimately, this will allow researchers to query and navigate between multiple data collections and make discoveries that were previously not possible.

UniCarbKB offers a unique approach to access a comprehensive biocurated overview of existing glycoinformation. The initiative is driven as a community endeavour to promote data sharing in glycobiology and ensure its future development and growth.

130: MIRAGE: Minimum Information Required for A Glycomics Experiment

René Ranzinger, Carsten Kettner and Will S. York; Complex Carbohydrate Research Center, University of Georgia, United States
rene@ccrc.uga.edu

Recent technical advances in analytical glycomics and glycoproteomics have enhanced our ability to understand the critical roles that complex glycans play in tissue and cell development and in the progression of disease. These techniques can produce extremely large amounts of data, and only a small portion of this data is usually reported in publications or databases describing the results of glycomics or glycoproteomics experiments. Members of the glycomics community have agreed that defining a minimum set of information regarding the techniques used to obtain these experimental results would allow scientists to better appreciate the depth and scope of each analysis and to reproduce the results. Therefore, an initiative to develop an international standard for the Minimum Information Required for A Glycomics Experiment (MIRAGE) was established at the 2nd Beilstein Symposium on Glyco-Bioinformatics in Potsdam Germany in June, 2011. The MIRAGE working group (http://glycomics.ccrc.uga.edu/MIRAGE/) has recently finalized and published the glycomics MS guidelines and is currently working on guidelines for other experimental techniques, such as Glycan Array analysis, LC, HPLC and CE. The aim of MIRAGE project is not to constrain how experiments should be performed but rather to provide guidance regarding the information that is required to adequately describe each type of experiment. By listing the essential information to be provided, these guidelines are of great importance not just for authors and reviewers of publications but also for software and database developers. Developers can profit from the MIRAGE guidelines in that they serve as a foundation for defining technical specifications for applications or databases that generate or consume key information. However, the extensive set of information specified by the MIRAGE guidelines requires intelligent implementation strategies to simplify data annotation and submission.

131: Informatics Tools for Glycoscience Research

Kiyoko F. Aoki-Kinoshita; Soka University, Faculty of Engineering, Dept. of Bioinformatics, Hachioji, Tokyo, 192-8577, Japan
kkiyoko@soka.ac.jp

Advancements in glycomics technologies have enabled more high-throughput generation of data such as glycan structure characterization of specific cells and tissues, and lectin- and glycan-binding affinities. In turn, the need for applicable software and tools has increased, and many software and web resources are being developed to allow such data to be analyzed efficiently and effectively.

The RINGS (Resource for INformatics of Glycomes at Soka) web resource at http://www.rings.t.soka.ac.jp provides a number of such tools. One of the first data mining tools for analyzing glycan structure patterns is called Profile PSTMM. This model augmented the linear Markov model originally used for amino acid sequences to include branched structures that had dependencies between siblings. Thus wider-range patterns that were expected to be found in glycan structures could be extracted rather efficiently. Another tool is the Glycan Miner Tool, which mines large numbers of glycan structures to find common substructures of sufficient size and diversity. There is also the Glycan Pathway Predictor (GPP) which is based on a mathematical model for predicting *N*-glycan biosynthesis for a number of glycosyltransferases. In addition to these tools, a number of utilities for converting between various glycan structure text formats are also available.

Many other algorithms have also been developed, such as for analysis of glycan biosynthesis at a systems level and motif finding using glycan array data. Some of these have been made available as free software as well.

132: Enhancement of the search function in JCGGDB

Toshihide Shikanai, Masako Maeda, Noriaki Fujita, Yoshinori Suzuki, Hongling Wen, Madoka Ishizaki, Hiromichi Sawaki, Hiroyuki Kaji, Hisashi Narimatsu; Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan

As entering the final year of JST/NBDC integrated database project in 2013, we are driving the development of JCGGDB (Japan Consortium for Glycobiology and Glycotechnology DataBase) to achieve the integration of Japanese databases as well as international collaboration.

JCGGDB was selected as a promotion program in the project, aiming to complete the integration of all the glycan-related databases in Japan and build user-friendly search systems. As a part of the project, we also intend a DB integration within Asia and construct ACGG-DB (Asian Communications of Glycobiology and Glycotechnology DataBase) in cooperation with Asian countries.

So far we have consolidated data from various Japanese institutes into JCGGDB and developed a couple of search functions such as cross-search by keyword entry and integrated search by glycan structures.

In addition, "Glyco"-Keywords have been collected using text-mining technique to analyze the frequency of keywords as well as the relationship between them. Collected data is also used to organize the synonyms and similar terms of glycan structures. We are developing retrieval techniques which enables to display those co-occurring words as Related Words in the DB and exchange links with related databases individually.

Working closely with Japanese and Asian institutes, we will continuously develop base technologies for the DB integration, facilitate interactions between databases in the field of glycoscience as well as other associated study areas, and build bioinformatics tools to support experimental study. Our goal is to create contents which could be easily and intuitively understood by every user.
This work was supported by Integrated Database project in JST/NBDC in Japan.

JCGGDB web site : http://jcggdb.jp

133: CSDB and other carbohydrate databases

Philip Toukach; N.D. Zelinsky Institute of Organic Chemistry, Leninsky prospekt 47, Moscow, 119991 Russia
netbox@toukach.ru

Nowadays, the orientation in a currently acquired volume of glyco-related information is impossible without special features of informatics. Therefore, the progress of glycobiology strongly depends on a presence of an information environment including data on structures, properties and functions of carbohydrates, as well as on taxonomy and properties of their biological sources. The main approach to create such environment is development of carbohydrate databases. In contrast to genomics and proteomics, informatization of glycomics is still suffering from incompatibility between the existing projects. In the present mini-review I report a comparative analysis of currently active carbohydrate databases, with Russian Carbohydrate Structure Databases[1] (CSDBs) emphasized.

Glyco-databases providing wide coverage are most demanded, among them meta-database GlycomeDB, GLYCOSCIENCES, GlycoSuiteDB, CFG Glycan Database, KEGG, JCGGDB, GlycoBase-Dublin, UniCarbKB, Bacterial&Archaeal CSDB, and Plant&Fungal CSDB (currently in development) and other. Historically first carbohydrate database, CCSD (Carbbank) pretended to have complete coverage of structures published before 1996, when its support was ceased. Collection and digitizing of primary data are the most time-consuming stages of a database development, and therefore almost all modern projects somehow use the Carbbank data.

Analysis of the distinctive features of various projects allows to establish the criteria of database evaluation: types of data stored, completeness of coverage, data quality, functions provided to users, interface (usability, stability and performance), integration with other projects, and database architecture. Although the last criterion is invisible to users, it has a strongest impact on a database usefulness, since architectural mistakes hamper maintainability, upgradability and error control, and continuously increase the cost of a project.

The minimal types of data stored and processed in a glyco database are a primary molecular structure and taxonomical and bibliographic annotations. Many databases store analytical data, such as NMR or MS spectra. Storage of biochemical, genetic, medical and other related data is often supported, but their coverage remains poor. Of major carbohydrate databases, KEGG lacks for taxonomical annotations, and GlycomeDB for bibliographic annotations. The databases with stored NMR spectra provide a spectral coverage of 5– 25 % of the published data.

Higher coverage significantly increases the value of a database, since even a negative answer to a search request presents valuable scientific information. Restricted potential of automatization of a search for suitable publications limits the acquisition of primary data and, therefore, the coverage. Nowadays only Bacterial CSDB reports almost complete (>75 %) structural coverage within a chosen compound class, namely glycans from procaryotic microorganisms. Since 2005 it has accumulated ca. 10 000 structures assigned to 5 000 microorganisms in 4 000 publications.[2] Newly established Plant&Fungal CSDB aims at achieving this level of coverage in 2016, and currently deposits re-annotated data published before 1996.

[1] CSDB web-site (2012) Bacterial, plant and fungal Carbohydrate Structure Databases, http://csdb.glycoscience.ru
[2] Ph.V. Toukach (2011) Bacterial Carbohydrate Structure Database 3: Principles and Realization *J Chem Inf Model* **51**, 159–170.

To keep the coverage actual, periodic updates are needed, which assumes one- or two-year lag between publication and deposition of data. A universal solution for keeping the data actual is a requirement of obligatory upload of every published structrure to a database prior to publication with subsequent provision of the obtained IDs to an editorial. Such approach has been realized in genomics for long but is still missing in glycomics. One of the reasons for that is an insufficient standardization of glycan description languages originating from high chemical variativity of carbohydrates. This problem, as well as limited cross-project compatibility, can be overcome by source-independent data framework, such as RDF. Several databases, including CSDB, GlycomeDB, UniCarbKB and other provide export of data as RDF triples according to an experimental version of GlycanRDF ontology formulated at Biohackathon 2012, Japan.

The process of data posting can hardly be automatized not only at the level of publication selection but also at the level of article interpretation. As a result, all chemical and biological databases contain errors. These errors originate from (in occurrence-descending order): annotators' failures, other imported databases, original publications, architectural inconsistencies, bugs in import and auto-annotation software. According to our investigation,[3] most records in Carbbank contain errors, and more than one third of records contain two or more errors. The most abundant error type is an incorrect taxonomical annotation of a structure. Significant gaps in the Carbbank coverage were also discovered. As most of the modern projects use the Carbbank data, these errors are being reproduced. Some of them can be revealed and sometimes corrected automatically. Such control is present in a number of databases; however, only a retrospective expert analysis of publications can provide really high data quality. Two thirds of CSDB budget is devoted to manual literature processing.

Database functionality is its capability to process various search requests, combine and refine them using diverse logics and other types of queries. *E.g.*, "*find all structures published from 2001 to 2005, that contain either an α-Gal(1–>3)KDO fragment or a monosaccharide-bound lysine or alanine, except synthetic structures or those found in gamma-proteobactertia, and display their* ^{13}*C NMR spectra*". In contrast to a search for bibliography, taxonomy, keywords, text fragments and similar data, a search for structural fragments in bigger molecules (as well as for structures or spectra resembling a specified one) requires more meticulous programming and computational power, making the inner database architecture critical for the performance of such queries. In the mid-2000s, developers of GLYCOSCIENCES.de formulated "Ten golden rules of carbohydrate database development", which summarized the experience of the German and Russian groups. The key points of this document include usage of a connection table for inward structure representation, maximal possible indexation, minimum of free text data (which, regretfully, are present in virtually every project), and unambiguously controlled vocabularies for as much data types as possible. An attempt to separate the monosaccharide vocabulary from glyco-databases was made within MonosaccharideDB. Nowadays it provides full coverage on monosaccharides present in mammalian glycans.

Possibility of correct processing of structural data is directly related to the format of both internal and user structure descriptions. Incapabilities and inter-incompatibility of glycan description languages have been limiting the progress of glycoinformatics for decades. The main criteria of carbohydrate language efficiency are: 1. unambiguity and uniqueness of every chemically distinct structure, including non-carbohydrate moieties; 2. support of all structural features of carbohydrates and glycoconjugates (single and multiple repeats, cyclic and combined glycans, glycolipids, glycoproteins, non-carbohydrate and untypical constituents, phospho- and sulfo-linkages, cyclic esters, amide and ether linkages, *etc.*); 3. support of underdetermined structures at the level of monomers and their configurations, stoichiometry, substitution positions, and chain topology; 4. computer-readability with no need for ambiguous parsing, as in the case of Extended JUPAC, and human-readability required for tracking of errors that appear during human processing of data dumps; 5. compatibility with other formats (presence of converters that help language learning and cross-database operations), *e.g.* monomer vocabulary widely recognized by glycobiologists. The CSDB Linear and GlycoCT languages possess most of these features. However, the former does not support nested repeats and have limited aglica support, and the latter is not human-readable and supports carbohydrate moieties only. Glycomics still lacks a standard language except the JUPAC, which is highly imperfect.

The functionality can be extended by carbohydrate-related services, such as conformation map simulation, spectra prediction, search for structural motifs *etc.* CSDB project provides a generator of structural variety restricted by user constraints determined from simple experiments (*e.g.* number of residues per repeat and other data). This approach is a gateway to theoretical structural elucidation and ranking based on experimental data, and can be applied to this or to any other glycan database. Every structure in the generator output is a subject to either database-driven averaging or fully theoretical simulation of properties. Among these properties are the NMR spectra. As realized in CSDB, ^{13}C NMR simulation combines both environment-dependent database search and statistical

[3] K.S. Egorova, Ph.V. Toukach (2012) Critical analysis of CCSD data quality. *J Chem Inf Model* **52**, 2812–2814.

processing, and empirical incremental prediction of chemical shifts. To find a chemical shift in a database a twelve-step generalization of atomic environment is applied. As well as the other related projects (CASPER, BIOPSEL), this feature is aimed at dramatic simplification of natural carbohydrate structural studies.

Modern quality standard in informatics implies that both user and administrative interfaces are intuitive, well-documented and freely accessible *via* Internet. Intuitiveness includes structure input and output formats, which users should not have to study. Standalone services for structure input and visual editing are of extreme usefulness, as they allow users of any database to stay within the interface which they got used to. Cross-project integration implies not only common interface of search requests but also automated data interchange *via* API. It concerns interactions with non-carbohydrate databases as well, at least with NCBI Taxonomy and NCBI Pubmed. First two projects that reported protocols for automated data exchange were GLYCOSCIENCES.de and Bacterial CSDB, and since then the development of glyco-related web-services has intensified.

Within the Bacterial CSDB project, we tried to develop the database architecture and to realize it in software free of disadvantages of other glyco-databases. Since then Bacterial & Archaeal CSDB has been maintained and regularly updated. In 2012, we started its expansion to plant and fungal carbohydrates, being the pre-last step to creation of a complete database of natural glycans, which, we hope, will ideologically replace Carbbank.

WorkshopII : Proteoglycans

134: Keynote Lecture: Exploiting the heparan sulphate interactome: from biology to synthetic chemistry to therapeutic applications

J. E. Turnbull; Centre for Glycobiology, Institute of Integrative Biology, University of Liverpool, Liverpool, L69 7ZB, UK
j.turnbull@liverpool.ac.uk

Heparan sulfates (HSs) are complex sulfated polysaccharides present on almost all cell surfaces in multicellular organisms. They have variant structures which represent a molecular code that is a subset of the glycome called the 'heparanome'. These sulfation patterns confer the ability to interact selectively with a wide interactome of proteins that influences many cellular processes important in the development, regulation and repair of tissues. Understanding the chemical biology of these enigmatic molecules is now becoming possible through a variety of tools, reagents and approaches including saccharide libraries, microarray methods and novel sequencing approaches. We are developing and exploiting semi-synthetic and synthetic chemistry strategies to produce targeted libraries for activity screening to decode the molecular basis of the functional diversity of HS. This has yielded new insights into this code in a variety of biological contexts, including neural development, degeneration and repair, and cancer metastasis. This has led to a translational pathway of biomedical applications, including discovery of novel drug leads for Alzheimers disease and cancer metastasis, and potential interventions to improve neural cell transplantation for nerve repair.

135: Synthesis of Heparan Sulfate Hexa- to Dodecasaccharides as Inhibitors of the Alzheimer's Disease Target β-Secretase

Ralf Schwörer[1], Jeremy E. Turnbull[2], Peter C. Tyler[1], Olga V. Zubkova[1]; [1]Carbohydrate Chemistry, Callaghan Innovation Research Limited, Lower Hutt, 5040, New Zealand; [2]Institute of Integrative Biology,University of Liverpool, Liverpool, L69 3BX, UK.
peter.tyler@callaghaninnovation.govt.nz

Heparan sulfates (HS) are a class of sulfated polysaccharides which function as dynamic biological regulators of the functions of diverse proteins. The structural basis of these interactions however remains elusive, and chemical synthesis of defined structures represents a challenging but powerful approach for unravelling the structure-activity relationships of their complex sulfation patterns. HS has been shown to function as an inhibitor of the beta-site cleaving enzyme β-secretase, a protease responsible for generating the toxic A-beta peptides that accumulate in Alzheimer's disease (AD), with 6-O-sulfation identified as a key requirement. Here we demonstrate a novel generic synthetic approach to HS oligosaccharides applied to production of a library of 16 hexa- to dodeca-saccharides, targeted at β-secretase inhibition. Screening of this library has provided new insights into structure-activity relationships for optimal β-secretase inhibition, and yielded a number of potent non-anticoagulant inhibitors with potential for development as leads for treatment of AD through lowering of A-beta peptide levels.

136: Developing an enzymatic approach to synthesize heparin and heparin

Jian Liu; Division of Chemical Biology and Medicinal Chemistry,Eshelman School of Pharmacy; University of North Carolina, Chapel Hill, NC 27599

Heparan sulfate is a sulfated glycan that exhibits essential physiological functions, and heparin is a clinically used anticoagulant drug. Interrogation of the specificity of heparan sulfate-mediated activities demands a library of structurally defined oligosaccharides. Synthesis of heparan sulfate using enzymes provides a promising approach because of the high regioselectivity of heparan sulfate biosynthetic enzymes. The synthesis of heparan sulfate involves 15 different enzymes, including sulfotransferases, an epimerase and glycosyltransferases. Up to now, a number of oligosaccharides with different sulfation patterns and sizes were synthesized. These oligosaccharides are now used to probe the biosynthetic mechanism of heparan sulfate and heparin, improving the synthesis of heparin drugs, and understanding the interaction of heparan sulfate with proteins. The availability of structurally defined heparan sulfate oligosaccharides will significantly advance the ability to understand the biology of heparan sulfate.

137: RAGE: A proteoglycan-associated receptor

Ding Xu[*1], Jeffrey Young[1], Kevin D. Corbett[1,3], Walter J. Chazin[4], Lars C. Petersen[2] and Jeffrey D. Esko[1]; [1]Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California, San Diego, La Jolla, CA 92093, [2]Laboratory of Structural Biology, National Institute on Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, [3]Ludwig Institute for Cancer Research,[4]Department of Biochemistry, Center for Structural Biology, Vanderbilt University, Nashville, TN 37232

Many cell surface receptors rely on heparan sulfate for activity, but depend on different modes of interaction. Fibroblast growth factor (FGF) receptors form 2:2:2 complexes with heparin and FGF ligands. Based on crystallization studies, the non-reducing end of each heparin chain docks with one FGF and a canyon defined by the two receptors. In contrast, vascular endothelial growth factor (VEGF) receptors depend on heparan sulfate for activity presumably due to interaction with a heparin-binding coreceptor, such as neuropilin-1, or for stabilization of receptor oligomers induced by ligand binding. In this system, interaction of the ligand with heparin is not required. Recently, we described a third model based on the receptor for advanced glycation end products (RAGE), which also requires heparan sulfate for activity. Here we show that RAGE undergoes a heparin-dependent, ligand-independent oligomerization, generating a stable hexamer of the RAGE extracellular domain. Site-directed mutagenesis mapped two separate heparan sulfate-binding sites in the Ig-like V and C1 domains of RAGE. A hydrophobic dimeric interface essential for the formation of hexamer was also identified in the V domain. Crystallization of RAGE V-C1 in the presence of a heparin dodecasaccharide revealed a hexameric structure in the crystal lattice, consistent with complex composed of a trimer of dimers. A solution structure consistent with a hexamer was also observed by small angle X-ray scattering. Mechanistically, the data suggests that heparan sulfate binds to a positively charged cleft created by two subunits of the dimer, stabilizing an otherwise weak hydrophobic dimeric interface and promoting dimer formation and assembly into a hexamer. A monoclonal antibody targeting the hydrophobic interface blocks receptor signaling, demonstrating that oligomerization is required for signaling. Thus, RAGE defines a third mode of interaction in which heparan sulfate is a subunit of the holoreceptor.

138: Receptor for Advanced Glycation End-products (RAGE) Functions as Receptor for Sulfated Glycosaminoglycans (GAGs): Anti-RAGE Antibody or Sulfated GAGs Inhibit Lung Metastasis of Tumor Cells

Kazuyuki Sugahara, Jun Takahashi, Shuji Mizumoto; Hokkaido University Graduate School of Life Science, Sapporo, Japan 001-0021
k-sugar@sci.hokudai.ac.jp

Altered expression of chondroitin sulfate (CS) and heparan sulfate (HS) at the surfaces of tumor cells plays a key role in malignant transformation and tumor metastasis. Previously we demonstrated that a Lewis lung carcinoma (LLC)-derived tumor cell line with high metastatic potential had a higher proportion of E-disaccharide units, GlcUA-GalNAc (4,6-*O*-disulfate), in CS chains than low metastatic LLC cells and that such CS chains are involved in the metastatic process. The metastasis was markedly inhibited by the pre-administration of CS-E from squid cartilage rich in E units or by pre-incubation with a phage display antibody specific for CS-E. However, the molecular mechanism of the inhibition remained to be investigated.

In this study the receptor molecule for CS chains containing E-disaccharides expressed on LLC cells was revealed to be receptor for advanced glycation end products (RAGE), which is a member of the immunoglobulin superfamily predominantly expressed in the lung. Interestingly, RAGE bound strongly to not only E-disaccharide, but also HS-expressing LLC cells. Furthermore, the colonization of the lungs by LLC cells was effectively inhibited by the blocking of CS or HS chains at the tumor cell surface with an anti-RAGE antibody through intravenous injections in a dose-

dependent manner. These results provide the clear evidence that RAGE is at least one of the critical receptors for CS and HS chains expressed at the tumor cell surface and involved in experimental lung metastasis and that CS/HS and RAGE are potential molecular targets in the treatment of pulmonary metastasis. Recent advances of the work will also be discussed.

139: Heparan Sulfate Is Required For Prostate Cancer Initiation And Progression

Xuanyang Li[1], Alison Nairn[1], Tamas Nagy[2], Fen Wang[3], Yu Yamaguchi[4], Kelley Moremen[1], Lianchun Wang[1]; [1]Complex Carbohydrate Research Center and Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602, USA; [2]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA; [3]Center for Cancer and Stem Cell Biology, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX 77030, USA; [4]Sanford-Burnham Institute for Medical Research, La Jolla, California 92037,USA

Prostate cancer (PCa) is one of the most prevalent forms of malignancy and the second most common cause of cancer-related death in men. The failure in treatment of this disease is our inability to prevent and control PCa growth and metastasis. A better understanding of the mechanisms underlying PCa pathogenesis will greatly enhance our effort to cure this life-threatening disease. Heparan sulfate (HS) is a linear, sulfated polysaccharide, and expresses abundantly in prostate and PCa tissues. Intriguingly, the HS content and sulfation modifications appear to increase when the prostate becomes malignance, suggesting that HS may critically modulate PCa pathogenesis. In current study, we specifically ablated Ext1, the enzyme that initiates HS biosynthesis, in mouse prostate at late development stage. The Ext1 ablation does not affect prostate development and function, instead, it protects the mice from tumorigenesis and invasion in a spontaneous PCa mouse model. Tissue staining showed that the Ext1 deficiency attenuated PCa cell proliferation, increased apoptosis, and blocked PCa stem/progenitor cell differentiation and epithelial-mesenchymal transition. The Ext1 deficiency PCa tissues also showed significant attenuation of fibrinosis, inflammation, MMP-9 expression and hypoxia. In summary, our studies demonstrate that HS functions via multiple mechanisms to promote PCa tumorigenesis and invasion, and also reveal that targeting HS may represent a novel and effect approach to cure PCa.

140: Role of high endothelial venule-expressed heparan sulfate in chemokine presentation and lymphocyte homing

Hiroto Kawashima[1] and Koichiro Tsuboi[1]; [1]Laboratory of Microbiology and Immunology, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
kawashih@u-shizuoka-ken.ac.jp

Lymphocyte homing to peripheral lymph nodes (PLNs) is mediated by multi-step interactions between lymphocytes and high endothelial venules (HEVs). Heparan sulfate (HS) has been implicated in the presentation of chemokines on the surface of HEVs during this process. However, it remains unclear whether this cell surface presentation is required for chemokine function *in vivo*. In this study, we generated conditional knockout (cKO) mice lacking *Ext1*, which encodes a glycosyltransferase essential for HS synthesis, by crossing $Ext1^{flox/flox}$ mice with *GlcNAc6ST-2-Cre* transgenic mice expressing Cre recombinase in HEVs. Immunohistochemical studies indicated that HS expression was specifically eliminated in PLN HEVs but retained in other blood vessels in the cKO mice. The accumulation of a major secondary lymphoid tissue chemokine, CCL21, on HEVs was also abrogated without affecting *CCL21* mRNA levels, indicating that HS presents CCL21 on HEVs *in vivo*. Notably, a short-term lymphocyte homing assay indicated that lymphocyte homing to PLNs was partially diminished in the cKO mice by 30 to 40 %, and the lymphocytes could attach to and transmigrate through HEVs that lacked HS *in vivo*. The residual lymphocyte homing to PLNs in cKO mice was completely abrogated by pertussis toxin which inactivates Gi subunit-coupled heterotrimeric G proteins involved in chemokine receptor signaling, suggesting that soluble chemokines detected in the sera and PLN organ culture supernatants of cKO mice are involved in the residual lymphocyte homing. Furthermore, contact hypersensitivity responses were also partially diminished in the cKO mice. These results suggest that chemokine presentation by HS on the surface of HEVs facilitates lymphocyte homing and immune responses but is not absolutely required for chemokine function.

WorkshopIII-Young Glycoscientists' Symposium

141: Structural glycobiology of influenza virus receptors in respiratory tissues of humans and other animal models

Nan Jia[1], John M. Nicholls[2], Anne Dell[1], Stuart M. Haslam[1];[1]Division of Molecular Biosciences, Imperial College London, London SW7 2AZ, UK; [2]Department of Pathology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong SAR, China
nan.jia07@imperial.ac.uk

Influenza is a virus that infects both birds and mammals, including pigs, ferrets, mice and humans. The seasonal flu has been a public health concern for years and has the potential to cause global pandemics. Additionally, infected animals are slaughtered to prevent further spreading of the disease, leading to great economic loss. Although the exact mechanism by which the influenza becomes transmissible in human populations and between animal reservoirs remains incompletely understood, the initial interaction between the virus and the host cell is crucial. More specifically, it is the binding between viral surface proteins hemagglutinin and sialic acid residues displayed by glycoproteins and glycolipids that are expressed on the host plasma membrane, which triggers cellular recognition, attachment and viral entry events.

Sialic acids link to their adjacent monosaccharides *via* α2, 3 or α2, 6 glycosidic bonds and this linkage difference together with the change from Neu5Ac to Neu5Gc, are key factors defining the species barrier. The regional distributions of the two types of the glycosidic bonds along the respiratory tract, which is the infection site of mammalian species, have been demonstrated mostly by lectin staining studies. To better understand the functionality of host glycan receptors, we carried out complementary studies where structural information was extracted from animal tissues. Samples were processed to yield purified N- and O- linked as well as glycolipid derived glycan molecules, which were subsequently analysed by mass spectrometric instrument. Additional linkage information was further acquired by enzymatic digestion and GC-MS experiments.

We have produced glycomic profiles of several respiratory tissues, such as lung and trachea from different animal species. The ultimate aim of the study is to investigate how the receptor tropism present by different hosts could affect the infectivity of various influenza strains. Moreover, our structural data provides fundamental information of cellular receptors, based on which the structural heterogeneity of glycans along the respiratory tract can be compared among different animal models and which can be used for the production of glycan arrays that resemble the spectrum of glycans present in the respiratory tract.

142: Improved O-glycan Analysis Methods

Radoslaw P. Kozak[1], Louise Royle[1], Richard A. Gardner[1], Gerhild Zauner[2], Emanuela Lonardi[2], André M. Deelder[2], Daryl L. Fernandes[1] and Manfred Wuhrer[2];
[1] Ludger Ltd. Culham Science Centre, Oxfordshire, OX14 3EB, United Kingdom; [2] Biomolecular Mass Spectrometry Unit, Department of Parasitology, Leiden University Medical Center, Postbus 9600, 2300 RC, Leiden, The Netherlands
rad.kozak@ludger.com

The analysis of O-glycans is essential for better understanding of their functions in biological processes. Although many techniques for O-glycan release have been developed, in our hands, the hydrazinolysis method has proven to be the best for producing O-glycans with free reducing termini in high yield. This release technique allows the glycans to be labelled with a fluorophore and analysed by fluorescence detection. Under the hydrazinolysis release conditions, an unwanted side reaction is observed which causes the loss of monosaccharides from the reducing terminus of the glycans. This side reaction is known as peeling. The reduction of this glycan degradation step is necessary in order to obtain information on the initial oligosaccharide composition. Using bovine fetuin and bovine submaxillary gland mucin (BSM) we demonstrated that peeling can be greatly reduced when the sample is buffer exchanged prior to hydrazinolysis with either solutions of 0.1 % trifluoroacetic acid (TFA) or low molarity (100, 50, 20 and 5 mM) ethylenediaminetetraacetic acid (EDTA). The presented technique for sample preparation prior to hydrazinolysis greatly reduces the level of undesirable cleavage products in O-glycan analysis and increases the robustness of the method.

We further evaluate the use of O-glycans obtained by hydrazinolysis from various biological sources for the preparation of natural O-glycan arrays. To this end, O-glycans released from BSM and porcine stomach mucin (PSM) and human saliva are labelled with 2-aminobenzamide, fractionated by hydrophilic interaction liquid chromatography (HILIC) UPLC with fluorescence detection, and characterized by MALDI-TOF(/TOF)-MS. When necessary, O-glycan fractions are subjected to a second-dimension reverse phase separation to obtain homogeneous fractions. Subsequently, O-glycans are printed onto epoxy-activated glass slides and probed with well-characterized carbohydrate-binding proteins for the

detection of structural determinants as well as for the evaluation of lectin specificities.

143: Quick and convenient diagnostic method for Guillain-Barré syndrome using sugar-chain immobilized fluorescent nano-particles

Hiroyuki Shinchi[1], Nobuhiro Yuki[2], Hideharu Ishida[3], Koichi Hirata[4], Masahiro Wakao[1], Yasuo Suda[1]; [1]Graduate School of Science and Engineering, Kagoshima Univ., Kagoshima, 890-0084, Japan; [2]Development of Medicine, National University of Singapore, Singapore, 117599, Singapore; [3]Faculty of Applied Biological Science, Gifu Univ., Gifu, 501-1193, Japan; [4]Dokkyo Medical Univ., Tochigi, 321-0293, Japan
k9561067@kadai.jp

Guillain-Barré syndrome ranks as the most frequent cause of acute flaccid paralyis, often being misdiagnosed as having stroke. Patients frequently carry autoantibodies against gangliosides, which are usually detected by ELISA. However, it takes several days to receive the assay results from the diagnostic laboratory. Toward the rapid point of care diagnostic kit, we developed a novel method for the detection of the anti-ganglioside antibodies in serum using sugar-chain immobilized fluorescent nano-particles (SFNPs) containing component sugar-chains of gangliosides.

CdTe/CdS core/shell quantum dots (QDs), possessing high photo-stability and attractive optical properties, were used as fluorescent nanoparticles. SFNPs were prepared by the surface ligand exchange reaction using sugar-chain ligand conjugate which was obtained by the condensation reaction of the chemically synthesized sugar moiety of ganglioside with our original fluorescent linker molecule. Immobilization of sugar chain onto the nano-particle was confirmed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS).

The prepared SFNPs were then used for the detection of anti-ganglioside antibodies in sera from patients with Guillain-Barré syndrome. SFNPs were mixed with an appropriate amount of serum and left for a few hours. After centrifugation of the mixture, fluorescent precipitates were observed only in the Guillain-Barré syndrome patient's serum. It was confirmed by SDS-PAGE that the precipitate contained IgG, suggesting the specific complex of SFNP with anti-ganglioside antibodies. Since our developed method detects serum anti-ganglioside antibodies in a few hours, it can be utilized as a rapid and conventional diagnostic method.

144: NMR analyses of conformational dynamics and interactions of oligosaccharides by employing paramagnetic probes

Ying Zhang [1,2,3], Takumi Yamaguchi [1,2,3], Sayoko Yamamoto [1,3], Yoshinori Uekusa[1,3], Maho Yagi-Utsumi [1,3] and Koichi Kato [1,2,3]; [1]Institute for Molecular Science and Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences and [2]Department of Functional Molecular Science, The Graduate University for Advanced Studies (SOKENDAI), 5-1 Higashiyama, Myodaiji, Okazaki, 444-8787, Japan, [3]Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, 467-8603, Japan
yzhang@ims.ac.jp

Oligosaccharides play important physiological and pathological roles in biological systems. For better understanding the molecular basis of the mechanisms underlying oligosaccharide functions, it is quite desirable to describe their conformational dynamics and interaction systems at atomic level. However, these are still the remaining challenges, because high conformational flexibility of oligosaccharides hinders the application of conventional analytical methods such as X-ray crystallography.

Nuclear magnetic resonance (NMR) spectroscopy has immense potential to deal with such dynamics issues in various ranges of spatial and time scales. For conformational characterization of flexible oligosaccharides, their structures should not be dealt with as a single well-defined global free energy minimum but as an ensemble of low energy conformers. To overcome the major limitation of traditional NMR methods for the insufficiency of conformational information of oligosaccharides provided by nuclear Overhauser effect data, we have been developing an NMR methodology for evaluating a dynamic ensemble of oligosaccharide conformations by employing paramagnetic effects in conjunction with molecular dynamics (MD) simulation. Paramagnetic effects were induced by unpaired electron providing long-distance information on conformations and dynamics of oligosaccharides.

By applying this approach, we have successfully characterized the 3D structure of flexible GM3 trisaccharide (αNeu5Ac-(2-3)-βGal-(1-4)-βGlc) and branched GM2 tetrasaccharide (βGalNAc-(1-4)-[αNeu5Ac-(2-3)]-βGal-(1-4)-βGlc) which share the common core structure of gangliosides forming an integral part of cellular membranes. The interbranch interactions responsible for the distinct conformational ensembles between the GM3 and GM2 oligosaccharides were elucidated by the paramagnetic-assisted NMR

analyses in conjunction with MD simulations. The results demonstrated that this methodology offers a valuable tool for the characterization of flexible oligosaccharides conformational dynamics. Furthermore, we extended this methodology to analyze oligosaccharides interaction systems, such as carbohydrate-carbohydrate and carbohydrate-protein interactions. For example, the weak interactions between gangliosides were observed through the analyses of inter-molecular paramagnetic effect offered by NMR measurements. This methodology opens a new prospect for conformational analyses of dynamic structures of oligosaccharides toward decoding glycocodes from the 3D structural aspects.

145: Expression and characterization of the first snail-derived UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase

Christopher Taus[1], Chantal Lucini[1], Takeshi Sato[2], Kiyoshi Furukawa[2], Reingard Grabherr[3] and Erika Staudacher[1]; [1] Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria, [2]Laboratory of Glycobiology, Nagaoka University of Technology, Nagaoka, Japan, [3] Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
Christopher.Taus@boku.ac.at

Mucin-type O-linked protein glycosylation is initiated by the UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase (ppGalNAcT) enzyme family. To date, several members of mammalian ppGalNAcTs, as well as various ppGalNAcT isoforms from *Caenorhabditis elegans* and *Drosophila melanogaster* have been identified and described. The large number of different ppGalNAcT isoforms with varying acceptor-substrate specificities *in vitro* suggests that O-glycosylation *in vivo* is a very complex process. In this study, we isolated and characterized the first snail-derived ppGalNAcT from *Biomphalaria glabrata* embryonic (Bge) cells.

Due to limited mollusk sequencing data, our first approach was to create a full-length cDNA library from the Bge cell line. Using sense and antisense degenerated primers designed to highly conserved sequences of the ppGalNAcT enzyme family, a corresponding fragment from snail origin could be amplified by homology-based PCR. Within three experimental steps we could isolate the full-length ppGalNAcT gene. The snail glycosyltransferase has high homology to other ppGalNAcTs and contains all domains common to this enzyme family. Based on the amino acid sequence, there is no N-glycosylation site, whereas O-glycosidase treatment prior to lectin blot analysis indicates the occurrence of O-glycosylation. The novel ppGalNAcT shows a pH-optimum at pH 6.0-6.5, divalent cations are required, with manganese being optimal at 10 mM, and 37 °C as best incubation temperature. All tested acceptor peptides were glycosylated immediately with one but during extensive incubation also with more GalNAc residues. Different acceptor quality of the tested peptide substrates indicate that the amino acid sequence plays an important role for glycosylation efficiency.

Better insights in the glycobiology of snails will increase the understanding of glycan biosynthesis and may also result in new strategies for pest control, as well as a better understanding of molecular host-parasite interactions.

This project is supported by the Austrian Science Fund (FWF): [P22118-B20].

146: Fucosylated glycan epitopes of the malaria mosquito *Anopheles gambiae*

Simone Kurz[1], Jonas King[2], Rhoel Dinglasan[2], Michael Tiemeyer[3] and Iain B. H. Wilson[1]; [1] Department of Chemistry, University of Natural Resources and Life Sciences (BOKU), A-1190 Vienna, Austria, [2] Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205, USA, [3] University of Georgia, Complex Carbohydrate Research Center, Athens, Georgia 30602, USA
simone.kurz@boku.ac.at

The most efficient vector of human malaria is the mosquito *Anopheles gambiae*. Two fundamental steps are required for the *Plasmodium* parasite development and transmission. Both steps, binding of parasite in insect vector and in vertebrate host cells, have been connected to the presence of oligosaccharide structures (glycans).

By homology searching of the *A. gambiae* genome, we found that the mosquito possesses three fucosyltransferases potentially involved in N-glycan biosynthesis. All three fucosyltransferases were cloned and expressed in *Pichia pastoris* GS115 cells.

The recombinant fucosyltransferases were further purified by Affi-Gel Blue and Ni-NTA affinity chromatography. Various optima (pH, temperature and ion dependence) have been determined in MALDI-TOF MS based assays. The characterisation of all three fucosyltransferases shows that in comparison to many other glycosyltransferases, the mosquito fucosyltransferases have no absolute requirement for any special divalent cation cofactor. The recombinant mosquito fucosyltransferases were then used in apo-transferrin

remodelling experiments to create positive controls for Western and Lectin Blot analysis.

Furthermore, immunofluorescence staining of mosquito sections with selected antibodies (anti-HRP) and lectins (*Aleuria aurantia* lectin) were performed to determine if the mosquito fucosyltransferases are also active *in vivo*. The staining patterns indicate the presence of *in vivo* fucosyltransferase products.

In addition, N-glycan analysis as well as Western Blot analysis of mosquito larvae and whole protein lysates could confirm the occurrence of fucosylated N-glycan epitopes. These experimental data as well as forthcoming analysis of the O-glycans are the basis for further exploration of the glycomic potential of this organism.

147: The Ribosome-inactivating Proteins (RIPs) from elderberry (*Sambucus nigra*) in the Battle Against Cancer??

Chenjing Shang[1], Mariusz Madej[1], Winnok De Vos[2], Els J.M. Van Damme[1]; [1]Biochemistry and Glycobiology, Dept. Molecular Biotechnology, Gent University, Gent, 9000, Belgium; [2]Cell Systems and Imaging, Dept. Molecular Biotechnology, Gent, 9000, Belgium
chenjing.shang@ugent.be

The family of type-2 ribosome-inactivating proteins (RIPs) groups chimeric proteins, composed of an A-chain with N-glycosidase activity and a B-chain with carbohydrate-binding/lectin activity. The type-2 RIPs from *Ricinus communis* and *Abrus precatorius* are well known as extremely toxic proteins. In the last decade it has been shown that the cytotoxicity of type-2 RIPs relies on the binding of the B-chain to carbohydrate structures on the cell surface, as such facilitating the endocytosis process of the ribosome-inactivating proteins in the cell.

Elderberry (*Sambucus nigra*) expresses several type-2 RIPs as well as lectins, differing from each other in their molecular structure and carbohydrate-binding properties. We studied the cytoxicity of several RIPs/lectins from elderberry towards human cells. To this end, transformed (HeLa cervix carcinoma) and mortal (human dermal fibroblasts) cell types were treated with different concentrations of elderberry proteins, after which viability was scored by means of PrestoBlue spectrophotometric assays and morphological observation. Using confocal microscopy, the internalization and intracellular localization of the proteins was determined. Our findings show clear differences among the elderberry proteins tested, depending on the lectin binding properties of the proteins and their interaction with glycans present on the cell surface. The data revealed that RIPs from elderberry are much less toxic than the classical RIPs from *Ricinus* and *Abrus*. In addition, fibroblasts were found to be less susceptible to some of the elderberry proteins compared to HeLa cells. Hence, these proteins are promising candidate molecules as cancer therapeutics… Future experiments will focus on the possible application of elderberry RIPs as a potential tool in the battle against cancer.

148: Biological activity of the tandem-repeat type Galectin-8 is related to the length of the linker region

Myriam Friedel[1], Sabine André[2], Hans-Joachim Gabius[2], Reinhard Schwartz-Albiez[1]; [1]German Cancer Research Center (DKFZ) Heidelberg, Germany; [2]Ludwig-Maximilian Universität Munich, Germany
myriam.friedel@dkfz.de; r.s-albiez@dkfz-heidelberg.de

The tandem-repeat type Galectin-8 (Gal-8) has two carbohydrate recognition domains (CRD) which are connected by a linker peptide of defined length (Gal-8S: 34aa, Gal-8L: 76aa). Several different functions of Gal-8, like its role in angiogenesis and a pro-apoptotic effect on lymphoctes, have been described so far. But knowledge about the influence of the linker region on the biological activity is sparse. We investigated the functional role of human recombinant Galectin-8 (hGal-8) with regard to its two different isoforms. We report that the human short, but not the long linker isoform may play a regulatory role in angiogenesis. In an *in vitro* angiogenesis assay (tube formation assay), hGal-8S in contrast to hGal-8L was able to enhance angiogenesis of human endothelial cells (HUVEC) on a feeder layer of human fibroblasts in combination with VEGF. In comparison, hGal-1 and hGal-3 only displayed small positive effects in this angiogenesis assay. With regard to its effect on lymphocytes, we could now show that hGal-8L in contrast to hGal-8S exerts a pronounced pro-apoptotic influence on human B leukemic cells of pre-B-cell origin (REH and Nalm-6). The different functional effects could at least partly be due to the fact that these isoforms vary in their ability to multi- or dimerize in solution. Using microscale thermophoresis measurement to quantitatively measure interactions of two molecules in solution, we were able to show that hGal-8L has a significantly stronger ability to dimerize in solution than hGal-8S. These observations may point to the fact that the length of the linker between the two CRDs influences the biological action of hGal-8. It has to be elucidated whether these findings can also be applied for other tandem-repeat galectins like for example human Galectin-9.

This work is funded by the “Deutsche José Carreras Leukämie-Stiftung”.

149: GD1a activates MAPK pathway *via* calcium signaling to suppress NOS2 expression in mouse osteosarcoma cells in an EGFR-independent manner

Xiao-Yan Yang, Lan Zhang, Xiao Zhang, Sadako Yamagata and Tatsuya Yamagata; Laboratory of Tumor Biology and Glycobiology, Shenyang Pharmaceutical University, 103 WenHua Rd, Shenyang, 110016 China
yangxiaoyan.syphu@gmail.com

Inducible nitric oxide synthase (NOS2) has been shown to increase during inflammation and tumor progression. Our previous work revealed that NOS2 was responsible for the malignant nature of murine osteosarcoma FBJ-LL and that NOS2 expression was enhanced by silencing ERK1 that mediated the suppression of NOS2 by ganglioside GD1a cells (Cao T, *et al.*, Ganglioside GD1a suppression of NOS2 expression *via* ERK1 pathway in mouse osteosarcoma FBJ cells. ***J Cell Biochem*, 110**, 1165–1174, 2010). In the present work, we will show that GD1a in FBJ-LL cells suppresses NOS2 expression through calcium signaling that activatesgrowth factor receptor-bound protein 2 (GRB2) followed by MAPK pathway activation. In untreated cells, NOS2 expression was suppressed through MAPK pathway that is activated by EGFR, whose signal is mediated by GRB2, but in GD1a-activated cells, the GD1a signal did not include EGFR activation. When we investigated molecules involved in MAPK pathway, we found that silencing MEK2, but not MEK1, augmented NOS2 expression in untreated FBJ cells and impaired the GD1a signal. Furthermore, silencing (GRB2) resulted in an increase in NOS2 in untreated cells and abrogated the GD1a signal. In addition, EGF suppressed NOS2 expression through activation of EGFR, GRB2, and MEK2 in FBJ-LL cells. However, the GD1a suppression of NOS2 was not cancelled by treating cells with the EGFR inhibitor, AG 1478, or by silencing EGFR; though these treatments cancelled the effects of EGF on NOS2 suppression. An anti-GRB2 antibody precipitated phosphorylated EGFR in the sample from EGF-activated cells, but not in the sample from GD1a-treated cells. These results indicated that, in FBJ-LL cells, GD1a stimulated an unidentified signaling molecule to stimulate calcium influx that activated the GRB2/MEK2/ERK1 pathway and could suppress NOS2 without EGFR involvement. In contrast, in untreated cells, EGFR mediated the activation of GRB2/MEK2/ERK1, which then suppressed NOS2 expression. We have also shown that in murine Lewis lung carcinoma cells GD1a suppressed NOS2 expression *via* EGFR-independent signal pathways.

150: Characterization of Cell Surface Glycosylation in Ovarian Cancer Leads to Identification of Dysregulation in *MGAT3* and *ST6GAL-1* Gene Expression

Merrina Anugraham[1], Sheri Nixdorf[2], Francis JACOB[3], Viola Heinzelmann-Schwarz[3], Nicolle H. Packer[1]; [1]Macquarie
University, Sydney, 2109, Australia, [2]University of New South Wales, Sydney, 2052, Australia, [3]University Hospital Basel, Basel, 4031, Switzerland
merrina.anugraham@mq.edu.au

Ovarian cancer is the fifth most common cause of cancer worldwide with the highest mortality rate among all gynaecological cancers. Following diagnosis, the standard therapy includes maximal cytoreductive surgery and platinum-based chemotherapy of which more than 80 % of patients undergo relapse due to chemotherapy resistance. This poor prognosis is primarily attributed to difficulties in detecting the disease at an early stage, lack of noticeable early symptoms and inadequate screening methods. The consequences of aberrant glycosylation are now widely established in cancer, as evidenced by alterations in glycan structures. However, the extent to which these glycosylation changes are influenced by aberrant epigenetic regulation of gene expression is less extensively studied in ovarian cancer and remains crucial in understanding the development and progression of this disease.

To address this effect, we first analysed the cell surface glycosylation of human ovarian surface epithelial (HOSE 6.3 and HOSE 17.1) and ovarian cancer cell lines (SKOV 3, IGROV1, A2780 and OVCAR 3). Membrane glycoproteins were enriched using Triton-X114 membrane partitioning. The *N*-linked glycans were released by PNG*ase* F, converted to alditols and analyzed using nano-liquid chromatography on porous graphitized carbon and electrospray ionization mass spectrometry. Glycan structures were characterized based on their molecular masses and MS/MS fragmentation patterns. We identified characteristic glycan features that were unique to the ovarian cancer cell lines, namely the 'bisecting GlcNAc' type *N*-linked structures and increased levels of sialylated oligosaccharides, predominantly with an alpha-2,6 sialic acid linkage. These *N*-glycan changes were then verified by examining their gene transcript levels (*MGAT3* and *ST6GAL-1*) using RT-PCR. We further evaluated the potential epigenetic influence on the *MGAT3* coding gene by treating the cell lines with 5-Azacytidine (5-Aza), a DNA methylation inhibitor. For the first time, we provide evidence to support the role of DNA hypomethylation of *MGAT3* gene which lead to the observation of the unique 'bisecting GlcNAc' type *N*-glycans in serous ovarian cancer cells.

It is envisaged that the specific *N*-glycans detected by mass spectrometry and their complex association with epigenetic programming in ovarian cancer could potentially be used for the development of novel anti-glycan drug targets and clinical diagnostic tools.

151: Accumulation of β1,6-N-acetylglucosamine-branched N-glycans Promoted PTPRT's Dimerization and Decreased its Catalytic Activity

Jingjing Qi[1], Na Li[1], Kun Fan[1], Zengxia Li[1,2,3], Liying Wang[1,2,3], Xiliang Zha[1,2,3*];
[1]Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai 200032, China; [2]Key Laboratory of Glycoconjugate Research, Ministry of Health, Shanghai 200032, China; [3]Key Laboratory of Molecular Medicine, Ministry of Education, Shanghai, 200032, China.
xlzha@shmu.edu.cn (Xiliang Zha)

Receptor-like protein tyrosine phosphatases (RPTPs) are type I transmembrane proteins whose catalytic activities are regulated by dimerization. The intrinsic mechanisms involved in dimerizing processes remain obscure. In this study, we identified receptor protein tyrosine phosphatase rho (PTPRT) as a novel substrate of GnT-V. Addition of β1,6 GlcNAc branches promoted PTPRT's dimerization through galectin-3 binding manner. Increased dimerization subsequently decreased PTPRT's catalytic activity, trigging nuclear export of phorsphorylated Y705 signal transducer and activator of transcription 3 (STAT3). Collectively, our results provide new evidence that aberrant regulation of PTPRT and STAT3 compromise an important part in GnT-V mediated metastasis.

152: Profiling the O-GlcNAcylation of the Kinome using Kinase Microarrary

Guanghui Han[1], Johnathan Neiswinger[2], Heng Zhu[2], Gerald W. Hart[1]; [1]The Johns Hopkins University, Department of Biological Chemistry; [2]The Center for High-Throughput Biology, Johns Hopkins University School of Medicine
ghan6@jhmi.edu

Mutations and dysregulation of kinases play causal roles in human disease development, signaling, and metabolism. Understanding the function of kinases is of an outstanding interest for biomarker discovery and also necessary for the development of agonists and antagonists for the use in disease therapy. O-linked beta-N-acetylglucosamine (O-GlcNAcylation) is a post-translational modification known to regulate different aspects of a proteins function including localization, activity, and stability. Like phosphorylation, O-GlcNAcylation, which modifies serine and threonine residues on nuclear and cytoplasmic proteins, is a ubiquitous, reversible process that regulates numerous cellular processes. Recent evidence indicates that site-specific crosstalk between O-GlcNAcylation and phosphorylation and the O-GlcNAcylation of kinases play an important role in regulating cell signaling. Hence, it is very important to investigate the O-GlcNAcylation of the kinome. Previously, we identified 42 kinases that are substrates for O-GlcNAcylation using an *in vitro* OGT assay with [H^3] radiolabel on a functional kinase array. However, using [H^3] has serve senstitive limiations suggesting a more sensitive approach is needed to probe large libraries of proteins. Herein, using a novel kinase microarray coupled with an *in vitro* OGT labeling assay with immuno fluorophore detection technique, we report a simple, yet sensitive, strategy by which this method can be used to profile the entire kinome for O-GlcNAcylated proteins.

153: Glycomic Investigation of Neuromuscular Junction Disorders by Mass Spectrometry

Qiushi Chen[1], Juliane S. Müller[2], Steven H. Laval[2], Hanns Lochmüller[2], Stuart Haslam[1], Anne Dell[1];
[1]Division of Molecular Bioscience, Imperial College London, South Kensington Campus, London SW7 2AZ, UK; [2]Institute of Human Genetics, Newcastle University, Newcastle upon Tyne NE1 3BZ, UK.
q.chen11@imperial.ac.uk

Glutamine-fructose-6-phosphate transaminase 1 is the first and rate-limiting enzyme of the hexosamine biosynthetic pathway. It catalyzes the reaction of glutamine and fructose-6-phosphate to produce glutamate and glucosamine-6-phosphate, supplying the substrate for uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) synthesis. It is universally acknowledged that the supply of UDP-GlcNAc is critical for the biosynthesis of several glycoconjugates and has an influence on branching of N-glycans. It is reported that *GFPT1* mutations result in congenital myasthenic syndrome (CMS) which is a type of neuromuscular junction disorders in which the synapses that form between motoneurons and skeletal muscle fibers that transmit the impulse resulting in muscle contraction have impaired function. Patients from two European countries (Germany and Spain) have been found to have CMS, due to mutations in the *GFPT1* gene. *In vitro* cultured myoblasts from affected patients were glycomically investigated using highly sensitive Mass Spectrometry. The result showed that in the patients the tetra-antennary glycans are substantially reduced compared with those glycans in controls. The reduction in tetra-antennary glycans in patients was compensated for by longer polylacNAc antennae. We conclude that *GFPT1* mutations in CMS patients have an influence on tetra-antennary N-glycan biosynthesis.

154: *Pseudomonas aeruginosa* changes the *N*-glycan profile of the sputum proteins from cystic fibrosis patients

Vignesh Venkatakrishnan[1], Morten Thaysen-Andersen[1], Helena Nevalainen[1], Nicolle H. PACKER[1]; [1]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, 2109, Australia
vignesh.venkatakrishnan@mq.edu.au

Cystic fibrosis (CF) is a prevalent autosomal recessive disease characterized by chronic infection and inflammation in the lungs. The damaged lungs continue to secrete mucus which is the primary site of microbial infection and *Pseudomonas aeruginosa* is one of the major infecting pathogens among CF patients. Alteration in glycosylation of mucins (heavily *O*-glycosylated proteins) has been shown to play a role in bacterial binding to the mucous in the lungs of CF patients. However, there has been little reported on the *N*-glycosylation of the protein complement of CF sputum. We have characterized the *N*-glycans from sputum collected from CF, and other non CF, patients with lung infection to determine any correlation between the *N*-glycan structures and the microorganisms which commonly cause CF-specific lung infection. Sputum samples were reduced, alkylated and the *N*-glycans were released enzymatically using PNGase F enzyme and analysed on porous graphitized carbon (PGC)-LC-ESI-MS/MS.

The major difference in the *N*-glycans of CF sputum, compared to non-CF, sputum was the presence of unusual pauci-mannose glycans. To test whether this was due to pathogenic infection, different clinical *P.aeruginosa* strains isolated from CF sputum samples were inoculated into non-CF derived sputum and incubated at 37 °C for 3 days. All the isolated *P.aeruginosa* strains trimmed the complex *N*-glycans in the non-CF sputum to pauci-mannose structures similar to those seen in CF sputum. However, other *P. aeruginosa* cultured laboratory strains such as PAO1 and AES1-R did not affect the *N*-glycan structures. A Biolog plate assay determined that the isolated bacteria did not use these types of trimmed sugars (sialic acid, N-acetylglucosamine and galactose) as a carbon source for their growth. These data suggests that *Pseudomonas* may be modifying the *N*-glycan structures on proteins to promote bacterial adhesion in CF associated lung infection.

155: ManLAM of virulent Mycobacterium tuberculosis induces IL-10-producing Breg cells

Chun-Hui Yuan[1], Xiao-Lei Tang[2], Qin Pan[3], Xiao-Lian Zhang*; Wuhan University School of Medicine, Department of Immunology, Donghu Road 185, Wuhan 430071, China.
zhangxiaolian@whu.edu.cn

Mannose-capped Lipoarabinomannan (ManLAM) as a kind of lipoglycan which is the main component of *Mycobacterium.tuberculosis* (***M. tb***) cell wall, and of importance, putatively referred as an immune suppressor that facilitate *M.tuberculosis* escape. However the relationship between ManLAM and IL-10-producing Breg cells remains elusive. Here we found that ManLAM purified from Virulent *M.tb* H37Rv induced IL-10-producing Breg cells both *in vivo* and *in vitro* and that these Breg cells contained both $CD5^-$ and $CD5^+$ B subset. We next detected these Breg cells' cytokine profile, and found that they mainly secreted IL-4, moreover, these IL-10-producing Breg cells induced by ManLAM negatively regulated IL-4 secretion of $CD4^+$ T cells in co-culture assay, and this special function could be suppressed by IL-10 antibody. Together these results suggest that, IL-10-producing Breg cells induced by ManLAM belong to 'Be2' subtype and this is the first report demonstrating that ManLAM from Virulent *M.tb* H37Rv induces CD5+ and $CD5^-$ IL-10-producing Breg cells which might act as a new immune suppressor facilitating virulent *M. tb* immune escape.

Poster Session I-Glycoproteomics

156: A Novel Approach to Enriching Azido Labeled Glycopeptides from Metabolically Labeled Cells

Sheng Wang, Xiu Zhang, Wenxian Xie, Xia Zou, Yan Zhang; Ministry of Education Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, P.R. China, 200240
yanzhang2006@sjtu.edu.cn

Protein glycosylation is usually difficult to analyze due to the low proportions of glycopeptides and the heterogeneity in the structure of glycans. But recently, an azide-labeling strategy has emerged as a promising approach to help decipher the myths of glycosylation. In this study, we have synthesized disulfide- and terminal alkyne- modified magnetic silica particles (DA-MSPs) and developed a novel method for enrichment of azido labeled glycopeptides based on bioorthogonal chemistry. In our results, the adsorption capacity of these particles is calculated to be over 4 pmol/mg, and the enrichment recovery rate is over 40 %, and specific enrichment can be realized when the ration of interference BSA peptides to targeting standard glycopeptide is 100:1. Further, Jurkat cells were cultured with Ac_4GalNAz and the membrane and cytoplasmic proteins were collected. Metabolic labeling with Ac_4GalNAz introduces a bioorthogonal chemical reporter into mucin-type *O*-

linked glycopeptides that can react with alkyne probes for detection or enrichment. After covalently capture and reductively release azido-glycopeptides *via* click chemistry and dithiothreitol treatment, the glycoproteins were isolated. The efficiency of cell labeling and glycoprotein enrichment was validated by fluorescence detection and lectin blot respectively. And the enriched glycoproteins were separated by 2D lectin blotting or by on-line 2D nano-HPLC and analyzed with LTQ-Orbitrap mass spectrometry.

157: Purification of Novel α-Galactoside-binding Lectins from Aquatic Animals and their Distinct Cell Regulatory Activities

Imtiaj Hasan[1,2], Yasuhiro Koide[1], S. M. Abe Kawsar[1,3], Ryo Matsumoto[1,4], Yuki Fujii[5], Yukiko Ogawa[5], Shigeki Sugawara[6], Masahiro Hosono[6], Kazuo Nitta[6], Yasuhiro Ozeki[1]; [1]Graduate school of Yokohama City University, Yokohama, 236-0027 Japan; [2]Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205 Bangladesh; [3]Department of Chemistry, University of Chittagong, Chittagong-4331, Bangladesh; [4]Institute for Environmental and Gender-Specific Medicine, Juntendo University, Urayasu, Chiba, 279-0021 Japan; [5]Department of Functional Morphology and Microbiology, Faculty of Pharmaceutical Science, Nagasaki International University, Sasebo, Nagasaki, 859-3243 Japan; [6]Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, Sendai, *981-0905* Japan
ozeki@yokohama-cu.ac.jp

An α-galactoside-glycosphingolipid, globotoriaosyl ceramide (Gb3; Galα1-4Galβ1-4Glc-Cer) is focused to be the trigger for cell regulations through the interaction with the glycan-binding receptors. As glycan-binding subunit of Vero toxin that is secreted from pathogenic bacteria, such as O157 acts for the incorporation of the toxin into Gb3 ceramide expressing digestive cells, Gb3-binding lectins isolated from marine invertebrates and fishes were also reported to perform direct cytotoxic activity, up- and down-regulation of cytokines and are the important membrane proteins on cell surface.

Two distinct cell regulative activites through Gb3 and lectins that recognize the glycan is discussed in this presentation. MytiLec is an α-galactoside-binding lectin isolated from Mediterranean mussel, *Mytilus galloprovincialis* with a highly novel primary structure consisted of 149 amino acids and cytotoxic activity against Burkitt lymphoma Raji cells expressing Gb3. On the other hand, another Gb3-binding lectin, SAL from Catfish (*Silurus asotus*) eggs that is a member of SUEL-lectin family showed dow- regulation of multidrug resistance associate protein-1 (MRP1) of the Raji cells instead of the direct cell death occurred by MytiLec. However, the cells which are treated by SAL were effectively killed with low concentrated anti-cancer drugs by the deficiency of MRP1.

In addition to above studies, we found another α-galactoside-binding lectin from Sea hare (*Aplysia kurodai*) eggs by the combined use of melibiosyl-agarose and lactosyl-agarose columns. This analytical procedure showed the presence of two different lectins in the eggs which recognize α- and β-galactoside, respectively. Different from MytiLec and SAL, Sea hare lectins were assumed to be glycoproteins. We are now comparing the cell regulation activities with these lectins against Raji cells. The discovery of plural α-galactoside-binding lectins and the elucidation of the distinct cell regulatory mechanisms through the lectin-glycan interaction will provide invaluable findings for glycan-dependent cell signaling.

158: Comparative Analysis of the surface *N*-linked Glycoproteomes of HC-04 and HepG2 hepatocyte cell lines by a highly sensitive online integrated system

Dingyin Tao, Jonas G. King, Philipp Jost, Justin Boddey and Rhoel R. Dinglasan[‡]; Johns Hopkins Bloomberg School of Public Health and the Malaria Research Institute, Baltimore, Maryland, 21205, USA; Technical University of Munich, Munich, Germany; Walter and Eliza Hall Institute, Parkville, Australia, [‡]Corresponding author: Rhoel R. Dinglasan, PhD, MPH.
rdinglas@jhsph.edu

Liver cell lines are important models for understanding hepatocyte biology as well as the pathogenesis of infectious diseases such as malaria and hepatitis. Several critical aspects of the basic biology of these cell lines remain unknown, including the composition of their plasmalemma surface proteome and extracellular glycocalyx, and how these cell surface molecules interact with pathogens and respond to their environment. Here, we focused on analyzing the surface glycoproteins of two important human liver cell lines: HC-04, which has been shown to support human malaria liver stage development and HepG2, a widely-used cell line that will not support infection with human malaria parasites. Although the determinants of susceptibility to malarial infection are unknown, we hypothesize that a differential response to culture conditions are likely to modulate surface protein expression density and repertoire, which may influence susceptibility. The HC-04 and HepG2 surface glycoproteins were labeled and enriched by DIBO-AHA pull-downs prior to SDS-PAGE separation and in-gel digestion. To

overcome technical limitations due to the small amount of surface glycoproteins, we developed a highly sensitive online integrated system, and in a proof of principle study, analyzed the surface *N*-linked glycoproteomes of the HC-04 and HepG2 cell lines in response to titrated concentrations of hepatocyte growth factor. The online system is composed of a (i) weak anion exchange (WAX) column, (ii) a PNGase F immobilized enzymatic reactor (PIMER) and a Polaris chip, which was used for glycopeptide enrichment by **E**lectrostatic **R**epu**l**sion-hydrophilic **I**nteraction **C**hromatography (ERLIC) followed by deglycosylation, and (iii) peptide analysis by RPLC-MS/MS. In comparison with the conventional offline method, the total analysis time was shortened from days to several hours, and the sample amount for each analysis was reduced to 1~2 micrograms. Furthermore, the contamination risk was reduced since all preparation steps were online after in-gel digestion. In addition, we captured aglycosylated peptide fragments during ERLIC enrichment, which could be used for glycoprotein quantification analysis by the label-free method developed in our previous work. The differentially expressed glycoproteins identified between the HC-04 and HepG2 cell lines provide insight into the impact of growth conditions on surface glycoproteomes of these important hepatocyte lines.

159: Characterization of *O*-Glycans of Ovomucin Isolated from Hen Egg White Based on the One-pot Procedure by Electrospray Ionization Mass Spectrometry

Weibo Xing, Chengjian Wang, Xiaohua Li, Zhongfu Wang, Linjuan Huang; Educational Ministry Key Laboratory of Resource Biology and Biotechnology in Western China, Life Science College, Northwest University, Xi'an 710069, China
huanglj@nwu.edu.cn

Mucin-type glycans of glycoproteins are extensively believed to play important roles in many biological processes, such as immune responses, protection of the gastrointestinal tractus, and egg-sperm interaction. Detailed investigations of the structure diversity of the *O*-glycans from various glycoproteins can expand our still very limited knowledge of *O*-glycosylated proteins and facilitate the research on the structure-function correlations of mucin-type glycans. This study is focused on the comprehensive characterization of the *O*-glycans released from a bioactive glycoprotein, ovomucin, which possesses anti-viral, anti-bacterial and anti-tumor bioactivities and is supposed to have potential applicability to medicine, nutriology and health care. Ovomucin was isolated from chicken egg white, and then its *O*-glycans were released with Carlson's reductive β-elimination and the one-pot procedure we reported previously (the procedure allows simultaneous *O*-glycan release and 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization), separately. After comprehensive analysis of the obtained glycan samples by electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS), we found 14 kinds of hen egg ovomucin *O*-glycans, of which 5 are neutral and 9 are sialylated or sulfated. Additionally, it was also found that the Carlson's method could induce some *N*-glycans to cleave from ovomucin along with the *O*-glycans, whereas the one-pot procedure could only release the *O*-glycans from ovomucin, showing the superiority of the one-pot method over the conventional reductive β-elimination in releasing specificity for *O*-glycans. These analysis results could provide a basis for the further investigations on the structure-function correlations of ovomucin as well as its deep exploitation.

160: Multivalent Hydrazide Functionalized Magnetic Nanoparticles for Glycopeptides Enrichment and Identification

Qi C. Cao[1, 2,3], Wan T. Ying[3*], Xiao H, Qian[1, 3]; [1]School of Pharmacy, Pharmaceutical University, Shenyang 110016, China;[2]The affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang 110032,China; [3]State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing 102206, China;[*] Correspondence Author
qianxh1@163.com (Xiao H, Qian);proteomics@126.com (Wan T. Ying)

Abstract: Among the common approaches for global glycopeptides enrichment and identification, hydrazide chemistry is well recognized. However, the mainly commercial hydrazide functionalized products are composed of single layer hydrazide functional group, therefore, they can only provide limited affinity to the glycopeptides for highly complex proteomic samples. In this work, we introduced a novel multivalent hydrazide functionalized magnetic nanoparticle coated with hydrazide modified non-cross linked polymer chains. Such material was synthesized *via* surface initiated atom transfer radical polymerization (SI-ATRP) technique. The density of hydrazide groups on the surface of these nanoparticles were 10 folds higher than that of conventional single layer magnetic particles. The multivalent hydrazide functionalized particles were demonstrated to enrich glycopeptides with high sensitivity from a peptide mixture of Bovine Fetuin and Bovine Serum Albumin at a mass ratio of 1:100. The recovery of glycopeptide was investigated to be 73 %, and the glycopeptides binding capacity was proven to be 25 μg/mg. Finally, the novel hydrazide functionalized particles were applied to enrich N-linked glycosylated peptides from 50 μg mouse liver tissues. In total, 116 unique

glycopeptides belonged to 91 different glycoproteins have been identified, and the result further demonstrated the multivalent particles' potential for selective glycopeptide isolation in complex proteomics samples.

161: In-depth profiling of the sialylated N-glycoproteome of human plasma using optimized strategy based on metal oxide enrichment

Wantao Ying, Xinyuan Zhao, Xiaohong Qian; State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing 102206, China
yingwtll@yahoo.com.cn

Glycosylation is an important post-translational modification of proteins and plays a crucial role in both cellular functions and secretory pathways. Sialic acids (SAs), a family of 9 carbon-containing acidic monosaccharides, often terminate the glycan structures of cell surface molecules and secreted glycoproteins and perform an important role in many biological processes. Hence, in-depth profiling of the sialylated glycoproteome may improve our knowledge of this modification and its effects on protein functions. Here, we systematically investigated different strategies to enrich the SA proteins in human plasma using a newly developed technology that utilizes titanium dioxide for sialylated N-glycoproteome profiling by mass spectrometry. Our results showed that using a combination of a filter-aided sample preparation (FASP) method, TiO2 chromatography, multiple enzyme digestion and two-dimensional reversed-phase peptide fractionation led to in-depth profiling of the SA proteome. In total, 982 glycosylation sites in 413 proteins were identified, among which 37.8 % were newly identified, which established the largest database of sialic acid-containing proteins from human plasma.

162: Multiplex Profiling of Glycoproteins Using Novel Bead-based Lectin Array

Hong Li, Hong Wang, Wei Zhang, Liming Wei, Hongxiu Yu, Pengyuan Yang; Fudan University, Shanghai, 200032, P.R. China
pyyang@fudan.edu.cn

Lectin array is becoming important in profiling targeted glycan/glycoprotein, but weak interaction between lectin and glycan causes low sensitivity of the approach. This study aims to develop a bead-based lectin array for improving the sensitivity of glycosylation profiling. Lectins are chemically coupled to fluorescent dye coated microbeads, and glycan-lectin recognition is carried out three-dimensionally. The performance of this platform was evaluated, and the limit of detection of lectin *Ricinus communis* agglutinin 120 (RCA120) was 50 pg/mL (1 pM) of asialofetuin, providing the bead based lectin microarray with the highest sensitivity among the reported lectin microarrays. Furthermore, multiplexed assay was performed, which allowed the simultaneous detection of multiple carbohydrate epitopes in a single reaction vessel. The glycosylation patterns of hepatocellular carcinoma associated immunoglobulin G were analyzed, and increased (α-1, 6) core fucosylation and (α-2, 6) sialylation patterns were observed, which may provide significant clinical evidence for disease diagnosis.

163: Optimal sample choice for the analysis of protein antennary fucosylation as screening tool for HNF1A-MODY

Novokmet M[1], Thanabalasingham G[2], Kattla J[3], Huffman J[5], Rudan I[5,6] Wright AF[4], Campbell H[4], Owen K[2], Rudd PM[3], McCarthy MI[2], Lauc G[1,7]; [1]Genos Ltd, Glycobiology Laboratory, Zagreb, Hondlova 2, Croatia, mnovokmet@genos.hr, [2]Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, Oxford, UK [3] National Institute for Bioprocessing Research and Training, Dublin-Oxford Glycobiology Lab.,Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland, [4] MRC Human Genetics Unit; Institute of Genetics and Molecular Medicine, Western GeneralHospital, Edinburgh, UK, [5] Centre for Population Health Sciences, The University of Edinburgh Medical School, [6] University of Split School of Medicine, Šoltanska 2, Split, Croatia, [7] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

Maturity-onset diabetes of the young (MODY) is a dominantly inherited form of non-insulin dependent diabetes caused by mutations in several genes. A subtype of MODY is caused by mutations in HNF1A, a nuclear transcription factor which appears to be one of the key regulators of metabolic genes. Recently we showed that deleterious coding mutations in HNF1A have profound effects and that antennary fucosylation of plasma proteins is significantly decreased in HNF1A-MODY patients. The proportion of HPLC peak DG9 in the sum of DG8 and DG9 (HAFU index) roughly indicates the level of antennary fucosylation of triantennary glycans in plasma. Low values of this index appeared to be very indicative of HNF1A-MODY. HNF1A-MODY patients could be nearly completely separated from Type 1 diabetes, Type 2 diabetes, GCK-MODY and general population on the basis of the HAFU index with Receiver-

Operator Characteristic (ROC) curves approaching 90 % specificity at 90 % sensitivity. In order to optimize and simplify collecting and shipping procedure for samples, we compared the analysis of standard plasma samples, dried blood spots that were generated from whole blood (venous blood) and dried blood spots generated from capillary blood by finger prick test. All analyzed sample types displayed only small differences in HPLC peaks DG8 and DG9 and preserved the information value of HAFU index.

164: Recent Technical Advances in Mass Spectrometry-based Glycoproteomic Applications

Kay-Hooi Khoo, Sz-Wei Wu, Chu-Wei Kuo, Shui-Hua Wang; Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan
kkhoo@gate.sinica.edu.tw

Despite intensive efforts over the last few years, mass spectrometry (MS)-based glycoproteomics remain difficult with many unsolved technical problems. We and others have advocated direct analysis of intact glycopeptides instead of deglycosylated peptides or trimmed down glycopeptides in order to answer the biological relevance of site-specific protein glycosylation. We are particularly interested in asking if a specific glycotopes of interest is restricted in distribution to only a few sites or a few glycoproteins, from a global glycoproteomic perspectives through a shotgun analysis. Over the years, we have worked on mAb-based affinity capture and other biophysical enrichment methods to target specific subsets of glycopeptides including the sulfated glycopeptides, investigated various modes of glycopeptide sequencing, and automated data analysis through development of software suites. We aim to determine if terminal glycotopes mapping can be more or equally effective at such a shotgun glycoproteomic level compared with glycomics. Through applications to single isolated glycoproteins, we demonstrated that novel glycosylation sites could be identified when data analysis is not constrained by inputs of predicted sites. On many occasions, unpredicted proteolytic cleavages produces peptide cores not matching anticipated peptide pools and therefore cannot be identified unless *de novo* sequenced. In general, either a software-driven two-tiers MS2/MS3 on ion trap or a combined HCD/ETD coupled with database search provides the most objective, non-biased high throughput analysis, which can be used in complementary. Likewise, a combination of two-step orthogonal enrichment is often more effective in producing the desirable glycopeptide pools. Analysis of sulfated sialylated glycopeptides remain daunting, which can be facilitated by chemical derivatization. We highlight here several aspects of technical advances and progress we have made over the last few years in glycoproteomics, exemplified by a diverse range of applications including one on endogenous EGFR from several lines of primary lung cancer cells.

165: Development of glyco-biomarker for liver disease

Makoto Ocho[1], Akira Togaychi[1], Hiroyuki Kaji[1], Atsushi Kuno[1], Etsuko Iio[2], Maki Sogabe[1], Yasuhito Tanaka[2], Yuzuru Ikehara[1], Masashi Mizokami[3], and Hisashi Narimatsu[1]; [1]Research Centre for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science And Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan; [2]Department of Virology and Liver, Nagoya City University Medical School, Nagoya, 467-8601, Japan; [3]Research Centre of Japan, Hepatitis and Immunology, Kohnodai Hospital, International Medical Centre, Ichikawa, 272-8516, Japan
makoto.ochou@aist.go.jp

Identification of new glyco-biomarkers predicting the liver disease progression and the presence of hepatocellular carcinoma (HCC) has been attempted. An accurate method for monitoring the progression of liver fibrosis, predicting liver cirrhosis (LC) and detecting HCC is urgently needed. We previously proposed a large number of candidate glycoproteins for monitoring liver disease progression which were identified by comprehensive analysis of serum samples.

In this study, we found liver disease-associated glycan profile on a candidate protein, which pointed out the presence of markedly developed HCC or progressed LC in the patients with HCV-/HBV- infected chronic liver disease. In order to clarify the relationship between the presence of the candidate molecule with aberrant glycosylation and the prevalence of progressed liver diseases, we analyzed this candidate marker by a clinical validation study. As a result, the increased amount of the candidate molecule with the aberrant glycosylation was more relevant to poor outcome of LC than hepatic carcinogenesis of the patients. While our result suggests that the biomarker is feasible to use in individualization for poor prognostic patients with a risk of LC progression, further clinical studies would yield more precious information.

This work was supported by a grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.

166: Development of novel marker for cholangiocarcinoma glyco-diagnosis

Atsushi Matsuda[1], Atsushi Kuno[1], Hideki Matsuzaki[1], Toru Kawamoto[2], Tatsuro Irimura[3], Toshihide shikanai[1], Yasuni Nakanuma[4], Masakazu Yamamoto[2], Nobuhiro Ohkohchi[5], Yuzuru Ikehara[1], Junichi Shoda[6], Jun Hirabayashi[1], Hisashi Narimatsu[1]; [1]Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, 305-8568, Japan; [2]Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, 162-8666, Japan; [3]Laboratory of Cancer Biology and Molecullar Immunology, Guraduate School of Pharmeceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan; [4]Department of Human Pathology, Graduate School of Medical Science, Kanazawa University, Kanazawa, 920-1192, Japan; [5]Department of Surgery, Graduate School of Comprehensive Human Science, University of Tsukuba, Tsukuba, 305-8575, Japan; [6]Field of Basic Sports Medicine, Sports Medicine, Graduate School of Comprehensive Human Science, University of Tsukuba, Tsukuba, 305-8575, Japan.
atsushi-matsuda@aist.go.jp

Cholangiocarcinoma (CC) is an aggressive malignant tumor arising from the epithelial lining of the biliary tract. Since the definitive CC diagnosis relies on invasive methods such as biliary cytology and biopsy, a noninvasive assay with high diagnostic accuracy has been keenly required. We previously reported that *Wisteria floribunda* agglutinin (WFA) is the best probe lectin which reliably distinguishes between the non-cancerous and cancerous region in tissue sections of CC by the lectin microarray and histochemical analysis. Here our aim is the development of a novel glycoprotein marker for reliable CC diagnosis by WFA-based glycoproteomics approach. We identified several WFA binding glycoproteins by immunohistochemistry and mass spectrometry analysis. Then, we verified these WFA-positive glycoproteins as the diagnostic marker candidates for CC using bile and serum specimens. Sialyl-MUC1, recognized with a specific monoclonal antibody, MY.1E12, was the primary candidate identified as one of the WFA-positive glycoproteins. A WFA-coated MY.1E12 sandwich ELISA was constructed for validation with bile and serum, and this marker could distinguish CC from other patients in bile specimens (Matsuda A., *et al.*, *Hepatology,* 2010). L1 cell adhesion molecule (L1CAM) was the second candidate by the WFA-assisted glycoproteomics from surgical CC tissue sections. We confirmed that WFA-positive L1CAM expressed specifically in the CC tissues and bile, and the amount was significantly different between the bile specimens of CC and benign bile duct disease. The combined use of WFA-sialyl MUC1 and WFA-L1CAM sufficiently improved the accuracy of CC (overall accuracy = 0.84, AUC = 0.93) in the biliary diagnosis. As the ultimate goal of this study is the serological diagnosis, we are performing further verification of these markers using serum by immunodetection and lectin microarray analysis. This combination assay using WFA-sialyl MUC1 and WFA-L1CAM will possibly be a useful serological test with enhanced reliability.

This work was supported a grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.

167: Glycoproteomics of serum proteins in hepatitis B and hepatitis C induced liver cirrhosis

Gautam Mondal[1], Yogesh K Chawla[2], Hirokazu Yagi[3], Koichi Kato[4], Bishnu P Chatterjee[1]†; [1]Department of Natural Sciences, West Bengal University of Technology, Salt Lake, Kolkata-700064, India, [2]Department of Hepatology, Post Graduate Institute of Medical Education and Research, Chandigarh -160012, India, [3]Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-diri, mizuho-ku, Nagoya 4678603, Japan, [4]Institute for Molecular Science and Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Okazaki Institute for Integrative Biosciences, 5-1 Higashiyama, Myodaiji, Okazaki-444-8787, Japan
cbishnup@gmail.com

Glycosylation is one of the most important events of post translational modification in many serum proteins which can provide information about the onset and progression of disease. Both hepatitis B virus (HBV) and hepatitis B virus (HCV) cause acute and chronic liver infection and most chronically infected individuals remain asymptomatic for many years. Although biopsy is the gold standard method for both hepatitis B virus induced liver cirrhosis (HBV-LC) and C virus induced liver cirrhosis (HCV-LC), it has several disadvantages. Therefore, a non-invasive, simple test would be highly desirable. During inflammation and other related disorders altered glycosylation patterns are observed in several acute phase proteins besides their alteration in expressions. Our aim was to monitor the changes in the expression level and glycosylation pattern of serum glycoproteins of hepatitis patients which could be used as serological markers for diagnosis. Alpha-1 acid glycoprotein (AGP) was isolated from the albumin depleted sera of HBV-LC and HCV-LC patients by affinity chromatography on mononclonal anti-AGP column. The unbound fraction by 2D gel electrophoresis gave several protein spots, which

were identified by MALDI-TOF-MS. Database search of MS-MS spectra identified six significantly altered serum glycoproteins: haptoglobin alpha 2 (Hp-α2) chain, haptoglobin beta 2 (Hp-β) chain, apolipoprotein A-1(ApoA-1), alpha-1 antitypsin (AAT), vitamin D binding protein (BTDBP) and immunoglobulin heavy chain (IGHA) respectively. This result was further validated by immunoblot using monoclonal antibody indicating possible increased expression of AAT and decreased level of Hp-α2 and Hp-β chain in both hepatitis patients with respect to control. Lectin blots showed decreased level of sialic acid and increased of fucosylation of serum glycoproteins which was assessed by *Maackia amurensis* agglutinin (MAA) and *Lens culinaris* agglutinin (LCA) respectively. The alteration of glycosylation in AGP from two groups of hepatitis patients was determined by HPLC mapping method followed by mass spectrometry and GALAXY database search. N-glycans released from AGP by hydrazinolysis were labeled with 2-aminopyridine and separated by three successive HPLC columns, *viz.,* DEAE, ODS and amide silica. We found decrease or absence of sialic acid in HBV-LC and increased fucosylation of neutral N-glycans in both hepatitis patents. Thus glycoproteomics could be helpful to search for novel glyco-markers in diagnosis of hepatitis virus induced liver cirrhosis.

168: *N*-glycan Analysis of Immunoglobulin G by Enzymatic Release with Remove-iT Endo S and LC-MS

Xiaofeng Shi; Elizabeth McLeod; Paula Magnelli; Alicia Beliek; Ellen Guthrie; New England Biolabs Inc., Ipswich, MA, 01938, USA
shi@neb.com

A growing number of monoclonal IgG antibodies are currently being developed as therapeutic agents. Critical for their structure and biological activity is the *N*-glycan moiety attached to the asparagine 297 residue in the constant domain. There are many variables in cell culture that can greatly influence the heterogeneity of the glycans on IgG. Therefore, it is critical to monitor the glycosylation profiles of these biotherapeutics in the production process. A platform that includes efficient and complete glycan release, fast workup, and compatibility with proteomics workflow for IgG glycan analysis is highly sought. Here we attempt to address these questions by using a novel endoglycosidase with a fused chitin-binding domain.

Remove-iT Endo S (also known as Endoglycosidase S) was cloned and purified from *Streptococcus pyogenes* and overexpressed as a fusion to the chitin binding domain in *E. coli.* Glycerol free IgG samples were enzymatically deglycosylated under native conditions using Remove-iT Endo S and PNGase F Glycerol Free. The deglycosylated IgG samples were then analyzed by SDS-PAGE gel shift analysis to estimate the degree of deglycosylation. In addition, the glycans released from IgG following deglycosylation by both enzymes were analyzed by nanoLC-TOF MS for glycan profiling.

The results show that Remove-iT Endo S has a high specificity for removing the *N*-glycan moiety of IgG after the first N-acetylglucosamine (GlcNAc) residue, leaving only the GlcNAc with or without a core fucose residue, whereas PNGase F cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from *N*-linked glycoproteins. Remove-iT Endo S is a more robust enzyme for the method described herein as it completely removes the sugar residues from IgG. Conversely, the PNGase F glycerol free digest does not result in a complete digestion under native conditions. Remove-iT enzymes can be easily eliminated from the digestion buffer by chitin magnetic beads, immediately followed by proteomics workflow.

169: Simplified Quantitative Glycomics Using the Stable Isotope Label Girard's Reagent P by Electrospray Ionization Mass Spectrometry

Chengjian Wang[1], Zhiyu Wu[1], Jiangbei Yuan[1], Bo Wang[1], Ping Zhang[2], Ying Zhang[1], Zhongfu Wang[1], Linjuan Huang[1]; [1]Educational Ministry Key Laboratory of Resource Biology and Biotechnology in Western China, Life Science College, Northwest University, Xi'an 710069, China; [2]Chemistry and Chemical Engineering School, Xianyang Normal University, Xianyang 712000, China
huanglj@nwu.edu.cn

Glycosylation alteration of proteins has proved to be associated with many diseases, such as cancer, type 2 diabetes, and congenital disorders of glycosylation (CDG). To monitor the changes in the abundance and structure of the glycan moieties of various glycoproteins, quantitative glycomics based on stable isotope labeling coupled with mass spectrometric analysis represents an emerging and promising technique. However, this technique is undermined by the time-consuming post-processing steps and the complexity of the mass spectra of isotope-labeled glycans arising from the presence of multiple metal ion adduct peaks, which result in a decrease of detection sensitivity and an increase of difficulties in data interpretation. In this study, a facile strategy for the relative quantitative analysis of *N*-glycans using the stable isotope label Girard's reagent P (GP) has been developed. This procedure features efficient labeling of reducing glycans with protium- or deuterium-substituted (d0/d5-) GP under non-reductive conditions and subsequent

direct detection by mass spectrometry (MS) without any post-processing steps. The obtained intensity ratio between the MS peaks of the d0- and d5-GP derivatives of each glycan allows rapid and high-throughput relative quantitative analysis. More importantly, the GP-derivatives exhibit only $[M]^+$ type ion peaks in their MS profiles, excluding the interference from multiple complex ion adduct peaks such as $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ and simplifying exceedingly the interpretation of their MS data. We have demonstrated the reliability and high sensitivity of the method using lactose as a model glycan by electrospray ionization mass spectrometry (ESI-MS). The relative quantitative analysis of the *N*-glycans released from bovine pancreas ribonuclease B and chicken egg white albumin revealed the good linearity and high reproducibility of the procedure within a 100-fold molar ratio range. The excellent applicability of the novel method was validated *via* the comparative analysis of the *N*-glycans released from bovine and porcine immunoglobulin G (IgG) by ESI-MS as well as of those from mouse and rat serum by ESI-MS and hydrophilic interaction liquid chromatography coupling with mass spectrometry (HILIC-MS).

170: A Robust Method for the Relative Quantitative Analysis of N-Glycan

Weiqian Cao, Wei Zhang, Jiangming Huang, Pengyuan Yang*; Fudan University, Shanghai, 200032, China
pyyang@fudan.edu.cn

With the development of glycoproteomics, relative quantification of glycosylation has received considerable research attention, which is important for discovering glycosylation changes and finding potential biomarker. High throughput glycan quantitation was developed to investigate quantitative glycan changes in structure type, composition, linkage and conformation of glycans from different biological samples. Similar to protein quantitation, MS based isotopic label is also a powerful tool for glycan quantitation.

In this reach, we developed a novel relative quantitation method for glycans. The novel sodium borohydride assisted enzymatic ^{18}O labeling added the reducing end of all released glycans with 3 Da. After this label, the mass gap increased to 3 Da, and the partial overlap of isotope envelopes was largely reduced. Moreover, the glycan became more stable, and the labeled ^{18}O would never exchange with ^{16}O again in normal water. We further tested the stability and overlap of this label, which showed good results. By this label reaction, we optimized the enzymatic glycan ^{18}O labeling, and made the method more suitable for quantitative glycomics by mass spectrometry.

171: An efficient approach to prepare boronic acid functional core-shell polymer nanoparticles for enrichment of glycoproteins *via* RAFT media precipitation polymerization

Jianxi Liu[1,2], Kaiguang Yang[1], Yanyan Qu[1,2], Lihua Zhang[1]*, Zhen Liang[1] and Yukui Zhang[1]; [1]Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic R. & A. Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China;[2] Graduate School of the Chinese Academy of Sciences, Beijing100039, China.
lihuazhang@dicp.ac.cn

Glycosylation is one of the most common post-translational modifications of proteins, and plays an important role in a variety of biological activities. Therefore, the global profiling of N-linked glycosylationsites is a prerequisite to study the biological functions of glycoproteins. Due to low abundance and poor ionization capacity, the selective enrichment of glycoproteins or glycopeptides before MS analysis is imperative. Herein, we developed a novel approach to prepare hydrophilic core–shell polymer nanoparticles. The hydrophilic core was formed by distillation precipitation polymerization (PP) method using methacrylic acid (MAA) as the monomer, and N,N-methylenebisacrylamide (MBAA) as the cross-linker. The functional shell was prepared by reversible addition/fragmentation chain-transfer (RAFT) with 3-acrylamidophenylboronic acid (APBA) and MBAA, instead of by traditional free radical polymerization (TFRP). The new strategy presented here could obviously simplify the preparation procedure. The obtained boronate affinity polymer nanoparticles, poly(MBAA-co-MAA)@(MBAA-co-APBA), showed good stimuli-response towards cis-diol containing molecules, and the maximum adsorption capacity for horseradish peroxidase (HRP) was 76.25 mg/g. The results demonstrated that poly(MBAA-co-MAA)@(MBAA-co-APBA) nanoparticles prepared by RAFT media PP might be promising in profiling the glycosylation site occupancy and corresponding glycan heterogeneity for glycoproteome analysis.

172: An integrated platform for quantitative glycoproteome analysis

Yejing Weng[1,2], Yanyan Qu[1,2], Lihua Zhang[1*], Huiming Yuan[1], Hao Jiang[1,2], Zhigang Sui[1,2] and Yukui Zhang[1]; [1]Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic R. & A. Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; [2]Graduate School of the Chinese Academy of Sciences, Beijing 100049, China.
lihuazhang@dicp.ac.cn

Protein N-glycosylation is one of the most prevalent and complex post-translational modifications, which plays an important role in various biological process, especially in cancer progression and immune responses. Therefore, the quantitative profiling of N-linked glycosylation sites occupancy is of important biological and clinical significances. However, for quantitative glycoproteome analysis, traditional offline protocols often suffer from drawbacks such as long analysis time, sample loss and manual manipulation.

Herein, a novel integrated platform for quantitative glycoproteome analysis was established, by which glycopeptides was first captured by a click chemistry maltose based hydrophilic interaction chromatography (HILIC) column, online solvent exchange by a nitrogen assisted HILIC-RPLC interface, deglycosylated by a hydrophilic PNGase F immobilized enzymatic reactor (IMER), trapped on a C18 column, labeled by isotope dimethyl reagents, and analyzed by MALDI-TOF MS/MS. To evaluate the performance of such an integrated platform, the digests of IgG with the coexistence of 50 times (mass ratio) BSA were analyzed, and 15 fmol deglycosylated glycopeptide from IgG was selectively detected with high signal to noise ratio. Furthermore, the quantitative accuracy and precision were demonstrated by comparing the signal intensity ratio of light labeling deglycosylate glycopeptide (m/z 1186) with heavy labeling deglycosylated glycopeptide (m/z 1190). The relative standard deviation (RSD) triplicated analysis was 5.7 %, and the experimental ratio of both peptides was kept as 1:1, in accordance with the theoretic value. All these results demonstrated that the integrated platform was of great promising for both qualitative and quantitative N-linked glycosylation sites profiling. The further on-line hyphenation of such a platform with ESI-MS/MS is undergoing, to achieve high-throughput analyses of biological samples.

173: Boronic acid functionalized graphene oxide composites for specific recognition of N-linked glycopeptides

Jiang Bo[1,2], Yang Kaiguang[1], Liang Zhen[1], Zhang Lihua[1]*, Zhang Yukui[1]; [1]National Chromatographic R. & A. Center, Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian 116023, China. [2]State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China.
lihuazhang@dicp.ac.cn

Glycosylation is one of the most important post-translational modifications in proteomes. Because of its high efficiency and high sensitivity, MS technology shows immense potential for the detailed structure characterization of protein glycosylation. Unfortunately, due to the inherent low abundance of glycopeptides and the microheterogeneity of each glycosylation site, it remains a great challenge for the comprehensive understanding of glycoproteome by MS based strategy. Therefore, the specific enrichment and purification of glycopeptides is essential prior to MS analysis.

Herein, for the first time, we developed a facile method to prepare novel boronic acid functionalized graphene oxide composites, GO/PEI/Au/MPB, and investigated the performance on the selective enrichment of N-linked glycopeptides. Due to the high hydrophilicity of such composites, even the mass ratio of BSA to ASF was increased to 100:1, the signal of glycopeptides was still high. Our experimental results further demonstrated the robustness and reliability of such material to capture glycopeptides from complex samples.

174: Gold nanoparticles modified hydrophilic polymer monoliths functionalized with boronate acid for specific capture of glycoproteins

Ci Wu[1,2], Yu Liang[1], Qun Zhao[1,2], Yanyan Qu[1,2], Zhen Liang[1], Lihua Zhang[1]*, and Yukui Zhang[1]; [1]Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic R. & A. Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China.
lihuazhang@dicp.ac.cn

Glycoproteome plays a crucial role in numerous biological processes. However, the main bottleneck for glycoproteome study is the low abundance of glycoproteins in complex samples. Recently, various boronate affinity monoliths have been springing up for glycoproteins enrichment. However, monoliths applied in boronate affinity chromatography are still meeting some challenges. One is the nonspecific adsorption of proteins, which could reduce the enrichment selectivity and the recovery of glycoproteins. The other is the limited enrichment capacity owing to the low surface area of monoliths. Therefore, the development of hydrophilic boronate affinity monolithic columns with high surface area is significant for the enrichment of glycoproteins with high efficiency and selectivity.

In this study, a novel kind of hydrophilic boronate affinity monoliths was successfully synthesized by co-assembling 4-mercaptophenylboronic acid (MPBA) with 2-mercaptoethylanine (MPA) on a gold nanoparticles modified hydrophilic monolithic column, prepared by the thermal initiated polymerization of poly (ethylene glycol) diacrylate and glycidyl methacrylate. The matrix showed the low nonspecific

adsorption of proteins because of good hydrophilicity. Gold nanoparticles modified on such matrix could obviously increase the surface area. With MPBA and MPA co-assembled, such a new affinity monolithic column could specifically capture horseradish peroxidase (HRP) from the mixture of HRP and BSA, even at a ratio of 1:1000 (m/m). The binding capacity of ovalbumin reached 0.39 mg•g^{-1}, higher than that obtained on other boronate affinity monoliths reported. In addition, high recovery of glycoproteins on the prepared boronate affinity monolith (85 %) was achieved. Moreover, such a column was successfully employed to specifically capture glycoproteins from egg white, demonstrating the great promising for glycoproteome research.

175: Preparation of amide functionalized hydrophilic monolith by *in situ* photo-polymerization for highly selective enrichment of glycopeptides

Hao Jiang[1,2], Huiming Yuan[1], Yu Liang[1], Yanyan Qu[1,2], Qi Wu[1,2], Lihua Zhang[1]*, Yukui Zhang[1]; [1]National Chromatographic Research and Analysis Center, Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Science, 457 Zhongshan Road, Dalian 116023, China, [2]Graduate School of Chinese Academy of Sciences, Beijing 100039, China
lihuazhang@dicp.ac.cn

For glycoproteome analysis, glycopeptide enrichment is an indispensable step prior to MS analysis. Recently, various methods have been developed, such as lectins, boronic acid affinity, hydrazide chemistry and hydrophilic interaction chromatography (HILIC), among which HILIC based methods have been drawn much attention due to low bias, good reproducibility and mild operation conditions. However, since the surface property on traditional HILIC materials is not homogenous, it is necessary to develop novel materials to improve the selectivity of glycopeptide enrichment.

In this study, a novel kind of amide functionalized monolith was synthesized by the *in situ* photo-copolymerization of acrylamide, methylene diacrylamide and N-vinyl-2-pyrrolidone in a UV transparent capillary. The performance of such a monolithic column was evaluated by the enrichment of glycopeptides from the tryptic digests of immunoglobulin G (IgG). With the captured glycopeptides analyzed by MALDI-TOF MS, 16 glycopeptides from IgG digests could be identified, demonstrating the high efficiency for glycopeptide enrichment. Furthermore, the selectivity of glycopeptide enrichment was also investigated by mixing the digests of bovine serum albumin and IgG with the ratio of 10000:1 (m/m), and five glycopeptides were unambiguously identified, further indicating the high selectivity and good specificity of such material. Moreover, the monolithic column was also applied for the glycosylation sites profiling of 6 μg proteins extracted from human serum (HS), corresponding to 100 nL original HS, and 78 unique glycosylation sites were identified, indicating its great potential for glycoproteome analysis.

176: Tandem dimethyl and solid-phase ^{18}O stable isotope labeling for N-glycoproteome quantitation

Yanyan Qu[1,2], Yejing Weng[1,2], Kaiguang Yang[1], Lihua Zhang[1], Zhen Liang[1], Yukui Zhang[1]; [1]Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic R. & A. Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China.
lihuazhang@dicp.ac.cn

N-glycosylation is one of the most common and complex posttranslational modification of proteins. Besides the global mapping of N-linked glycosylation sites, recently much attention has been paid on N-glycosylation occupancy quantitation at specific sites.

To date, a variety of chemical labeling approaches, *i.e.*, dimethyl labeling, isotope-coded affinity tags, and tandem ^{18}O stable isotope labeling (TOSIL), have been used for the relative quantitation of protein glycosylation. In contrast to other strategies, TOSIL, which incorperates three ^{18}O atoms into the heavy labeled glycopeptides during tryptic and N-glycosidase F (PNGase F) mediated hydrolysis, could obviously improve the distinguishment of glyco- and nonglycopeptides, and also the isotope distribution overlapping between differently labeled glycopeptides. However, the false positive rate of the quantification results might be increased by the limited ^{18}O-labeling efficiency, back exchange of ^{18}O and interference of residual active trypsin that incorporates ^{18}O into peptides during PNGase F deglycosylation.

Herein, we presented a new tandem dimethyl and solid-phase ^{18}O stable isotope labeling strategy (TDSOSIL), by which glycopeptide pairs were first labeled with light/heavy dimethyl after selectively enrichment, and then labeled with light/heavy oxygen atoms during deglycosylation by PNGase F immobilized enzymatic reactor (PNGase F IMER), generating enlarged 6, 10 or 12 Da difference between the glycopeptide pairs. It was demonstrated that the developed PNGase F mediated solid-phase ^{18}O labeling could dramatically minimize the back exchange of ^{18}O labeled peptides, and overcome the spontaneous

nonenzymatic ^{18}O labeling. In combination with the high-efficient dimethyl labeling, the accuracy of N-glycosylation quantitication and qualitation was improved. By such strategy, 146 N-glycosites were quantified with equal amount syngeneic mouse hepatocarcinoma ascites (HCA) cell digest labeled with light/heavy isotope, and 97 % of which had the H/L ratios in the range of 0.50–2.00 with RSD <30 %, indicating the great promise of TDSOSIL strategy for N- glycosylation quantitation. This strategy is now being applied for the differential glycoproteome analysis of highly and lowly metastatic typed HCA cell lines, the result of which will provide important technical support for the revealment of metastatic mechanism and the further discovery of potencial biomarker for desease diagnosis and drug therapy.

Poster Session I-Glyco(bio)technology

177: Analysis of highly complex glycan mixture derived from human fluid by MALDI-TOF/TOF, nano-LC/Chip Q-TOF and UPLC-Triple Quadruple Mass Spectrometry

Su Hee Kim[1], Bum Jin Kim[1], Jae Han Kim[2] and Hyun Joo An[1*]; [1]Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon, Korea, [2]Department of Food and Nutrition, Chungnam National University, Daejeon, Korea
sugar@cnu.ac.kr

Depending on the ionization methods and the type of mass analyzer, various mass spectrometric analyses have been developed. Due to their unique analytical advantages, appropriate mass spectrometric method has to be chosen practically depending on the purpose. While MALDI-TOF enables the quick view of whole glycan profile in the samples, nanoLC/Chip Q-TOF can provide the insight of isomers which MALDI-TOF is intrinsically ignored. UPLC-Triple Quadruple offers fast and quantitative analysis comparing with nano-LC/Chip Q-TOF, however, relatively low mass accuracy and resolution are disadvantages. Although the use of appropriate MS is crucial for the efficient analysis of complex glycan mixtures, data comparability between each MS has to be evaluated prior to the MS data interpretation and comparison. In this study, we assess the qualitative and quantitative comparability of each mass spectrometry using human milk oligosaccharides (HMO). HMO is a highly complex glycan mixture with a similar structure to the O-glycan. Extraction of free oligosaccharides was performed by following steps including the removal of lipids by Folch method and ethanol protein precipitation. To get rid of αand βisomers, free oligosaccharides were reduced to alditols. Extracted free oligosaccharides were further purified by solid phase extraction enriched 20 % and 40 % (in 0.05 % TFA) ACN fraction. Through an efficient analytical method, we found 64 free oligosaccharides by MALDI-TOF MS and 110 free oligosaccharides from nano-LC/Chip Q-TOF MS. The validity of the quantitative data was confirmed by analysis using the UPLC-Triple Quadrupole MS with multiple reaction monitoring (MRM) mode. Following this analytical method we have wide application various dairy streams, such as goat milk, unprocessed milk and low-fat bovine milk. In the dairy industry, this study can be applied as an analytical platform to composition profile and isomer specific glycan quantify free oligosaccharides using suitability Mass Spectrometry.

178: SimianTools: A software suite for the interpretation of high throughput glycomics mass spectrometry data

René Ranzinger, Ki Tae Myoung, Mindy Porterfield, Michael Tiemeyer and William S. York; Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA
rene@ccrc.uga.edu

Over the last decades the approaches for analyzing and identifying glycan structures using mass spectrometry have evolved from the study of single purified glycans towards the analysis of complex mixtures. However, most interpretation of the resulting datasets is still done manually. But comprehensive analysis of complex biological samples, producing several thousand mass spectra, consumes significant amount of time which consequently reduces the number of samples that can be analyzed. Therefore, development of high-throughput glycomics analysis software is crucial to efficiently tackle the large amount of data produced. SimGlycan® is a commercial software system which facilitates the interpretation of MS based glycomics data. However, the output of this product is not amenable to high-throughput studies designed to compare glycomics changes across many samples. Therefore we have developed a suite of programs that build on the SimGlycan® functionality and extend the usefulness of this tool.

SimGlycanDatabaseBot is a program that enables the generation of customized SimGlycan® databases which can subsequently be used for the interpretation of experimental data. The large, publicly available glycan databases that contribute to the database utilized by SimGlycan® contain many redundancies and partial erroneous structures that can result in invalid annotations. Our software allows the user to create a database by importing own structures or by selecting a subset of GlycomeDB structures to be imported into the new database. *SimianTools* is a program we have developed to enhance the evaluation of

results generated by SimGlycan®. After importing annotations generated by SimGlycan®, *SimianTools* generates graphical glycan representations and adds several statistical measures and specific identifiers to assist users in the evaluation of the total dataset. The program also supports side by side comparisons of results from several different analyses, making it a useful tool to find changes in glycan expression. All results can be exported in Excel format to allow post-processing of the information using third-party programs.

Poster Session I-Structural & Chemical Glycobiology and Glycomics

179: Cell-Selective Metabolic Glycan Labeling Based on Ligand-Targeted Liposomes

Ran Xie, Senlian Hong, Lianshun Feng, Jie Rong, and Xing Chen; Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
xingchen@gmail.com

Cell-surface glycans play key roles in mediating various molecular recognition events; aberrant glycosylation is implicated in disease progression. Therefore, probing the dynamic changes of glycan biosynthesis and structures is of great importance for augmenting our understanding of glycobiology and improving disease diagnosis and therapeutics. The metabolic glycan labeling was an appealing approach to incorporate specially designed carbohydrate analogs into the glycans, which enables the detection and imaging of the glycans in cells and living animals. However, one major bottleneck of this method is cell-type selectivity. Herein, we present the cell-specific metabolic glycan labeling using ligand-targeted liposomes to deliver unnatural sugars to target cells in a cell-surface receptor dependent manner. In this strategy, sugar analogs are encapsulated in ligand-targeted liposomes. The ligands bind to specific cell-surface receptors that are only expressed or up-regulated in target cells, which mediate the intracellular delivery of azidosugars *via* endocytosis. The delivered azidosugars are metabolically incorporated into cell-surface glycans and detected or imaged using a bioorthogonal reaction. The application of this strategy includes the facile introduction of myriad alternative ligands, as well as the cell-specific or tissue-specific imaging and detection of glycosylation *in vivo*.

Acknowledgements
We thank Prof. P. Wu for providing the initial batch of BTTAA ligand. This work was supported by the National Natural Science Foundation of China (No.21172013, No. 91127034) and the National Basic Research Program of China (973 Program) (No. 2009CB930303, No. 2012CB917303).

180: Detection and Isolation of Dendritic Cells Using Lewis X-functionalized Magnetic Nanoparticles

Sara H. Rouhanifard[1], Ran Xie[2], Guoxin Zhang[3], Xiaoming Sun[3], Xing Chen[2] and Peng Wu[1]; [1]Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China, [2]Department of Biochemistry Albert Einstein College of Medicine, Yeshiva University, 1300 Morris Park Ave, Bronx, NY 10461, [3]State Key Laboratory of Chemical Resource Engineering, P.O. Box 98, Beijing University of ChemicalTechnology, Beijing 100029, P. R. China
xingchen@gmail.com

Dendritic cells (DCs) are professional antigen-presenting cells that serve as messengers between innate and adaptive immunity. Due to their unique roles, DCs are under active investigations as the target for antigen delivery in vaccination against human immunodeficiency virus (HIV), cancer and autoimmune diseases. However, there's yet no perfect strategy for a cost-effective, high-selective and time-saving detection and isolation of DCs from a complex cell population.

Adhesion molecules such as DC-specific intracellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) on the surface of DCs play a critical role in establishing contact between the activated DCs and the naïve T cells through interactions with intercellular adhesion molecule-3 on T cells. DC-SIGN is a member of the C-type lectin receptors and can recognize antigens bearing mannose-rich or fucosylated glycans, as well as Lewis X (LeX). Here, we present the fabrication of magnetic nanoparticles coated with multivalent LeX glycans using the Cu (I)-catalyzed azide-alkyne cycloaddition. The resulting nanoparticles are selective and biocompatible, serving as a highly efficient tool for DC capture detection and enrichment.

Acknowledgements
This work was supported partially by a DuPont Young Professor Award and the National Institutes of Health to P.W. (R01GM093282), the National Natural Science Foundation of China to X.C. (No. 21172013, and No. 91127034) and the National Basic Research Program of China (973 Program) to X.C. (No. 2009CB930303, and No. 2012CB917303).

181: Functional Similarities and Differences between New Lectin Systems in Human Organism: Protein Hormone and Probiotic Bacterial

Mikhail V. Lakhtin, Vladimir M. Lakhtin, Andrey V. Aleshkin, Stanislav S. Afanasiev, Vladimir A. Aleshkin; Department of Medical Biotechnology, G.N. Gabrichevsky Research Institute for Epidemiology & Microbiology, Moscow, 125212, Russia
Lakhtinv@yandex.ru

New lectin systems (LS) as well as Lectins-Glycoconjugates (L-GC) relationships on solid surfaces are of increasing interest for glycol(bio)technology. The aim was to find functional similarities and differences between new hierarchic LS in human organism on the examples of protein hormone (EPO) and probiotic bacterial L (PBL) isolated from the cultural fluids of industrial probiotic lactobacilli and bifidobacteria of human origin.

Human erythrocytes and yeasts were used in agglutination assays. Pseudopolysaccharides (biotinylated or not) as GC were used (www.lectinity.com). Commercial human recombinant EPO preparations or PBL containing concentrates separated by IEF-PAG were blotted on Immobillon P and visualized using Streptavidin-Peroxidase or immune sandwich labeled with peroxidase followed by chemiluminescent registration in the presence of chemiluminescent peroxidase substrate in optimal conditions of live imagination in the Dark Room of BioChemi System (UVP, Calif.). Proteins were visualized by fluorescent dye SYPRO Ruby protein blot stain (Bio-Rad).

1. Both LS described and L-GC/GC-L interactions were adaptive to the test systems (soluble surroundings and solid phase used). L specificity as well as ranging GC depended on the choice of testing: agglutination (activation) or adhesion (stabilization), cell type (human or microbial), natural or artificial, heterogenic or homogenic. GC ranging depended on non-aggregated or aggregated L state. In the latter case EPO specificity to sialylated GC was appeared. 2. In case of PB molecular protein associates on the blot, at least three different layers (mask, protein and subprotein) were detected; LS mosaics within protein distributions were registered as strain and GC dependent. 3. Similarities of GC recognition by both LS types were established (α-L-Fuc-PAA, LacNAc-PAA). 4. Simultaneous recognition of GC system and discrimination of simple antigenic GC by LS are demonstrated.

Results are useful for further understanding LS and L-GC multilayer assembling and disassembling on solid surfaces.

182: Neolectins with tailored valency as tools in Glycobiology

Julie Arnaud [1], Julie Claudinon [2], Kevin Tröndle [2], Annabelle Varrot [1], Winfried Römer [2], Aymeric Audfray [1] & Anne Imberty [1]; [1]CERMAV-CNRS, Grenoble, 38041, France; [2]BIOSS Centre for Biological Signalling Studies, Freiburg, 79104, Germany
imberty@cermav.cnrs.fr

The ability of lectins to specifically recognize glycoconjugates on the surface of cells makes them a useful tool in biomedical diagnosis associated with change of glycosylation (inflammation, cancer…) Also, lectins are useful research tools for studying membrane trafficking since lectin-glycosphingolipid interactions can lead to membrane bending and endocytosis. However, only a limited number of lectins are available, limiting their use in biotechnology and research.

RSL is a fucose-binding lectin from the bacterium *Ralstonia solanacearum* that adopts a ß-propeller fold formed by trimer association and presenting six binding sites. RSL has been chosen as the paradigm for designing neolectins since it is easy to produce, has a high affinity for fucose and present six symmetrical binding sites. Alteration of the symmetry of the ß-propeller architecture is used to produce neoRSLs (nRSLs) with controlled valency in order to improve diversity in the development of technological tools and to understand the endocytosis mechanism. Several mutants have been produced in order to modify the valency of RSL.

The R17A neo-RSL stochiometry has been reduced to three residues per ß-propeller, while neither the affinity for monosaccharides nor the avidity to surfaces is altered. However, when the neolectin is tested on giant unilamellar vesicle, it shows that is not capable of forming membrane invaginations contrary to the wild type protein RSL, demonstrating a strong effect of the degree of multivalency on membrane dynamics and internalization. Neolectins with modified valency therefore appears as tools of choice for studying membrane dynamics and intracellular trafficking.

183: Chitosan Coupling Makes Bacterial Biofilm Susceptible to Antibiotics

Amin Zhang, Haibo Mu, Jinyou Duan*; College of science, Northwest A&F University, Yangling 712100, Shaanxi, China
jduan@nwsuaf.edu.cn

Bacterial biofilms are prevalent in natural, industrial and hospital settings and are inherently resistant to both antimicrobial agents and host defenses. Herein we demonstrate that covalent

coupling of antibiotics to cationic polymers (chitosan) can efficiently break down established biofilms and prevent biofilm formation, and as a result, make biofilm bacteria more susceptible to antibiotics. This conjugation is suitable to treat biofilms formed by a broad spectrum of gram-positive, but not gram-negative organisms. Mechanistic insight demonstrates chitosan conjugation renders antibiotics access into biofilms, thereby available to interact with biofilm bacteria. It appears that the amount of antibiotics, the specialized molecular size and highly positive charge in the conjugate are the key for maintenance of high anti-biofilm efficiency. Thus, this work represents an innovative strategy that antibiotics covalently linked to carbohydrate carriers can overcome antibiotic resistance to biofilms, and might provide a comprehensive solution to combat biofilms in industrial and medical settings.

184: Metrology for the characterization of Biomolecular Interfaces for Diagnostic Devices (BioSurf)

Véronique Blanchard[1], Aryaline Kamalakumar[1], Rudolf Tauber[1], Wolfgang Unger[2] and Paul Dietrich[2]; [1]Institute of Clinical Chemistry and Pathobiochemistry, Charité Medical University, Augustenburger Platz 1, 13353 Berlin, Germany, [2]BAM -Bundesanstalt für Materialforschung und -prüfung, Unter den Eichen 44-46, 12203 Berlin, Germany
veronique.blanchard@charite.de

In-vitro diagnostics are vital for the drive for cost effective healthcare, point of care monitoring and personalized medicine. The diagnosis and management of medical conditions is becoming increasingly reliant upon the detection and measurement of biochemical markers, or targets. BioSurf is a european project that aims at developing measurement methods for the research, development and quality control of biomolecular interfaces in diagnostic devices, and establish accurate, traceable and comparable methods to determine the amount of biomolecular probe immobilized at both planar and nanoparticle interfaces. It investigates novel approaches for the measurement of biointerfacial structures that can be correlated with activity and binding efficiency and methods to measure and predict the activity of immobilized probes. With the goal of supporting high throughput and multiplexed diagnostic methods, BioSurf is also assessing the capabilities of emerging techniques capable of detecting many targets simultaneously. TOF-SIMS uses a pulsed primary ion beam to desorb and ionize species from a sample surface. This technique allows determine the elemental and molecular species on a surface or in depth and to visualize the distribution of individual species on the surface.

The quality, reproducibility and shelf life of biomolecular interfaces will be presented with the above-mentioned techniques using high-mannose N-glycans and Concavalin A as a example.

185: High-throughput substrate profiling of the UDP-GalNAc: polypeptide *N*-acetylgalactosaminyltransferase using human proteome microarrays

Wenxian Xie, Xing Li, Zhijue Xu, Jing Wang, Shumin Zhou, Shengce Tao and Yan Zhang; Ministry of Education Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, 200240, China
yanzhang2006@sjtu.edu.cn

Mucin type *O*-glycosylation is arguably the most abundant and complex type of protein glycosylation. A large family of UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase (ppGalNAc-T, EC 2.4.1.41) control and regulate the initial step in the biosynthesis of mucin-type *O*-glycan. To date, there are 15 members of human ppGalNAc-T family having the GalNAc transferase activity *in vitro*. They display tissue-specific expression and the specificity substrate activities. Several ppGalNAc-T isoforms have been reported to be important for many cellular and developmental processes under physiological or pathological conditions by modifying specific target proteins. However, by now we have poor knowledge on understanding how many proteins will be GalNAc *O*-glycosylated and how individual ppGalNAc-T selects the target protein and how to decide the *O*-GalNAc modification site. To find more comrades, we developed a strategy to identify potential substrates of ppGalNAc-Ts globally by taken advantage of the high-throughput capability of protein microarray and the high specificity of click chemistry. We took ppGalNAc-T2 as an example and successfully identified 226 candidates as potential substrates. Gene ontology analysis showed that membrane-associated proteins were highly enriched. Intriguingly, other categories of protein, such as transcriptional factors and cytoskeleton related proteins were also significantly enriched. Among these potential substrates, 17 selected proteins were successfully glycosylated by ppGalNAc-T2 *in vitro*, and also validated by HPA lectin blotting. The *in vitro O*-GalNAc modified sites of p53 were successfully identified by mass spectromentry. Moreover, the overexpressed p53 in U2OS cells could be found *O*-GalNAc glycosylated, which is detected by HPA and VVA Lectin blotting. In this study, we have systematically screened potential substrates of ppGalNAc-T2 on proteome-wide and found some interesting potential substrates located in nucleus and cytoplasm. Our strategy could be easily applied for other ppGalNAc-Ts, and this will greatly facilitate the construction of the complete ppGalNAc-T and substrates network for all human ppGalNAc-Ts, and eventually help us to unveil the mysterious function of ppGalNAc-T systematically.

186: An Extra Fragment within the GH Module of an Endo-β-agarase from *Flammeovirga* sp. MY04 is Involved in the Degradation of Agarose

Wen-Jun Han[1, 2], Jing-Yan Gu[1], Hui-Hui Liu[1], Fu-Chuan Li[2, ξ], Zhi-Hong Wu[1], Yue-Zhong Li[1, ξ]; [1] State Key Laboratory of Microbial Technology, the School of Life Science, Shandong University, Jinan 250100, P. R. China; [2] National Glycoengineering Research Center, the School of Life Science, Shandong University, Jinan 250100, P. R. China
fuchuanli@sdu.edu.cn; lilab@sdu.edu.cn

Agarase hydrolyzes agarose into a series of oligosaccharides with repeating disaccharide units. The glycoside hydrolase (GH) module of agarase is known to be responsible for its catalytic activity. However, variations in the composition of the GH module and its effects on enzymatic functions have been minimally elucidated. The *aga*G4 gene, cloned from the genome of the agarolytic *Flammeovirga* strain MY04, encodes a 503-amino acid protein AgaG4. Compared with elucidated agarases, AgaG4 contains an extra peptide (N246-G302) within its GH module. Heterologously expressed AgaG4 (rAgaG4) was determined to be an endo-type β-agarase. The protein degraded agarose into neoagarotetraose and neoagarohexaose at a final molar ratio of 1.5: 1. Neoagarooctaose was the smallest substrate for rAgaG4, whereas neoagarotetraose was the minimal degradation product. Removing the extra fragment from the GH module led to the inability of the mutant (rAgaG4-T57) to degrade neoagarooctaose, and the final degradation products of agarose by the truncated protein were neoagarotetraose, neoagarohexaose and neoagarooctaose at a final molar ratio of 2.7: 2.8: 1. The optimal temperature for agarose degradation also decreased to 40 °C for this mutant. Bioinformatic analysis suggested that tyrosine 276 within the extra fragment was a candidate active site residue for the enzymatic activity. Site-swapping experiments of Y276 to 19 various other amino acids demonstrated that the characteristics of this residue were crucial for the AgaG4 degradation of agarose and the cleavage pattern of substrate.

187: Ribosome-Inactivating Proteins (RIPs) in Apple (*Malus sp.*)

Chenjing Shang, Willy J. Peumans, Els J.M. Van Damme; Lab: Biochemistry and Glycobiology, Dept. Molecular Biotechnology, Gent University, Gent, 9000, Belgium
chenjing.shang@ugent.be

Ribosome-inactivating proteins (RIPs) are a group of plant proteins, which possess highly specific N-glycosidase activity and are capable of catalytically inactivating eukaryotic ribosomes through the removal of a specific adenine residue from a highly conserved loop of the large rRNA. The family of RIPs is classically divided into type-1 RIPs, which consist only of an N-glycosidase domain and type-2 RIPs in which the RNA N-glycosidase domain is linked to a C-terminal carbohydrate-binding domain.

Until now there are no reports of ribosome-inactivating proteins in edible fruits. Recent searches in the fully completed genome sequences of different edible plant species revealed the occurrence of RIP related sequences. Furthermore, transcriptome databases available for apple (Malus sp.) provided evidence for the expression of these RIP related sequences. We cloned the RIP related sequences from apple, and the corresponding proteins were expressed and purified using different eukaryotic expression systems. The ribosome-inactivating proteins have been characterized for what concerns their molecular structure and RNA N-glycosidase activity. In addition, the carbohydrate-binding properties of the type 2 RIP were analyzed in detail using glycan arrays. Future experiments will focus on the physiological role of apple RIPs in the plant and their possible involvement in plant defense.

188: Recombinant human butyrylcholinesterase produced in plants: the synthesis of biologically active multi-sialylated glyco-forms

[1]Jeannine D. Schneider, [1]Alexandra Castilho, [2]Laura Neumann, [2]Friedrich Altmann, [1]Andreas Loos, [3]Latha Kannan, [3]Tsafrir S. Mor, and [1]Herta Steinkellner [1]Institute of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria; [2]Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria;[3] The Biodesign Institute, Arizona State University, Tempe, Arizona, USA
jeannine.schneider@boku.ac.at

Protein-based drugs can often not be produced satisfactorily by the established cell-based production platforms. This is not the least because of a complex *N*-glycosylation pattern. An example is human serum protein butyrylcholinesterase (BChE), which is a heavily sialylated protein that is being considered as a potential bioscavenger for organophosphate nerve agents. Sufficient amounts of active enzyme cannot be obtained from current sources. Plants are being recognized as a platform for the expression of recombinant proteins with complex *N*-glycosylation. Importantly, glycoengineering demonstrates the enormous plasticity of plant cells to tolerate modifications towards human-like structures

Nicotiana benthamiana, a tobacco-related plant species, was used as a host for the transient expression of recombinant human BChE (rBChE). The protein was collected from the extracellular space (apoplast) 4 days post infiltration (dpi) of the respective cDNA constructs and subjected to *N*-glycan analysis. While rBChE expressed in wild-type plants carries mainly complex structures with plant-specific *N*-glycans (*i.e.* core á1,3-fucose and â1,2-xylose), the enzyme derived from the glycosylation mutant ΔXTFT exhibits human-type GnGn structures. Co-expression of BChE with six mammalian genes required for *in planta* protein sialylation (*i.e.* GNE, NANS, CMAS, CST, GalT, ST) resulted in the generation of a rBChE that carries di-sialylated structures. Moreover, addition of GnT-IV (which carries out branching) to the infiltration mix produced rBChE decorated with tri-sialylated *N*-glycans. Notably, these structures were achieved by transient expression using multigene vectors within 4 dpi. Surprisingly, rBChE purified from total cellular extracts also exhibits significant amounts of endoplasmatic reticulum-typical oligomannosidic structures. These structures might arise from incorrect subcellular targeting of rBChE, as indicated by subcellular localisation experiments. Finally, *in vitro* activity assays exhibited biological activity of the various rBChE glycoforms. In summary, our results establish a proof of principle study for the *in planta* generation of rBChE with a di- and multisialylated glycosylation pattern, a prerequisite for clinical applicability. We present a highly flexible and time-efficient procedure for the generation of complex *N*-glycan structures on pharmaceutically interesting proteins that allows advanced structure-function studies, potentially leading to drugs/vaccines with enhanced efficacies.

189: Engineering of sialylated mucin-type *O*-glycosylation in plants

Martina Dicker[1], Alexandra Castilho[1], Laura Neumann[2], Daniel Maresch[2], Herta Steinkellner[1] Friedrich Altmann[2], Richard Strasser[1]; [1]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, 1190, Austria; [2] Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, 1190, Austria;
martina.dicker@boku.ac.at

Current mammalian cell-based expression systems for recombinant pharmaceutical proteins typically produce a mixture of heterogenous glycoforms that are neither identical to human glycans nor optimized for enhanced efficacy. In terms of glycosylation, plants offer certain advantages compared to other organisms as the *N*-glycosylation pathway of plants can easily be modified towards the generation of homogenous human-type *N*-glycans and a typical mammalian mucin-type *O*-glycosylation pathway does not exists at all. In this project, we focus on the generation of customized *O*-glycan structures on recombinant proteins produced in plants. By transient expression of human erythropoietin fused to an IgG heavy chain fragment (EPO-Fc) in leaves of *Nicotiana benthamiana* plants together with mammalian glycosyltransferases we were able to produce the cancer-associated Tn, T and STn antigens as well as the di-siaylated core 1 structure on the single *O*-glycosylation site of EPO-Fc. For the generation of sialylated *O*-glycans, we introduced an entire mammalian biosynthetic pathway into *N. benthamiana*, comprising the coordinated expression of the genes for (i) biosynthesis, (ii) activation, (iii) transport, and (iv) transfer of Neu5Ac to *O*-linked GalNAc. The formation of the different *O*-linked-glycans on EPO-Fc was confirmed by LC-ESI-MS. Notably, engineering of the *O*-glycosylation pathway did not interfere with the formation of *N*-linked glycans on EPO-Fc and by combining our approaches for customized *N*- and *O*-glycan engineering we were able to produce multi-sialylated EPO-Fc *in planta*. In summary, our studies demonstrate that *N. benthamiana* are amenable to *N*- and *O*-glycosylation engineering and are a valuable platform to produce glycoproteins with defined glycans for therapeutic use and structure-function studies.

References:

Castilho, A., Neumann, L., Gattinger, P., Strasser, R., Vorauer-Uhl, K., Sterovsky, T., Altmann, F., Steinkellner, H. (2013) Generation of Biologically Active Multi-Sialylated Recombinant Human EPOFc in Plants. PLoS ONE 8: e54836.

Castilho, A., Neumann, L., Daskalova, S., Mason, H.S., Steinkellner, H., Altmann, F., Strasser, R. (2012) Engineering of Sialylated Mucin-type O-Glycosylation in Plants. The Journal of Biological Chemistry 287, 36518–36526.

190: Effects of Chitosan Oligosaccharides on Improving Cold Resistance in Crops

Meng Y. Wang[12], Wen X. Wang[2], Heng. Yin[2], Li S X. Huang[12], Xiao M. Zhao[2], Yu G. Du[2]; [1]University of Chinese Academy of Sciences, Beijing, 100049, China; [2]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China
duyg@dicp.ac.cn

Chitosan oligosaccharides (COS) has the high solubility, which make them especially attractive in a number of useful applications. The effects of COS on growth, antibacterial properties and drought resistance of crops have been

investigated. Recently, it has been demonstrated that COS has the effect on improving cold resistance in wheat. In test field, the wheat seedlings treated with COS suffered less damage after late spring coldness compared to the control. The samples of COS treament group in test field show a increased activity of multiple enzymes and the content of the total chlorophyll up to 10 %. In the lab research, wheat seedlings suffered zero degrees Celsius temperature stress, and the COS treament group show a lower growth in electric conductivity and MDA level to control, while total chlorophyll, water solutble sugar (WSS) and reducing sugar increased. But no significant changes in the concentration of proline among different treatments has been detected. By accumulating the secondary metabolites production, COS enhances the resistance ability of wheat seedlings to low temperature stress. The concentration of chitosan oligosaccharides at 75 mg/L show the most effective results. The obtained results prove that COS could promote photosynthesis and metabolism and provide safety for low temperature cold damage in crops. Moreover, COS is friendly to environment, and the crops treated with the COS have no side effects to human.

191: Heterochitooligosaccharides: Enzymatic Preparation and Analysis by MALTI-TOF MS

Yong Zhao, Jianping Zhang, Yuguang Du; Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian, 116023, China.
zy1805@dicp.ac.cn

Oligosaccharides derived from chitin and chitosan exhibit various biological functions and have been attracting more and more attentions. They can be used as elicitors in agriculture, immune regulator in animal science, and to decrease cholesterol in healthy food and so on.

These useful oligosaccharides have been obtained by acid or enzymatic hydrolysis of chitin and chitosan followed by chromatographic separation. However, oligosaccharides between chitin and chitosan oligosaccharides—so called by heterochitooligosaccharides are still unknown to us. In our study, we developed a method by which heterochitooligosaccharides with average degree of acetylation 25 %, 35 % and 50 % could be prepared. They were derived from water soluble chitin with different degree of acetylation by an endo-type chitosanase. They were further analyzed by MALTI-TOF MS. It was believed that deeper studies are necessary to understand the relationship between their structure and unique biological activities and other application aspects.

192: Sulfonyl Hydrazine-functionalized Polymer as a Specific Capturer of Reducing Glycans from Complex Samples for High-throughput Analysis by Electrospray Ionization Mass Spectrometry

Chengjian Wang, Xiaohua Li, Jiangbei Yuan, Zhongfu Wang, Linjuan Huang; Educational Ministry Key Laboratory of Resource Biology and Biotechnology in Western China, Life Science College, Northwest University, Xi'an 710069, China
huanglj@nwu.edu.cn

Glycosylation represents one of the most common post-translational modifications of proteins and is involved in various important biological processes, such as molecular recognition, cell adhesion, and signal transduction. Qualitative and quantitative analysis of structurally diverse glycans of various glycoproteins can provide a basis for the investigations into their complex structure-function relationships. However, the glycan analysis is usually troubled with tedious sample processing steps prior to detection, including deproteination, desalting and removal of some other impurities, which result in a considerable sample loss and increase the difficulties of quantitative analysis. Herein we report a facile and versatile procedure for the specific and quantitative capture of reducing glycans from complex samples using sulfonyl hydrazine-functionalized polystyrene (SHPS) beads. This method allows efficient covalent conjugation of the aldehyde group of reducing glycans with the active sulfonyl hydrazide group of the SHPS beads under anhydrous conditions, resulting in the formation of a sulfonyl hydrazone bond. The unreactive non-glycan components in the samples, such as proteins, salts and some other impurities, can be completely removed in one step by washing the glycan-coated SHPS beads repeatedly. The regeneration of the reducing glycans can be performed *via* a mild hydrolysis of the sulfonyl hydrazone bond on the washed SHPS beads based on reversible hydrazide chemistry. The suggested procedure is compatible with all of the current techniques for the derivatization or detection of reducing glycans. We have obtained the fundamental data of this method, including the optimized reaction conditions, the glycan recovery ratio, the quantitative capability and the reproducibility. Additionally, the method was successfully applied to the glycan analysis of a series of complex biological samples, such as milk, human blood plasma and fetal bovine serum, demonstrating the good applicability of this novel procedure.

193: Production of human saposin B with mannose-6-phosphate-type *N*-glycans in the methylotrophic yeast *Ogataea minuta*

Toru Watanabe[1], Ikuo Kawashima[2,3], Yuki Takaoka[1], Akihiko Kameyama[1], Seiji Matsuda[4], Hitoshi Sakuraba[3,5] and Yasunori Chiba[1,3]; [1] Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki 305-8566, Japan; [2] Molecular Medicine Project, Tokyo Metropolitan Institute of Medical Science, Tokyo 156-8506, Japan; Departments of [3]Clinical Genetics and [5]Analytical Biochemistry, Meiji Pharmaceutical University, Tokyo 204-8588, Japan; [4]Division of Anatomy and Embryology, Department of Integrated Basic Medical Science, Ehime University School of Medicine, Ehime 791-0295, Japan.
y-chiba@aist.go.jp

Saposin B is a small and non-enzymatic proteinaceous cofactor that mediates degradation of sulfatide by arylsulfatase A and globotriaosylceramide by α-galactosidase A in lysosomes, where saposin B extracts the targeted sphingolipids from membranes and forms a soluble protein-lipid complex that is recognized by the enzymes. The inherited defect of saposin B accumulates sphingolipids predominantly sulfatide in the lysosome, which leads to a metachromatic leukodystrophy-like disease, saposin B deficiency.

The lysosomal dysfunction of sphingolipidoses is a consequence of a deficiency of lysosomal enzymes and their activators and results in the accumulation of sphingolipids. Recently, enzyme replacement therapy with recombinant enzymes has been developed for some sphingolipidoses, including Fabry disease (GLA deficiency). Here we report the production of a recombinant human saposin B (SapB) using the methylotrophic yeast *Ogataea minuta*, which is a cofactor that mediates the degradation of sulfatide and globotriaosylceramide in lysosomes. By overexpressing SapB gene and *OmMNN4* under the control of the alcohol oxidase promoter in *O. minuta*, we succeeded in producing a large amount of SapB containing more than 95 % of mannosylphosphorylated *N*-glycans. PNGase F treatment suggested that phosphorylated *O*-glycan is absent in the SapB. The recombinant SapB activated the degradation of globotriaosylceramide by GLA *in vitro* and was well incorporated into cultured human fibroblasts *via* mannose 6-phosphate receptors. The incorporation of SapB with mannose-6-phosphate glycan was dose-dependent; however, non-glycosylated SapB was not incorporated, even at a concentration of 20 μg/ml. The recombinant SapB holds promise as a replacement therapy for SapB deficiency and as an enzyme activation therapy for Fabry disease.

194: Natural Oligosaccharides Library Preparation

Wang wenxia[1], Li Shuguang[1], Tuo Yaqin[1], Guo Zhimou[1], Yin Heng[1], Shi Bo[2], Du Yuguang[1,*]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning 116023, China; [2]Feed research Institute Chinese Academy of Agriculture Sciences, Beijing, 100081, China
duyg@dicp.ac.cn

Oligosaccharides from natural products have promising bioactive ability in a wide range of diseases prevention and treatment through the alternative complement pathway; however, the study of oligosaccharide is technically challenging as no effective sequencing tool and obtained method. In this article, 60 kinds of polysaccharide from natural products were degraded by chemical or enzymatic method. They include marine polysaccharides like carrange, Chinese herbal polysaccharides like Poria cocos polysaccharide, fungal polysaccharides like Agaricus brasiliensis polysaccharide and other plant polysaccharides like Abelmoschus manihot medic polysaccharide. The distribution of degraded oligosaccharides was further analyzed through high performance liquid chromatography, hydrophobic interaction liquid chromatography or fluorophore-assisted carbohydrate electrophoresis method. Afterward according to the linkage type of monosaccharide unit, oligosaccharide composition and monomer were isolated through preparative high performance liquid chromatography. Finally a natural oligosaccharide library including more than 1,000 kinds of oligosaccharide component and monomer was established. The library offer an opportunity to identify new active oligosaccharide functional subunits and revealing oligosaccharide structure-activity relationship, therefore, the library have value for novel carbohydrate-based medicines discovery and development.

195: Differences and Similarities Between New Probiotic Bifidobacterial and Lactobacillus Lectin Systems Interacting to Glycoconjugates

Mikhail V. Lakhtin, Vladimir M. Lakhtin, Andrey V. Aleshkin, Stanislav S. Afanasiev, Vladimir A. Aleshkin; Department of Medical Biotechnology, G.N. Gabrichevsky Research Institute for Epidemiology & Microbiology, Moscow, 125212, Russia
Lakhtinv@yandex.ru

New Lectins-Glycoconjugates (L-GC) relationships on membranes are of importance for glycol(bio)technology. The aim was to describe functional similarities and differences between probiotic bacterial L (PBL) on the examples

of L identified in cultural fluids of industrial lactobacillus (LL) and bifidobacterial (LB) strains.

Pseudopolysaccharides, artificial antigens and peptidoglycans (biotinylated or not) were used as GC (www.lectinity.com). Lectin systems (LS) of strains of *Lactobacillus helveticus, L.amylovorus, Bifidobacterium adolescentis, B.angulatum, B.bifidum, B.gallinarum, B.longum* and *B.pseudocatenulatum* were investigated. PBL containing crude concentrates were separated by IEF-PAG in gradients pH 2–6 or 4–8, electroblotted on Immobillon P and visualized using GC-biotin followed by Streptavidin-Peroxidase treatments. Chemiluminescence of activated highly sensitive peroxidase substrate in optimized conditions of live imagination kinetic regimes was registered using Dark Room of BioChemi System (UVP, Calif.). Protein distribution was supported by fluorescent dye SYPRO Ruby protein blot stain (Bio-Rad) visualization.

Different LS recognizing α–D-mannan (Man: phosphorylated or not), α-L-fucan (Fuc), peptidoglycans (MDP), asialylated mammalian gut mucin-like (GalNAc); antigens Tn, blood group A (A_{di}) and/or Forssman (F_s) were identified among bacterial acidic proteins. The found LS were able to discriminate GalNAc-containing GC (GalNAc, LacNAc, A_{di} and F_s). Different L mosaics in tracks were involved in simultaneous recognition of different GC types (for example, F_s+MDP, Fuc+A_{di}). LS (as major 1-2 or major+minor components) were characterized as genera, species and strains fundamental features.

Results indicate important for biotechnology significant differences between LB and LL in GC specificities in identical testing conditions. The data support our findings on PBL interactions to eukaryotic and prokaryotic cells, synergistic antimicrobial action of LB and LL against fungal and Gram positive pathogens.

196: Enzymatic synthesis of *N*-acetylneuraminc acid derivatives of 5-bromo-4-chloroindolyl 4,7-*di*-*O*-methyl-β-D-galactopyranoside to determine α-2,3- and α-2,6-specific neuraminidase activity

Juana Elizabeth Reyes Martinez, Robert Sardzik, Josef Voglmeir, Sabine L Flitsch*; Manchester Institute of Biotechnology, School of Chemistry, The University of Manchester, Manchester M1 7DN, U.K. Corresponding author: Tel: +44(0)161-306-5172
juana.reyesmartinez@postgrad.manchester.ac.uk.

Sialic acid (*e.g. N*-acetylneuraminic acid (Neu5Ac)) plays a significant role in many biological and pathogenic processes such as cell-cell recognition, signal transduction, viral entry into the host cell and immune response. Neu5Ac can most commonly be found on glycoproteins as a terminal sugar linked to β-galactose residues with either α-2,3- or α-2,6-linkage. Influenza viruses which bind Neu5Ac are probably the most studied pathogens due to the easy spread among humans causing a great number of hospitalizations and economic losses each year. The linkage recognition is an important aspect in the invasive process of those viruses.
The majority of methods used to determine the linkage selectivity of neuraminidases require expensive reagents, specialised equipments and well trained technicians. Using a one-step enzymatic approach we synthesized α-2,3- and α-2,6-Neu5Ac modified 5-bromo-4-chloro-3-indolyl-β-D-galactopyranosides as novel substrates for probing neuraminidase activity and specificity. A colorimetric method based on an indigo-derivative formation has been established. Upon incubation of these substrates with an active neuraminidase an indolyl compound is released in the presence of β-galactosidase which quickly undergoes oxidation to form 5,5′-dibromo-4,4′-dichloro-indigo. The sialidase activity can be easily detected by blue coloration of the sample. Spectrophotometric techniques can be used for quantification.

The enzymatic syntheses were performed using trans-sialidase from *Trypanozoma cruzi* (TcTS) for α-2,3- linkage formation and sialyltransferase from *Photobacterium damsela* (ST6Pd) for α-2,6-linked Neu5Ac. The Neu5Ac addition to 5-bromo-4-chloro-indolyl-β-D-galactopyranoside was confirmed by MALDI-ToF MS and HPLC. The two novel substrates were successfully used for hydrolytic activity evaluation of 3 bacterial sialidases (*S. typhimurium* neuraminidase, *C. perfringens* neuraminidase, *A. ureafaciens* neuraminidase). In addition we also confirmed the presence of neuraminidase activity and specificity of *B. pumilus* and *A. aurescens* towards α-2,3 linkage sialoglycoconjugates.

197: The Study on Oligochitosan Inducing Cotton Resistance against Verticillium dahliae kleb

Xiao M. Zhao[12], Heng. Yin[12], Wen X. Wang[12], Meng Y. Wang[123], Yu G. Du[12]; [1]Liaoning Provincial Key Laboratory of Carbohydrates Dalian, 116023, China, [2]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China; [3]University of Chinese Academy of Sciences, Beijing, 100049, China
zhaoxm@dicp.ac.cn

Verticillium wilt of cotton is difficult to control by chemical pesticide. Oligochitosan can induce cotton resistance against verticillium dahliae kleb. Oligochitosan can promote root

growth of cotton, improve resistance of cotton root cap cell against toxin secreted by verticillium dahliae kleb, activate resistance genes expression. Oligosaccharides also increase accumulation of lignin on cotton root cells.

198: Advance in Application of Oligochitosan on Crops

Xiao M. Zhao[12], Heng. Yin[12], Wen X. Wang[12], Meng Y. Wang[123], Yu G. Du[12]; [1]Liaoning Provincial Key Laboratory of Carbohydrates Dalian, 116023, China, [2]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China;[3]University of Chinese Academy of Sciences, Beijing, 100049, China
zhaoxm@dicp.ac.cn

Oligochitosan was prepared from enzymatic hydrolysis of chitosan (the degree of N-acetylation is below 5 %) and separated with membrane in our Laboratory. Oligochitosan can induce defense responses of plant. Our recent research results showed that oligochitosan could activate plant cold resistance,thus Improve plant fruit-set rate.low concentrations of oligochitosan promoted the germination of seeds and growth of crops. Oligochitosan can increase crop yield and quality.

199: Systematic Analysis of Drug Glycosylation Critical Quality Attributes (GCQAs)

Louise Royle, Radoslaw P. Kozak, Li Phing Liew, Robert J. Emery and Daryl L. Fernandes; Ludger Ltd. Culham Science Centre, Oxfordshire, OX14 3EB, United Kingdom
rad.kozak@ludger.com

Glycosylation can have a significant effect on the clinical safety and efficacy of biopharmaceuticals. Issues with glycans have caused great financial, legal and regulatory problems for those companies who have not dealt effectively with their product's glycosylation. Regulatory authorities are now tightening the requirements for biopharmaceutical companies to characterise, control and compare the glycosylation of their therapeutics. However, measurement and control of drug glycans can be difficult to achieve due to their complexity and heterogeneity. Consequently, changes in glycosylation are the major cause of batch variability for most glycoprotein therapeutics.

At Ludger we use a systematic approach to greatly reduce the risks of suffering from problems with glycosylation. This system aligns with current and emerging regulatory guidelines from FDA, EMA and ICH and has three broad steps:

1. **GCQAs**. Specification of Glycosylation Critical Quality Attributes (GCQAs) (*i.e.* those glycosylation parameters that most influence the drug product's safety and efficacy profiles).
2. **Glycoprofiling**. Implemention of appropriate, affordable glycoprofiling modules to measure the GCQAs throughout the drug's life cycle.
3. **Interpretation and Corrective Action**. Interpretation of the glycoprofiling data and taking appropriate action if the product falls out of specification (OOS) or trends towards OOS.

This poster focuses on the glycoprofiling modules of step two.

A wide range of analytical techniques are available for glycan structure analysis and profiling—but it can be difficult to select the most appropriate of these modules for a particular drug. The types of glycoprofiling analyses required may also change at different points during a drug's life cycle. For example a wide range of complementary methods may be required for initial characterisation of glycosylation and identification of the GCQAs, but once they have been identified a simpler, faster method may be sufficient for quality control. To reduce regulatory problems it is crucial that reliable, robust, validated methods are used. We present here some straightforward strategies that drug developers and biomanufacturers can use for detection and quantification of common biopharmaceutical GCQAs such as sialylation, core fucosylation, antennary composition, lactosamine extensions, alpha-galactose and *N*-glycolylsialic acid. The emphasis is on methods that allow compliance with emerging drug regulations.

200: Metabolic Engineering of *Escherichia Coli* for Mannose Production

Hailong Cao[1], Yuguang Du[1]; [1]Dalian Institute of Chemical Physics, Zhongshan Road 457, Dalian, 116023, China
duyg@dicp.ac.cn

A whole-cell catalyst for the conversion of fructose to mannose was developed in *Escherichia coli BL21(DE3)*. The gene of NAD-dependent mannitol-1-dehydrogenase (MTD) which catalyzes the conversion of mannitol to mannose was synthesized and cloned into pET23b for optimal expression in *Escherichia coli BL21 (DE3)*. The mannitol dehydrogenase (MDH) from *Leuconostoc mesenteroides* was co-expressed with MTD in *E.coli BL21 (DE3)*. Using the created recombinant *E. coli* strain

as a whole-cell catalyst, a high conversion rate of fructose to mannose was achieved.

201: Efficient Adhesion-based Plasma Membrane Isolation for Cell Surface *N*-glycan Analysis

Ji-Young Mun, Kyung Jin Lee, Hoon Seo, Ohsuk Kwon and Doo-Byoung Oh*; Systems & Synthetic Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, South Korea
dboh@kribb.re.kr, Tel: 82-42-860-4457

Glycans, as attached to proteins and lipids on the cell surface, play crucial roles in various physiological phenomena such as cell-cell communication, differentiation and development. However, analysis of cell surface glycans requires difficult and laborious processes for the isolation of plasma membrane from other cellular components. Especially, efficient removal of endoplasmic reticulum (ER) and Golgi membranes are required since the high amount of high-mannose type glycans mainly located in ER has hampered the analysis of complex-type glycans on the cell surface. In the present study, we employed the simple adhesion-based method for the isolation of plasma membrane to analyze the *N*-glycans coating on the cell surface efficiently. The purity of the isolated plasma membranes was evaluated by fluorescence imaging using organelle-specific probes and optimal isolation conditions were established to minimize the contamination of ER and Golgi fractions. Cell surface *N*-glycans of Chinese hamster ovary (CHO) cells obtained by this adhesion-based plasma membrane isolation method were analyzed using matrix-assisted laser desorption/ionization time-of-flight and high-performance liquid chromatography, which were compared to *N*-glycan profiles derived from the corresponding total cell fractions. Complex-type *N*-glycans capped with terminal sialic acids were observed as major populations in plasma membrane fractions whereas high-mannose type glycans mainly detected in the profiles of total cells. Moreover, when compared with widely used plasma membrane isolation methods based on ultracentrifugation or cell compartment kit, our adhesion-based method showed the highest efficiency to detect complex-type *N*-glycans with the smallest amount of high-mannose type glycans. All these results indicated that the adhesion-based plasma isolation provides easy, rapid and efficient preparation method for the analysis of cell surface glycans, which will contribute to elucidating the biological implications of their changes in cell-cell communication, differentiation and development.

202: Enhancement of N-glycan Isomer Separation for Glycomic Characterization of Biotherapeutics and Biosimilars

Min Kyung So, Myung Jin Oh, Serenus Hua and Hyun Joo An*; GraduateSchool of Analytical Science Technology, ChungnamNationalUniversity, Daejeon, Korea
hjan@cnu.ac.kr, Tel:82-42-821-8547,Fax:82-42-821-8541

Glycosylation is largely influenced by the quality, safety, and potency of biopharmaceutical products.

Isomer separation for proper characterization of glycans in biotherapeutics, such as EPO (erythropoietin) or mAbs is particularly important because different isomers lead to different biological functions. PGC (porous graphitized carbon) is widely employed for effective isomer-specific separation and enrichment of glycans based on polarity, size, and three-dimensional structure. PNGase F treatment releases N-glycans as aldehydes. However, PGC separates aldehyde sugars into α and β anomers, increasing the complexity of isomer-specific separations. Here, we aimed to optimize glycan reducing conditions to get rid of α and β anomers by reducing aldehydes to alditols using sodium borohydride. Glycans released from ribonuclease B (high–mannose type), IgG (complex type), fetuin (highly sialylated) and EPO were used to optimize reducing conditions for all glycan types. After releasing N-glycans using PNGase F, released glycans were purified by SPE (solid phase extraction) and reduced by sodium borohydride. Reduced glycans were purified and enriched by SPE. Reduced N-glycans were easily identified by MALDI/TOF MS due to the two Da mass increase resulting from conversion of the reducing terminal to an alditol. To obtain isomer-specific information, glycans were analyzed before and after reduction using chip-based PGC nano-LC/MS. We found less peaks in chromatograms after reducing N-glycans,suggesting the removal of α and β anomers. For example,in common N-glycan$[Hex]_5[HexNAc]_4[Fuc][NeuAc]_2$, four chromatographic peaks were observed before reducing. After reduction, only two chromatographic peaks were observed, both at different retention times from the previous four peaks. Isomer-specific MS/MS also revealed different fragmentation patterns associated with each chromatographic peak. There are a number of biosynthetic explanations for the different isomers; for example, the fucose may be attached to either the N-glycan core or one of the antennae; or, the sialic acids may be either alpha-2,3 or alpha-2,6-linked.

We successfully applied and demonstrated the feasibility of our reducing method with various biopharmaceuticals products including EPO or mAbs. This analytical platform will provide a powerful analytical tool for isomer-specific separation of glycans on PGC and be globally used in the biopharmaceutical field.

203: Gas1 Engineering for Selection of a Yeast Strain with Improved Protein Secretion

Hoon Seo[1, 2], Dong Hyeun Hwang[1], Ji-Young Mun[1], Jin Young Gil[1], Kyung Jin Lee[1], Ohsuk Kwon[1], Sang Ki Rhee[2] and Doo-Byung Oh[1]; [1]Systems & Synthetic Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon, South Korea, [2]Department of Medicinal Biotechnology, Soon Chun Hyang University, Asan, 336-745, Korea
dboh@kribb.re.kr

Gas1 protein is a beta(1,3)-glucanosyltransglycosylase playing an essential role in the assembly of cell wall as localized on the yeast surface through a glycosylphsphatidylinositol (GPI) anchor. When *GAS1* gene was disrupted in several yeasts including *Saccharomyces cerevisiae* and *Pichia pastoris*, the resulting mutant strains were reported to exhibit hypersensitivity to cell wall-perturbing reagents and temperature-sensitive phenotype together with increased capability of protein secretion due to the loosed cell wall structure. In the present study, functional complementation of cell wall-defective phenotype of *GAS1*-deletion mutant using recombinat expression of Gas1 protein was employed to generate a screening system for a strain with improved capability of protein secretion. We constructed the expression vectors encoding fusion proteins with *N*-terminal secretory protein of interest linked to Gas1 proteins without signal sequence. After these vectors were transformed into *GAS1*-deletion mutant, the growths of the resulting transformants were tested on the agar plate containing cell wall-perturbing reagent. Only the strains expressing Gas1 proteins fused to well secreted proteins showed restored growth phenotype under cell-wall stress condition. Currently, we are exploring the possiblity that this system can be used to enrich yeasts with improved secretion capability after genome-wide random mutagenesis, which would lead to the development of Gas1-based screening system for super secretory yeast.

204: Rapid oligosaccharides sequencing by exoenzymatic digestion on the basis of microfluidic device

Benwei Zhu[1, 2], Runtao Zhong[1, 3], Bingcheng Lin[1, 3], Yuguang Du[*1, 3]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China; [2]University of Chinese Academy of Sciences, Beijing, 100049, China; [3]Research Center of Lab on a Chip, Dalian, China
duyg@dicp.ac.cn

Glycosylation is the most significant procedure of the post-translational modifications. The structural analysis of oligosaccharides attached on the surface of the glycoprotein is essential for understanding the vital process and developing efficacious drugs as well as antibodies. Exoglycosidase digestion coupled with matrix-assisted laser desportion/ionization mass spectrometry (MALDI-MS), all through the way, has been demonstrated to be an effective method for oligosaccharides sequencing. But the major disadvantage of the sequential digestion is that it requires the repeated isolation, which not only waste much more time and efforts, but the incubation of enzyme and oligosaccharides mixture takes a dozen hours.

To address the circumstances mentioned above, we report a novel method for oligosaccharides sequencing, which combines enzymatic digestion with microfluidic devices. The mixture of digestion reaction is compartmentalized into aqueous picoliter-volume droplets, dispersed in inert carrier oil. Each droplet is an enzymatic reaction vesssel with highly efficient hydrolysis (~37 °C) due to rapid mixing and enhanced mass transfer within microdroplets, as well as high uniformity of droplet size and temperature. After enzymatic digestion, the products are recovered and characterized by matrix-assisted laser desportion/ionization mass spectrometry (MALDI-MS). By performing microfluidic droplet-based reaction, the time required for glycosidase digestion could be shortened to several hours, which is very important for oligosaccharides sequencing and analysis.

205: Inactivation of the gene *rmla* remodels *Burkholderia kururiensis* exopolyssacharide reducing ability to promote rice growth and colonization

Letícia Hallack, F[1]; Priscila A.V. Oliveira[1]; Luciana L. Penha[1]; Bruna G. Coutinho[2]; Daniel Passos da Silva[2]; Jose Osvaldo Previato[1]; Lucia Mendonça-Previato[1]; [1] Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2] International Centre for Genetic Engineering & Biotechnology, Trieste, Italy
lehallack@gmail.com; luciamp@biof.ufrj.br

Burkholderia kururiensis is a diazotrophic endophytic bacterium isolated from internal tissues of several plants. Interaction studies showed that *B. kururiensis* is able to promote growth and increase grain yield of rice plants. The molecular mechanisms involved in the endophytic bacteria-grass interactions are unknown, but a specific relationship between bacterium exopolysaccharide (EPS), capsular polysaccharide (CPS) or lipopolysaccharide (LPS) and host plant has been proposed.

B. kururiensis M130 produces large amounts of acidic EPS. Structural studies of this polymer showed the presence of a linear pentasaccharide repeats units with the following structure: –>4)-alpha-D-Glcp-(1–>2)-alpha-L-Rhap-(1–>4)-alpha-

D-GlcpA-(1–>3)-beta-L-Rhap[2OAc]-(1–>4)-beta-D-Glcp-(1–>.

The monosaccharide rhamnose (Rha) is commonly found in bacterial EPS that interact with plants. Some studies have shown the direct involvement of L-rhamnose in the interaction processes. Four enzymes (RmlA-D) are present in the dTDP-L-rhamnose biosynthetic pathway, and D-glucose-1-phosphate thymidylyltransferase (RmlA) catalyzes the first step of dTTP and D-glucose-1-phophate (D-Glc-1-P) to dTDP-D-glucose (dTDP-D-Glc) and PPi

In this study we performed a functional knockout in the biosynthesis of L-rhamnose. In order to investigate the function of the EPS in the rice x bacteria interaction, the *B. kururiensis rmla* gene was interrupted, generating the mutant rmla. Structural analysis of EPS of the mutant strain showed the presence of a new EPS composed of tetrasaccharide repeat units containing two residues of D-rhamnose, instead L-rhamnose, and two residues of heptose. Gnobiotic experiments with the rice were done to comparatively evaluate the endophytic colonization ability of the mutant strain. It was observed a three-fold reduction on the capacity to promote plant growth, also the ability to colonize roots and aerial parts of the rice plant was reduced.

These data demonstrate the involvement of EPS in the endophytic colonization of the *B. kururiensis* and rice, adding further insight into the possible molecular mechanisms involved in this interaction.

206: Refining the specificities of anti-I and anti-i antibodies using neoglycolipid-based array technologies and applications in the immuno-sequencing of poly-*N*-acetyllactosamine backbones

Chao Gao[1], Angelina S. Palma[1,2], Wengang Chai[1], Yan Liu[1], Robert A. Childs[1], Ten Feizi[1]; [1]Glycosciences Laboratory, Department of Medicine, Imperial College London, Lodon, W12 0NN, UK; [2]REQUIMTE,Departamento de Química, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
c.gao11@imperial.ac.uk

Monoclonal autoantibodies in sera of patients with cold agglutinin syndrome recognize developmentally-regulated carbohydrate antigens termed I and i on erythrocytes and other cell types. Their characterization and demonstration of their power as sequence-specific reagents ante-dated similar work with hybridoma-derived antibodies generated to onco-developmental antigens. Anti-I and anti-i recognize branched and linear sequences of poly-*N*-acetyllactosamine type, respectively, and they are unique reagents for detection of un-substituted forms of these sequences. This presentation will be concerned with neoglycolipid (NGL)-based arrays to define more precisely the sizes of the I and i antigens and apply these antibodies as immuno-sequencing reagents.

In the original studies of Ten Feizi and colleagues, the specificities of several anti-I and -i antibodies were elucidated in a succession of analyses that in essence involved *inhibition of binding,* using as inhibitors structurally-defined synthetic or natural oligosaccharides, and also glycolipids. Methodologies included quantitative inhibition of precipitation or haemagglutination, and inhibition of binding in radioimmunoassays. These antibodies proved to be invaluable in monitoring changes that occur in the branching patterns of carbohydrates in the course of embryonic development, cell differentiation and malignancy. Among the antibodies, anti-I Ma has been studied in greatest detail. On the branched poly-*N*-acetyllactosamine chains, it recognizes the trisaccharide domain Galβ1-4GlcNAcβ1-6R (where R is Gal or GalNAc). Anti-I Ma is the prototype of one group of anti-I. A second group recognize mainly the Galβ1-4GlcNAcβ1-3 domains of branched chains, with individual antibodies differing in their additional requirements for all or part of the Galβ1-4GlcNAcβ1-6 branch. These differences are advantageous in the use of these antibodies as tools for analysis of backbone sequences of glycans.

The NGL-based microarray system enables antigenic analyses of a series of oligosaccharides at low fmol levels, and represents a substantial miniaturization relative to the mmol of oligosaccharides required for inhibition studies. We now observe that for optimal binding, anti-I Ma requires a longer sequence than tetrasaccharide (Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAc). We will describe results of a re-evaluation of anti-I/i specificities using macro and microarrays and application of the antibodies, in conjunction with glycosidase treatments and mass spectrometry, as immuno-sequencing reagents to characterize novel carbohydrate antigens on *O*-glycans and glycolipids.

207: Lectin microarray assists development of the glycodiagnostic systems for direct measurement

Atsushi Kuno[1], Takashi Sato, Atsushi Matsuda, Yuzuru Ikehara, Hisashi Narimatsu; [1]Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan
atsu-kuno@aist.go.jp

“Glycodiagnosis” is a new paradigm of clinical diagnosis based on the quantitation of glyco-alteration well reflecting

disease progression. To detect such changes in glycosylation, all of the currently available glycomics techniques (*e.g.*, mass spectrometry, liquid chromatography, capillary electrophoresis) require at least 3 h of sample preparation before analysis. Alternative technologies based on a lectin–antibody sandwich assay had been proposed for detection of proteins bearing disease-specific glyco-alterations. Most of them detect the changes in fucosylation of *N*-linked glycans, which are well-known to be associated with liver disease. However, the fucose-binding lectins force us the essential of preliminary enrichment of the target protein, and thus are inappropriate in practical use for serological diagnosis. In this study, we present a new methodology with two technical advances to overcome these problems in glycodiagnosis associated with clinical implementation: (1) We adopted a microarray-based method with a unique subtraction process for easy selection of the most robust lectin. In fact, the method efficiently led us to WFA to establish the direct measurement system. (2) We selected M2BP as a potential glycobiomarker that shows a fibrosis-related glyco-alteration which we proved for the first time. The diagnostic utility of M2BP is greatly owing to a favorable density and orientation of the disease-related glycan on the homomultimer resembling a "sweet-doughnut". These characteristic structures contribute to a major increase of the avidity of M2BP for the plated WFA in ELISA. The resulting glycan–lectin interaction (*i.e.*, WFA-M2BP interaction), which is remarkably strong and specific, made it possible to develop the rapid (17 min) and highly sensitive assay and realized "on-site diagnosis". This unique methodological approach is applicable to develop other glycodiagnostic tools, thus it will revolutionize the use of glycodiagnosis in clinical medicine and potentially provide a framework for the development of a new generation of biomarker assays.

This work was supported by a grant from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

208: Molecular cloning and functional expression of lectin from *Datura stramonium* seeds

Suguru Oguri, Kaori Tanaka, Keisuke Nishimoto, Takahiro Murakami, Hikaru Sakamoto; [1]Department of Bioproduction, Faculty of Bioindustry, Tokyo University of Agriculture, Hokkaido, 099-2422, Japan
S-oguri@bioindustry.nodai.ac.jp

The *Datura stramonium* agglutinin (DSA) is a chitin-binding lectin purified from Jimson weed (*D. stramonium*) seeds. DSA binds selectively to the branch structure on complex-type N-glycans containing N-acetyllactosaminyl structures. It has been used to analyze structural changes of sugar chains during oncogenesis. Thus, DSA has been used for an essential tool in glycobiology. DSA is a dimer composed of two subunits linked by disulfide bonds. It was reported that an affinity-purified DSA fraction was a mixture of three different isolectins which arise from AA, AB and BB combination of the both subunits. The A- and B-subunits had similar but not identical *M*r values (32,000 and 28,000, respectively). At present, cDNA of DSA had not been isolated and its molecular structure remains unknown. In order to isolate the DSA gene, we analyzed the N-terminal and the internal amino acid sequences from the isolated B subunit of DSA (DSA-B). Based on the sequences, the degenarate primers were designed and RACE was conducted. The isolated lectin cDNA (*dsa-b*) contained an open reading frame encoding 279 amino acids, including peptides that were sequenced. The deduced sequence consisted of two distinct motifs: (1) a Cys-rich carbohydrate binding domain composed of four conserved chitin-binding domains; (2) an extensin-like domain of 37 residues containing 4 SerPro4-6 motifs inserted between the second and third chitin-binding domains. Although each chitin-binding domain contained 8 Cys residues at the conserved positions, only the second chitin-binding domain contained an extra Cys residue, which may participate in dimerization through an intra-disulfide bridge formation. The molecular mass of the native DSA-B was determined as 68,821 Da by MALDI-TOF MS. The molecular mass of the isolated subunit of DSA-B was 37,593 Da and that of the deglycosylated form was 26,320 Da, as pyridilethylated derivatives. This correlated with the estimated molecular weight of the deduced sequence. Transgenic *Arabidopsis* plants overexpressing *dsa-b* possessed hemagglutinating activity. The recombinant DSA was produced as a homo-dimeric glycoprotein with similar molecular mass compared to native one. Moreover, N-terminus of the purified recombinant DSA was identical with that of the native DSA-B. These findings confirmed that the cDNA encoded DSA-B.

209: Unique gangliosides synthesis by sialyltransferases from marine bacteria

Hisashi Kamimiya[1], Yusuke Suzuki[1], Anila Mathew[1], Shinobu Watarai[2], Toshiki Mine[3], Takeshi Yamamoto[3], and Yasunori Kushi[1]; [1]College of Science and Technology, Nihon University, Tokyo, 101-8308, Japan; [2]Graduate school of Life and Environmental Science, Osaka Prefecture University, Osaka, 599-8531, Japan; [3]Product Science Division, Japan Tobacco Inc., Kanagawa, 227-8512, Japan.
h.kamimiya@gmail.com

Sialyltransferases (ST) are enzymes that transfer *N*-acetylneuraminic acid (NeuAc) from the common

donor substrate cytidine 5′-monophospho-NeuAc (CMP-NeuAc), to acceptor substrates. Many STs and the genes encoding them have been obtained from various sources including mammalian, bacterial and viral sources. Recently, we had discovered new bacterial STs from marine bacteria belonging to genus *Photobacterium* and closely related to genus *Vibrio*, both of which are gram-negative bacteria. One of the authors have previously cloned these STs and enzymatically characterized them using fluorescence-labeled oligosaccharides, and they were found to be β-galactoside α2-3 or α2-6ST, which catalyzes the incorporation of NeuAc from CMP-NeuAc into the terminal galactose residue in carbohydrate chain at position 3 or 6.

To further characterize these STs, we tested ganglioside synthesis by STs using various glycosphongolipids (GSLs) as substrates. Bacterial STs showed affinity to neolacto- and lacto-series GSLs, particularly in neolactotetraosyl ceramide (PG, nLc_4Cer, Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1'Cer). Gangliosides synthesized from nLc_4Cer by α2-3 and α2-6ST were structurally characterized by several analytical and immunological methods, and they were identified as IV^3αNeuAc-nLc_4Cer (S2-3PG) and IV^6αNeuAc-nLc_4Cer (S2-6PG). In addition, these STs also showed affinity to asialoganglio-series GSLs. As a results, GA1 (Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1'Cer) abd GA2 (GalNAcβ1-4Galβ1-4Glcβ1-1'Cer) were found to be good substrates for these STs. Ganglioside generated by the catalytic activity of α2-3ST was identified as GM1b (NeuAcα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1'Cer). On the other hand, when enzyme reaction by α2-6STs were performed using substrates GA1 and GA2, very unique gangliosides were generated. By using several analytical procedures, the generated gansgliosides were identified as NeuAcα2-6GA2 (S2-6GA2) and NeuAcα2-6GA1 (S2-6GA1), respectively. Furthermore, the above synthesized ganglioside, S2-6GA2, showed binding activity to the influenza A virus {A/panama/2007/99 (H3N2)} at a similar level to purified S2-3PG and S2-6PG from mammalian sources. These results suggest that bacterial STs have unique features, including substrate specificities restricted not only to neolacto-series, but also to asialoganglio-series GSLs, and catalytic potentials for unique ganglioside synthesis. This demonstrates that effective *in vitro* ganglioside synthesis could be a valuable tool for selectively synthesizing NeuAc modifications. We are currently preparing transformed mammalian expression systems using STs gene from marine bacteria.

210: Characterisation and manipulation of N-glycans in insect cells

Rhiannon Stanton[1], Dieter Palmberger[1] and Iain B. H. Wilson[1]; [1]Department für Chemie, Universität für Bodenkultur, Vienna, 1190, Austria.
rhiannon.stanton@boku.ac.at

Anti-carbohydrate antibodies are present when foreign glycans are detected by the immune system and thus be a problem if recombinant glycoproteins, such as antibodies, are used as therapeutic agents. It is also of importance that the recombinant antibodies have the correct glycosylation in order to prevent clearance from the serum. By screening for the glycans from insect cells, anti-carbohydrate antibodies, lectins and their binding partners can be detected.

Using glycans from natural sources can be problematic because of the low amounts generated. However, even though only small quantities are extracted, these can still be used in glycomic experiments. This work demonstrates how glycans isolated from natural sources (in this case glycans from the *Trichoplusia ni* insect cell line High Five) can be used in glycan microarray screens. First, N-glycans can be isolated from the glycopeptides from the cells, labelled and purified prior to characterisation by a variety of HPLC and MS methods. Labelled N-glycans were then applied to glass slides prior to detection by lectins and anti-horseradish peroxidase.

As *T. ni* cells tend to be an excellent choice for expression of antibodies, engineering of these cells is a pre-requisite for humanisation of the glycans and avoidance of potential immune responses. The recently-described SweetBac technology can be used to produce modified antibodies with altered glycosylation; in particular, introduction of a nematode *N*-acetylglucosaminyltransferase II and a mammalian galactosyltransferase results in a high proportion of biantennary N-glycans on recombinant antibodies derived from *T. ni* cells.

211: Generation of Complete Series α-2,8-sialic Acid Oligosaccharides from Polysialic Acid Produced by *Escherichia coli* CCTCCM208088

Li Zhu [1], Xiao-bei Zhan[1,2,*], Hong-Tao Zhang[2], Jiang-Rong Wu[2], Zhi-Yong Zheng[2], Chi-Chung Lin[2]; [1] Jiangsu Rayguang Biotech Co. Ltd., Wuxi, Jiangsu 214125, China; [2] Key Laboratory of Carbohydrate Chemistry and Biotechnology of Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, Jiangsu 214122, China;
xbzhan@yahoo.com (X.-B. Zhan)

Polysialic acid (PSA) mainly exists in cell surface serves to modulate the distance between cells and involved in a number of plasticity-related responses in adult central nervous system. However, PSA-signal protein recognition is the key step for PSA playing its role. To exploration the minimal oligosaccharide for signal protein, it is necessary to acquiring complete series of α-2,8-sialic acid oligosaccharides. In this work, PSA was treated with 0.1 M HoAc at 40 °C, 60 °C and 80 °C for 30, 60, 90, 120, 160 and 190 min respectively and then adjusted all solution to neutral with 0.5 M NH_4HCO_3. All the hydrolysis products were desalted further with G10 column. Separation step were done using HPLC with evaporative light—scattering detector (ELSD) and Click mail column. The optimal condition for acquiring complete series sialic acid oligosaccharides from 3 to 15 mer is at 60 °C for 160 min.

212: SPRi-based Lectin Array Chip Powerful Glycoprotein Analysis Tool

John Zhang [1], Yue Yuan [1], Trevor Johnston [1], Christopher Lausted [3], Bridget Sexauer [1], Andrew Weber [1], Hong Liu [2], Jingsong Zhu [2]; [1] Plexera LLC, 401 Terry Avenue North, Seattle, WA 98109, USA; [2] National Center for Nanoscience and Technology, Haidian, Beijing, 100190, China; [3] Institute of SystemBiology, 401 Terry Avenue North, Seattle, WA 98109, USA
Cro@plexera.com

Lectins are well-characterized, highly-specific sugar-binding proteins that can be produced economically for a wide range of applications. Microarrays of these sugar-binders allow multiplexed high-throughput protein analysis in small sample volume. The carbohydrate-specific binding proteins serve many different biological functions and play a key role in cells and protein recognition pathways. They also are involved in immune system response by recognizing carbohydrates that are found exclusively on pathogens or alternatively formed carbohydrates on cancer cells. Their ability to bind to soluble extracellular and intercellular glycoproteins allows them to be excellent glycoprotein screening agents. Our first SPRi-based Lectin microarray chip contains 41 of the most common lectins targeting seven classes of typical sugar motifs. This high-throughput glycoprotein biomarker discovery platform termed ***Lectin-NanoChip™*** is able to identify and monitor glycosylation changes in a different types of samples rapidly. Surface Plasmon Resonance Imaging (**SPRi**) also offers label free detection of the glycan and its respective sugar subtypes. Additionally, the affinity of different glycan can be determined *via* binding constant determination supplied by our analysis tool. Therefore, lectin array combining with SPRi technology is a great platform for glycoproteins characterization. The ***Lectin-NanoChip™*** can be applied to a vast glycoform characterization fields that include but not limited to these following: disease-relevant glycol-biomarkers discovery, cell surface glycome profiling, pathogen recognition, bacterial tropism identification, cancer stem cell markers detection and altered glycan structure classification.

213: A high throughput screening of ligands to multiple targets by Surface Plasmon Resonance Imaging (SPRi) Platform, *PlexArray*®

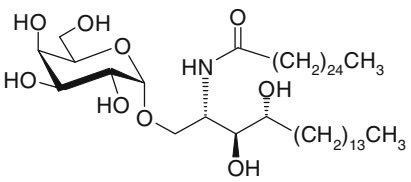
John Zhang [1], Christopher Lausted [3], Trevor Johnston [1], Yue Yuan [1], Bridget Sexauer [1], Andrew Weber [1], Hong Liu [2], Jingsong Zhu [2]; [1] Plexera LLC, 401 Terry Avenue North, Seattle, WA 98109, USA; [2] National Center for Nanoscience and Technology, Haidian, Beijing, 100190, China; [3] Institute of SystemBiology, 401 Terry Avenue North, Seattle, WA 98109, USA
Cro@plexera.com

Researchers have been seeking new and improved methods for multiplex, label-free and real-time monitoring of bio-molecular interactions. Surface Plasmon Resonance Imaging (**SPRi**) is a versatile method for observing such interactions. This system enables the measurement of a wide range of targets that includes kinases, peptides, DNA, antibodies and adhesive molecules in both single and multiple assay formats. Plexera's unique platform combines array with SPRi technology to monitor high-throughput interactions up to five thousand molecules. The powerful platform can be applied for high-throughput, label-free, fast biomarker screening, personalized diagnostics development, drug discovery,and various other proteomic studies. Our SPRi array platform has become a powerful tool for studies on multiplexing interactions of many different biomolecues. So far, cytokines, lectins, peptides, DNA, kinases and even expressing proteins on cells have been successfully validated by this real-time bioassay. The tool can be applied to many different biological functional studies, such as epitope mapping, cytokine and glycan profiling, as well as active kinase interactions. Most importantly, it is able to select targets for small molecule binders of interesting. Thus, we can accelerate the speed for discovering novel biomarkers dramatically.

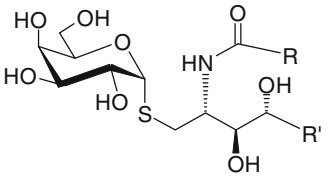
214: Synthesis of Thioglycoside Analogues of KRN7000

Xiangming Zhu[1,2], Ravindra T. Dere[2], Junyan Jiang[1];
[1]College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua 321004, China; [2]Centre for Synthesis and Chemical Biology, School of Chemistry and Chemical Biology, University College Dublin, Dublin 4, Ireland
xiangming.zhu@ucd.ie

α-Galactosylceramide, KRN7000, has been utilized in studies for the treatment of many diseases including cancer, diabetes, malaria, and hepatitis B, and in most cases impressive activities have been observed.[1] This compound can stimulate NKT cells to release a variety of cytokines *in vitro* and *in vivo*, which are recognized subsequently by other cells of the immune system and may have a widespread influence on immune responses. Nevertheless, the efficacy of KRN7000 has been limited because it stimulates production of a mixture of T_H1 and T_H2 cytokines, which are antagonistic to each other. Therefore, many efforts have been devoted in the past decade to synthesize its analogues with the hope of developing novel lead compounds with better cytokine-inducing selectivities and appropriate potency as immunostimulatory agents.[2]



KRN7000



R, R' = Hydrocarbon chain

Thioglycoside analogues

We present here the first total synthesis of thioglycoside analogue of KRN7000,[3] and convenient synthesis of other thioglycoside analogues of α-galactosylceramide carrying the truncated sphingoid chain.[4] A great advantage of the used synthetic strategy is that the anomeric stereochemistry was introduced at an early stage in the synthesis by use of a galactosyl thiol building block, and the strategy would also be applicable to other α-*S*-galactosylceramides of interest. Also, the synthesis efficiency was greatly increased in the synthesis of the truncated analogues by employing Mitsunobu reaction to retrieve an unwanted diastereoisomer.

The subsequent bioassay demonstrated that the thioglycoside analogue of KRN7000 possesses similar potency to KRN7000 in human NKT cell activation, and stimulates cytokine release in the same manner as well.[5] As such, the thioglycoside analogue may be superior to KRN7000 as a parent compound for developing immune stimulants for humans in view of its bioactivity, stability, ease of synthesis, and flexibility for making other analogues.

Acknowledgement: This work was supported by Research Frontier Program of Science Foundation Ireland and the Natural Science Foundation of Zhejiang Province (R4110195).

215: Polysaccharide isolated from *Lonicera japonica Thunb*, a novel inhibitor of amyloid beta peptide aggregation

Peipei Wang, Jianping Fang, Qin Liu, Kan Ding*;
Glycochemistry & Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai,201203,China
kding@mail.shcnc.ac.cn

The Alzheimer's disease (AD) is becoming more prevalent in ageing populations worldwide, yet there are currently no effective treatments. Compelling evidence indicates that aggregates of amyloid-beta (Aβ) peptides plays a central role in the in the development of AD due to a cascade of harmful event that cause cellular dysfunction and neuronal death. Therefore, inhibition Aβ accumulation is thought to be an effective strategy for the prevention and/or treatment of patients with AD. Recent studies reported that several polysaccharides from Chinese medicinal herbs had a neuroprotective effect against the toxicity of Aβ peptides, such as these from *Antrodia camphorate*, *Nerium indicum* and *Lycium barbarum*. However, little is known about the molecular mechanism underlying their bioactivity. In the present study, a homogeneous polysaccharide, named as LJW0F2, with an average molecular weight of 37.1 kDa was purified from *Lonicera japonica Thunb*, by anion-exchange chromatography and gel permeation chromatography. Using monosaccharides composition analysis, methylation analysis, IR and NMR spectroscopy, LJW0F2 was elucidated to an alpha-D-(1→4)-glucan with an alpha-(1→4) linked branch attached

to C-6 position. Its inhibitory effect on Aβ42 aggregation was monitored by fluorescence spectroscopic analysis with thioflavin T (ThT) and the morphological changes were investigated by atomic force microscopy (AFM). The results showed that LJW0F2 could significantly inhibit Aβ42 aggregation in a dose-dependent way, where LJW0F2 inhibited more than 90 % of aggregation at the concentration of 100 μg/ml. AFM showed that regular fibril aggregates formed by Aβ42 significantly decreased in the presence of polysaccharide LJW0F2. To the best of our knowledge, this is the first report showing that the exogenous plant-derived polysaccharides can directly target Aβ42 and block Aβ42 aggregation, which maybe a potentially therapeutic agent for Alzheimer's disease.

216: Structural Characterization of polysaccharides from *Lonicera japonica Thunb* and their protective effects on islet β cells

Peipei Wang, Lina Lu, Qin Liu, Kan Ding*; (Glycochemistry & Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai,201203,China)
kding@mail.shcnc.ac.cn

Abstract: Flos lonicerae is the flower of *Lonicera japonica Thunb* and has been commonly used as traditional Chinese medicine with heat-clearing and detoxifying effects. Polysaccharides isolated from those flowers of *Lonicera japonica Thunb* have been shown many biological activities, *e.g., antibacterial, antiviral, antioxidant and anti-inflammatory.* However, only few polysaccharides with those bioactivities were homogeneous and their structure activity relationships (SAR) were still unknown. To further exploit and utilize of this medicinal herb, the fine structure of polysaccharides from those flowers of *Lonicera japonica Thunb* will be necessarily investigated. In the present study, two homogeneous polysaccharides, LJW2F1 and LJW2F2 were isolated from those flowers of *Lonicera japonica Thunb* with hot water extraction and further purified by ion-exchange chromatography and gel permeation chromatography, respectively. Using monosaccharide analysis, methylation analysis, IR and NMR spectroscopy, their structures were evaluated. LJW2F1 had a very complex structure, containing galactose, rhamnose, arabinose, xylose, glucose and galacturonic acid. LJW2F2 was elucidated to be a linear poly-(α1→4)-a-D-galactopyranosyluronic acid with an average molecular weight of 7.2 kDa. Bioactivity test indicated that the acid pectic polysaccharides, LJW2F1 and LJW2F2 could remarkably protect islet β cell-line INS-1 from high glucose induced cell apoptosis. Furthermore, the protective effect of LJW2F2 was more significant than that of LJW2F1. These results suggested that the content of uronic acid had significant effect on this bioactivity of polysaccharides. This is the first report of the fine structure of polysaccharides from those flowers of *Lonicera japonica Thunb.* However, their protective mechanism of INS-1 cells needs further study.

217: Preparation and Antitumor Activity of Sulfated Polysaccharides

Qun Dong, Xinyan Ni, Kan Ding; Glycochemistry and Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China
qdong@mail.shcnc.ac.cn (Q. Dong); kding@mail.shcnc.ac.cn (K. Ding)

Sulfated polysaccharides play critical roles in various biological events in a variety of organisms, especially in higher and lower animals and in algae. They can be obtained either by isolation from biological tissues or by chemical modification of non-sulfated polysaccharides. Sulfated polysaccharides have been reported to show different biological activities, such as antiviral, antitumor, anti-angiogenesis, immune activation/inhibition, and antioxidation, *etc.* Although the antitumor activity of some sulfated polysaccharides has been reported in different pharmacological models, the mechanism and the structure-activity relation remains largely unknown. Sulfated polysaccharides can inhibit tumor growth *via* several different pathways: directly inducing apoptosis of cancer cells, inhibiting tumor angiogenesis, or activating immune response against cancer. In this study we prepared different types of sulfated polysaccharides by chemical modification of non-sulfated polysaccharides, including Aga-S from agarose, Dex-S from dextran, ALGH-S from alginate, CFAA2-S from galactomannan, CFBB2-S from homogalacturonan, CRAA2-S from amylose, and CRAB1-2-S from amylopectin. Then their antitumor activity was examined against different cancer cell lines, and their immunomodulatory activity was also tested on NF-κB activation/inhibition experiments. The results showed that sulfated amylopectin exhibited a strong NF-κB activation, while sulfated alginate exhibited NF-κB inhibition. According to *in vitro* antitumor tests, the sulfated polysaccharides showed different degree of inhibition to different cancer cells. For PANC-1 pancreatic cancer cells, significant inhibition activity was observed in the order: CFAA3-S>CFBB2-S >CRAA2-S >ALGH-S> Dex-S. Aga-S showed no inhibition on PANC-1 but it can inhibit other pancreatic cancer cell lines, such as BXPC-3 and ASPC-1. All these sulfated polysaccharides showed no significant inhibition against proliferation of SMMC 7721 liver cancer cells. The results indicated that the immunomodulatory and antitumor activity of sulfated polysaccharides are strongly dependent on the types and structure of polysaccharides,while the action of the polysaccharides showed cell specificity response. Further study is still urgently needed for understanding the

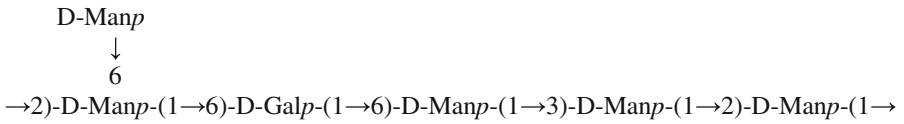
complex relationship between the structure of sulfated polysaccharides and their antitumor activity.

218: Immunological Effects and Chemical Structure of *Antrodia cinnamomea* Polysaccharides

Feng-Ling Yang[1], Shih-Hao Zhan[1], Yu-Liang Yang[2], Kuo-Feng Hua[3], Jung-Feng Hsieh[4], and Shih-Hsiung Wu[1]; [1]Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; [2]Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan; [3] Department of Biotechnology and Animal Science, National Ilan University, Ilan, Taiwan; [4] Department of Food Science, Fu Jen Catholic University, Taipei, Taiwan
yang111@gate.sinica.edu.tw

Antrodia cinnamomea is used as traditional Chinese medicine. Water-soluble polysaccharide of mycelia of *A. cinnamomea* was separated by size-exclusion chromatography and assayed the cytokine stimulation.

The molecular weight of the biological active polysaccharide of *A. cinnamomea* is around 70 to several hundred kilodaltons. The major sugar component is mannose (86 %), and galactose as minor (14 %). The sugar linkages include 1,2; 1,3; 1,6; 1,2,6-, and terminal mannosyl-linkages and a 1,6-galactosidic linkage. This polysaccharide can stimulate tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in dendrite cells. The proposed structure of *Antrodia cinnamomea* polysaccharide is:



219: Structural Characterization of Two Homogalacturonans from the Recycled Residue of Green Tea and Anti-complementary Activity of Their Sulfated Derivatives

Huijun Wang[1,2], Songshan Shi[1,2], Bin Bao[1,2], Chao Zhu[1,2], Shunchun Wang[1,2,*]; [1]Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China; [2]Shanghai R&D Center for Standardization of Traditional Chinese Medicines, Shanghai201203, China
shunchunwang@126.com

The residue of green tea, which was commonly discarded by tea polyphenols manufacturer, was collected. Two homogenous water-soluble polysaccharides (TPSR4-2B and TPSR4-2C) were obtained from the recycled residue of green tea. Their average molecular weights were estimated to be 41 kDa and 28 kDa, respectively. A combination of composition, methylation and configuration analysis, as well as NMR spectroscopy, indicated that both TPSR4-2B and TPSR4-2C were poly-(1-4)-α-D-galactopyranosyluronic acid in which 30.5±0.3 % and 28.3± 0.5 % of uronic acid existed as methyl ester respectively. Two sulfated derivatives (Sul-R4-2B and Sul-R4-2C) at C-2 and/or C-3 of Gal*p*A from TPSR4-2B and TPSR4-2C were prepared after sulfation with 2:1 of chlorosulfonic acid and pyridine. The anti-complementary assay showed that Sul-R4-2B and Sul-R4-2C demonstrated a stronger inhibitory effect (CH_{50}: 5.4±0.6 μg/mL for Sul-R4-2B; CH_{50}: 5.2±0.4 μg/mL for Sul-R4-2C) on the complement activation through the classic pathway, compared to that of heparin (CH_{50}: 8.9±0.3 μg/mL). In contrast, native TPSR4-2B and TPSR4-2C had no such effects. These results suggested that the sulfated derivatives of homogalacturonans from green tea residue might be considered as a promising candidate of an anti-complement agent in the treatment of many diseases with characteristic of excessive activation of complement system, such as system lupus erythematosus, rheumatoid arthritis, and acute respiratory distress syndrome.

220: Top-down synthesis of a library of high mannose-type glycans: preparation of various high mannose glycans from a tetradeca-saccharide precursor by glycosidase digestions

Akira Seko[1], Ichiro Matsuo[2], Yoichi Takeda[1], Akihiko Koizumi[1], Masakazu Hachisu[1], Maki Takatani[1], Junichi Aikawa[3], and Yukishige Ito[1,3]; [1]JST, ERATO, Wako, 351-0198, Japan; [2]Gunma Univ, Kiryu, 376-8515, Japan; [3]RIKEN ASI, Wako, 351-0198, Japan
seko@glt.jst.go.jp

High mannose-type glycans play important roles in assisting in proper folding of nascent glycoproteins in the ER quality control (QC) system. The glycans serve as a signal reflecting folding states of attached polypeptide chains. To clarify molecular interaction of the glycans with various QC-associated proteins, it is necessary to provide a series

of structurally definite high mannose-type glycans. Here we show top-down synthesis of a library of high mannose-type glycans, which features trimming of the common precursor, a synthetic tetradeca-saccharide precursor by various glycosidase digestions.

This method is based on chemical synthesis of a tetradecasaccharide precursor that consists of Man9-oligosaccharide, capped with sugar residues at the three non-reducing termini, and fluorescent BODIPY residue at the reducing terminal. The capping sugars were chosen to be α1,3-linked Glc, β1,4-linked Gal, and β1,6-linked GlcNAc at the termini of A-, B-, and C-arms, respectively. These capping enabled selective cleavage of five α1,2-linked Man residues by *Aspergillus saitoi* α1,2-mannosidase (α1,2-Man'ase). In order to cleave these capping selectively, we examined several glycosidases, and found that the α-Glc, β-Gal, and β-GlcNAc capping were selectively removed by rat liver α-glucosidase-II (G-II), *Aspergillus oryzae* β-galactosidase (β-Gal'ase), and jack bean β-*N*-acetylhexosaminidase (β-HexNAc'ase), respectively. For the trimming of single α1,2-linked Man on the A-arm, which contains two sequential α1,2-linked Man residues, we used Golgi-resident, *Branchiostoma floridae* endo-α-mannosidase (endoMan'ase) under the presence of α1,3-linked Glc residue. This enzyme could be produced in *E. coli* cells in a high yield. Simply by chenging the order of glycosidase digestions, we could separately prepare glycan isoforms. For example, M8A was prepared by the sequential digestions of β-HexNAc'ase, endoMan'ase, and β-Gal'ase, while M8B was prepared by those of β-Gal'ase, α1,2-Man'ase, β-HexNAc'ase, and G-II. So far, thirty-one high mannose-type glycans have been successfully prepared from the common precursor. The library of high mannose-type glycans will be applied to various studies for binding specificities of lectins and substrate specificities of glycosidases and glycosyltransferases involved in the QC system in the ER.

221: The Presence of Immunoglobulin Specific for N-linked Glycan without Terminal Galactose in Rheumatoid Arthritis

Xiukun Xu,Yanping Wang, Yunsen Li*; Laboratory of Cellular and Molecular Tumor Immunology, Jiangsu Key Laboratory of Infection and Immunity, Institutes of Biology and Medical Sciences, Soochow University, Suzhou 215123, China yunsenli@suda.edu.cn

Previous studies have reported that altered N-glycosylation occurs in rheumatoid arthritis (RA), particularly the reduction in galactose residues in IgG. To further observe the N-glycans in RA disease, we showed profiling approaches to N-glycan of total immunoglobulin from RA and normal serum, employing electrospray ionization mass spectrometry. Through comparing the N-glycans from 25 RA patients and 21 healthy controls, we identified about 55 N-glycans and found that the most significant change seen in RA was decreased levels of mono-galactosyl bi-antennary N-glycans, which is also consistent with the previous reports focusing on the N-glycan in IgG. For instance, many articles have proved many anti-antibodies specific for IgG0 are presence in RA serum, so our hypothesis was that there may exist some antibodies or immonogloblins that are specific for redundant N-glycans without terminal galactose. Therefore, high performance liquid chromatograph (HPLC) with both ultraviolet and fluorescence detector was used for further detection of co-culture of total immunoglobulin and standard N-glycans with PA labeled, and our result confirmed our former hypothesis. We speculate that our preliminary results will provide a promising method both for studies of RA mechanisms and diagnosis.

222: Preparation and characterization of guluronic acid oligosaccharides degraded by a rapid microwave irradiation method

Ting Hu[a], Chunxia Li[a,b], Xia Zhao[a,b*], Guangsheng Li[a], Guangli Yu[a,b], Huashi Guan[a,b]; [a]Key Laboratory of Marine Drugs, Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China [b]Shandong Provincial Key laboratory of Glycoscience and Glycoengineering, Ocean University of China, Qingdao 266003, China

Guluronic acid oligosaccharides (GOS) with degree of polymerization (DP) ranging from 1 to 10 were prepared by a rapid and non-acidic microwave degradation method. Polyguluronic acid, fractionated from alginate hydrolysate, was dissolved in dilute ammonia water at a concentration of 20 mg/mL (pH=5) and then hydrolyzed under microwave irradiation (1600 W) at 130ºC for 15 min to produce GOS mixture. The GOS mixture was separated by a Bio-Gel P6 column and ten fractions were obtained. Each GOS fraction was further characterized by electrospray ionization mass spectrometry, ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR spectroscopy techniques. The data showed that the GOS fractions were saturated oligoguluronates with general molecular formula $C_{6n}H_{8n+2}O_{6n+1}$ (n=1−10). This microwave degradation method was not only convenient, less time consuming, and environment-friendly, but also produced GOS with high yield (71 %) and eliminating a desalting procedure compared to conventional acid hydrolysis method.

223: Aberrant Fucosylation of Glycosphingolipids in Human Hepatocellular Carcinoma Tissues

Yanping Wang,Jian Zhu, Yunsen Li*; Laboratory of Cellular and Molecular Tumor Immunology, Jiangsu Key Laboratory of Infection and Immunity, Institutes of Biology and Medical Sciences, Soochow University, Suzhou 215123, China
yunsenli@suda.edu.cn

Glycosylation promoting or inhibiting tumor cell invasion and metastasis is of crucial importance in current cancer research. Tumor-associated carbohydrate antigens are predominantly expressed on the tumor cell surface. Glycosphingolipids (GSLs) are members of this family. In this study, we performed glycosphingolipidomic assays on neutral GSLs obtained from solid hepatocellular carcinoma (HCC) tissues and paired peritumoral tissues by linear ion trap-electrospray ionization mass spectrometry. The expression of a group of fucosylated neutral GSLs was found to be higher in the tumor tissues, as their proportion of total cellular GSLs in the tumor tissue was 3.3-fold higher than that in the peritumoral tissue ($P<0.01$). Moreover, we finished the qualitative analysis of these fucosylated GSLs by comparing theirMS fragments with standardsand/or reference data. Seven types of fucosylated GSLs were identified, including$Fuc\alpha_2Gal\beta_3GlcNAc\beta_3Gal\beta_4Glc\beta_1Cer$(TypeIHantigen),$Fuc\alpha_2Gal\beta_4GlcNAc\beta_3Gal\beta_4Glc\beta_1Cer$(Type II H antigen),$Fuc\alpha3(Gal\beta_4)GlcNAc\beta_3Gal\beta_4Glc\beta_1Cer$ (Le^x), $Fuc\alpha_4(Gal\beta_3)GlcNAc\beta_3Gal\beta_4Glc\beta_1Cer$($Le^a$),$Fuc\alpha_2Gal\beta_3(Fuc\alpha_4)GlcNAc\beta_3Gal\beta_4Glc\beta_1Cer$($Le^b$), $Fuc\alpha_2Gal\beta_4(Fuc\alpha_3)GlcNAc\beta_3Gal\beta_4Glc\beta_1Cer$($Le^y$),and Fucα2Galβ3GalNAcβ3Galα4GlcNAcβ3Galβ4-Glcβ1Cer(Globo H). And these aberrant GSLs mainly contained terminal $Fuc\alpha_2Gal$- topology. This result may lead to improved immunotherapy of HCC and contribute to the understanding of the role of aberrant fucosylated GSLs in development and progress of HCC in following studies.

224: Structural analysis of extracellular polysaccharide from micro-algae *Dunaliela tertiolecta*

Bon Geun Goo[a], Yong Il Park[b], Andriy Synytsya[c], Choul-Gyun Lee[d], Jae Kweon Park[a]; [a] Department of Pharmaceutical Science, Gachon University, Yeonsu-gu, Yeonsu-dong, 406-799 Incheon, Republic of Korea [b] Department of Biotechnology, The Catholic University of Korea, Bucheon 420-743, Republic of Korea [c] Department of Carbohydrate Chemistry and Technology, ICT Prague, Technická 5, 166 28 Praha 6 Dejvice, Czech Republic [d] Department of Biological Engineering, Inha University, Incheon 402-751, Republic of Korea

Extracellular polysaccharide (EPS) was isolated from defatted micro-algae *Dunaliela tertiolecta* and defined as linear (1 -> 4)-a-D-glucan based on monosaccharide composition, enzymatic and spectroscopic analyses. Optimization and characterization of acidic and enzymatic hydrolyses of EPS have been performed for its potential use as a renewable biorefinery material. The hydrolytic methods were improved to assess the effect of substrate specificity, reaction time, pH, ionic strength and temperature on efficiency of glucose production. EPS was effectively converted into glucose within one-step enzymatic or acidic hydrolysis under optimized conditions. Over 90 % recovery of glucose was achieved for both hydrolytic approaches. High potential production of EPS and high yield conversion of this substrate to glucose may allow further exploration of microalga *D. tertiolecta* as a potential biomass producer for biotechnological and industrial exploitation of bioethanol.

225: A Novel Lectin from Mussel *Mytilus trossulus* Induces Cytokines Production

Aleksandra S. Kondrashina, Irina V. Chikalovets, Oleg V. Chernikov; G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, 690022, Russian Federation
kondrashina.alex@gmail.com

Lectins are proteins or glycoproteins with specific binding affinity for carbohydrate moiety of glycoproteins or glycolipids on cell surface. Many lectins also possess various biological activities *in vitro* and *in vivo*, and some lectins bind to specific carbohydrate receptors on cells, which can activate the receptors and thereby induce intracellular signalling cascades leading to alterations in cellular behaviour. If the target cells are involved in the innate immune system, the lectin binding can result in specific cellular responses, including cytokine secretion.

The lectin MTL (*Mytilus trossulus* lectin) was purified from the mantle of bay mussel *Mytilus trossulus* by affinity chromatography on PSM-agarose and following gel filtration. The purified lectin was homogeneous on SDS-PAGE with apparent molecular weight of 18 kDa and 17 kDa on MALDI.

MTL agglutinated all human blood types. The hemagglutinating activity of MTL was independent of the divalent cation Ca^{2+}. Significant MTL activity was observed between pH 9–10 and up to 60 °C. In hemagglutination inhibition assays, N-acetyl-D-galactosamine and D-galactose were the most potent inhibitors among the monosaccharides tested. Among the glycoproteins, PSM and fetuin were

inhibitors as well. Isoelectric point of the protein was determined by capillary isoelectric focusing to be 6.09 ± 0.01.

MTL have been investigated for its *in vitro* effect on the cytokine profile (IFN-γ, TNF-α, IL-10, IL-4) of unstimulated or stimulated with LPS whole human blood cells. MTL at high concentrations (80 μg/mL) enhanced the synthesis of proinflammatory cytokines in stimulated and unstimulated cells but at low concentration (5 μg/mL) possesses immunomodulating action, reducing the IL-10 overexpression in stimulated cells. MTL didn't render any influence on production of IL-4. The obtained data allow to assume MTL role as the factor stimulating production of analogs cytokines in an organism of a mollusk. Besides, in different physiological and pathological conditions lectin can render both inhibiting and stimulating action for maintenance of an immune cellular homeostasis and inflammation regulation.

This research was supported by the grant from the Russian Foundation for Basic Research (no. 12-04-31208\12), a grant from the RAS Presidium (no. № 12-I-П6-10) under the project "Molecular and Cellular Biology".

226: Synergistic effects of ginseng pectic polysaccharides on inhibition of L-929 cell migration

Yuying Fan, Guihua Tai, Yifa Zhou*; School of Life Sciences, Northeast Normal University, 130024, Changchun, Jilin Province, China
zhouyf383@nenu.edu.cn; fanyy033@nenu.edu.cn

The structures of ginseng pectic polysaccharides related to the cell migration inhibitory effects in our previous study, but the underlying mechanisms are less known. In present study, rhamnogalacturonan I (RG-I) -rich pectins prepared from ginseng pectin were tested for their effects on L-929 cell migration. The combination of homogalacturonan (HG) and RG-I-rich pectins exerted stronger effect than either HG- or RG-I-rich pectin alone. Further studies revealed that the effects of HG- and RG-I-rich pectins were dependent on pretreatment of the cells using pectin samples. The pretreatment caused alterations in cell morphologies such as cell size and shape, focal adhesion, and the organization of actin filaments, suggesting that HG and RG-I pectins exert synergistic effects on cell migration and they probably act by different way. Morphological data and quantitative cell adhesion and spreading assays showed that HG- and RG-I-rich pectin treatment decreased cell adhesion and cell spreading on the substratum, suggesting that HG- and RG-I-rich pectins might exert their effects on cell migration *via* decreasing cell adhesion and cell spreading. The experimental results that L-929 cells expressed little galectin-3 (Gal-3) and that lactose, an inhibitor of Gal-3, did not block the activities of HG- and RG-I-rich pectins, implicated that cell migration inhibited by pectin did not correlate to Gal-3.

227: Anti-HRP epitopes in lower eukaryotes

Stefan Mereiter, Alba Hykollari, Birgit Schiller, Katharina Paschinger, Shi Yan and Iain Wilson; Department of Chemistry, University of Natural Sciences and Applied Life Sciences, A-1190 Vienna, Austria
stefan.mereiter@boku.ac.at

HRP (horseradish peroxidase) is a well characterized plant protein carrying N-glycans with beta-1-2-xylose and alpha-1-3-fucose on its core region. Those Anti-HRP antibodies which are specifically binding this N-glycan epitope are also cross-reacting to similar structures and are therefore a standard tool for analyzing N-glycans in other organisms. It already has been shown that anti-HRP is capable of cross-reacting with neurons in Drosophila melanogaster due to its core-fucose. We have now shown that HRP-epitopes are as present in other lower eukaryotes, due to the presence of either a beta-1-2-xylose, in the case of the unicellular parasites Acanthamoeba and Trichomonas, or an alpha-1-3-fucose in the N-glycan core region as in Dictyostelium discoideum and Caenorhabditis elegans. A mix of Western blotting, HPLC and mass spectrometric approaches have been used to define the presence of these glycan modifications in these organisms. Even though these glycan structures are evolutionary widespread their function has not yet been elucidated.

228: Generation, Structural Validation and Utilization of A Large Library of Diverse Xyloglucan-derived Oligosaccharides for Analytical Purposes

Sami T. Tuomivaara[1,2], Utku Avci[1], Michael G. Hahn[1,3], and William S. York[1,2]; [1]Complex Carbohydrate Research Center, [2]Department of Biochemistry and Molecular Biology, [3]Department of Plant Sciences,University of Georgia, Athens, GA 30602.
stuomiva@uga.edu

Xyloglucans are among the most structurally complex polysaccharides in the plant cell wall and exhibit structural diversity at all levels of biological hierarchy. Xyloglucan structure consists of a backbone of β(1,4)-linked D-glucopyranosyl residues with frequent and regularly situated side-chains. Nearly twenty side-chain structures with lengths up to four glycosyl residues have been described to date. The biological and functional significance of this

diversity and complexity is largely unknown. The physical and chemical recalcitrance of plant cell walls creates additional hurdles for the extraction and analysis of its components. Consequently, xyloglucan analysis poses many challenges that are common in the field of glycobiology, including the generation and purification of homogenous preparations for functional assays from complex matrices. Well-defined oligosaccharides are indispensable tools for functional analyses and would greatly advance the analytical possibilities in xyloglucan analysis, and in understanding many aspects of xyloglucan biology, including synthesis, turnover, localization, and interactions with other components in the plant cell wall. We have undertaken a task to generate, mostly by enzymatic hydrolyses, a large library of xyloglucan-derived oligosaccharides that can be used as standards in various chromatographic and mass spectrometry applications as well as in validating substrate specificities of xyloglucan-active glycosyl transferases and glycosyl hydrolases, and defining the epitopes recognized by xyloglucan-binding monoclonal antibodies. We show that it is possible to generate diverse library of oligosaccharides and to purify them in preparative amounts for analytical purposes. We then demonstrate several analytical applications for this oligosaccharides collection. First, we utilize the oligosaccharides to analyze the epitopes of nearly thirty monoclonal antibodies that were generated against xyloglucan polymers from various plant sources. The characterized monoclonal antibodies show clear differences in their binding specificities towards the oligosaccharides and thus are useful probes of xyloglucan structure in various experimental applications, including glycome profiling and *in muro* immunofluorescence experiments. Additionally, we utilize the oligosaccharides to explore mass spectrometric, chromatographic and spectrophotometric methods as quantitative tools in oligosaccharide analysis.

229: Changes in content, density and localization of gangliosides in obstructive cholestatic rat livers and the influence of activity of heme oxygenase-1

Smid Vaclav[1,2], Petr Tomas[1], Vitek Libor[1,2], Smid Frantisek[1], Muchova Lucie[1]; [1]Institute of Medical Biochemistry and Laboratory Diagnostics, [2]4th Department of Internal Medicine – Gastroenterology and Hepatology, General Teaching Hospital and First Medical Faculty, Charles University in Prague, 121 08, Czech Republic
venca.smid@gmail.com

Accumulation of bile acids in cholestasis is linked to liver alternation through mechanisms comprising oxidative stress, bile induced apoptosis as well as inflammatory cell-mediated liver cell necrosis. While induction of heme oxygenase (HMOX) protects liver against these stressors, gangliosides form mechanically and chemically resistant domains (rafts) in the cellular membrane and shield hepatocytes against detergent effect of bile acids.

The aim of this study was to determine changes in topography of ganglioside GM1 (GM1) in cholestatic rat livers, as well as to assess the relationship between heme oxygenase-1 activity and ganglioside content, density and localization in cholestatic conditions.

Wistar female rats were intraperitonealy pretreated with hemin (HMOX-inductor) orSn-mesoporphyrin (HMOX-inhibitor) followed by bile duct ligation (BDL, 5 days) or sham operation. Serum markers of cholestasis, liver histology, total lipid sialic acid (total gangliosides), amount of *a-* and *b- series* of gangliosides, GM1 topography and density were determined. Expression of key enzymes in gangliosides synthesis, were evaluated (GalT2, Sia2).

BDL, as well as HMOX inhibition, resulted in severe histopathological cholestatic injury compared to controls - increased area of ductular proliferation ($P<0.001$) and amount of bile infarcts ($P<0.05$). Compared to it, activation of HMOX decreased the area and intensity of ductular proliferation ($P<0.05$). Total sialic acid content was higher in BDL and after HMOX activation ($P<0.001$). BDL led to 2.4 fold increase of b-series of gangliosides. High concentrations of bile acids as well as modulation of HMOX activity led to redistribution of GM1 within the hepatocyte, exactly from the cytoplasm to the sinusoidal part of hepatocyte membrane ($P<0.001$; <0.05 respectively). Moreover, HMOX inhibition resulted in a significant increase in GalT2 expression compared to controls ($P<0.01$), while HMOX induction had an opposite effect ($P<0.05$).

Supported by GAUK 516912, SVV-2011-262513.

230: Mass Spectrometry-based glycan profiling for characterization of liver tissue from wild type and genetically modified mice mimicking hepatocellular carcinoma

Tianhua Wang[1], Lyn Chiin Sim[1], Jianxin Huo[1], Muriel Bardor[1, 2] and Kong-Peng Lam[1]; [1]Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, #06-01 Centros, Singapore 138668. [2]Université de Rouen, Laboratoire Glycobiologie et Matrice Extracellulaire Végétale EA 4358, Institut de Recherche et d'Innovation Biomédicale (IRIB), Faculté des Sciences, 76821 Mont-Saint-Aignan Cédex, France.
wang_tianhua@bti.a-star.edu.sg

Liver cancer (hepatocellular carcinoma, HCC) is among the leading cancers affecting people in South-East Asia, China,

Japan and Korea. In Singapore, it is the 4th most frequently occurring and third most fatal cancers in males. If untreated, most patients do not survive beyond 6 months after diagnosis. Surgery is the only treatment that allows for a reasonable survival beyond 5 years. However, most liver cancers are diagnosed at advanced stages when it is usually too late for surgery. As a consequence of late detection, the general prognosis for liver cancers is poor. Glycosylation changes are a universal feature of malignant transformation and tumor progression, possible readouts for changes in the expression of genes during oncogenic transformation and progression which may underlie alterations of intracellular signaling occurring as cells gradually lost control in proliferation, adhesion and apoptosis.

A genetically knockout mice were generated and found to be more prone to develop chemically induced HCC. In this study, we profiled the N- and O-linked glycans extracted from the liver tissue from this knockout mouse and compared it to that of the wild type mouse so as to help understand the glycosylation changes occurring in HCC which may lead to better appreciation of the onset of HCC. For that, we have developed in the institute a glycomics platform which allows us to do glycan profiling starting from tissue. With optimized sample preparation protocol and MALDI-TOF MS analysis, we are able to profile the N-linked glycan structures of the liver tissues from both wild type and genetically knock-out mice. Some of the ions identified correspond to possible glycan structures not reported in previous literature. The more abundant species among them were subjected to tandem MS analysis for further structural elucidation while attempts for comparative liver glycomics are being made to pinpoint salient differences in the glycan profiles between the wide-type and knock-out mice.

231: Betel nut lectin: quantum dot nanoconjugate in the identification of serum glycoproteins

Urmimala Chatterjee[1], Partha P Bose[2], Shuvendu Singha[1], Gautam Mondal[1], Bishnu P Chattejee[1]*; [1]Department of Natural Sciences, West Bengal University of Technology, Salt Lake, Kolkata-700064, India [2]Department of Biotechnology, National Institute of Pharmaceutical Education and Research, Hajipur, Bihar-844101, India
*cbishnup@gmail.com

Betel nut (*Areca catechu*) extract was found to contain two lectins of different carbohydrate specificities. One bound fetuin while the other recognized asialofeutin. The former was purified by affinity chromatography on fetuin-Sepharose column. The homogeneity of the lectin was judged by 10 % SDS-PAGE and showed molecular mass of approximately 75 kDa. The lectin was Ca^{2+} dependent and was active from pH 6.5 to 7.0 and stable upto 25 °C. It precipited N-linked serum glycoproteins. The lectin agglutinated mammalian erythrocytes including human irrespective of any blood group system. The heamagglutination titer was found to be more with pronase treated human erythrocytes. Heamagglutination-inhibition assay was performed with serum glycoproteins *viz*., transferrin, fetuin, alpha 2HS glycoprotein, ceruloplasmin, lactoferrin, and immunoglobulins. Of them transferrin and immunoglobulins were found to be better inhibitor requiring 0.1 mg/ml. Betel nut lectin was conjugated with quantum–dot nanoparticles, which was tested by TEM, XRD and DLS. The QD-lectin conjugate detected high level of expression of transferrin in alcoholic liver cirrhosis patients' sera than those of normal individuals.

232: Characterization of novel glycophosphosphingolipids found in insect parasite protozoa

Tatiana C. Garcez[1], Marilza Corrêa[1], Jorick Vanbeselaere[2], Bernadette Coddeville[2], Emmanuel Maes[2], Yann Guérardel[2], Lucia Mendonça Previato[1], Jose Osvaldo Previato[1]; [1]Laboratório de Glicobiologia, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av Carlos Chagas Filho 373, 21941 902-Rio de Janeiro, RJ, Brasil. [2]Unité de Glycobiologie Structurale et Fonctionnelle, Université de Lille 1, 59 655- Villeneuve d'Ascq, France
luciamp@biof.ufrj.br

The protozoan flagellates of genus *Crithidia* are monoxenous trypanosomatids parasite able to colonize the gut epithelium of their insect hosts. Lipoarabinogalactan is the predominant cell surface glycoconjugate of *Crithidia* species so far studied. However, recent studies by our group have found that *Crithidia deanei* and *Crithidia desouzai* produce lipofucoglucan instead of lipoarabinogalactan.

Although fucosylated glycoinositolphospholipids (GIPLs) or fucosylated polysaccharides have never been described in protozoan parasites of the Trypanosomatidae family, it was observed that L-Fucose can replace D-Arabinose in lipophosphoglycan (LPG) when *Leishmania major* is cultured with 50 mM of L-Fucose. To gain insights into the biological significance of fucosylated glycotopes in trypanosomatid parasites, we began the structural analysis of a highly fucosylated GIPLs isolated from *C. deanei.*
To establish the structure of this glycophosphosphingolipid, it was isolated by hot aqueous phenol extraction and

purified by hydrophobic chromatography. Monosaccharide composition of purified GIPLs determined by gas chromatography–mass spectrometry showed that the glycan structure is composed of fucose (Fuc), glucose (Glc), mannose (Man), galactose (Gal), *N*-acetyl-glucosamine (GlcNAc) and glucosamine (GlcN) in a ratio of 5:4:2:1:1:1, respectively. The sphingolipid anchor is linked through an inositol phosphate bond to the glycan domain. The compositional analysis revealed that the sphingolipid anchor consist of C-21 phytosphingosine *N*-acylated with 2-hydroxystearic acid or lignoceric acid, as the major component; with smaller amounts of C-22 phytosphingosine *N*-acylated with 2-hydroxysteric acid or lignoceric acid; and C-20 phytosphingosine *N*-acylated 2-hydroxysteric acid or lignoceric acid. NMR spectroscopy and Electrospray Mass Spectrometry analysis showed that the glycan of *C. deanei* GIPLs is a branched structure, with a backbone containing the following monosaccharide sequence: βGal-βGlcNAc-βGlc-βGlc-βGlc-βGlc-αMan-αMan-αGlcN. The side chains are composed of αFuc-αFuc-αFuc or αFuc.

Taken together, the results suggest that the *C. deanei* GIPLS could be useful in studies related with the biological significance of fucosylated epitote in the trypanosomatid x insect host interactions.

233: Chemoenzymatic synthesis of mono- and di-fluorinated Thomsen-Friedenreich (T) antigens and their sialylated derivatives

Jun Yan[1], Xi Chen[2], Fengshan Wang[1] and Hongzhi Cao[1]; [1]National Glycoengineering Research Center, School of Pharmaceutical Science, Shandong Universtiy, Jinan, 250012, China; [2]Department of Chemistry, University of California, Davis, California, 95616, USA
hzcao@sdu.edu.cn

The Thomsen–Friedenreich antigen (TF or T-antigen, Galβ1–3GalNAcαSer/Thr) is one of the most common tumor-associated carbohydrate antigens (TACAs). Recently, T-MUC1 glycopeptide analogs containing one or two fluorine substituents on the sugar were synthesized. They have been conjugated to a tetanus toxoid carrier protein and the conjugate vaccines elicited strong and specific immune responses in mice. These "foreign" fluorinated TACA based vaccines not only provided enhanced immunogenicity and metabolic stability but also improved bioavailability. However, the chemical synthesis of fluorinated T-antigens and other Galβ1–3-GalNAc-containing O-glycans is challenging. A highly efficient two-step one-pot two-enzyme protocol for the preparation of fluorinated T-antigens was developed by adding two enzymes sequentially to accommodate their distinct pH preferences. The substrate promiscuity of a recombinant galactokinase (EcGalK), a novel *Bifidobacterium infantis* D-galactosyl-β-1–3-*N*-acetyl-D-hexosamine phosphorylase (BiGalHexNAcP), and an α-2–3-sialyltransferase from *Pasteurella multocida* (PmST1) allows the high-yield chemoenzymatic synthesis of fluorinated T- and ST-antigens. In addition, the high expression levels in the *E. coli* expression systems of these enzymes and a *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS) permit their application in large-scale synthesis.

234: Crystal structure of anti-polysialic antibody single chain Fv fragment in complex with alpha2-8 linked oligosialic acids

Masamichi Nagae[1], Akemi Ikeda[1], Shinya Hanashima[1], Ken Kitajima[2], Chihiro Sato[2], and Yoshiki Yamaguchi[1]; [1]Structural Glycobiology Team, Systems Glycobiology Research Group, RIKEN Global Research Cluster, RIKEN-Max Planck Joint Research Center, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan; [2]Bioscience and Biotechnology Center and the Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
mnagae@riken.jp

Polysialic acid is a linear homopolymer typically of α2-8 linked sialic acids on gangliosides and glycoproteins. Cell surface polysialic acid plays important roles in cell adhesion and differentiation events in a manner that is often dependent on the degree of polymerization (DP). Anti-oligo/polysialic acid antibodies have DP-dependent antigenic specificity, and such antibodies are widely utilized in biological studies for the detection and differentiation of oligo/polysialic acids. Although a murine monoclonal antibody mAb735 has a preference for long polysialic acid, the atomic details of polysialic acid recognition mechanism of this antibody still remain unclear. Here we report the crystal structure of mAb735 single chain Fv fragment (scFv735) in complex with octa-sialic acid at 1.8 Å resolution. In the asymmetric unit, two scFv735 molecules cooperatively grab one octa-sialic acid. In each complex, all the complementarity determining regions except for L3 interact with three sequential sialic acid residues out of eight residues. A striking feature is that ordered water molecules bridge the gap between antibody and ligand. The dihedral angles of the three sialic acid residues interacting with scFv735 are not uniform, indicating that mAb735 does not favor the previously suggested helical conformation. Importantly, both reducing and non-reducing ends of the bound ligand are completely exposed to solvent. We suggest that mAb735 gains its apparent high affinity for long polysialic acid chain by recognizing every three sialic acid unit within it.

235: Synthesis of *Mycobacterium* Peptidoglycan (PGN) Fragments Containing *N*-Glycolylmuramic Acid

Qianqian Wang[1], Fukase Matsuo[1], Yukari Fujimoto[1], Koichi Fukase[1]; [1]Graduate School of Science, Osaka University, Osaka, 560-0043, Japan;
yukarif@chem.sci.osaka-u.ac.jp

Peptidoglycan (PGN) is a glycoconjugate that constitutes bacterial cell wall, which has been known as a potent immunopotentiator and an adjuvant for antibody production. PGN consists of polysaccharide chains, which are cross-linked with peptides. Our group has synthesized series of PGN partial structures and revealed the ligand structures of several PGN-binding proteins including the innate immune receptor proteins Nod1 and Nod2; Nod1 recognizes diaminopimelic acid (DAP)-containing peptides, whereas Nod2 recognizes the MurNAc-L-Ala-D-Gln. The polysaccharide of PGN in many bacteria is a β(1-4) glycan composed of alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc), while *Mycobacterium* PGN contains *N*-acetylglucosamine and a mixture of *N*-glycolylmuramic acid (MurGlyc) and *N*-acetylmuramic acid (MurNAc). Because biological activities of PGN fragments containing MurNGlyc have not been elucidated well, we started the synthetic study of PGN fragments having MurNGlyc. *Mycobacterium* PGN has also *meso*-DAP as an amino acid at the branched position, and we also developed the efficient synthetic method for it.

The synthesis of orthogonally protected (2*S*,6*R*)-DAP was first examined. Because it is a key intermediate and crucial moiety for Nod1 receptor recognition, an efficient and highly stereoselective synthesis for *meso*-DAP was developed by using chemoenzymatic method. It is advantageous for the short reaction steps and mild conditions comparing with previously reported methods. DAP-containing tripeptide (L-Ala-D-iso-Gln-*meso*-DAP) and tetrapeptide (L-Ala-D-iso-Gln-meso-DAP-D-Ala)) were obtained efficiently with the newly developed method. We then synthesized a series of the fragment structures of *Mycobacterium* PGN containing one of the following saccharide structures; MurNAc, MurNGlyc, 1,6-anhydro-MurNAc or 1,6-anhydro-MurNGlyc. For the introduction of the glycolic group, we examined the selective amidation reaction for shorter reaction steps.

The compounds will be used for the evaluation of their immunostimulatory activities *via* Nod1 or Nod2 receptors and also other PGN recognition proteins. The present study will contribute to the understanding of immune-responses in *Mycobacterium*-related diseases.

236: Typing of Blood-Group Antigens of Neutral Oligosaccharides by Negative-Ion Electrospray Ionization Tandem Mass Spectrometry

Hongtao Zhang[1], Shuang Zhuang[2], Guanjun Tao[2], Yibing Zhang[3], Barbara Mulloy[3], Xiaobei Zhan[1*], Wengang Chai[3*]; [1]The Key Laboratory of Carbohydrate Chemistry and Biotechnology of Ministry of Education, School of Biotechnology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, China; [2]Testing and Analysis Centre, State Key Laboratory of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, China; [3]Glycosciences Laboratory, Department of Medicine, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom
w.chai@imperial.ac.uk; xbzhan@yahoo.com

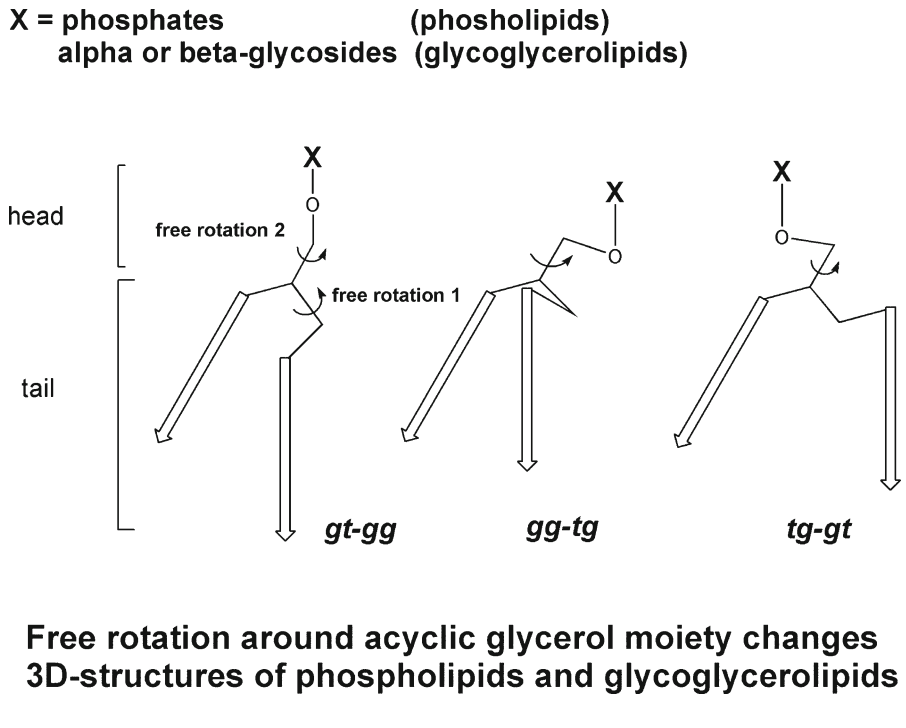
Blood group antigens, such as those containing fucose and bearing the ABO(H)- and Lewis-type determinants expressed on the carbohydrate chains of glycoproteins and glycolipids, and also on unconjugated free oligosaccharides in human milk and other secretions, are associated with various biological functions. We have previously shown the utility of negative-ion electrospay ionization tandem mass spectrometry with collision-induced dissociation (ESI-CID-MS/MS) for typing of Lewis (Le) determinants, *e.g.* Le^a, Le^x, Le^b, and Le^y on neutral and sialylated oligosaccharide chains. In the present report we extended the strategy to characterization of blood-group A-, B- and H-determinants on type 1 and type 2, and also on type 4 globoside chains to provide a high sensitivity method for typing of all the major blood-group antigens, including the A, B, H, Le^a, Le^x, Le^b, and Le^y determinants, present in oligosaccharides. Using the principles established we identified two minor unknown oligosaccharide components present in the products of enzymatic synthesis by bacterial fermentation. We also demonstrated that the unique fragmentations derived from the D- and ^{0,2}A-type cleavages observed in ESI-CID-MS/MS, which are important for assigning blood-group and chain types, only occur under the negative-ion conditions for reducing sugars but not for reduced alditols or under positive-ion conditions.

237: Conformational properties of alpha- and beta-glycolipids as *Mycoplasma* cytoplasmic-membrane components

Mengfei Yuan[1], Ryoko Matsunaga[1], Hirofumi Dohi[1], Sachie Matsuda[2], Kazuhiro Matsuda[2], Yoshihiro Nishida[1]; [1]Advanced Integration Science, Graduate School of Chiba University, Matsudo-648, 271-8510, Japan; [2]M. Biotechnology, Fukazawa 2-1-3-1103, Setagaya, Tokyo, 158-0081, Japan.
Ynishida@faculty.chiba-u.jp

Pathogenic *Mycoplasmas* such as *M. pneumoniae* and *M. fermentans* utilize either alpha or beta-glycosyl glycerolipids as constituents of cytoplasmic membrane together with phospholipids. These glycolipids are made of saturated fatty acid like C16 and C18 and, therefore, may be discriminated from ubiquitous beta-galactolipids in plant chloroplast thylakoid-membrane. In the present study, we examined 3D structures of synthetic alpha- and beta-glycolipids having defined fatty acids (C2 or C16) with proton NMR spectroscopy and compared the results with those of phosphatidylcholine having C16 fatty acid (DPPC).

Our NMR analysis was based on the complete assignment of any diastereomeric protons at the glycerol *sn*-1 and 3 positions as well as at the sugar C-6 position. Time-averaged distributions of three staggered conformations (*gg*, *gt*, *tg*) were estimated from vicinal coupling constants of diastereomeric protons for these acyclic positions. The analysis showed that the acyclic glycerol moiety in beta-galactolipids keeps a conformational property very close to that of DPPC; the polar head-group takes three staggered conformations randomly (*gg* = *g t* = *tg*), while the hydrophobic tail group takes *gt* and *gg* conformations out of the possible three conformers. The observed unique property was little affected by glycerol acyl groups (C2 and C16) and solvents (D_2O, CD_3OD, $CDCl_3$). Conformations at the sugar C6 position were changed significantly by solvents and also by glycerol acyl group to some extents. Conformations of alpha-glycolipids (C16) showed a similar tendency to those of beta-glycolipids and DPPC, while the time-averaged populations changed slightly by substituent groups at the sugar C-6 position.



.

238: Mass spectrometry based sulfoglycomics of lymphoid tissues

Shin-Yi Yu[1], Chu-Wen Cheng[2], Ming-Yi Ho[2], Kay-Hooi Khoo[2], Reiji Kannagi[1]; [1]Institute of Biomedical Sciences and [2]Institute of Biological Chemistry, Academia Sinica, Nankang, Taipei 115, Taiwan

Sulfate modification on carbohydrate modifies the physico-chemical properties of carrier, and alters its cognate recognition by specific lectins, which is involved in normal physiological process and diseases such as chronic inflammation. Additionally, precise positioning of sulfate on these glycans can determine whether they serve as ligands for one or more glycan binding proteins. One of the better-studied system is the homing of lymphocytes to secondary lymphoid organs, which is dependent on L-selectin correctly recognizing specific sulfated glycotopes presented on sialomucins lining the high endothelial venules of peripheral lymph nodes and the mucosa-associated lymphoid tissue. Few sulfated glycotopes were characterized due to limited availability of monoclonal antibodies. Moreover, antibody detection cannot provide information on the structural and conformational context of the underlying glycan carriers, which may dictate their actual *in vivo* physiological functions.

Advanced mass spectrometry offers a precise and high sensitivity mapping of the glycomic repertoire of a particular tissue or cell at any one pathophysiological stage. Due to low abundance and multiple negatively charges of sulfate, the sulfated glycome is often refractory to MS-based glycomic mapping. Previously, we have established a viable workflow for sulfoglycomic analysis based on optimized sample preparation, detection by MALDI-MS mapping and MS/MS sequencing. To progress further, nano-ESI-MS and MS/MS are now shown to afford a more comprehensive MS/MS analyses and detection of multiply sulfated glycans without losing the extra sulfates during MS ionization. A series of diagnostic fragment ions for precise location of sulfates was identified based on either MALDI-MS/MS or HCD-MS/MS analysis of a panel of chemically synthesized sulfo-glycan standards. In recent applications to peripheral lymph nodes from different sulfotransferase knockout mice, we have successfully identified the location of sulfate, their carriers, as well as discovering novel sulfated glycotopes. These findings attest to the practical utility of our sulfoglycomic approach, which will be presented here.

239: Total cellular glycomics: a glycomic approach to describe cells and streamline the discovery of cellular biomarkers

Yasuro Shinohara, Naoki Fujitani, Jun-ichi Furukawa, Jinhua Piao; Graduate School of Advanced Life Science and Frontier Research Center for Post-Genome Science and Technology, Hokkaido University, Sapporo 001-0021, Japan yshinohara@sci.hokudai.ac.jp

Cell surfaces are coated with a variety of intricately arranged glycoconjugates such as glycoproteins, glycolipids and proteoglycans. Therefore, elucidating the expression profiles of glycans derived from various classes of glycoconjugates is important to understand cellular glycosylation homeostasis and systems biology glycomics. We have established a series of methodologies for the analysis of N- and O-glycans derived from glycoproteins, glycosphingolipid glycans, glycosaminoglycans, and free oligosaccharides using mass spectrometry and liquid chromatography. Procedures to analyze each class of glycan were then combined to visualize the entire complement of sugars in the cellular glycome, so-called total cellular glycomics. As an example, analysis of total cellular glycomics analysis of the wild-type CHO cell line and its lectin-resistant mutant (Lec 1 and Lec 8) was performed. Perturbation of a glycan synthetic pathway(s) can cause unexpected glycan expression profiles, which may be difficult to predict without the use of glycomics. When this technique was applied to various human cells including embryonic stem cells, induced pluripotent stem cells and various cells derived from normal and carcinoma cells, total cellular glycomes were found to be highly cell type-specific, demonstrating their utility as unique cellular descriptors. Total cellular glycomics can streamline the discovery of cellular biomarkers as demonstrated by the identification of known pluripotency biomarkers as well as novel candidate biomarkers.

References

1. Furukawa, J., Fujitani, N., Araki, K., Takegawa, Y., Kodama, K., and Shinohara, Y. (2011) Anal. Chem. 83, 9060–9067
2. Fujitani, N., Takegawa, Y., Ishibashi, Y., Araki, K., Furukawa, J., Mitsutake, S., Igarashi, Y., Ito, M., and Shinohara, Y. (2011) J. Biol. Chem. 286, 41669-41679
3. Takegawa, Y., Araki, K., Fujitani, N., Furukawa, J., Sugiyama, H., Sakai, H., and Shinohara, Y. (2011) Anal. Chem. 83, 9443-9449
4. Fujitani, N., Furukawa, J., Araki, K., Fujioka, T., Takegawa, Y., Piao, J., Nishioka, T., Tamura, T., Nikaido, T., Ito, M., Nakamura, Y., and Shinohara, Y. (2013) Proc. Natl. Acad. Sci. USA, 110, 2105-2110

240: Structural Analysis of a Heteropolysaccharide from Saccharina Japonica by ESI -CID-MS/MS

Weihua Jin[1,2,3], Jing Wang[1,3], Wenjing Zhang[1,2], Quanbin Zhang[1,3]; [1]Institute of Oceanology, Chinese Academy of Science, Qingdao 266071, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China; [3]Nantong Marine Science and Technology R & D Center, IOCAS, Jiangsu 226006, China
qbzhang@qdio.ac.cn

A fucoidan extracted from *Saccharina japonica* was fractionated by anion exchange chromatography. The most complex fraction F0.5 was degraded by dilute sulphuric acid and then separated by use of an activated carbon column. Fraction Y1 was fractionated by anion exchange and gel filtration chromatography while Fraction Y2 was fractionated by gel filtration chromatography. The fractions were determined by ESI-MS and analyzed by ESI-CID-MS/MS. It was concluded that F0.5 had a backbone of alternating 4-linked GlcA and 2-linked Man with the first Man residue from the nonreducing end accidentally sulfated at C6. In addition, F0.5 had a 3-linked glucuronan, in accordance with a previous report by NMR. Some other structural characteristics included GlcA 1→3 Man 1→4 GlcA, Man 1→3 GlcA 1→4 GlcA, Fuc 1→4 GlcA and Fuc 1→3 Fuc. Finally, it was shown that fucose was sulfated at C2 or C4 while galactose was sulfated at C2, C4 or C6.

241: The *Pseudomonas Aeruginosa* RetS periplasmic sensor domain reveals an intriguing CBM-like fold

Florence Vincent[1], Adam Round[2], Aline Reynaud[1], Christophe Bordi[3], Alain Filloux[4] and Yves Bourne[1]; [1]Architecture et Fonction des Macromolécules Biologiques, UMR6098, CNRS et Universités Aix-Marseille I et II, 163 avenue de Luminy, 13288 Marseille, France; [2] Institut de Biologie Structurale J.P. Ebel 41, rue Jules Horowitz 38027 Grenoble Cedex 1, France; [3]Laboratoire d'Ingénierie des Systèmes Macromoléculaires, UPR9027, CNRS-IMM, Université de la Méditerranée, 31 Chemin Joseph Aiguier, 13402 Marseille cedex 20, France; [4]Imperial College London, Division of Cell and Molecular Biology, Centre for Molecular Microbiology and Infection, South Kensington Campus, Flowers Building, London SW7 2AZ, UK.
florence.vincent@afmb.univ-mrs.fr

Bacterial two-component regulatory systems (TCSs) sense environmental stimuli to adapt the lifestyle of microbial populations. For many TCSs the stimulus is a ligand of unknown chemical nature. Pseudomonas aeruginosa utilizes the closely related RetS and LadS sensor kinases to switch between acute and chronicinfections. These sensor proteins antagonistically mediate biofilm formation through communication with a central TCS, GacA/GacS. Recently, it was shown that RetS modulates the GacS sensor activity by forming RetS/GacS heterodimers. LadS and RetS are hybrid sensors with a signalling domain consisting of a 7-transmembrane (7TMR) region and a periplasmic sensor domain (diverse intracellular signalling module extracellular 2, DISMED2). The 2.65 Å resolution crystal structure of RetS DISMED2, called RetSp, reveals three distinct oligomeric states capable of domain swapping. The RetSp structure also displays two putative ligand binding sites. One is equivalent to the analogous site in the structurally-related carbohydrate carbohydrate binding module (CBM) but the second site is located at a dimer interface. These observations highlight the modular architecture and assembly of the RetSp fold and give clues on how homodimerization of RetS could be modulated upon ligand binding to control formation of a RetS/GacS heterodimer. Modelling the DISMED2 of LadS reveals conservation of only one ligand binding site, suggesting a distinct mechanism underlying the activity of this sensor kinase.

Poster Session II-Biosynthesis & Metabolism of Glycoconjugates

242: N-glycosylation of β-1,3-glucanosyltransferases is vital for cell wall synthesis in *Aspergillus fumigatus*

Zhao Wan and Jin Cheng; State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, CHINA
jinc@sun.im.ac.cn

Gel1p and Gel2p have been described as β-1,3-glucanosyltranferase working with β-1,3-glucan synthase (Fks1p) to synthesize β-1,3-glucan chains in *Aspergillus fumigatus*. All of them are detected to possess N-glycan chains.

α-mannosidaseI(Cwh41) has been found as the key enzyme to initiate synthesis of N-glycan processing pathway and deletion of *cwh41* gene can cause a defective N-glycan processing of the proteins and a temperature-sensitive deficiency of cell wall integrity (CWI) in *A.fumigatus*.

According to these, We hypothesized that loss of natural N-glycosylation (*e.g.* in *Δcwh41*) would impair the cell wall integrity of *A.fumigatus*,and some enzyme which

related to cell wall synthesis such as Gel1p,Gel2p and Fks1p could not work as usual. To verify this prediction, *Δgel1Δcwh41* and *Δgel2Δcwh41* double disruptions were constructed, and the phenotypes were observed. The single *Δcwh41* mutant and two double mutants in *A.fumigatus* showed the similar growth condition and much more sensitive than the *Δgel1* or *Δgel2* single mutant strains. Then N-glycosylation-site-mutant revertants of different mutants were also constructed by site-directed mutagenesis,named $\Delta gel1^{\text{Gel1-NM}}$,$\Delta gel2^{\text{Gel2-NM}}$,$\Delta gel1\Delta cwh41^{\text{Gel1-NM}}$ and $\Delta gel2\Delta cwh41^{\text{Gel2-NM}}$. According to the comparison results we found that the revertants without N-glycosylation-sites displayed similar growth condition as the corresponding knock out strains revealing that the N-glycosylation is a necessary process of *A.fumigatus* life.

243: One-pot three enzymes catalyzed synthesis of UDP-sugars and their derivatives

Jun Liu[a,b], Yang Zou[a,b], Mengyang Xue[a,b], Xueer Zhao[a,b], Junkai Dong[b], Wenjun Wang[c], Jie Shen[c], Peng George Wang[a], Min Chen[a,b]*; [a] National Glycoengineering Research Center, Jinan, Shandong 250100, People's Republic of China; [b] The State Key Laboratory of Microbial Technology and School of Life Sciences, Jinan, Shandong 250100, People's Republic of China; [c] College of Pharmacy Nankai University, Tianjin 300071, People's Republic of China
chenmin@sdu.edu.cn

Monosaccharide nucleotides play key roles in the biological systems. The availability of structure variety of nucleotide sugars is particularly important for the characterization study on glycoconjugates and glycosyltransfersases. Enzymatic synthesis of these nucleotide sugars displays more advantages than chemical method. Therefore, finding enzymes with highly active and broad substrates specificity become a key factor to obtain these nucleotide sugars. In this study, a promiscuous USP (AtUSP) was cloned from *Arabidopsis thaliana*; a UTP-glucose-1-phosphate uridylyltransferase (GalUSpe4) and a galactokinase (GalKSpe4) were cloned from *Streptococcus pneumoniae* TIGR4 and were successfully used to synthesize UDP-sugars and their derivatives in an efficient one-pot reaction system. The properties of the one-pot reaction were annotated. GalKSpe4 had relaxed activity towards galactose derivatives with modifications on the C-6, 4- or 2-positions. Additionally, GalKSpe4 can also tolerate glucose while glucose derivatives with modifications on the C-6, 4- or 2-positions were unacceptable. More interestingly,GalKSpe4 can phosphorylate L-mannose in moderate yield (43 %), while other L-sugars such as L-Gal cannot be recognized by this enzyme. AtUSP has different tolerance for C4 and C2 derivative of Gal-1-P compared to GalUSpe4. This provides abundant information for better understanding of the enzymatic synthesis of nucleotide sugars.

244: Snail glycosylation—a continuous challenge

Chantal Lucini[1], Christopher Taus[1], Reingard Grabherr[2] and Erika Staudacher[1]; [1] Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria; [2] Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
Erika.staudacher@boku.ac.at

Glycosylation abilities of snails deserve attention, because snail species serve as intermediate hosts in the developmental cycles of some human and cattle parasites. In analogy to many other host-pathogen relations, the glycosylation of snail proteins may likewise contribute to these host-parasite interactions. Furthermore due to the large heterogeneity of structures and their unusual modifications the class of gastropods is a fascinating target for investigation.

In previous studies we have successfully analyzed the N- and O-glycan patterns of a number of snail species (Gutternigg *et al.* (2007) Glycoconjugate J. 24, 475–489, Stepan *et al.* (2012) Glycoconjugate J. 29, 189–198). We found structural elements of mammals, plants, insects, nematodes and trematodes in snail N-glycans and small O-glycans with galactoses and mannoses, sometimes modified by methyl groups and fucoses, linked to a protein bound N-acetylgalactosamine residue. Going now for the corresponding glycosyltransferases is an extra challenge as only few sequencing data exist from mollusk origin. Starting with cDNA libraries of several snail species (*Arion lusitanicus, Achatina fulica, Biomphalaria glabrata*) we could identify a fucosyltransferase involved in N-glycosylation and enzymes involved in O-glycosylation, a T-synthase and a polypeptide N-acetylgalactosaminyltransferase (for more information about the latter one see the presentation of Ch. Taus). The cloning strategy, expression and characterization of these enzymes are presented here. All these novel glycosyltransferases show decent homology to previously described enzymes with similar specificity and fit well into the phylogenetic tree.

This is the first detailed description of glycosyltransferases from the phylum of mollusks.
This work was supported by the Austrian Science Fund (FWF) [grant number P22118-B20].

245: Identification and characterization of endo-β-*N*-acetylglucosaminidase from methylotrophic yeast *Ogataea minuta*

Satoshi Murakami[1], Yuki Takaoka[1], Hisashi Ashida[2], Kenji Yamamoto[3], Hisashi Narimatsu[4] and Yasunori Chiba[1]*; [1]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8566, Japan; [2]Department of Science and Technology on Food Safety, Faculty of Biology-Oriented Science and Technology, Kinki University, Kinokawa 649-6493, Japan; [3]Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Nonoichi 921-8836, Japan; [4]Research Center for Medical Glycoscience, AIST, Tsukuba 305-8566, Japan
y-chiba@aist.go.jp

Endo-β-*N*-acetylglucosaminidase (EC 3.2.1.96) catalyzes the digestion of *N*,*N*′-diacetylchitobiose core of asparagine-linked oligosaccharides on glycopeptides and glycoproteins. In 4 yeast strains, *Ogataea minuta*, *Candida parapolymorpha*, *Pichia anomala*, and *Zygosaccharomyces rouxii,* we identified endo-β-*N*-acetylglucosaminidase homologous sequences by database searches, and also a corresponding enzyme activity was confirmed in crude cell extract obtained from each strain. The *O. minuta* endo-β-*N*-acetylglucosaminidase (Endo-Om) -encoding gene was directly amplified from *O. minuta* genomic DNA and sequenced. The Endo-Om-encoding gene contained a 2319-bp open reading frame; the deduced amino acid sequence indicated that the putative protein belonged to glycoside hydrolase family 85. The gene was introduced into *O. minuta*, and the recombinant Endo-Om was overexpressed and purified. When the enzyme assay was performed using an agalacto-biantennary oligosaccharide as a substrate, Endo-Om exhibited both hydrolysis and transglycosylation activities. Endo-Om exhibited hydrolytic activity for high-mannose, hybrid, biantennary, and (2,6)-branched triantennary *N*-linked oligosaccharides, but not for tetraantennary, (2,4)-branched triantennary, biantennary with bisecting GlcNAc, and core-fucosylated biantennary *N*-linked oligosaccharides. Endo-Om also was able to hydrolyze *N*-glycans attached to RNase B and human transferrin under both denaturing and non-denaturing conditions. Thus, the present study reports the detection and characterization of a novel yeast endo-β-*N*-acetylglucosaminidase.

246: Expression of Poly-*N*-acetyllactosamine on the Surface of Human Hepatocarcinoma Cells and Its Enzymatic Mechanism

Hao Qiu[1], Wei P. Wang[1], Mei F. Jin[2], Mei H. Miao[2], Hui L. Chen[3]; [1]Department of Biochemistry and Molecular Biology, Medical School of Soochow University, Suzhou 215123, China; [2]Affiliated Children's Hospital, Soochow University, Suzhou 215006, China; [3]Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai 200032, China.

By using FITC-labeled tomato lectin (*Lycopersicon esculentum)* as the probe, it was discovered that poly-*N*-acetyllactosamine (PolyLacNAc, [Galβ1,4Gnβ1,3]n) was moderately expressed on the cell surface of H7721 human hepatocarcinoma cell line. After the treatment of 20 ng/ml EGF (epidermal growth factor) for 72 h, the expression of PolyLacNAc was highly increased. Oppositely, it was significantly decreased after the treatment of 10^{-5} M of ATRA (*all-trans* retinoic acid) for 72 h. To elucidate the enzymatic mechanism of the altered expression of PolyLacNAc, five glycosyltransferases related to the synthesis of PolyLacNAc were studied, including β6/β4-*N*-acetylglucosaminyltransferase-V/IVb (GnT-V/IVb), the branching enzymes in *N*-glycan synthesis; β4-galactosyltranferase-5 (β4GalT-5), the main enzyme transferring the galactose residue at the outside of the β6 branched *N*-acetylglucosamine; as well as β3-*N*-acetylglucosaminyltransferase-2 (β3GnT-2) and β4-galactosyltransferase-1(β4GalT-1), two enzymes participated in the synthesis of [Galβ1,4Gnβ1,3]n in H7721 cells (this cell line does not express β3GnT-8, which is considered as the main enzyme for PolyLacNAc synthesis in other carcinoma cells such as column cancer). It was found by RT-PCR method that the expressions of GnT-V and GnT-IVb were elevated about 3 or 2 fold after the treatment of EGF, while they were declined apparently after treated with ATRA. β4GalT-5 was also up- or down-regulated by the treatment of EGF or ATRA respectively as GnT-IVb. The expression of β3GnT-2, the rate-limiting enzyme in PolyLacNAc synthesis, was approximately doubled after EGF and decreased to very low level after ATRA, while the expression of β4GalT-1 was changed slightly with no statistic significance after both EGF and ATRA treatment. Two other glycosyltransferases, β2-*N*-acetylglucosaminyltransferase-I (GnT-I) and β3-*N*-acetylglucosaminyltransferase-5 (β3GnT-5) selected as the

negative controls, were almost unchanged after the treatment of EGF and ATRA. Among the four glycosyltransferases with altered expressions, GnT-V was most elevated by EGF, while GnT-V and β3GnT-2 were most declined by ATRA. However, which one is most important as a leading enzyme in the regulation of PolyLacNAc synthesis, will be further investigated.

247: Essentiality and a colorimetric assay of mycobacterial MurA

Liming Xu[1], Dongting Wu[1], Qi Zheng[1], Liang Liu[1] and Yufang Ma[1, 2]; [1]Department of Biochemistry and Molecular Biology, Dalian Medical University, Dalian 116044, China; [2]Liaoning Provincial Core Lab of Glycobiology and Glycoengineering, Dalian Medical University, Dalian 116044, China
yufang_ma@hotmail.com

The cell wall core of mycobacteria consists of mycolic acid, arabinogalactan, and peptidoglycan. The peptidoglycan is a structural basis for sustaining the morphous and osmotic pressure of mycobacteria. The pepetidoglycan is a reticular molecule that is the connection of N-acetylglucosamine and N-acetylmuramate in turn, then crosslink with oligopeptide. UDP-N-acetylmuramate is a sugar donor of N-acetylmuramate. The formation of UDP-N-acetylmuramate from UDP-N-acetylglucosamine is catalyzed by two enzymes (MurA and MurB) and MurA, UDP-N-acetylglucosamine enolpyruvyle transferase catalyzes the first reaction. By catalysis of MurA enzyme, the enolpyruvyle group is transferred from phosphoenolpyruvate to the 3′-OH of UDP-N-acetylglucosamine to form UDP-N-acetylenolpyruvylglucosamine. The metabolic pathway of UDP-N-acetylmuramate does not exist in mammalian cells. Therefore, MurA may be a potential target to develop anti-tuberculosis drugs.

In this study, *M. semgmatis murA* gene knockout strain was constructed by DNA homologous recombination. The changed growth rate, cell morphology and structure of *M. semgmatis murA* gene knockout strain clarified the essentiality of *murA* gene for mycobacterial growth. Therefore, it strongly supports that *murA* gene is a potential drug target.

To investigate the catalytic mechanism of both *M. smegmatis* MurA and *M. tuberculosis* MurA, expression vectors were constructed respectively and MurA proteins were overexpressed. A colorimetric assay of MurA enzyme was established and the analyses of MurA kinetics are in progress.

This work was supported by the National Basic Research Program of China (2012CB518803) and Research Fund for the Doctoral Program of Higher Education (20112105110002).

248: Roles of N-acetylglucosaminyltransferase III in Epithelial-to-mesenchymal Transition Induced by TGF-β1

Qingsong Xu[1], Jianguo Gu[2*] and Yuguang Du[1*]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; [2]Division of Regulatory Glycobiology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, Sendai Miyagi, 981-8558, Japan

The epithelial-to-mesenchymal transition (EMT) plays crucial roles in the embryonic development, wound healing, tissue repair and cancer progression. In the present study, we demonstrate that transforming growth factor β (TGF-β1) down-regulated expression of *N*-acetylglucosaminyltransferase III (GnT-III) during EMT. The treatment with TGF-β1 resulted in a decrease in E-cadherin expression and GnT-III expression as well as its product, the bisected N-glycans, which was confirmed by E4-PHA lectin blot in human MCF-10A and mouse GE11 cells. To understand roles of GnT-III expression in EMT, the MCF-10A cell was stably transfected with GnT-III. Of particular interesting, overexpression of GnT-III partially, but did inhibit EMT induced by TGF-β1, which were confirmed by cell morphological changes of phase-contrast, immunochemical staining patterns of E-cadherin and actin. In addition, GnT-III modified E-cadherin, which contributed to prolong E-cadherin turnover on cell surface examined by biotinylation and pulse-chase experiments. Consistently, GnT-III expression inhibited β-catenin translocation from cell-cell contact into cytoplasm and nucleus. Furthermore, the transwell assay showed that GnT-III expression suppresses TGF-β1-induced cell motility. Taken together, these observations are the first to clearly

demonstrate that GnT-III plays important roles in EMT, and explain a molecular mechanism for inhibitory effects of GnT-III on cancer metastasis.

249: *M. tuberculosis* Rv1096, a novel peptidoglycan deacetylase

Shufeng Yang [1, 2], Jian Kang[2], Wenli Zhang[2], Yufang Ma[2]; [1] Department of Biochemistry and Molecular Biology, Dalian Medical University, Dlian, 116044, China; [2] Department of Microbiology, Dalian Medical University, Dlian, 116044, China
yufang_ma@hotmail.com

Tuberculosis is a major chronic infectious disease in the world and caused by pathogen *Mycobacterium tuberculosis*. The bacteria in alveolar macrophages of lung are not digested by lysosome, therefore, they remain dormant for months, years, and even decades without increasing in number and without making the person sick. Once the bacteria are reactivated in the people with weakened immune systems, the people are at a greater risk for developing tuberculosis.

The cell wall of *M. tuberculosis* contains unusual polysaccharides, lipids and proteins. The modification of cell wall may prevent the fusion of the phagosome with the lysosome of macrophages. The evidences from *Streptococcus pneumonia* and *Listeria monocytogenes* showed that deacetylated peptidoglycan was resistant to degradation of lysosome. Bioinformatic analysis revealed that *M. tuberculosis* Rv1096, a cell wall protein, was homologous to peptidoglycan deacetylases from *S. pneumonia* and *L. monocytogenes*.

Here, we constructed two types of expression vectors: pColdII-Rv1096 and pVV2-Rv1096. The Rv1096 protein was expressed from *E. coli* ER2566/pColdII-Rv1096 and *M. smegmatis* mc^2155/pVV2-Rv1096 and was purified. The peptidoglycan was prepared from *M. smegmatis* mc^2155 and incubated with the purified Rv1096 protein. The acetyl group released by enzyme reaction was measured by using a acetic acid detection kit. In addition, *M. smegmatis* mc^2155/pVV2-Rv1096 showed resistance to lysozyme compared to wild type *M. smegmatis* mc^2155 in the exponential growth phase. Therefore, we conclude that Rv1096 is a novel peptidoglycan deacetylase. The characterization of Rv1096 is under way in our laboratory.

This work was supported by the National Basic Research Program of China (2012CB518803) and Research Fund for the Doctoral Program of Higher Education (20112105110002).

250: Construction and characterization of *M. smegmatis glmM* gene knockdown strain

Jian Kang[1], Liming Xu[1], Yi Xin[2, 3] Yufang Ma[1, 3]; [1]Department of Biochemistry and Molecular Biology, Dalian Medical University, Dalian 116044, China; [2]Department of Biotechnology, Dalian Medical University, Dalian 116044, China; [3]Liaoning Provincial Core Lab of Glycobiology and Glycoengineering, Dalian Medical University, Dalian 116044, China
yufang_ma@hotmail.com

Due to the essential roles of *M. tuberculosis* cell wall, the enzymes involved in the biosynthesis of the cell wall are potential targets for new anti-tuberculosis drugs. The cell wall core of *M. tuberculosis* consists of mycolic acid, arabinogalactan, and peptidoglycan and a disaccharide linker (L-Rha-D-GlcNAc-P) connects mycolated-arabinogalactan to peptidoglycan. UDP-GlcNAc is a glycosyl donor of the linker and also a precursor of peptidoglycan. The phosphoglucosamine mutase (GlmM) which catalyses the second step in UDP-GlcNAc biosynthetic pathway. Our previous results from *M. smegmatis glmM* gene knockout strain confirmed that *glmM* gene was essential for the growth of mycobacteria. Therefore, GlmM could be a potential target of anti-tuberculosis drugs.

To gain insights into the functions of *glmM* gene in mycobacteria, a tetracycline inducible *M. smegmatis glmM* gene knockdown strain was constructed by using antisense RNA technology. The growth, morphology, biofilm formation and antimicrobial susceptibility of the *glmM* gene knockdown strain were detected to elucidate the biological roles of GlmM protein.

We found that after induction with tetracycline, the expression of GlmM protein in the *glmM* gene knockdown strain was significantly decreased, resulting in a decline of cell growth. The morphology of the *glmM* gene knockdown strain exhibited a wrinkled cellular surface and an enlarged shape. Furthermore, insufficient GlmM protein reduced the biofilm formation and increased the sensitivity to isoniazid and ethambutol. In addition, we also performed two-dimensional gel electrophoresis for both wild-type *M. smegmatis* and the *glmM* gene knockdown strain and found some different protein spots between them. These spots will be identified by MS/MS and confirmed by qRT-PCR or Western blot.

This work was supported by the National Basic Research Program of China (2012CB518803) and National Natural Science Foundation of China (30970067).

251: UDP-N-acetylglucosamine pyrophosphorylase is essential for cell wall biogenesis in *Aspergillus fumigatus*

Ting Du, Cheng Jin; State Key Laboratory of Mycology,Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China
duting2008@163.com

As an important cell wall composition, chitin is synthesized from uridine diphosphate-N- acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is also a sugar donor for the synthesis of glycoproteins, lipopolysacharide and GPI anchors. UDP-N-acetylglucosamine pyrophosphorylase (UAP1), a member of the large family of nucleotide diphosphate sugar pyrophosphorylases, catalyses the last step in eukaryotic biosynthesis of UDP-GlcNAc, converting UTP and GlcNAc-1P to the sugar nucleotide.

In this study, Af*uap1*, a gene encoding UAP1p, was identified in the opportunistic pathogen *Aspergillus fumigatus*. A conditional inactivation mutant was constructed because loss of *uap1* was lethal. Under the suppression condition the mutant showed increased sensitivity to the cell wall interference and abnormal conidial germination. Our results suggested that suppression of the *uap1* gene in *A. fumigatus* led to retarded growth and cell wall defect. Further analysis demonstrated that down-regulation of the *uap1* gene resulted in a reduction of α-glucan and chitin in cell wall. Although the amounts of glycoprotein and β-glucan in mutant strain were similar with the wild-type ones, GlcNAc released from these proteins was decreased by 23 % and mannose was increased by 59 %.

These results indicate that the *uap1* is essential for cell wall synthesis in *A. fumigatus.* The insights of the influence of this gene to cell wall will aid the future exploitation of this genetically validated enzyme as a potential target for the discovery of novel antifungal compounds.

252: GDP-Mannose transport in *Cryptococcus neoformans* is mediated by two distinct proteins and is dispensable for viability

Zhuo A. Wang, Matthew Williams, and Tamara L. Doering; Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110, USA
zhuowang@borcim.wustl.edu, doering@borcim.wustl.edu

Cryptococcus neoformans is an opportunistic pathogen responsible for cryptococcal meningoencephalitis. Every year this disease kills over 600,000 people who are immunocompromised due to AIDS or other conditions. The fungal pathogen is surrounded by a polysaccharide capsule, which is its major virulence factor. The capsule is composed primarily of two polysaccharides, glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal); mannose comprises over half of the capsule mass. *C. neoformans* also extensively utilizes mannose in cell wall synthesis and glycosylation of proteins and lipids.

GDP-mannose (GDP-Man) is produced in the cytosol by the sequential actions of phosphomannose isomerase, phosphomannomutase, and GDP-Man pyrophosphorylase. However, most of the glycan synthetic reactions for which this compound serves as the donor occur in the Golgi complex. This highly charged compound thus requires specific nucleotide sugar transporters to convey it to the site of these biosynthetic reactions, similar to the case for many other nucleotide sugars. Transport of GDP-Man is of particular interest in the context of a microbial pathogen, however, since mammalian cells lack this capability.

We previously identified two GDP-mannose transporters in *C. neoformans*, which we termed Gmt1 and Gmt2. Biochemical studies of each protein expressed in *Saccharomyces cerevisiae* showed that both are functional, with similar kinetics and substrate specificities. Surprisingly, microarray experiments indicated that the genes encoding Gmt1 and Gmt2 are transcribed with distinct patterns of expression in response to variations in growth conditions. To investigate potential functional differences between these two transporters, we first generated a double mutant strain. We were surprised that this strain was viable, since the single gene encoding a GDP-Man transporter in *S. cerevisiae* is essential. We next compared cell growth, colony morphology, protein glycosylation, and capsule phenotypes of the Gmt single and double mutants. In all of these studies, the *gmt1* mutant showed significant phenotypic differences from *gmt2* mutant, suggesting the two proteins play different roles in cryptococcal biology. This hypothesis was supported by nonidentical subcellular localization of the Gmts, as defined by immunofluorescence microscopy. We also found that the double mutant exhibited severe defects in capsule synthesis and protein glycosylation, and was completely avirulent in mouse models of cryptococcal infection.

253: The insect CMP-sialic acid synthetases: New features in the enzyme activity and intracellular localization

Di Wu[1,2,3], Akiko Fujita[1], Kayo Hamaguchi[1,3], Anne Harduin-Lepers[4], Philippe Delannoy[4], Vladislav M. Panin[5], Chihiro Sato[1,2,3], Ken Kitajima[1,2,3]; [1]Bioscience & Biotechnology Center, Nagoya University; [2]Program for Leading Graduate Schools IGER, Nagoya University; [3]Graduate School of Bioagricultural Sciences, Nagoya University, 464-8601, Japan; [4]Univ of Lille, Villeneuve d'Ascq, France; [5]Texas A&M Univ., Texsas, US.
kitajima@agr.nagoya-u.ac.jp.

Sialic acid (Sia) is a family of nine-carbon sugars with a carboxyl group, consisting of *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), deaminoneuraminic acid (Kdn), and their modified forms. Although many studies have revealed the importance of sialylation in prokaryotes and higher animals, sialylation in invertebrates has not been well studied. The objective of this study is to gain insights into sialylation in insects, focusing on the CMP-Sia synthetases (CSSs), which catalyze the synthesis of donor substrates for sialyltransferases. The following results have revealed new features of insect CSSs in their activity and intracellular localizaion: (i) The CSS cDNAs were cloned from *Tribolium castaneum* (red flour beetle), *Aedes aegypti* (yellow fever mosquito), and *Drosophilla melanogaster* (fluit fly). Amino acid sequence alignment of these CSSs showed that they share high sequence identities with the vertebrate CSSs in the N-terminal catalytic domain, while they lack the extra C-terminal domain common to vertebrate CSSs; (ii) The bacterially expressed recombinant insect CSSs had no *in vitro* activity to Neu5Ac, Neu5Gc, or Kdn. In contrast, the *in vivo* activity was detected with these insect CSSs using a CSS-impaired CHO mutant cell line LEC29.Lec32, which is negative in the surface expression of polysialic acid (polySia). The transfectant LEC29.Lec32 cells with the insect CSS cDNA plasmids were changed to be polySia-positive; (iii) To determine the intracellular localization of insect CSSs, the Azami-Green fluorescent protein-conjugated CSSs (AG-CSSs) were expressed in CHO cells. As previously reported, *Drosophila* CSS was localized in the Golgi. In contrast, *Aedes* CSS and *Tribolium* CSS were localized in cytosol and nucleus. These results indicate that the insect CSSs are functional in the enzyme activity at least in mammalian cells. Surprisingly, intracellular localization of CSS appears not to be common among insect species. Underling mechanisms for regulating the intracellular localization in insect cells remain to be elucidated.

254: Purification and Characterization of a Fucoidan Degrading Enzyme from *Sphingomonas paucimobilis* PF-1

Woo Jung Kim, Doo Jin Choi, Jisun Lee, Chang Won Lee, Seul Lee, Ji Won Choi, Yong II Park; Department of Biotechnology and The Research Centre for Biopharmaceutical Lead Molecule (GRRC), The Catholic University of Korea, Bucheon, Gyeonggi-do, 420-743, Korea
yongil382@catholic.ac.kr

A fucoidan degrading enzyme activity has been produced from *Sphingomonas paucimobilis* PF-1 (KCTC 11130BP) using Miyeokgui fucoidan (MF), isolated from Korean *Undaria pinnatifida sporophyll*, as a sole carbon source of culture medium. The enzyme was purified to an apparent homogeneity mainly by sonic disruption of cells, ammonium sulfate precipitation, DEAE-Sepharose column chromatography and chromatofocusing, with a final recovery of 3.2 % and a specific activity of 2.142 U/mg protein. The purified enzyme (tentatively named FNase S) appeared as a single band on Native PAGE gels with a molecular mass of approximately 130 kDa. However, the SDS-PAGE gels gave 3 separate protein with molecular masses of approx. 130 (designated as S1), 70 (S2) and 60 (S3) kDa, respectively, suggesting a multi-protein complex nature of this enzyme. The first 10 N-terminal amino acid sequences of S1 (SXPEAASLPG), S2 (SPQFDVVXIG), and S3 (SLQFDVVVIG) showed high homology (over 80–90 identity) with the N-terminal regions of ATPase, core domain (EGF 27867), carbohydrate kinase, PfkB family protein (YP003854323) and dihydrolipoyl dehydrogenase (ZP08017938), suggesting that these three proteins may delineate a new family of glycoside hydrolases. The optimum conditions for enzyme activity on MF were pH 6.0–7.0 and 40–45 °C. The activity was stable within pH 5.5–8.0 and most stable at 40–45 °C for 1 day at pH 6.0. The enzyme was activated by Mn^{2+} and Na^{+} at the concentration of 1 mM. The enzyme was specific (almost equally active) towards MF, commercial fucoidan (Sigma) and alginate, but exhibited very low activity towards heparin, starch, laminarin and dextran. Apparent K_m, V_{max} and K_{cat} values for MF were 1.7 mM, 0.62 μmol/ml · min, and 0.38 S^{-1}, respectively. The purified FNase S depolymerized MF into at least more than 7 distinct low-molecular weight galactofuco-oligosaccharides, ranging from 318 to 3,312 Da, with no production of monosaccharides, suggesting that this enzyme is an endo-acting fucoidanase and may be an attractive material for not only industrial applications utilizing low-molecular weight galactofuco-oligosaccharides but also structural analysis of fucoidan.

255: Fluorescence-based assays for plant glycopolymer biosynthesis

Irina Ivanova, Stephan Goetz, Sergey A. Nepogodiev, Robert A. Field*; Department of Biologycal Chemistry, John Innes Centre, Norwich, NR4 7UH, UK
irina.ivanova@jic.ac.uk

Glycosylphosphatidylinositol (GPI) anchors are simple glycolipids that are involved in the post- translational attachment of arabinogalactan proteins to the plasma membrane of plant cells. Despite their importance in plants, little work has been done to elaborate the structural or and biosynthetic pathway leading to GPI anchorts in plants. To our knowledge, the only plant GPI structure that has been determined is from *Pyrus communis*. Consequently, the biosynthetic pathway for the assembly of plant GPI anchors and their attachment to arabinogalactan proteins has only been predicted on the basis of bioinformatics analysis. Therefore, the aim of this project is to assess the biosynthetic pathway using non-radioactive methodology

The initial goal of this project is the establishment of fluorescence-based methodologies which will include the incorporation of fluorophore into glycoside acceptors or the post-biotransformation attachment of fluorescent labels to glycosides using "click chemistry". As with any new methodology it will be necessary to validate and benchmark against the established radiochemical assays, which have long been used to determine the structure and biosynthetic pathways of GPI anchors in *Trypanosoma brucei*. These new fluorescence-based methodologies will then be used to investigate aspects of plant GPI anchors biosynthesis, building on ongoing work in the Field group on plant cell wall biosynthesis.

256: Ubiquitous occurrence and biosynthesis of sulfated sialic acids in mammals

Thanyaluck Phitak[1], Hiroshi Fujita[2], Chihiro Sato[1,2], Ken Kitajima[1,2]; [1]Bioscience & Biotechnology Center; and [2]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601; [3]Department of Pharmaceutical Sciences, Kitasato University, Tokyo 108-8641, Japan
kitajima@agr.nagoya-u.ac.jp

It is well known that sialic acids (Sias) show structural diversity in which various modifications occur on three fundamental Sia species, *i.e.*, *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), and deaminoneuraminic acid (Kdn). We previously found sulfated Sia (SiaS) residues in glycolipids and glycoproteins of sea urchin gametes, and demonstrated that they are involved in sperm-egg interaction at fertilization. In mammals the presence of SiaS has been demonstrated in bovine gastric glycolipids and human and mouse tissue homogenates. However, nothing has been known about the biosynthesis and biological functions of SiaS. We have thus sought to understand biological significance and biosynthetic pathway of SiaS residues. In this study, we first developed chemical and immunochemical detection methods of SiaS: The DMB labeling-based fluorometric high-performance liquid chromatography (HPLC) analysis, and immunochemical detection using two monoclonal antibodies: mAb.3G9 specific for the 8-O-sulfated Neu5Ac (Neu5Ac8S) residue, and mAb.2C4 specific for the Neu5Ac8S/Neu5Gc8S residues. Immunohistochemistry and Western blotting showed that SiaS was present in various human and mouse tissues. On the fluorometric HPLC, not only NeuAc8S/9S and Neu5Gc8S/9S, but also Kdn8S/Kdn9S were detected in certain mouse and rat tissues. These results indicate that various SiaS residues ubiquitously occur in mammals. To understand the biosynthesis of the SiaS, we are now searching for the sialate sulfotransferase (SulT-Sia) by direct purification from sea urchin gametes, PCR cloning based on the motif structures of sulfotransferases, and expression cloning using mAb.3G9. Preliminary results showed the presence of a critical gene for expression of cell surface Neu5Ac8S residues.

257: Systematic identification of *in vivo* target proteins specific for a glycosyltransferases isozyme, β1,4-galactosyltransferase-I

Daisuke Sugahara[1], Hiroyuki Kaji[1], Kazushi Sugihara[2], Masahide Asano[2], Hisashi Narimatsu[1]; [1]Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan; [2]Division of Transgenic Animal Science, Advanced Science Research Center, Kanazawa University, Kanazawa, Ishikawa 920-8640, Japan.
d-sugahara@ks.kyorin-u.ac.jp

Recently, as a major focused issue in the glycobiology study field, clarification of the biological role of glycans is receiving specific attention. Model organisms containing deletion or mutation in a glycosyltransferase-gene exhibit various physiological abnormalities, suggesting that a specific glycan motif on certain glycoproteins control the molecular

function of the glycoproteins *in vivo*. In order to determine proteins responsible for the abnormalities and also their underlying molecular mechanisms, it is highly desirable to identify the target proteins for the glycosyltransferase responsible for the biosynthesis of the glycan motif. However, presence and co-expression of other isozymes interfere with the identification of the target proteins specific for the isozyme. The fact that isozymes could produce one particular glycan motif under the physiological condition complicates the situation.

Here, we present a systematic approach to identify the *in vivo* target proteins of a particular glycosyltransferase isozyme on a proteomic scale. To identify the target proteins specific for β1,4-galactosyltransferase-I (β4GalT-I) under the co-expression of other β4GalT isozymes, we determined and compared presence of Galβ1,4-terminated glycans on individual proteins of wild-type and *β4GalT-I*$^{-/-}$ mice. By utilizing a lectin-mediated affinity capture of glycopeptides carrying Galβ1,4-terminated glycans, isotope-coding glycosylated site specific tagging (IGOT) and shotgun LC/MS analysis, we achieved comprehensive identification of proteins carrying Galβ1,4-terminated glycans in mouse liver. Glycoproteins that were present in the wild-type mice, but not in the *β4GalT-I*$^{-/-}$ mice, were identified as plausible candidates for the β4GalT-I-specific target proteins, because the β4GalT-I-specific target proteins is assumed not to be β1,4-galactosylated in the *β4GalT-I*$^{-/-}$ mice. Among 1,176 proteins identified in this study, 181 proteins were identified as the target proteins. Bioinformatic analysis of the identified proteins offered some unique information on the molecular characteristics of the target proteins. Our approach is a powerful means to move forward the elucidation of the biological role of glycans and the understanding of the mechanism that controls assembly of a particular glycan motif on specific proteins *in vivo*.

258: Function study of TGFβ1、β1,4- galactosyltransferaseIin embryo implantation and the correlation of them on other implantation factors expression

Yunpeng Xie[1], Ting Gong, lin Zhang, Cheng Zhang, Ying Kong *; [1]Department of Biochemistry and Molecular Biology, Dalian Medical University . Dalian,116044,China . kongying@dlmedu.edu.cn

The mammalian embryo implantation is an extremely complex process. The blastocyst invasive and the establishment of endometrial receptivity are keys to ensure the successful implantation of the embryo. β1, 4 - galactosyltransferase enzyme I (β1,4-GalT-I) is divided into two phenotypes, which are short-chain and long-chain. Short-chain β1,4-GalT-I, distributed in the Golgi apparatus, exercises the functions of glycosyltransferase. Long-chain β1,4-GalT-I, located located on the cell membrane,acts as a cell adhesion molecule playing an important role in cell adhesion, sperm-egg recognition, neurite growth, tumor migration process, *etc.* TGF-β1 is one of the transforming growth factor-β superfamily members, two-way function to promote and inhibit of cell proliferation, and will display different biological roles for the different target cells, as well as different functional states of the same target cells. In the study, we investigate the function of TGF-β1, β1,4-galactosyltransferaseIin embryo implantation and the correlation of them on other implantation factors expression. Significantly, The expression of total β 1, 4-GalT-I reached the peak in estrogen role for the concentration of 10^{-4} ug/ul, time for 12 h by RT-PCR in RL95-2 cells (analog implantation of endometrial cells)($P<0.05$); The expression of total β 1, 4-GalT-I reached the peak in estrogen role for the concentration of 10^{-4} ug/ul, time for 12 h by RT-PCR in RL95-2 cells($P<0.05$). After RL95-2 cells were treated with *β1,4-GalT-I* gene over-expression plasmid transfection, the expression of the total *β1, 4-GalT I* gene and long-chain *β1,4-GalT-I* gene were significantly increased($P<0.05$); But the expression of the *TGF-β1* gene was not changed obviously. After RL95-2 cells were treated with *β1,4-GalT-I* gene interference plasmid,the expression of the total *β1,4-GalT-I* gene and long-chain *β1,4-GalT-I* expression were significantly decreased($P<0.05$); But the expression of the *TGF-β1* gene was not changed obviously. We hypothesize hormones might regulate the implantation of the embryo through the β1,4-GalT-I and β1,4-GalT-I might not affect the expression of TGF-β1.

(This work was supported by National Natural Scientific Grant No.30970646,PR China)

259: Glycosylation of protodioscin by *Arthrobacter* sp.

Jingyuan Liu[1, 2], Li Lu[1], Liping Kang[1, 2], Heshui Yu[1, 2], Yang Zhao[1], Chenqi Xiong[1], Liyan Yu[3], Baiping Ma[1]*; [1]Beijing Institute of Radiation Medicine, Beijing 100850, China; [2] Tianjin University of Traditional Chinese Medcine, Tianjin 300193, China; [3]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China mabaiping@sina.com

Steroidal saponins are known as a main kind of constituents in traditional Chinese medicine with activities such as inhibiting platelet-aggregation, anti-dementia, antibacterial and cytotoxicity. It was reported that sugar

chains play important roles on bioactivities of steroidal saponins. Therefore, studies on glycosylation of steroidal saponins, which are seldom reported, should be an important part in medical research. A strain of *Arthrobacter* sp. was found possessing glycosylation activity on steroidal saponins. Protodioscin, a steroidal saponin from *Dioscorea nipponica* Makino, could be converted to the corresponding fructosylation product by *Arthrobacter* sp. in this study. The biotransformation mixture was isolated by column chromatography on MCI and ODS silica-gel after being extracted by water-saturated n-butanol. One product was purified and its structure was elucidated by spectroscopic analysis of HR-ESI-MS, ^{1}H NMR, ^{13}C NMR, HSQC, ^{1}H-^{1}H COSY and HMBC and the acid hydrolysis. A fructosyl was added to the substrate at the position of C_6-OH of 26-*O*-β-D-glucopyranosyl. This study provided a new way to the glycosylation of steroidal saponins.

Fig. 1 Biotransformation of protodioscin by *Arthrobacter* sp.

260: Rational design of synthetic pathway in Escherichia coli for direct N-acetylneuraminic acid production from glucose

Junhua Kang, Pengfei Gu, Qian Wang, Qingsheng Qi*; State Key Laboratory of Microbial Technology, National Glycoengineering Research Center, Shandong University, Jinan, 250100 P. R. China
* Corresponding author: Tel & Fax: +86-531-88365628; qiqingsheng@sdu.edu.cn

N-acetylneuraminic acid (NeuAc) recently drew much attention owing to its applications in many aspects. In this study, we achieved recombinant *Escherichia coli* strains for the production of NeuAc from glucose. Genes involved in NeuAc synthetic pathway were overexpressed to construct a basic strain for next genetic manipulation. Mutation and overexpression of *glmS* gene improved NeuAc production from 0.12 g l^{-1} to 0.26 g l^{-1}. Afterward, deletion of GlcNAc degradation genes in *E. coli* resulted in 0.30 g l^{-1} NeuAc accumulation. Genes encoding acetate kinase, pyruvate oxidase and lactate dehydrogenase were eliminated to block pyruvate metabolic bypass, which enhanced the metabolic flux to NeuAc synthesis. The engineered *E. coli* produced 1.36 g l^{-1} NeuAc. Finally, *nanATEK* encoding enzymes of NeuAc catabolic pathway were eliminated together, which resulted in 1.62 g l^{-1} NeuAc production. In a 5 l fermentor study, after 4 days cultivation 7.85 g l^{-1} NeuAc was obtained. This process offered an efficient method to produce NeuAc in *E. coli* with glucose as carbon source.

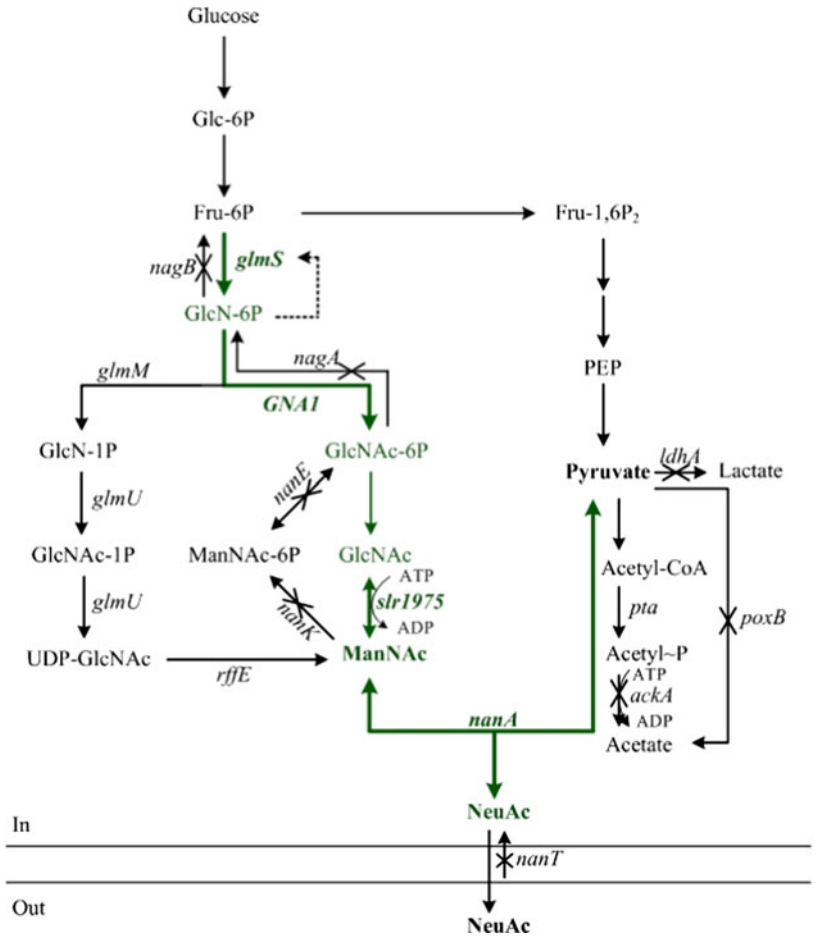


Figure. schematic presentation of NeuAc metabolic pathway in engineerd *E. coli.* The enzymes and substrates are described in the context. Dotted line means feedback inhibition. X indicated the genes were knocked out.

Poster Session II- Carbohydrate & Disease

261: Expression of chondroitin sulphate sulphation motifs and SLRPs in articular cartilage of Kashin-Beck disease

Jinghong Chen[1], Wei Wang[1], Mingxiu Luo[1], Siyuan Li[1], Zengtie Zhang[1], Boquan Yu, Clare E Hughes[2], Bruce Caterson[2], Junling Cao[1*]; [1]Institute of Endemic Diseases, Xi'an Jiaotong University College of Medicine; Key Laboratory of Environment and Genes related to Diseases (Xi'an Jiaotong University), Ministry of Education, Xi'an 710061, Shaanxi, P.R. China. [2]Connective Tissue Biology Laboratories, Division of Pathophysiology and Repair, School of Biosciences, Cardiff University, Cardiff, Wales, UK CF10 3AX
jixiang46@163.com

Objective: To clarify the expression changes of these Proteoglycans incuding aggrecan and SLRPs and association with the catabolism of the proteoglycans in KBD cartilages.

Methods: Samples of articular cartilage were divided into 2 groups: control children (5 samples from 5 cases), and children with KBD (5 samples from 5 cases). The morphology and pathology of hand joint cartilage of KBD children were examined by light microscopy and the expression of proteoglycans was determined by histochemical staining. A panel of well-characterized antibodies that recognize glycosaminoglycan or protein (linear or neoepitope) sequences was used to detect proteoglycans, such as aggrecan and glycosaminoglycan or the small leucine-rich proteoglycans (SLRPs) family proteins biglycan, decorin, karatocan in cartilages using immunohistochemistry.

Results: There was lesser amounts of toluidine blue staining and aggrecan in the extracellular matrix of the articular cartilage of KBD. Similarly, increased positive staining of 2B6 in KBD child cartilages was observed throughout the depth of cartilage, while increased staining of 3B3(+) from middle to deep zone of articular cartilag, suggesting that

6-sulphated and 4- sulphated N-acetyl galactosamine residues expression is increased in KBD cartilages. In contrast, There was a reduced intensity of 1B5 and 3B3(+) staining seen in the deep zone of KBD child cartilages, suggesting a decrease proteoglycans such as aggrecan or reduce the 0-sulphation patterns along CS/DS stubs. Interestingly, there was increased 7D4 and 5D4 staining from middle to deep zones, suggesting an initiation of an osteoarthritis-like lesion. Expression of keratocan and biglycan was prominent increased in the superficial and upper middle zone of the KBD cartilage samples, while expression of decorin was decreased which was only detected in the superficial zone or upper middle zone of the KBD cartilage samples, indicating additional molecular pathways that might compromise the integrity of the collagen matrix.

Conclusion: The study provides a characterization of glycosaminoglycan epitopes associated with the proteoglycans of cartilage, illustrating disrupt CS-GAG metabolism in ECM of the articular cartilages in KBD patients. And, the SLRPs family proteins biglycan, decorin and keratocan may play a role in the development of KBD, possibly contribute to cartilage potentially degradation in KBD.

*** Joint corresponding authors:** Prof. Junling Cao, Email: caojl@mail.xjtu.edu.cn

262: Sulfatide/miR-223/Sp1/integrin αV Pathway Stimulates Migration of Hepatocellular Carcinoma Cells

Yi Wei Dong, Qianqian Cai, Rong Wang, Xing Zhong Wu; Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Key Lab of Glycoconjugate Research, Ministry of Public Health, 138 Yi Xue Yuan Road, Shanghai 200032, P.R. China;
xz-wu@shmu.edu.cn

Hepatocellular carcinoma (HCC) is a common cause of cancer-related deaths worldwide, The roles of miR-223 and its regulation in HCC metastasis remain elusive. We here show distinct down-regulation of miR-223 in cancerous tissues verse matched adjacent non-tumor tissues in 57 patients with HCC. In highly metastatic MHCC97H cells miR-223 was expressed at a lower level than low metastatic MHCC97L cells. Ectopic expression of miR-223 had a negative effect on cell migration. Further experiments identified that Sp1 was a direct target of miR-223.and selectively stimulated the mRNA synthesis from integrin αV subunit gene whose promoter contains functional recognition sites for Sp1. Sustained expression of miR-223 suppressed the level of Sp1 and integrin αV expression. An inverse correlation between miR-223 and integrin αV was observed in HCC samples from 57 patients. Intriguingly, expression of miR-223 was suppressed by sulfatide, leading to Sp1 and integrin αV expression. The suppression of miR-223 by sulfatide was associated with reduced histone H3 and C/EBPα recruitment to the promoter of miR-223 gene. *In vivo* tumorigenicity and metastasis assays, miR-223 was proved to effectively inhibit HCC growth and metastasis in nude mice. These data support differential expression of miR-223 in HCC and demonstrate sulfatide/miR-223/Sp1/integrin αV pathway in HCC, which might be broadly employed by the intrinsic cell fate determining cell motility to promptly stimulate metastasis.

263: Fucoidan targeted to CTGF and inhibited glucose induced rat messangial cell proliferation

Jiejie Hao[1,2], Xin Liu[1,2], Xiaoliang Zhao[1,2], Guangli Yu[1,2]*; [1]Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China, Qingdao 266003, China, [2]Shandong Provincial Key Laboratory of Glycoscience and Glycotechnology, Ocean University of China, Qingdao 266003, China
glyu@ouc.edu.cn

Fucoidans, which were complex sulfated polysaccharides extracted from brown algae and some marine invertebrates, have been extensively investigated because of their various biological activities, *e.g.* anticoagulant, antiviral, antitumor and antidiabetes activities, and these activities differ according to the sulfate content, monosaccharide composition, glycosidic linkage and molecular mass. In the present study, we purified 12 fucoidans from different brown alge and showed that the fucoidan (FV2) from ***Fucus vesiculous*** could specifically interact with connective tissue growth factor (CTGF) by glycoarray technology. And the binding strength of fucoidan to CTGF not only depended on the backbone structure and positions of sulfate groups but also the branching sugar residues. We found that the repeated [→4Fuc2Sα1→3Fuc2S/4Sα1→] backbone of fucoidan was essential for binding to CTGF, and the binding strength was positively correlated with the molecular weight (120 to 380kD), and was enhanced by some hybrid xlyose and galactose residues. Furtherly, the effect of FV2 on diabetic kidney disease was investigated through mesangial cell model. Results showed that FV2 could significantly inhibit the high glucose induced HBZY-1 messangial cell proliferation and extracellular matrix (ECM) production. The secretory levels of collagen IV and fibronectin 1 were distinctly reduced by FV2 as compared with high glucose induced model group (1.17±0.12 *vs.* 2.19±0.16, and 1.23±0.15 *vs.* 2.37±0.21, respectively).

264: Mechanism study underlying the role of microRNAs in sulfated polysaccharide WSS25-inhibited angiogenesis

Fei Xiao, Kan Ding *; Glycochemistry & Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China; *To whom correspondence should be addressed: Kan Ding, Glycochemistry & Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China, Tel.: +86 21 50806928; Fax: +86 21 50806928;*
kding@mail.shcnc.ac.cn

WSS25 is a α-1,4-linked sulfated polysaccharide which inhibits angiogenesis. However, the mechanism underlying the regulation of angiogenesis by WSS25 is not well understood. Using microRNA (miRNA) microarray analysis, 25 miRNAs are found to be upregulated and 12 downregulated by WSS25 in human microvascular endothelial cells (HMEC-1), including microRNA-210 (miR-210) and microRNA-885-3p (miR-885-3p). Interestingly, Dicer, a key enzyme for miRNA biosynthesis, is downregulated by WSS25 in HMEC-1 cells. Further studies indicate that HMEC-1 cell tube formation and miR-210 are suppressed while Ephrin-A3 is enhanced by the silencing of Dicer. In contrast, HMEC-1 cell tube formation and miR-210 are induced while Ephrin-A3 is suppressed by Dicer overexpression. Moreover, miR-210 is downregulated while Ephrin-A3 is upregulated by WSS25 in HMEC-1 cells. HMEC-1 cell migration and tube formation are arrested, while Ephrin-A3 expression is augmented by anti-miR-210. In addition, HMEC-1 cell tube formation is significantly attenuated or augmented when Ephrin-A3 is overexpressed or silenced, respectively. Nevertheless, the tube formation blocked by WSS25 is partially rescued by manipulation of Dicer, miR-210, and Ephrin-A3. The above data indicates that WSS25 inhibits angiogenesis *via* suppression of Dicer, leading to downregulation of miR-210 and upregulation of Ephrin-A3. We also show that miR-885-3p dramatically suppresses angiogenesis *in vitro* and *in vivo*. MiR-885-3p inhibits Smad1/5/8 phosphorylation and downregulates DNA-binding protein inhibitor ID-1 (Id1), a proangiogenic factor, by targeting bone morphogenetic protein receptor, type IA (BMPR1A), leading to impaired angiogenesis. Overexpression or silencing of BMPR1A affects angiogenesis in a Smad/Id1-dependent manner. Furthermore, miR-885-3p impairs the growth of HT-29 colon cancer cell xenografts in nude mice by suppressing angiogenesis through disruption of BMPR1A and Smad/Id1 signaling. These results support a novel role for miR-885-3p in tumor angiogenesis by targeting BMPR1A, which regulates a proangiogenic factor. In summary, our research provides the first demonstration concerning the role of Dicer and miRNAs probed by polysaccharide in mediating angiogenesis. These findings add to new evidences that WSS25 not only modulates angiogenesis factors, but also influences miRNAs function. This study advances our understanding of the actions of WSS25 in angiogenesis and even provides further insight into potential WSS25-based new drug development in terms of miRNAs.

265: Stereoselective synthesis and biological evaluation of 2-deoxy-α-glycosides and oligosaccharides

Guofang Yang, Qingbing Wang, Jiafen Zhou, Xiaosheng Luo, Jianbo Zhang* and Jie Tang; Department of Chemistry, East China Normal University, Shanghai 200241, China
jbzhang@chem.ecnu.edu.cn

2-Deoxy glycosides and oligosaccharides has recently received considerable attention because of many natural products composed of 2-deoxysugars are known to exhibit antimicrobial, anticancer, and antibiotic activities. For example, 2, 6-dideoxy hexopyranoses are common structural units of antitumor agents or antibiotics, such as chromomycin (1) and olivomycin (2). The synthesis of 2-deoxy glycosides; however, can be difficult to achieve due to the lack of directing group at the C-2 position and the acid lability of the resulting 2-deoxy glycosides. Thioglycoside, activated oxygen derivatives, glycosyl halides, and glycals have been used as donors in the formation of either α- or β-2-deoxyglycosides. These glycosyl donors work well in the glycosylations with excellent anomeric selectivities in indirect strategy, which involves the introduction of a temporary directing group such as halides, phenylseleno, and sulfenyl derivatives at C-2, and the reductive removal of the group after the glycosylation step has been completed. Obviously, additional steps are needed and toxic reagents were often applied in the removal step. Compared with the indirect strategy, using 2-deoxyglycopyranosyl donors in a direct way for the formation of 2-deoxyglycosyl linkages would be more efficient and desirable. Early works in this area employ glycosyl halides, glycosyl phosphites, and trichloroacetimidates as donors for the formation of β-selective glycosylation in the presence of mild activators. On the other hand, α-2-deoxyglycosides have been obtained under acid-catalyzed activation of glycals, anomeric ethers, anomeric esters, glycosyl halides, thioglycosides and glycosyl ortho-alkynylbenzoates. Despite a variety of methods available, the direct synthesis of 2-deoxyglycopyranosides from 2-deoxyglycosyl donors, with high stereoselectivity, still remains a challenge because the above methodologies often demand specified donors, promotors or harsh reaction conditions. An

efficient and mild method for stereoselective synthesis of 2-deoxy-containing glycosides and oligosaccharides has been developed in our group. Stable and convenient donors, 2-deoxy glucosyl and galactosyl acetates were reacted with phenols, alcohols, thioalcohols and glycosyl acceptors in the presence of catalytic TMSOTf at 0 °C with excellent α-selectivity (4:1 to above 19:1) and high yields (up to 99 %). Trisaccharides were obtained in one-pot synthesis. The structure and stereochemistry of all the glycosidation products were characterized by high resolution NMR and MS data. Preliminary biological evaluation revealed some of the oligosaccharides have good anticancer bioactivity against human cancer cells lines (K562, A549, MM7721).

The authors gratefully thank financial supports from the Natural Science Foundation of Shanghai (11ZR1410400).

266: GM3 but not GM2 interaction with tetraspanin CD82 leads to inhibition of EGFR-phoshorylation –dependent colon cancer cell sw620 motility

Xiaohua Huang[1#, 2], Ying Li[1#], Jianing Zhang[1], Yuefei Xu[1], Yuxiang Tian[1], Keli Ma[1*]; [1] Department of Biochemistry and Molecular Biology, Dalian Medical University, Dalian 116044, China; [2] Department of Clinical Biochemistry, College of Laboratory Medicine, Dalian Medical University,Dalian 116044, China
[1*]Corresponding author: makeli666@yahoo.com.cn Tel: +86-411-86110311(O), 86-15998663363(mobile), Fax: +86-411-86110309
1 #These authors contributed equally to this work
******This work was supported by Grant from the National Program on Key Basic Research Project 973*

It has been documented that the metastasis suppressor CD82/KAI with gangliosides can exert synergistic inhibitory effect on cell mobility and migration. However, the precise mechanism has not been fully elucidated. In the present study, we want to known whether the EGFR signaling is involved in the mechanism by which CD82 and gangliosides synergistically inhibit the motility and migration of SW620 cells. SW620 cell is colon cancer cells with a high lymph node metastatic potential and a low expression of CD82, and a maior ganglioside composition GM2. By alteration of the ganglioside composition with P4 (D-1-threo-1-phenyl-2-palmi-Toylamino-3- pyrro-idino-1-propanol, an specific inhibitor of glucosylceramide synthesis), and/or addation of exogenous gangliosides, and/or overexpression of CD82 by transferring of CD82 cDNA, we found that (i) GM3 alone suppressed the EGF- stimulated cell motility and migration but GM2 didn't; CD82 alone dramatically inhibited the EGF- stimulated cell motility and migration; GM3 can enhance the inhibitory effect of CD82 on cell mobility and migration but GM2 didn't. These results suggested that, in this cell line, GM3, but not GM2 with CD82 synergistically inhibit the cell motility and migration.(ii) GM3 alone suppressed the EGF- stimulated phosphorylation of EGFR at the Tyr845 and Tyr1173; Overexpression of CD82 alone inhibited the EGF- stimulated phosphorylation of EGFR at the Tyr 1045. Addation of GM3 as well as overexpression of CD82 inhibited the EGF-stimulated phosphorylation of EGFR at the Tyr845, Tyr1173 and Tyr 1045. These results suggested that the mechanism for GM3 inhibiting cell migration and phosphorylation of EGFR is different from that of CD82. (iii) GM3 alone suppressed the EGF- stimulated phosphorylation of Akt at Ser473 and Thr308; Overexpression of CD82 alone inhibited the EGF- stimulated phosphorylation of ERK; these demonsdrated that CD82 combined with GM3 can reduced EGF- stimulated activity of PI-3K/AKT and ERK signaling pathway.

Taken together, all the exparimental data suggested that the synergistic inhibitory effect of GM3 and CD82 on cell mobility and migration involve the inhibition of phosphorylation of EGFR and the activity of PI-3K/AKT and ERK signaling pathway.

267: Modular Synthesis Methodology for the Generation of Heparan Sulfate Oligosaccharides

Ralf Schwörer[1], Jeremy E. Turnbull[2], Peter C. Tyler[1], Olga V. Zubkova[1]; [1]Carbohydrate Chemistry, Callaghan Innovation Research Limited, Lower Hutt, 5040, New Zealand; [2]Institute of Integrative Biology,University of Liverpool, Liverpool, L69 3BX, UK.
ralf.schwoerer@callaghaninnovation.govt.nz

Heparan sulfate (HS) is a linear polysaccharide with highly diverse functionality. Its disaccharide repeating unit consists of of D-glucosamine and D-glucuronic acid or L-iduronic acid and can be variously *O*- or *N*-sulfated or *N*-acetylated. HS is found as a proteoglycan on cell surfaces and in the extracellular matrix and mediates many important biological processes [1,2].

We have developed methodologies for the assembly of heparan sulfate oligosaccharides. The control of protecting groups enables selective sulfation of the oligosaccharide targets, as well as the selective functionalisation of the amino groups. The key element of our strategy is to establish the synthetically more difficult α-linkage first (85–95 % yield, α) to generate a set of disaccharide building blocks.

The anomeric configuration was confirmed by x-ray crystal structure analysis.

The use of Fmoc at the non-reducing and p-methoxyphenyl at the reducing end gives rapid access to donors as well as acceptors for oligosaccharide assembly. Chain elongation is achieved by glycosidation of disaccharide trichloroacetimidates onto the non-reducing end of the oligosaccharide chain, again in excellent yields with high stereoselectivity (78–97 % yield, β). The uronic acid functionalities are introduced late in the synthesis by oxidation on the fully assembled oligosaccharide.

Here we report the synthesis of defined hexa- to dodecasaccharides containing GlcNAc6S-UA or GlcNAc6S-UA2S disaccharides with the uronic acids being either D-glucuronic or L-iduronic, both with and without the uronic acid 2-*O*-sulfates as it may be beneficial for bioavailability and reduction of off-target effects to keep down the numbers of sulfate residues. *In vitro* activity assays using FRET peptides identified several lead compounds as effective BACE1 inhibitors (β-site APP cleaving enzyme 1).

[1] Bishop, J.R. *et al.*, 2007, *Nature* **446**, 1030–1037.
[2] Turnbull, J.*et al.*, 2001, *Trends Cell Biol* **11**, 75–82.

268: Lowered level of core fucosylation is a possible non-invasive predictive marker for chronic obstructive pulmonary disease (COPD)

Congxiao Gao[1, 2], Koichiro Kamio[3], Fumi Ota[1, 2], Takeo Ishii[3], Kozui Kida[3], Naoyuki Taniguchi[1, 2]; [1]RIKEN Alliance Laboratory, The Institute of Scientific and Industrial Research, Osaka University, Japan; [2]Disease Glycomics Team, RIKEN Advanced Science Institute, Japan; [3]Dept. of Internal Medicine, Division of Pulmonary Medicine, Infectious Diseases and Oncology, and Respiratory Care Clinic, Nippon Medical School Tokyo, Japan
cxgao@sanken.osaka-u.ac.jp, tani52@wd5.so-net.ne.jp

Deficiency in core fucosylation by the genetic disruption of α 1, 6- fucosyltransferase (*Fut8*) leads to lethal abnormalities and the development of emphysematous lesions in the lung (Ref. 1). The physiological relevance of core fucosylation in the pathogenesis of COPD was herein investigated using cigarette smoke (CS) exposed heterozygous knockout mice ($Fut8^{+/-}$)(Ref. 2). A marked decrease in FUT8 activity and elevated matrix metalloproteinase (MMP)-9 activities were observed at an early stage of exposure. Emphysema developed after a three-month-CS-exposure, which is half the time required for wild type mice. Moreover, we investigated whether reduced Fut8 levels leads to increased inflammatory response in human COPD and the disease progression among symptomatic current or ex-smokers with stable COPD or at risk outpatients (n=226) (Ref. 3). Although FUT8 activity increased with age among the at risk patients, this association was not clearly observed in the case of COPD patients. A faster annual decline of the forced expiratory volume in 1 s (FEV1) was significantly associated with lower FUT8 activity. Patients with lower FUT8 activity experienced exacerbations more frequently. These data suggest that reduced FUT8 activity is associated with the progression of COPD and serum FUT8 activity is a minimally invasive predictive biomarker for progression and exacerbation of COPD.

Ref. 1 Wang X *et al. Proc Natl Acad Sci. USA* (1995) 102, 15791–15796
Ref. 2 Gao C *et al. J Biol Chem*. (2012) 287:16699–16708
Ref. 3 Kamio K *et al. Biochem. Biophys. Res. Commun.* (2012), 424, 112–117

269: Expression of β-1, 4 galactosyltransferase V in rat Schwann cells *in vivo* and *in vitro*[*]

Meijuan Yan, Chun Cheng, Aiguo Shen;** The Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Nantong, Jiangsu Province 226001, P. R. China
shag@ntu.edu.cn

Glycosylation is one of the most important post-translational modifications. It is clear that the single step of β-1, 4-galactosylation is performed by a family of β-1, 4-galactosyltransferases (β-1, 4-GalTs), and that each member of this family may play a distinct role in different tissues and cells. In the present study, Real-time PCR revealed that the β-1, 4-GalT V mRNA reached peaks at 2w after sciatic nerve crush and 1w after sciatic nerve transection. Combined *in situ* hybridization for β1, 4-GalT V mRNA and immunohistochemistry for S-100 showed that β1, 4-GalT V mRNAs were mainly located in Schwann cells after sciatic nerve injury. In conclusion, β1, 4-GalT V might play important roles in the regeneration and degeneration of the injuried sciatic nerve. In other pathology, such as inflammation, we found that LPS administration affects β-1, 4-GalT

*[Acknowledgements] This work was supported by National Natural Science Foundation of Jiangsu Province(BK2011188), Post-doctoral Project in Jiangsu Province(1201028C), Doctor Fund of Nantong University(03080532)

V mRNA expression in sciatic nerve in a time- and dose-dependent manner, and β-1, 4-GalT V mRNA expressed mainly in Schwann cells. These results indicated that β-1, 4-GalT-V play an important role in the inflammation reaction induced by intraperitoneal injection of LPS. Similarly, we found that β-1, 4-GalT-V in Schwann cells and the binding with RCA-I on the Schwann cell surface *in vitro* were affected in a time- and concentration dependent manner in response to LPS stimulation, and the trend of the binding with RCA-I on the cell surface was similar to the trend of β-1, 4-GalT V. All these results suggest that β-1, 4-GalT V and Gal β1-4GlcNAc containing glycan structure plays an important role in inflammation. In addition, β-1, 4-GalT V production and overall lectin binding were drastically suppressed by U0126 (ERK inhibitor), SB203580 (p38 inhibitor), or SP600125 (SAPK/JNK inhibitor), which indicated that Schwann cells regulated expression of β-1, 4-GalT V and galactosylation of membrane glycoproteins after LPS stimulation were *via* ERK, SAPK/JNK, and P38MAP kinase signal pathway.

270: *Helicobacter Pylori* Infection Leads to Gastric Cancer with the coexpression of Lewis Y and CA724

Faisal Aziz[1], Xuesong Yang[1], Xiaoqi Wang[2], Yan Qiu[1];
[1]Department of Biochemistry and Molecular Biology, Dalian Medical University, Liaoning Provincial Core Lab of Glycobiology and Glycoengineering, Dalian 116044, China; [2]Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
yanqiu63@126.com

Helicobacter pylori (*H. pylori*), the gram-negative bacteria pathogen, is known as a major causative agent for the induction of chronic gastritis and gastroduodenal ulcer disease as well as gastric adenocarcinoma. This particular risk factor is ranked as a class I carcinogen by the International Research Agency on Cancer (IRAC). Although more than 50 % of the world population is colonized by *H. pylori*, only a few suffer from active diseases. Lewis Y (LeY) carried by the glycoproteins and glycolipids on the cell membrane is a difucosylated oligosaccharide which is highly expressed in 70–90 % of human epithelial cancers. CA724 is a sensitive and specific carbohydrate antigen of gastric cancer in Chinese population. To explore the potential correlation of *H. pylori* infection, LeY and CA724, a total of over 200 gastric patient's tissues and serums were evaluated by IHC and ELISA. Gastric primary cell culture from the surgical tissues of the gastric cancer patients was also developed to study the effect of anti-LeY antibody and celecoxib drug on the inhibition of gastric cancer cells by ICC, Western blot, FACS. We found a significant higher expression of LeY, CA724 and *H. pylori* infection copy in gastric cancer tissues as compared to those in the gastric ulcers and chronic gastritis ($p<0.05$). The significant correlation (R-0.86161, R-0.7420, R-0.7146) between LeY, CA724 and *H. pylori* ($p<0.01$) was shown in the gastric cancer tissues. Furthermore, the decreased expression of LeY and CA724 in anti-LeY antibody or celecoxib treated cells was corresponding to the lower infection of *H. pylori* and CA724 ($p<0.01$), and the cancer cell growth was also inhibited. **Conclusion**: Gastric cancer has a significantly positive correlation of *H. pylori* infection, LeY and CA724. The study suggests that *H. pylori* infection triggered gastric cancer may be more efficiently blocked by the coinhibition of LeY and CA724.

271: Gu-4 improves suvival in experimental sepsis by inhibiting the release and proinflammatory activity of HMGB1

HuiTing Zhou[1], Qing Li[2], ZhongJun Li[2], ZhiHui Zhao[1];
[1]JiangSu Province Key Laboratory for Molecular and Medical Biotechnology, College of Life Science, Nanjing Normal University, Jiangsu Province, China; [2]State Key Laboratory of Natural and Biomimetic of Pharmaceutical Sciences, Peking University, Beijing, China.
zhaozhihui_1964@yahoo.com.cn

High mobility group box 1(HMGB1) was first recognized as a nuclear protein that increases the chromatin remodeling and regulates transcription of many genes. During the past two decades, HMGB1 has been identified as an important "late" proinflammatory mediator due to its unique secretion pattern and lethal effects in sepsis. Therefore, preventing the active release and inhibiting the proinflammatory activity of HMGB1 become alternative ways for the treatment of sepsis. Here,we report the therapeutic effects of Gu-4, a lactosyl derivative, in sepsis and the underlying mechanism that related with HMGB1.

On an experimental rat model of sepsis caused by cecal ligation and puncture (CLP), *in vivo* treatment of Gu-4 prominently attenuated lung ingjury and improved the survival of the animals, which was closely related with the decrease of serum HMGB1 level. Data from our *in vitro* experiments showed that lipopolysaccharide (LPS) induced HMGB1 cytoplasmic translocation and release in RAW264.7 macrophages was significantly suppressed by Gu-4 treatment. Moreover, HMGB1-induced production of proinflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-

1β), was significantly decreased by Gu-4 treatment. Further studies by flow cytometry and Western blot analysis demonstrated that Gu-4 could effectively inhibit HMGB1-stimulated activation of CD11b and NF-κB signaling pathways.
Taken together, our results suggested that Gu-4 could exert therapeutic effects in experimental sepsis model, and the underlying mechanisms of which might lie in: 1) Gu-4 inhibits LPS-induced HMGB1 release; 2) Gu-4 suppresses proinflammatory activity of HMGB1.

272: Effect of dietary Chitooligosaccharides on the Reproduction and Embryo development in the mouse

Lun X. Wang[1], Yu G. Du[1,] *; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, PR China.

[Objective] The effect of Chitooligosaccharides (**COS**) on the reproductive capacity of animals has not been reported yet. The current study was to test the effect of dietary COS on the litter size and embryonic development in the mouse. [Method] The Kunming female and male mouse at 1 month after birth were fed with or without COS in the diet, which included COS group (300 mg/kg), SPM group (100 mg/kg COS, 100 mg/kg Jerusalem artichoke powder, and 100 mg/kg Polysaccharide yeast),and Control group (basal diet). The female was caged with male mice (1:1) when they were sexually mature. Litter size in successive two generations and embryonic development including total number of embryos and litter weight were recorded. [Result] In the first parity, both SPM supplement and COS supplement improved ($P<0.05$) total litter size compared with that in Control group (13.40 ±3.00/13.50 ±1.83/11.33 ±3.11, SPM group/COS group/Control group). In the second parity, SPM supplement significantly improved total litter size compared with that in Control group (13.75± 2.45/11.56±2.96). COS supplement improved one more litter compared with Control group (12.56±1.51/11.56± 2.96), although there was no significant difference between them. In addition, COS supplement improved ($P<0.05$) both total numbers of embryos (15.83± 1.94/11.71±0.95) and total numbers of live embryos (15.00±1.67/10.86±1.35) compared with Control group. COS supplement improved ($P<0.05$) both litter fetus weight (19.33±2.43/14.94±2.39) and litter placental weight(1.79±0.33/1.36±0.19) compared with Control group. [Conclusion] These results indicated that dietary COS can markedly improve the reproductive capacity and embryo development in the mouse.

273: The elevated expression of chondroitin sulphated proteoglycans in human hepatocellular carcinoma

Xiaoli Jia[1], Shuangsuo Dang[1], Siyuan Li[2], Bruce Caterson[3], Clare E. Hughes[3] & Junling Cao[4]; [1]The Department of Infectious Diseases, [2]The Department of Anaesthesia, The Second Hospital of Xi'an Jiaotong University, Xi'an, 710004, China; [3]The Department of Pathophysiology and Repair, School of Biosciences, Cardiff University, CF10 3AX, UK; [4]The Institute of Endemic Diseases, Medical School of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061,China
dang212@126.com

Introduction: In previous studies we found that there was an increase in chondroitin sulphated/dermatan sulphated glycosaminoglycan (CS-GAG) content in rat hepatocellular carcinoma (HCC) tissue, which is associated with higher CS proteoglycan (PG) expression in the tumor [Jia *et al.* World J Gastroenterol. 2012, 18(30): 3962–76]. However, the alteration of specific CSPG contents in human HCC is still largely unknown. In this study, we investigated the expression of CSPGs in human HCC tissues.

Materials & Methods: Twelve HCC and five normal adult human liver tissues were collected. Histological and immunohistochemical staining was used to investigate the expression and distribution patterns of CS/DS GAG and different CSPGs including aggrecan, versican, biglycan and decorin in the tissues. PCR and Western Blotting were used to measure the gene and protein levels of these CSPG members in the control and tumor tissues.

Results: Toluidine blue staining indicated a higher sulphated GAG content in the HCC tissues when compared with the normal liver tissues, suggesting higher PG expression in the tumor. Our further immunohistochemical staining results showed an elevated CSPG expression including aggrecan, versican, biglycan and decorin in the HCC tissues when compared with the control group, although their positive staining patterns were different. Interestingly, there was no positive staining for lumican and keratocan in liver tissues from both HCC and control groups. Quantitative PCR and Western blotting results demonstrated that there was a significant increase in aggrecan, versican, biglycan and decorin gene and protein expression in HCC tissues when compared with the normal liver tissues ($p\leq0.05$).

Conclusion: HCC liver tissues showed an altered expression and distribution patterns in these CSPGs in

comparison with the normal liver tissues. There was an elevated expression in CS/DS GAG contents in HCC tissues, which was associated with higher CSPG levels in the tumor. These results demonstrate that CSPGs play an important role in the onset and progression of HCC, and may provide potential therapeutic targets and clinical biomarkers for this commonly prevalent tumor in humans.

274: Binding Specificity of the H1N1 Swine-Origin Influenza A Virus (S-OIV)

Laura Neumann[1], Florian Krammer[2], Miranda de Graaf[3], Cornelis H. Hokke[4], Friedrich Altmann[1]; [1]University of Natural Resources and Life Sciences, Vienna, 1190, Austria; [2]Icahn School of Medicine at Mount Sinai, New York, 10029, USA; [3]ErasmusMC, Rotterdam, 3015GE, The Netherlands; [4]Leids Universitair Medisch Centrum (LUMC), Leiden, 2333ZA, The Netherlands
Friedrich.Altmann@boku.ac.at

Influenza A viruses are known to attack their hosts by an initial binding to a glycoconjugate containing receptor. It is widely known that the crucial element of the glycoconjugate for Influenza A virus binding is N-acetylneuraminic acid (sialic acid). Depending on the species origin hemagglutinins differ in their prevalence for sialic acids in different linkages (*e.g.* á2,6-linkage found in the human nasopharyngeal tract, á2,3-linked sialic acid found in avian tissues and á2,6- as well as á2,3-linked sialic acid in porcine tissues).

In this study we investigated the binding specificity of the recombinantly expressed H1 hemagglutinin from the H1N1 Influenz A virus (California/04/09) and several other hemagglutinins as controls to N-glycans isolated from mammalian tissue of the nasopharyngeal tract.

The H1 hemagglutinin (A/California/04/09) and as a control several other hemagglutinins, originating from different pandemic viruses, were recombinantly expressed in insect cells to obtain a soluble functionally active trimer.

To investigate the binding specificity we chose two different approaches.

In the first set of experiments N-glycans from different tissues were isolated, labeled with anthranilic acid or 2-aminobenzamide for fluorescent detection, separated by a multidimensional HPLC approach and printed on NHS-activated glass slides to produce glycan arrays. Theses glycan arrays were used to investigate distinct binding of the different hemagglutinins originating from several pandemic virus strains. Data was analysed with the free software Multi Experiment Viewer (MeV). Cluster analysis showed distinct binding patterns of different viruses according to their sialic acid linkage prevalence.

In a second approach affinity chromatography was employed to investigate retention of glycans to immobilized hemagglutinins. To this end, therefore we coupled the recombinant protein to NHS-activated sepharose and monitored retention of fluorescently labelled glycans by fluorescent detection, fractionation and subsequent Matrix Assisted Laser Desorption Ionisation-Time of Flight-Mass spectrometry (MALDI-TOF-MS) analysis.

275: Consistent Gene Regulations of Toll-Like Receptors in Cultured Cancer Cells by Carbohydrate-Specific RP215 Monoclonal Antibody and Anti-Antigen Receptors

Yiting Tang[1], Hao Zhang[1], Gregory Lee[1,2]; [1]UBC Center for Reproductive Health, University of British Columbia, Vancouver, V6H 3 N1, Canada; [2]Department of Pathology, Shantou University Medical College, Shantou, 515041, China
cyglee@yahoo.com

RP215 is a monoclonal antibody generated against a carbohydrate-associated epitope of glycoproteins designated as CA215 which consist mainly of immunoglobulin superfamily proteins expressed by cancer cells. Since RP215 was shown to induce apoptosis and inhibit tumor growth in nude mouse models, effects of RP215 and antibodies against immunoglobulins as well as T-cell receptors (anti-antigen receptors) were investigated on the gene regulations of cultured ovarian as well as cervical cancer cells. By using semi-quantitative RT-PCR, changes in expressions of a number of selected genes involved in proliferation, protein synthesis, cell cycle regulations as well as the innate immunity were analyzed upon various antibody treatments of cultured cancer cells. RP215 and anti-antigen receptors were found to regulate similarly a number of genes ($n=12$) including NFκB-1, IgG, P21, Cyclin D1, ribosomal P_1 and c-fos. Among toll-like receptor genes (TLR-2, -3, -4, -6, -7 and -9), the expressions of TLR-3, TLR-4 and TLR-9 were highly detected. RP215 and anti-antigen receptors were found to up-regulate TLR-3, whereas those of TLR-4 and TLR-9 were down-regulated. Based on these observations, it can be concluded that apoptosis of cultured cancer cells was induced similarly by either RP215, anti-human IgG or anti-T cell receptors through the regulations of the same set of genes with

few exceptions. The innate immunity of cancer cells can also be affected by RP215, anti-human IgG or anti-T cell receptors through the unidirectional mediations of certain toll-like receptors. Therefore, the anti-cancer therapy of RP215 Mab is in part, directly related to surface bound immunoglobulins and T-cell receptors, the expressions of which may be involved in the growth and proliferation of cancer cells. The linkage relationship of toll-like receptors in gene regulations of cancerous immunoglobulins as well as NFκB-1 transcription factor and others was first demonstrated in this study.

276: Synthesis of *N*-acyl and Ureido Derivatives of Diosgenyl Glycoside with Potent Antifungal and Antibacterial Activity

Daria GRZYWACZ[1], Henryk MYSZKA[1], Malgorzata DAWGUL[2], Wojciech KAMYSZ[2]; [1]Faculty of Chemistry, University of Gdansk, Gdansk, 80-952, Poland; [2]Faculty of Pharmacy, Medical University of Gdansk, Gdamsk, 80-416, Poland
E-mail: plaskiew@chem.univ.gda.pl

Saponins are natural glycosides which possess a wide range of pharmacological properties. They are used in traditional medicine as an antidiabetes and antihyperglycemia agents, medical material to treat malaria, helminthes infections and snake bites. Some of diosgenyl glycosides exhibit a wide spectrum of biological activities including antifungal, antibacterial and anticancer properties.

In view of the fact that fungi and bacterial infections are becoming more common and these species are more resistant to the treatment, we have made an attempt to find new substances active against those pathogens. Synthesized by us glycosides consists of diosgenin and D-glucosamine residue. The carbohydrate chain constitutes a hydrophilic part, while appropriate sapogenin is a hydrophobic fragment in this kind of glycosides. Our synthetic strategy is based on the preparation of glycosyl donors, coupling of the respective donors with diosgenin, deprotection of the NH_2 and OH groups and finally obtaining of *N*-acyl and ureido derivatives (Fig. 1).

In biological set of experiments we have investigated the antibacterial and antifungal effect of some *N*-acyl and ureido derivatives. Minimum inhibitory concentration (MIC) was determined for reference strains of the following bacteria: *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212, *R. equi* ATCC 6939, *S. aureus* ATCC 25923, *S. epidermidis* PCM 2118, *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. mirabilis* PCM 543, *P. vulgaris* PCM 2668, *P. aeruginosa* ATCC 9027 and the following fungi: *C. albicans* ATCC 10231, *C. tropicalis* PCM 2681, *C. lipolytica* PCM 2680. Synthesized saponins exhibit various degrees of activity against fungi and bacteria, *e.g.* some of them turned out active against *Candida* species.

This research was part-financed by the European Union within the European Regional Development Fund - grant UDA-POIG.01.01.02-14-102/09-03.

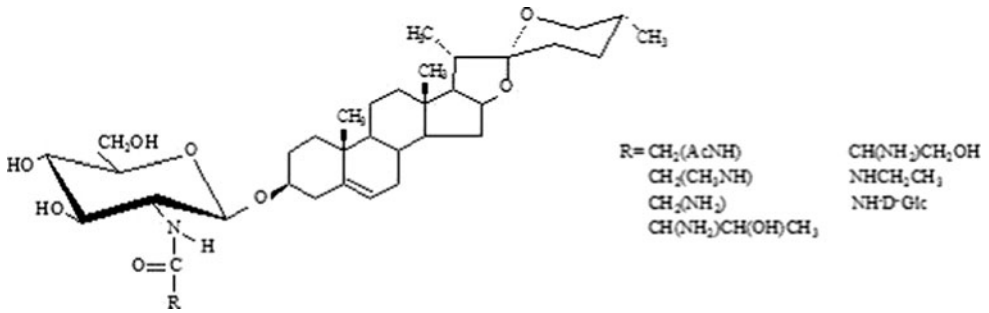


Fig. 1.

277: Conformational Studies of the Furanosides, Important Components of Bacterial Glycans

Dominik Walczak[1], Daria Grzywacz[1], Andrzej Nowacki[1], Beata Liberek[1]; [1]Facult of Chemistry, University of Gdańsk, Gdansk, 80-952, Poland
domelek@chem.univ.gda.pl

It is difficult to overstate the importance of the furanose ring in biology. These moieties are found as constituents of nucleic acids, bacterial, parasitic, and fungal cell wall polysaccharides, as well as other natural products. Importantly, oligosaccharides involving furanosyl constituents are present in various microorganisms whereas these are absent in the mammals glycans. This fact suggests that the enzymes involved in the metabolism of such sugars in bacteria, fungi and protozoa would constitute a good target for the design of new drugs.

The conformational preferences of oligosaccharides composed of furanose moieties are not well understood. This is due both to a lack of experimental data on oligofuranosides and the significant flexibility of the five-membered ring. To understand the conformational preferences of oligofuranosides it is necessary to well understand the conformational preferences of the single furanose ring. By understanding the conformational preferences of these smaller components, it is hoped that an understanding of the secondary and tertiary structure of the large biomolecules will also be attained.

Conformations of furanosides in solution are hard identified by NMR techniques because these are equilibrating rapidly and averaging of coupling constants occurs. However, when a furanoid ring is conformationally restricted, *e.g.* by a rigid second skeleton, it is possible to predict its conformation. Therefore, to study conformations of a furanoid ring we synthesized series of furanoses and furanosides having bicyclic structures with the five-membered rings fused at carbons C3-C4, C2-C3, and C1-C2, respectively (Fig. 1). The NMR studies of the synthesized compounds point that spectra of each groups of furanoses and furanosides are very characteristic and indicative of one specific conformation or configuration (Fig. 1).

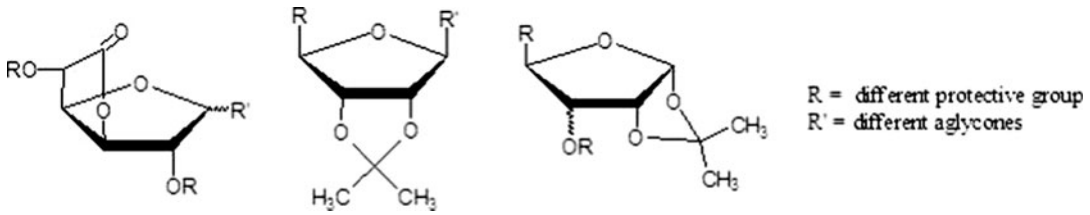


Fig. 1

278: The Inhibitory Effect of Marine Oligosaccharide Sulfate OMS against Influenza a H1N1 Virus

Wei Wang[1,2], Shi-Xin Wang[1], Chun-Xia Li[1,2], Guang-Li Yu[1,2], Hua-Shi Guan[1,2]; [1] Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China, Qingdao 266003, PR China; [2] Shandong Provincial Key Laboratory of Glycoscience & Glycoengineering, Ocean University of China, Qingdao 266003, PR China
wwwakin@ouc.edu.cn

Marine oligosaccharide sulfate OMS is a low molecular weight compound which obtained from alginate polysaccharide by acid degradation, fractionation and sulfated modification. The results of the cytopathic effect (CPE) inhibition assay and MTT assay showed that OMS with the average molecular weight of 3~5 KD could significantly inhibit the replication of influenza A H1N1 virus in MDCK cells. The cytotoxicity of OMS is very low, and the selectivity index (SI) of OMS *in vitro* is more than 15.0. Moreover, OMS could obviously inhibit the activity of influenza A H1N1 virus neuraminidase, and the 50 % inhibition concentration (IC_{50}) is less than 50 μg/ml. Furthermore, OMS could significantly alleviate the lung inflammation of BALB/C mice caused by the infection of influenza A virus (A/PR/8/34) at the dose of 40 mg/kg/day, and the inhibition rate of lung index was more than 33.9 %, which is comparable with the effect of positive drug oseltamivir phosphate. Compared to the model group, OMS could obviously decrease the lung viral load in virus infected mice ($P<0.01$), reduce the death rate of mice (death prevention rate >40 %), and prolong the survival time of mice. OMS could also enhance the production of interferon-γ in spleen 4 days post infection. In conclusion, the low molecular weight compound OMS can significantly inhibit the activity of influenza A virus neuraminidase and possess good anti-H1N1 virus effects *in vitro* and *in vivo*, which suggest that this compound merits further investigation as a potential anti-influenza A virus drug in the future.

279: Biological Activity of Diosgenyl 2-Amino-2-Deoxy-β-D-Glucopyranoside Hydrochloride and its *N,N*-Dialkyl Derivatives

Henryk Myszka[1], Daria Grzywacz[1], Malgorzata Dawgul[2], Wojciech Kamysz[2]; [1]Faculty of Chemistry, University of Gdansk, Gdansk, 80-952, Poland; [2]Faculty of Pharmacy, Medical University of Gdansk, Gdansk, 80-416, Poland,
myszka@chem.univ.gda.pl

Diosgenyl glycosides are steroid saponins isolated from a variety of plants, for example *Costus*, *Dioscorea*, *Paris*, *Solanum*, *Trillium*, *Yucca*. Some of them exhibit a wide spectrum of biological activities including antifungal, antibacterial and anticancer properties. The carbohydrate residue is covalently attached to the diosgenin backbone. Usually, in natural diosgenyl glycosides the first sugar connected to diosgenin is β-D-glucopyranose.

We have synthesized a diosgenyl glycosides containing D-glucosamine derivatives as a carbohydrate residue. Some of them were tested for their antifungal and antibacterial activity. (Fig. 1)

Antimicrobial activity against reference strains of bacteria and fungi (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Rhodococcus equi* ATCC 6939, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* PCM 2118, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* PCM 543 *Proteus vulgaris* PCM 2668 i *Pseudomonas aeruginosa* ATCC 9027; *Candida albicans* ATCC 10231, *Candida tropicalis* PCM 2681, *Candida lipolytica* PCM 2680, *Aspergillus niger* ATCC 16404) was studied.

Gram negative bacteria turned out to be resistant to the compounds at tested concentrations (0.5−1,024 mg/L), while the growth of gram positive strains and fungi was inhibited at concentrations 0.5−128 mg/L. Strains of *Candida sp*. were the most susceptible to saponins. Therefore MIC assay was performed for clinical isolates (*C. glabrata, C. krusei, C. tropicalis, C. parapsilosis*). The test was carried out also for conventional antifungal agents (amphotericin B, clotrimazole, fluconazole, itraconazole, natamycin, nystatin).

Among clinical strains of *C. krusei* and *C. tropicalis* we have identified numerous isolates resistant to tested compounds at applied concentrations (0.025−512 μg/mL). The saponins presented very strong activity towards clinical isolates of *C. glabrata* and *C. parapsilosis* comparable or stronger than conventional antimicrobials.

Afterwards the most active compounds were tested according to their hemolytic activity. Obtained glycosides were did not exhibit hemolytic activity towards human erythrocytes while applied at their microbiogically active concentrations. Results of presented study suggest potential application of saponins as future antifungal agents.

Part-financed by the European Union within the European Regional Development Fund—UDA-POIG.01.01.02-14-102/09-03.

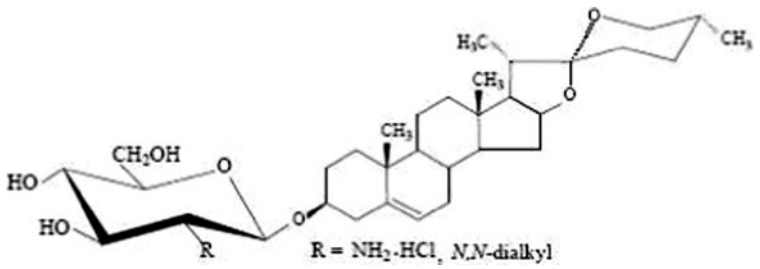


Fig. 1

280: Synthesis of Phosphono and Phosphate Derivatives of Hydroxyimino-D-alditols as New Potential Antifungal Agents

Magdalena Cyman[1], Henryk Myszka[1], Karolina Kwiatkowska[2], Slawomir Milewski[2], Beata LIBEREK[1]; [1]Faculty of Chemistry, University of Gdansk, Sobieskiego 18, 80-952 Gdansk, Poland; [2]Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Narutowicza 11/12, 80-233 Gdansk, Poland
cyman@chem.univ.gda.pl

In search for new effective antifungals we focus on two enzymes involved in biosynthesis of the fungal cell wall. The first enzyme is glucosamine-6-phosphate synthase (GlmS), which catalyzes transformation of D-fructose-6-phosphate (Fru-6P) to D-glucosamine-6-phosphate (GlcN-6P) in the chitin biosynthesis pathway. The second enzyme is phosphomannose isomerase (PMI) reported to play a crucial role in biosynthesis of many mannosylated structures, including cell wall components of fungi. PMI is aldose-ketose isomerase and catalyzes reversible isomerization of D-manno-6-phosphate (Man-6P) to D-fructose-6-phosphate (Fru-6P). Both enzymes are proposed as the targets for antifungal chemotherapy and a search for their selective inhibitors is continued.

Mechanisms of the reactions catalyzed by both enzymes are known and similar. The reaction performed by GlmS is believed to proceed through the formation of an imine intermediate **1**, whereas the reaction catalyzed by PMI proceeds *via* a *cis*-endiol intermediate **2** (Fig.1).

In search of mimetics of intermediates **1** and **2** we synthesize phosphono and phosphate derivatives of the hydroxyimino-D-glucitols ($\mathbf{3_{a\text{-}c}}$–$\mathbf{6_{a\text{-}c}}$). Similarity in the structures of the planed compounds to intermediates **1** and **2** allow us to assume that they can be the potential inhibitors of the both enzymes. Dimethyl and diethyl ester analogs ($\mathbf{3_{b,c}}$–$\mathbf{6_{b,c}}$) will have more lipophilic character, which make them easier to penetrate through the cytoplasmic cell membrane. It was proved that similar esters are hydrolyzed inside a cell. Here, the completed stages of our syntheses are presented.

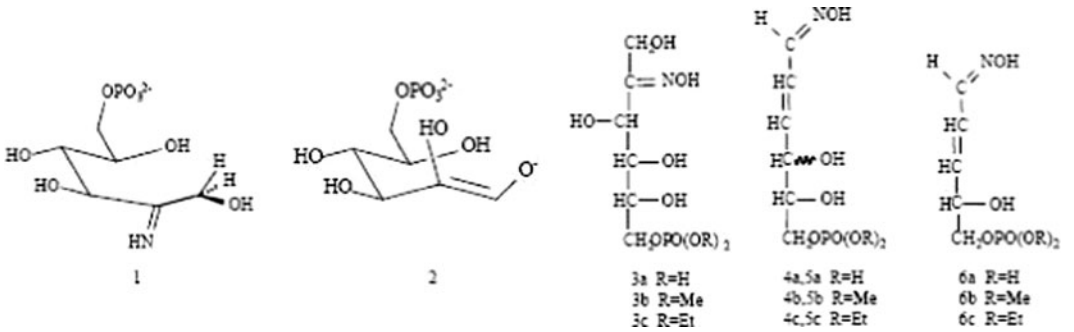


Fig.1

281: Convergent Approach to Glycoalkaloids: Syntheses of Solasonine and 25(*R*)-Solanine

Ming Li*, Yannan Peng, Pengwen Chen, Peng Wang; Key Laboratory of Marine Medicine, Education Ministry of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, Shandong, China
lmsnouc@ouc.edu.cn

Steriodal glycoalkaloids are rich in Solanaceae family including important agricutural crop plants such as potato, tomato and eggplant. According to the aglycon skeleton, they are classified into two basic types: the spirosolans and solanidans, which contain oxa-azaspirodecane and indolizidine subunit, respectively. The studies have shown that glycoalkaloids display various biological activities such as antitumor, antiheptatoxicity and molluscicide. A cream containing solansodine glycosides mainly composed by solamargine and solasonine has been marketed as "Curaderm" for solar keratosis. So far the syntheses of glycoalkaloids have been sporatically documented. Herein, we take solasonine and 25(*R*)-solanine as a representative of

spirosolan and solanidan glycosides, respectively, to illustrate our convergent syntheses of glycoalkaloids.

Our approaches to solasonine and 25(*R*)-solanine constitute preparation of aglycons of solansodine and 25(*R*)-solanidine, construction of solatriosyl imidate and their stereselective couplings followed by removal of protective groups. Solansodine and 25(*R*)-solanidine were achieved in 6 and 11 steps, respectively, by using the common intermediate of 3-*O*-*tert*-butyldiphenylsilyl-16-*O*-acetyl-26-azido-22-oxo-cholestane derived from the industrial diosgenin. The key steps involved E, F-ring openings of diosgenin and highly efficient reduction of azide by catalytic hydrogenolysis as well as reductive aminations. Acyl protected solatriosyl imidate was prepared from D-galactose, D-glucose and L-rhamnose following protection, deprotection and functional group manipulations as usual. Due to solatriosyl imidate bearing 1,2-linkage at the reducing end, thereby devoid of neighboring group assistance during glycosylation, its 1,2-*trans* stereselective couplings with aglycons are challenging. After extensive screenings of reaction conditions, the glycosylations of imidate with aglycons were realized under the promotion of $HB(C_6F_5)_4$ in a mixed solvent of $PhCF_3$/*t*-BuCN/CH_2Cl_2 and affored the desired glycoalkaloids with high efficiency and stereoselectivity, which were globally deprotected under Zemplén conditions to lead to solansonine and 25(*R*)-solanine. The synthetic glycoalkaloids were characterized by 1D and 2D NMR spectroscopy and the data are fully indentical with the reported. The developed method might be applied to the syntheses of other active glycoalkaloids and their analogues, which should facilitate in-depth investigations on structure-activity relationship of glycoalkaloids and their mode of action in detail.

Financial support from National Science Funding of China (21002095) is greatly appreciated.

282: Pectic polysaccharides from *Apocynum venetum L.* promote proliferation of beta-cells

Chengxin Sun, Xinzhi Li, Yuying Fan, Yifa Zhou; School of Life Sciences, Northeast Normal University, Changchun, 130024, China
zhouyf383@nenu.edu.cn

Diabetes mellitus is a chronic and insidious disease affecting much more people in the world. The blood glucose of patients could be controlled in a normal level under the treatment of commercial agents. However, all the hypoglycemic drugs have shown some side effects. It is reported that, due to oxidative stress, beta-cell dysfunction or reduction occurs both in type 1 and type 2 diabetes mellitus. Thus, it is more worthy of finding new hypoglycemic agents which can improve or promote the proliferation of beta-cells, especially with the natural original. Polysaccharides are well documented to have antioxidant capacities. We have scanned 10 kinds of polysaccharides from different hypoglycemic herbs. A water-soluble polysaccharide (ALP) was extracted from the leaves of *Apocynum venetum*. ALP could specifically promote the pancreatic beta-cells proliferation (INS-1 and RIN-m5F), but no effect on human colon carcinoma cells (HT29 and HCT116). Afterwards, ALP was further fractionated into ALPAP and other two fractions, using a combination of ion-exchange and gel filtration chromatography. Beta-cells proliferation assay showed that ALPAP presented stronger promotion than the other two fractions. HPLC analysis results indicated that ALPAP was homogenous with molecular weight of 18 kDa. The structural features of ALPAP were elucidated by periodate oxidation, partial acid hydrolysis, IR spectra and ^{13}C NMR spectroscopy. The results indicated that ALPAP is a homogalacturonan (HG) -rich pectin with RG-I domain. ALPAP stimulated beta-cells growth in a bell-shaped dose–response curve from 50 μg/ml to 5 mg/ml. After 1.0 mg/ml of ALPAP treatment for 48 h, the cell proliferation rate could reach up to 70 % compared with control. Flow cytometry data showed that ALPAP could elevate mitochondrial membrane potential of beta-cells, but no influence on cell cycle. All the results indicated that HG-rich pectic ALPAP was the main active fraction to stimulate beta-cells proliferation, which is probably mediated through enhancement on mitochondrial function.

283: Intracellular Localization and Phosphorylation of Galectin-4 is Controlled by Src Family Kinases

Hiroko Ideo[1], Ikue Hoshi[1], Katsuko Yamashita[1], and Masaru Sakamoto; [1]Mol. Biosci .of Medi, Sasaki Institute; [2]Dept. of Gynecology, Kyoundo hospital, Sasaki foundation, Tokyo, 101-0062, Japan.
ideo@po.kyoundo.jp

Galectins are members of a family of lectins defined by their affinity for β-galactoside–containing saccharides and are involved in regulating diverse biological phenomena . Galectin-4 is expressed abundantly in the epithelium of the alimentary tract and has 2 carbohydrate-recognition domains. Galectin-4 is a cytosolic protein that lacks a signal sequence but is externalized and binds to 3-*O*-sulfated glycoconjugates extracellularly. There is abundant evidence for the extracellular roles played by galectins as modulators of cell adhesion and signaling, although the secretion mechanism is not yet understood. Galectin-4 is also localized on the cell surface and binds

to sulfated glycosphingolipids and CEA, thereby modulating the adhesion of the cells. However, the mechanism of subcellular localization and externalization of galectin-4 has not yet been determined. It has been reported that galectin-4 recruits apical glycoproteins, including MUC-1, CEA, *etc.*, in DRMs, because these glycoproteins are depleted in DRMs isolated from galectin-4-knockdown HT-29 5M12 cells. This implies that galectin-4 plays an important role in trafficking by functioning as the carrier of these proteins.

A preliminary experiment using pervanadate (PV) showed that galectin-4 is tyrosine-phosphorylated in cells and suggested that Src kinases are involved. Cell transfection with galectin-4 and active Src plasmids showed that galectin-4 can be tyrosine phosphorylated by members of the Src kinase family. The C-terminal peptide YVQI of galectin-4 was found to play an important role in its tyrosine phosphorylation, and the SH2 domains of Src and SHP2 were found to bind to this peptide. Immunofluorescence analysis showed that the sub-membrane area of Src-activated cells was intensely stained by anti-galectin-4, indicating that Src kinase activity is important for subcellular localization of galectin-4. Furthermore, MUC1 derived from NUGC-4 cells was observed to bind to galectin-4, and externalization of the bound molecules from the cell to the medium increased in the hyperphosphorylated condition. These results suggest that localization of galectin-4 can be regulated by signaling molecules and that it may function intracellularly as an adaptor protein serving to modulate the trafficking of glycoproteins

284: Epigenetic modulation of HeLa cell membrane N-glycome by epigenetic inhibitors and reversibility of inhibition effects in a drug-free environment

Tomislav Horvat[1], Darko Barišić[1], Martina Deželjin[3], Ana Mužinić[4], Maja Herak Bosnar[3], Gordan, Lauc[2,4], Vlatka Zoldoš[1]; [1] University of Zagreb, Faculty of Science, Horvatovac 102a, Zagreb, Croatia; [2] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia; [3] Rudjer Boskovic Institute, Division of Molecular Medicine, Bijenicka 54, Zagreb, Croatia; [4] Genos Ltd, Glycobiology Laboratory, Planinska 1, Zagreb, Croatia
vzoldos@biol.pmf.hr

Deregulation of glycosylation occurs in a wide range of multifactorial diseases and aberrant glyco-phenotypes have been associated with specific pathophysiological states. Glycogenes are one of the groups of cancer-associated genes since changes in glycan structures are hallmarks of many cancers. Cancer-specific changes in glycan biosynthetic pathways are resulting from aberrant expressions of glycosyltransferases and glycosidases, which is often a result of epigenetic changes including DNA methylation and/or histone modifications (acetylation, methylation or phosphorylation). The aberrant gene expression pattern can be potentially restored using various epigenetic inhibitors. To test potential therapeutic usefulness of DNA methylation inhibitors, zebularine and 5-aza-2-deoxycytidine, and histone deacetylation inhibitors, trichostatin A and Na-butyrate, in inducing a reversal of undesired glyco-phenotypes, we developed an HPLC- based method for the analysis of glycan structures from HeLa cells embedded in polyacrylamide gels. In addition, we specifically investigated the preservation of altered glycan profiles over a prolonged period of time in a drug-free environment. Our results emphasize the importance of epigenetic control in the regulation of N-glycosylation, but also suggest the stability of complex biosynthetic pathways responsible for the establishment of glycan profiles in human cells in culture.

285: Dissecting the impact of *O*-GlcNAc modification on insulin action using different OGA inhibitors

Chin Fen Teo* and Lance Wells; Complex Carbohydrate Research Center and the Department of Biochemistry and Molecular Biology, the University of Georgia. 315 Riverbend Road, Athens, GA 30602, USA.
cfteo@uga.edu

O-GlcNAc modification is a ubiquitous and reversible glycosylation found on intracellular proteins. The spatial and temporal presence of *O*-GlcNAc is orchestrated by a pair of cycling enzymes, *O*-GlcNAc transferase (OGT) and β-*N*-acetylglucosaminidase (OGA), in response to a variety of cellular and environmental stimuli. Given that UDP-GlcNAc, the end product of the hexosamine biosynthetic pathway (HBP), is an obligatory donor substrate of OGT, *O*-GlcNAc is posed as an effector of excessive glucose flux through the HBP which in turn can lead to the development of insulin resistance, a hallmark of type 2 diabetes. Toward this end, previous studies have utilized PUGNAc, the first reported OGA inhibitor, albeit that can inhibit lysosomal hexosaminidases as well, to increase global *O*-GlcNAc levels and observed a correlation of elevated *O*-GlcNAc with the development of insulin resistance. This notion has been recently challenged by results from the Vocadlo's group, in which elevated global *O*-GlcNAc levels using a more selective OGA inhibitor, NButGT, did not lead to insulin resistance in cell culture or *in vivo*. In this study, we evaluated the impact of the *O*-GlcNAc modification on the pro-survival action of insulin under serum-deprivation induced apoptosis using three different OGA inhibitors, GlcNAcstatin-g (GNSg, another

OGA selective inhibitor), thiamet-G (TMG, a derivative of NButGT), and PUGNAc. We found that while PUGNAc inhibits the protective action of insulin, neither GNSg nor TMG negates the anti-apoptotic action of insulin. To address whether inhibition of the lysosomal hexosaminidase activity *via* PUGNAc, which would elevate global GM2 ganglioside levels, leads to its unique characteristic in blocking insulin action, we also examined the pro-survival role of insulin in the presence of a selective lysosomal hexosaminidase inhibitor, INJ2. We established that neither INJ2 alone nor the combination of OGA selective inhibitors with INJ2 mimic the inhibitory effect of PUGNAc. These results strongly suggest that the defect in insulin action upon PUGNAc treatment does not derive from its inhibition of OGA or lysososomal hexosaminidases, and that there is a third, yet unknown, target of PUGNAc that is the likely culprit in inhibiting the protective effect of insulin from apoptosis.

286: Structure and Immunological Activities of Mannogalactoglucans from *Lentinus edodes* Fruiting Bodies

Iteku B. Jeff[1,2], Tao Zhang[1], Yifa Zhou[1]; [1] School of Life Sciences, Northeast Normal University, 130024, Changchun, PR China; [2] Department of Biology, Kinshasa University, P.O. Box 190 Kinshasa, Democratic Republic of Congo
jeffitekubekomo@gmail.com (Iteku B. Jeff), zhouyf383@nenu.edu.cn (Yifa Zhou)

Lentinusedodes importance is attributed to both its nutritional value and medical application. Polysaccharides are the most important bioactive components in *Lentinusedodes*, with data from studies often focus on (1→3)-β-D-glucans.To investigate total active components of *Lentinusedodes*, we extracted *L. edodes*with cold and hot water as well as alkali to obtain the total polysaccharides. Among them, besides (1→3)-β-D-glucans and(1→6)-β-D-glucans, there are a series of mannogalactoglucans. Themannogalactoglucanswere obtained from WPLE (the hot water extracted polysaccharide), after freeze-thawing process and fractionation by size-exclusion chromatography into fourheteroglucansfractions: S-WPLE-II-a, S-WPLE-II-b, S-WPLE-III-a and S-WPLE-III-b,with M*w* (7.5–2,000 kDa). Chemical and spectroscopic, including 2D NMR, studies indicated that themannogalactoglucanscontained(1→6)-, (1→4)- and (1→3)-Glc*p*, (1→6)-Gal*p*, (1→3,6)- and (1→2,4)-Man*p* residues. But among four fractions, the ratios of each linkage are different. All these polysaccharides exhibited antitumor activities against S-180, HCT-116 and HT-29 cancer cells *in vitro* at a dose-dependent manner. Interestingly, highest M*w* fractions presented stronger antitumor activitiesthanthe native polymer, while the antitumor activities of lower M*w* fractions were lower than the native one. Suggesting that the antitumor activities of these polysaccharides are related with the monosaccharide content, M*w* andglycosidic linkage. The polysaccharides also showed selectively higher antitumor activity against suspended cells than adherent ones. Our results provided essential data for a better understanding of *L. edodes* polysaccharides. Our investigations also provided helpful information for careful selection of appropriateglucans is essential if we wish to investigate the effects of glucans clinically.

287: Plasma membrane-associated sialidase NEU3 confers neoplastic potential on colon cancer cells by regulating Wnt/β-catenin signaling

Kohta Takahashi[1], Masahiro Hosono[2], Keiko Hata[1], Tadashi Wada[1], Kazunori Yamaguchi[3], Kazuo Nitta[2], and Taeko Miyagi[1]; Divisions of [1]Cancar Glycosylation Research, and [2]Cell Recognition Study, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, Sendai, 981-8558, and [3]Division of Molecular and Cellular Oncology, Miyagi Cancer Center Research Institute, Natori, 981-1293, Japan.
tmiyagi@tohoku-pharm.ac.jp

The plasma membrane-associated sialidase NEU3 is a key enzyme for degradation of gangliosides, components of cell surface membranes. We previously demonstrated NEU3 to be markedly up-regulated in various human cancers including colon cancer, associated with augmented malignant properties including increased cell migration, invasiveness and cell survival. Here, we have found a molecular link between Wnt/β-catenin signaling and NEU3 expression in colon cancer cells by analyzing cancer stem-like characteristics and tumor initiating capability. NEU3-silencing in HT29 and HCT116 colon cancer cells resulted in significant decrease in clonogenic growth, chemoresistance to oxaliplatin, clonogenicity on soft agar and *in vivo* tumor growth, along with down-regulation of stemness genes, Nanog, Oct4, and Sox2, and Wnt–related genes Lgr5, Wnt 1 and Wnt3a. Analyses further revealed that NEU3 enhanced phosphorylation of the Wnt receptor LRP6 and consequently β-catenin activation by accelerating complex formation with LRP6 and recruitment of GSK3β and Axin, whereas its silencing exerted the opposite effects. As the null-activation mutant of NEU3, N88D and Y370C, could not accelerated the LRP6 phosphorylation, indicating that the glycolipid changes as results of catalytic reaction of NEU3 likely need for the acceleration. Indeed, analysis of lipids extraction of the cells by the Thin-Layer Chromatography (TLC), lactosylceramide (Lac-Cer) was decreased in NEU3-silenced HT29 cells, and exogenous

addition of the Lac-Cer accelerated the LRP6 phosphorylation. Under sphere-forming condition, when stemness genes were up-regulated, endogenous NEU3 expression was significantly increased, whereas NEU3 silencing suppressed sphere-formation. In fact, NEU3-silenced spheroids demonstrated reduced *in vivo* tumor formation in NOD-SCID mice, with down-regulation of stemness-and Wnt- related genes. Interestingly, microarray analyses in NEU3-silenced HT29 revealed that NEU3 affects Cripto-1 expression, that is primary target of Wnt/β-catenin signaling and directly regulated by Nanog and Oct4 in embryonic stem cells and colon carcinoma cells. These results suggest that NEU3 regulates maintenance of stem-like characteristics and tumorigenic potential of colon cancer cells *via* Wnt/β-catenin signaling.

288: Impairment of lipid metabolism in GD3 synthase knockout mice

Shuting Ji[1], Yuhsuke Ohmi[1], Yuki Ohkawa[1], Keiko Furukawa[1,2] and Koichi Furukawa[1]; [1]Department of Biochemistry II, Nagoya University Graduate School of Medicine, Nagoya, 466-0065, Japan; and [2]Department of Biomedical Sciences, Chubu University College of Life and Health Science, Nagoya, 487-8501, Japan
koichi@med.nagoya-u.ac.jp

The expression patterns and levels of acidic glycosphingolipids, gangliosides are different in tissues and organs, and high expression in central nervous system is observed. Although GD3 syntahse KO mice lack b-series gangliosides in whole body, there is no severe phenotype during the development and generation. When we analyzed serum samples from mice at the age of 15 weeks and 30 weeks, extremely low levels of leptin were observed in GD3 synthase KO mice compared with wild type mice.

Although the blood levels of leptin were significantly low, body weights of GD3 synthase KO mice were normal. Futher, when they were fed with high fat high sucrose diet, wild type and GD3 synthase KO mice showed similar obesity trend, and there were no significant differences in body weights. Then we analyzed histology of epididymal adipose tissues to assess the amount of total body fat and brown adipose tissues which relate with the burning of body fat. Consequently, white adipose tissues appeared normal, while the number of cells of brown adipose tissues were significantly increased in GD3 synthase KO mice. When high fat high sucrose was given, a futher rise of the cell number was observed. In addition, expression levels of UCP-1 gene increased in GD3 synthase KO mice at 10 weeks after birth, and the higher body temperature was observed. These results suggested that brown adipocytes of GD3 synthase KO mice are over-working. On the other hand, the gene expression of leptin was at the similar levels between GD3 synthase KO and wild type mice, although the protein levels of leptin showed significantly higher in GD3 synthase KO mice than wild type mice. In addition, immunohistochemistry of leptin revealed strong staining both in white and brown adipose tissues of GD3 synthase KO mice, suggesting that the secretion of leptin from adipose tissues were impaired in GD3 synthase KO mice.

In future, primary culture adipocytes of stromal vascular cells isolated from epididymal adipose tissues and brown adipose tissues will be used to clarify the molecular mechanisms for the morphological abnormalities and increased activity of brown adipose tissues, and the abnormal leptin secretion from white adipose tissues.

289: Analysis of polySia-NCAM biosynthesized by a mutated polysialyltransferase ST8SIA2/STX found in a schizophrenic patient

Masaya Hane[1,2,3], Ken Kitajima[1,2,3], Chihiro Sato[1,2,3]; [1]Department of Bioengineering Sciences, Graduate School of Bioagricultural Sciences, Nagoya University; [2]Bioscience and Biotechnology Center, Nagoya University; [3]Program for Leading Graduate Schools IGER, Nagoya University
hane.masaya@d.mbox.nagoya-u.ac.jp

Schizophrenia is a severe psychiatric disorder that affects approximately 1 % of the population worldwide. Causes are known to be multiple, genetic and environmental factors. However, the overall mechanism leading to schizophrenia remains unclear. A number of reports have shown the relationships between impaired expression of polysialic acid (polySia) and schizophrenia. PolySia is a homopolymer of sialic acid with a degree of polymerization of 8–400, and has anti-adhesive effects on cell-cell interactions due to its bulky polyanionic nature when present on neural cell adhesion molecule (NCAM). Recently, we demonstrated that polySia functions not only as an anti-cell adhesion molecule, but also as a reservoir scaffold for brain-derived neurotrophic factor (BDNF), a neurotransmitter (Dopamine) and fibroblast growth factor 2 (FGF2), which are biologically active molecules in neurogenesis and neural function. In 2006, Arai and his colleagues reported single nucleotide polymorphisms (SNPs) in the promoter region of ST8SIA2/STX in schizophrenic patients. We focused on a SNP of the ST8SIA2/STX gene, SNP7 (421G>A) on exon 4, which has been identified in a schizophrenic patient in their report because structural features of the polySia-NCAM in any case of the schizophrenia patients have not been clarified. Therefore, our objective is to gain an insight into the relationship between structure and function of polySia-NCAM biosynthesized by

ST8SIA2/STX. In this study, we analyzed the enzymatic activity of the mutated ST8SIA2/STX toward membrane-bound and secreted forms of NCAM, and chemically evaluated the polySia-NCAM biosynthesized by the mutated ST8SIA2/STX. In addition, we investigated the ability of polySia to bind BDNF and FGF2, using a surface plasmon resonance (SPR)-based method. We showed that polySia-NCAM biosynthesized by the mutated ST8SIA/STX contained less polySia with shorter chain length and that it exhibited impaired reservoir function for BDNF and FGF2 as compared with that synthesized by wild-type ST8SIA2/STX. Our findings suggest that the quantity and quality of polySia on NCAM is important for normal neuronal functioning.

290: Novel Glyco-biomarker for Epithelial Ovarian Cancer Selected by Glycoproteomic Approaches

Maki Sogabe [1], Hirofumi Nozaki[1], Tomomi Kubota[1], Atsushi Kuno[1], Hiroyuki Kaji[1], Akira Togayachi[1], Hayao Nakanishi[2], Toru Nakanishi[3], Nao Suzuki[4], Kazushige Kiguchi[4], Mikio Mikami[5], Yuzuru Ikehara[1], Hisashi Narimatsu[1]; [1]Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, 305-8568, Japan; [2]Division of Oncological Pathology, Aichi Cancer Center Research Institute, Nagoya, 464-8681, Japan; [3]Department of Gynecologic Oncology, Aichi Cancer Center Hospital, Nagoya, 464-8681, Japan; [4]Department of Obstetrics and Gynecology, St. Marianna University School of Medicine, Kawasaki, 216-8511, Japan; [5]Department of Obstetrics and Gynecology, School of Medicine, Tokai University, Isehara, 259-1193, Japan;
h.narimatsu@aist.go.jp

Epithelial ovarian cancer (EOC), is the leading causes of death among gynecological malignancies, and its incidence rate tends to increase is Japan. The indications of EOC are asymptomatic, so more that two-third of EOC cases are diagnosed in the advanced stages, thus, whose five-year survival rate is less than 30 %.

Lectin microarray analysis of conditioned media from ovarian, gastric, and colon cancer cell line showed that AAL signals were high in general. To select tumor biomarker candidates exhaustively, AAL column were used to collect glycopeptides for the analysis. In addition to the conditioned media, peritoneal washing (PW) from EOC and gastric cancer patients were subjected to IGOT-LC/MS analysis and more than 300 glycoproteins were found from EOC samples.

Validation study was performed with the lectin-agarose catch followed by Western Blotting analysis using the antibody for the candidates. Several glycoproteins were found in the lectin bound fraction of the PW from EOC patients. One of the candidate glycoprotein was validated for detail.

Sandwich ELISA system with the lectin and antibody for the glycoprotein was established. The ascites fluid from EOC showed higher ELISA signals than those from benign, including chocolate cyst and uterine endometrial carcinoma. Immunohistochemical study showed that both the lectin and antibody for the glycoprotein stained same cancerous part of the tissue from EOC patients.

Glycoproteomics tools, lectin microarray and IGOT-LC/MS, enable to find a novel EOC biomarker candidate, which is capable to distinguish EOC from other gynecological disease.

This work was supported by a grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.

291: Identification of glycoproteins carrying the sialyl lewis x antigen in human lung adenocarcinoma cells

Songlin Jiang[1], Naoki Ikeda[2] and Koichi Honke[1,3]; [1]Kochi System Glycobiology Center, Kochi University Medical School, Nankoku, Kochi 783-8505, Japan; [2]Dept. Surgery, Kinki-Chuo Chest Medical Center, Sakai, Osaka 591-8555, Japan; [3]Dept. Biochemistry, Kochi University Medical School, Nankoku, Kochi 783-8505, Japan

The interaction of the sialyl lewis x antigen on cancer cells with E-selectin on the endothelial cells initiates the cell adhesion process of cancer hematogenous metastasis. Therefore, expression of the sialyl lewis x antigen on cancer cells is thought to enhance cancer metastasis. Because sulfation and sialylation occur at the same position of the same precursor sugar chains, overexpression of a relevant sulfotransferase, Gal3ST-2 suppresses the expression of sialyl lewis x in human lung adenocarinoma-derived ABC-1 cells, and reduces the interaction with E-selectin and the hematogeneous metastasis in a nude mouse model. Since the two major sialyl lewis x-positive protein bands turn negative parallel to the above phenomena, we hypothesize that these proteins may be involved in the metastasis mechanism. In the present study, we have purified these glycoproteins and identified them by mass spectrometry-based proteomics analysis. Effects of siRNA knock down of these glycoproteins on expression of sialyl lewis x antigen and cancer metastasis by interaction with E-selectin are under study. Identified glycoproteins could serve as a new biomarker for clinical diagnosis of lung adenocarcinoma.

292: Silencing of GnT-V Potentiates the Antitumor Activity of Gemcitabine against Bladder Cancer Cells

Jianhui Fan[1], Ying Tang[2], Jingna He[1], Shujing Wang[1], Lifen Wang[2], Jianing Zhang[1*]; [1]Department of Biochemistry, Institute of Glycobiology, Dalian Medical University, Dalian, 116044, China; [2]Department of Pathology, Second Affiliated Hospital of Dalian Medical University, Dalian 116023,China.
jnzhang@dlmedu.edu.cn

Gemcitabine is a deoxycytidine analog used for the treatment of a wide range of solid tumors and has been widely accepted as the first-line treatment for patients with advanced bladder cancer. Its sensitivity is however often reduced due to the development of bladder cancer. N-acetylglucosaminyl-transferase V (GnT-V) is an enzyme that catalyses β1-6 branching of N-Glycans during synthesis of glycoproteins. Previous studies have shown that GnT-V was associated with tumorigenesis and metastasis. The functional roles of GnT-V in acquired resistance to gemcitabine remain unknown in bladder cancer. Our present study aimed to investigate the relationship between GnT-V expression and gemcitabine sensitivity in bladder cancer cells. GnT-V expression was studied by immunohistochemistry in 99 surgically resected bladder cancers, and the staining intensity was evaluated. High GnT-V expression in high grade tumor cells was found in 20 of 38 GnT-V overexpression cases ($p<0.05$), and was positively correlated with pathological grade and lymph vascular space. Consistently, GnT-V overexpression was examined in multiple human bladder cancer cell lines by RT-PCR and western blot assay. Furthermore, GnT-V expression was significantly downregulated by lentivirus induced RNAi in EJ bladder cancer cells and result in increased gemcitabine sensitivity with time and dose dependent way *in vitro*. More importantly, cellular administration of the GnT-V specific RNAi in EJ cells significantly inhibited the growth tumor cells implant solid tumor in nude mice and sensitized the tumors to gemcitabine treatment *in vivo*. These findings suggest that silencing GnT-V expression using lentivirus RNAi could potentially be an effective strategy to enhance the antitumor activity of gemcitabine in bladder cancer cells.

This work was supported by grants from the National Program on Key Basic Research Project (973 Program) (NO. 2012CB822103) and National Science Fund Committee (NSFC) (NO. 31000372, 31170774)

293: Therapeutic effects of glycosaminoglycans on elastase-induced emphysema in mice

Satoshi Kobayashi[1], Reiko Fujinawa[1], Shiho Kobayashi[1], Congxiao Gao[1], Fumi Ota[1], Shinobu Kitazume[1], Takashi Angata[2], Keiichi Yoshida[1], Takayuki Yoshida[3], Tomoko Betsuyaku[4], and Naoyuki Taniguchi[1]; [1]Disease Glycomics Team; [2]Glycan Recognition Team, Systems Glycobiology Research Group, Global Research Cluster, RIKEN-Max Planck Joint Research Center, RIKEN, Wako, 351-0198, Japan; [3]First Department of Medicine, Hokkaido University School of Medicine, Sapporo, 060-8638, Japan; [4]Division, Pulmonary Medicine, Keio University School of Medicine, Tokyo, 160-8582, Japan
skobayashi@riken.jp

Our previous studies indicated alpha 1,6 fucosyltransferase (Fut8) KO mice develop emphysematous changes due to the activation of matrix metalloproteinases (MMPs) by the dysregulation of transforming growth factor-beta signaling. There are increasing evidences to indicate that integrity and balance of extracellular matrix components are essential for normal lung function and the response to injury including emphysema. The inhibitors against MMPs are promising for the treatment of emphysema, but none of the suitable inhibitors are available at present because of the lack of specificity and the strong side effects.

Glycosaminoglycans (GAGs) are distributed in the bronchial walls and in airway secretions. They play a key role in many biological functions. We found that one of the GAGs, keratan sulfate (KS)-disaccharide (L4) inhibited MMP-9 activity *in vitro*. Thus, we determined whether exogenous GAGs, including L4 and hyaluronan (HA)-oligosaccharide, prevented or corrected the early stage of elastase-induced pulmonary emphysema *in vivo*. We intratracheally administered KS-I isolated from bovine cornea, L4, HA-tetramer (HA4) and -dodecamer (HA12) into C57BL/6 mice. Next day, we treated with porcine pancreatic elastase by intratracheal administration to induce emphysema. On the following day, we measured the accumulation of inflammatory cells and the production of tumor necrosis factor-alpha (TNF-α) and the activity of MMP-9 in the bronchoalveolar lavage (BAL) fluid. We found that KS-I significantly attenuated the accumulation of total inflammatory cells and the production of TNF-α in BAL fluid and the MMP-9 activity was suppressed in all groups except HA12-treated group as compared with the PBS-treated group. Furthermore, we also evaluated the progression of emphysema with micro-computed X-ray tomography (micro-CT) by calculation the percentage of the emphysema area to the total lung area. The analysis of micro-CT images showed that L4 and KS-I significantly suppressed the emphysema progression at 7

and 14 days after elastase administration as compared with the PBS-treated group. These results suggested that these GAGs, especially L4 and KS-I, might be effective against the lung inflammation and the early stage of emphysema formation.

294: Detection of disease associated sialoglycoconjugate specific IgG in the sera of children with acute lymphoblastic leukemia

Sujata Ghosh[a*], Sarika Kapoor[b], Ram Marwaha[b];
[a]Department of Experimental Medicine and Biotechnology, [b]Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh,160012, India.
suassi1@gmail.com

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. With existing treatment protocols, virtually all patients achieve remission and~70 % are eventually cured. However, patients in remission may have residual leukemic cells, which cannot be identified by the routine methods. Thus, the need of the hour is to develop clinically useful strategies to determine the treatment outcome and predict relapse. Change in glycosylation pattern especially aberrant sialylation in the cellular glycoconjugates is a prominent biomolecular alteration in cancer cells. The presence of disease associated glycoconjugate specific antibodies in the sera of cancer patients has been reported earlier. In the present study, we report the presence of significantly higher level of fetuin (taken as a broad spectrum sialoglycoconjugate) specific IgG (IgG_A) in the serum samples of children having ALL as compared to other non-neoplastic disorders. IgG_A level was found to be significantly higher as compared to the fetuin specific IgM and IgA level in the sera of ALL-patients. A remarkable reduction in the level of IgG_A was detected during therapy up to 1 year. To further confirm the sialoglycoconjugate specificity of IgG_A, different gangliosides [known to contain NeuAcα(2→3)Gal unit] were used and maximum specificity of IgG_A was found for GM2. IgG_A from the pooled sera of ALL-patients was isolated by sequential chromatography on Protein A-Sepharose CL-4B and Fetuin- Sepharose CL-4B. The purified IgG_A showed strong membrane positivity with CEM-CM3 cells as well as the lymphoblasts of ALL–patients. Further, the %age of IgG_A positive cells was more than 70 % in case of ALL-patients. A ~66 kDa band in the membrane fraction of CEM-CM3 cells as well as PBMCs of ALL-patients was found to interact specifically with IgG_A as well as *Maackia amurensis* agglutinin [specific for NeuAcα(2→3)Gal/GalNAc], the intensity of which was drastically reduced in presence of GM2. Taken together, IgG_A may have the potential to serve as a unique probe for detail investigation of childhood-ALL associated sialoglycoconjugate(s) present on the lymphoblasts. Further, due to high specificity of IgG_A to fetuin/GM2, it may be possible to develop a simple alternative diagnostic approach (fetuin/GM2 based ELISA) for childhood-ALL.

295: Potential importance of *Maackia amurensis* agglutinin in non-small cell lung cancer

Rakhee Chhetra[a], Sangeeta Mehta[b], Radhika Srinivasan[c], Suresh C. Sharma[b], Digambar Behera[d] & Sujata Ghosh[a];
[a]Department of Experimental Medicine & Biotechnology, [b]Department of Radiotherapy, [c]Department of Cytology & Gynaecological Pathology, Department of Pulmonary Medicine, Post Graduate Institute of Medical Education & Research,Chandigarh,160012, India

Malignant transformation is associated with alterations in cell surface carbohydrate architecture, which can be detected by lectins. Among various types of sugar residues, sialic acids are extremely important, since increased sialylation in the cellular glycoconjugates has been found to be associated with tumour progression and metastasis. Altered sialylation in cellular glycans was reported in case of non-small cell lung cancer (NSCLC). Although, drugs for treatment of NSCLC have been available for many years, but the use of such regimens are plagued by serious side effects because of their non-specific action on normal healthy cells. Thus, medical science is in need of prospective studies to develop newer targeted approaches for treatment of this life threatening disease. In this context, lectins are the potentially important biomolecules. *Maackia amurensis* agglutinin [specific for NeuNAcα (2-3) Gal/GalNAc] is gaining recognition as an important lectin, which can specifically distinguish cancer cells from normal cells and thereby it seems to have diagnostic potential in cancer of different origin. In the present study, we report that this lectin interacted strongly with human NSCLC cell lines as well as the tissue biopsies and cells obtained from fine needle aspirations of NSCLC patients, indicating the binding of this lectin to specific sialylated glycans on NSCLC cells. We have observed that this lectin selectively induced apoptosis in NSCLC cell lines but not in normal lung fibroblast cell line. The inhibition of *Maackia amurensis* agglutinin induced apoptosis in presence of GM2 and IgG_{MAA} indicated the importance of sugar binding epitope as well as antibody binding epitope of the lectin in inducing apoptosis in NSCLC cells. Our observation was further authenticated by studying this lectin induced apoptosis in the cells obtained by the fine needle aspiration of the tumour of NSCLC patients. Moreover, this lectin induced activation of apoptotic pathway in NSCLC cells was found to be accompanied by downregulation of Bcl-X_L, upregulation of Bax, release of cytochrome c

and activation of procaspase-3. Further, a synergistic effect of this lectin on paclitaxel induced cytotoxicity in NSCLC cell lines was also noted. Collectively our results have indicated the potentiality of *Maackia amurensis* agglutinin in NSCLC.

296: *N*-Glycans of IgG as a complementary biomarker to CA125 for ovarian cancer diagnosis

Yifan Qian[1], Yisheng Wang[2], Xingwang Zhang[1], Shifang Ren[1], Congjian Xu[2], Jianxin Gu[1]; [1]Key Laboratory of Glycoconjugate Research Ministry of Public Health; Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai, P.R. China, 200032; [2]Obstetrics and Gynecology Hospital, Department of Obstetrics and Gynecology of Shanghai Medical School, Fudan University, Shanghai, P.R. China, 200032
11211010023@fudan.edu.cn

Ovarian cancer (OC) is the most lethal malignancy of all gynecological cancers among women, causing more deaths of the female reproductive system. In general, elevated CA-125 level in blood is the most robust serum biomarker for OC detection. However, CA125 can give a false positive response in benign conditions due to its low sensitivity and specificity. The aim of this work was to find additional markers for the OC diagnosis which would complement the use of CA125 by analyzing the alteration of glycan of IgG in human blood. N-glycans were released from serum IgG by PNGase F digestion, purified in a solid phase extraction, permethylated und subsequently analyzed by MALDI-QIT-TOF MS. 31 ovarian cancer patients and 22 age-matched benign tumor patients were enrolled in this study. Statistical analyses were carried out using the SPSS 17.0 software. Statistical analyses show statistic significance difference between malignant and benign patients by combining the level of serum IgG-associated N-glycans with CA125. The results indicate that combinations of changes in the IgG glycans with CA125 might improve both sensitivity and specificity for acute ovarian cancer diagnosis.

297: Hydrolysis of secreted sialoglycoproteins in *ex vivo* and biochemical models of bacterial vaginosis

Warren G. Lewis[1], Lloyd S. Robinson[2], Amanda L. Lewis[2,3]; Departments of [1]Medicine, [2]Molecular Microbiology, and [3]Obstetrics and Gynecology, Center for Women's Infectious Disease Research, Washington University School of Medicine, St. Louis, MO, 63110 USA
geelewis@gmail.com, lewis@borcim.wustl.edu

Bacterial vaginosis (BV) is a common polymicrobial imbalance of the vaginal flora associated with a wide variety of obstetric and gynecologic complications including serious infections and preterm birth. As evidenced by high recurrence rates following treatment, interventions for BV are still lacking. Several hydrolytic activities, including glycosidases and proteases, have been previously correlated with BV and have been hypothesized to degrade host sialoglycoproteins that participate in mucosal immune functions. Sialidase activity is most predictive of BV status and correlates strongly with adverse health outcomes. Here we combine clinical specimens with biochemical approaches to investigate secretory immunoglobulin A (SIgA) as a substrate of BV-associated glycosidases and proteases. We show that BV clinical specimens hydrolyze sialic acid from SIgA, but not in the presence of the sialidase inhibitor dehydro-deoxy-sialic acid. The collective action of BV-associated glycosidases exposes underlying mannose residues of SIgA, most apparent on the heavily N-glycosylated secretory component of the antibody. Terminal sialic acid residues on SIgA protect underlying carbohydrate residues from exposure and hydrolysis by exoglycosidases (galactosidase and hexosaminidase). It is known that both IgG and SIgA are present in the human reproductive tract. We show that the IgG heavy chain is more susceptible to proteolysis than its IgA counterpart. Gentle partial deglycosylation of the SIgA secretory component enhanced susceptibility to proteolysis. Together, these data support a model of BV in which SIgA is subject to stepwise exodeglycosylation and enhanced proteolysis, likely compromising the ability of the reproductive mucosa to neutralize and eliminate pathogens.

Poster

298: Inhibiting activities of chitooligosaccharides on the growth of two different tumors

Haige Wu[1,2], Ziang Yao[2], Yuguang Du[1]; [1] Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian 116023, China; [2] Bioengineering College Dalian University, Dalian 116622, China
duyg@dicp.ac.cn

Anti-tumor activity of chitooligosaccharides (COS) has been reported previously, but its mechanism is not clear. In this research, two different animal models, Kunming mice bearing ascites tumor (S180) and hairless mice bearing human breast cancer (MCF-7) are used, tumor bearing mice are administered with different dose of COS, the growth of tumors are monitored. After administered with COS for

28 days, mice are sacrificed, tumor tissue are taken out and weighed, Elisa method is used to detect the contents of cytokines correlated with immunological regulation and angiogenesis in the serum of Kunming mice or hairless mice. The results show that COS can decrease the contents of VEGF (vascular endothelial growth factor) and VEGFR (vascular endothelial growth factor receptors) in both Kunming and hairless mice. In Kunming mice, COS can increase the thymus and spleen index, the contents of IL-2 and IL-6, therefore display immunological regulation activity. COS could inhibit the growth of tumor both in Kunming and hairless mice, but the inhibiting rate on S180 tumor in Kunming mice (maximum 65 %) is greater than that of on human breast cancer in hairless mice (maximum 47 %). Maybe this is because that in hairless mice COS couldn't display its immunological regulation activity. All the results indicate that COS have anti-tumor activity, and its anti-tumor activity is correlated with its anti-angiogenic and immunological regulation activities.

299: Ginseng pectin potentiates TRAIL-induced apoptosis through upregulation of death receptors

Hairong Cheng, Qi Sun, Guihua Tai, Yifa Zhou; School of Life Sciences, Northeast Normal University, 130024, Changchun, Jilin Province, China
chenghr893@nenu.edu.cn

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), is a potent cancer cell-specific apoptosis-inducing agent with little to no effect on normal tissues. However, acquired resistance of cancer cells to TRAIL is a roadblock, allowing them to evade the pro-apoptotic effects of TRAIL. Ginseng pectin exerts significant anti-tumor effect and potentiates anti-tumor effect of a series of chemotherapeutic drugs. In the present report, we investigated the effect of ginseng pectin on cancer cell apoptosis induced by TRAIL. We found that two homogenous homogalacturonan with small percentage of type-I rhamnogalacturonan domains (WGPA-3-RG and WGPA-4-RG) and two Heat-treated homogenous homogalacturonan (WGPA-3-HG and WGPA-4-HG) not only potentiated TRAIL-induced apoptosis in HCT116 colon cancer cells but also sensitized TRAIL-resistant colon cancer cells HT-29 to the cytokine. At a mechanistic level, ginseng pectin downregulated cell survival proteins, including Bcl-2, Mcl-1 and Bcl-XL, and upregulated pro-apoptotic protein Bax and the expression of TRAIL death receptors DR5. Gene silencing of DR5 by short hairpin RNA reduced the apoptosis induced by combination treatment of Ginseng pectin and TRAIL. Induction of DR5 by ginseng pectin was independent of p53 and Bax, but was dependent on production of reactive oxygen species (ROS), and sequestering of ROS abolished both upregulation of the receptor and potentiation of TRAIL-induced apoptosis. Taken together, our results provide the first mechanistic evidence that ginseng pectin treatment render cancer cells more sensitive to the cytotoxic activities of TRAIL, suggesting that Ginseng pectin can be given in combination with TRAIL, especially for those tumors that develop resistance to TRAIL.

300: Chitosan Oligosaccharides Protect Human Monocytes U937 from LPS-induced Inflammatory Damage through Blockade p38 MAPK Phosphorylation and Increasing O-GlcNAcylation of Proteins

Yu Li[1,2], Qiang Peng[1,2], Qingsong Xu[1], Yuguang Du[1]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China; [2]Graduate School of Chinese Academy of Sciences, Beijing 110864, China
articles1805@gmail.com

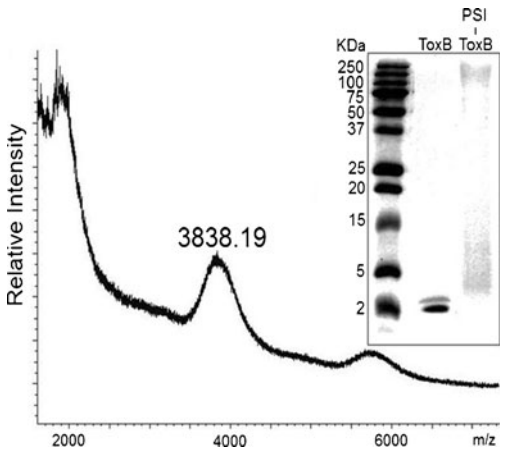
To investigate the effects of Chitosan oligosaccharides(COS) with different degree of polymerization(DP) on LPS-induced inflammation, U937 human monocytes were cultured with DP2-8(COS-A) and DP7-15(COS-B) respectively, which were prepared by our group. The results showed that both kinds of COS demonstrated obviously anti-inflammatory effects against LPS-induced over-expression of TNF-α and IL-8. Signal transduction studies indicated COS-A and COS-B efficiently down-regulated LPS-induced the phosphorylation of p38 MAPK. In several documented instances, phosphorylation and *O*-GlcNAc modification are reciprocal, occurring at the same or adjacent hydroxyl moieties. In this study, we also find COS-A and COS -B could significantly increase *O*-GlcNAc modification of proteins in LPS-induced U937 monocytes. Finally, we postulate that COS-A and COS-B may have anti-inflammatory effects *via* suppression of the expression levels of TNF-α and IL-8, regulated by p38 MAPK pathways and *O*-GlcNAcylation of proteins.

301: The Creation of Clostridium difficile Glycoconjugate Vaccines

Yuening Jiao, Zuchao Ma, Doug Hodgins, Brittany Pequegnat, Lisa Bertolo, Luis Arroyo, Mario A. Monteiro; Department of Chemistry, University of Guelph, Guelph, ON, N1G 2W1, Canada
jiaoy@uoguelph.ca

Clostridium difficile is the most common cause of antimicrobial-associated diarrhea in humans and may cause death. The bacterium expresses three phosphorylated polysaccharides, which we named PSI, PSII and PSIII. Due to the fact that PS-I is not expressed in great amount in vitro, we

developed a glycoconjugate containing a synthetic PSI pentasaccharide repeating unit carrying a linker at the reducing end, α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→O$(CH_2)_5NH_2$ by a linear synthesis strategy from four monosaccharide building blocks. The synthesized PSI pentasaccharide was conjugated to a subunit of *C. difficile* exotoxin B yielding a potential dual *C. difficile* vaccine. More significantly, sera from healthy horses were shown here to contain natural anti-PSI IgG antibodies that recognized the synthetic PSI pentasaccharide.



Poster Session II-Cell Biology

302: N-Acetylglucosaminyltransferase V triggers overexpression of MT1-MMP and reinforces the invasive/metastatic potential of cancer cells

Juhee Lee, Jeong Gu Kang, Kyoungjin Song, Seong Kook Jeon, Sejeong Oh, Yong-Sam Kim and Jeong-Heon Ko; Cancer Biomarkers Development Research Center, KRIBB, Daejeon 305-806, Korea
kang@kribb.re.kr, Tel:82-42-860-4135

N-Acetylglucosaminyltransferase V (GnT-V) is an enzyme that catalyzes the formation of a β1,6-N-acetylglucosamine (GlcNAc) side chain to a core mannosyl residue in N-linked glycoproteins. Besides its direct function of producing aberrant glycoproteins, it promotes cancer progression by its involvement in the stimulation of oncoproteins. Herein, we report that GnT-V guided the transcriptional activation of membrane-type matrix metalloproteinase-1 (MT1-MMP) in cancer cells. The activated MT1-MMP expression had dual effects on cancer progression. It not only promoted proteolytic activity for cancer cells *per se*, but also led to the activation of MMP-2. Consequently, the activation of the two MMPs triggered by GnT-V intensified the invasive potential. A quantitative analysis using clinical tissues revealed a relatively strong correlation between GnT-V overexpression and MT1-MMP upregulation. In this study, we report for the first time that GnT-V directs cancer progression by modulating MMPs in cancer.

303: Role of bacterial surface factors in adherence of enteric bacteria to plant glycolipid

Yannick Rossez[1], Ashleigh Holmes[1], Louise Isager Ahl[2], Henriette Lodberg Pedersen[2], Louise Birse[1], William GT Willats[2], Ian Toth[1] and Nicola Holden[1]; [1]Cellular and Molecular Sciences, James Hutton Institute, Dundee, Scotland, United Kingdom, [2]Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark.
Yannick.Rossez@gmail.com

It is generally recognized that bacterial attachment to plant or animal surfaces constitutes the first step in colonisation. This mechanism is mediated by interactions between adherence factors that are expressed on the bacterial cell surface, such as fimbriae or flagella, and host cell surface glycans. For example, *E. coli* type 1 fimbriae are well characterised structures for their interaction with mammalian epithelial cells and are known to recognise specific glycans present on host cell surfaces, such as α-D-mannose. *In planta,* recent papers have demonstrated the implication of curli fibers and flagella from Enteropathogenic *E coli* and *Salmonella* species respectively. However the molecular recognition pattern implicated in adhesion of human pathogen bacteria to plant tissues has yet to be described.

In this work plant glycan arrays have been used to investigate specific interactions with bacterial adherence factors. We found in this study a specific interaction between FliC, the flagella main structural subunit, and ionic plant membrane lipids. The work provides an improved understanding about the molecular mechanisms underpinning human foodborne pathogens with plants.

304: HSF1 and Sp1 are involved in regulation of FUT4 gene expression and cell proliferation in breast cancer cells

Xuesong Yang[1], Shuai Liu[1], Qiu Yan[1]; [1]Department of Biochemistry and Molecular Biology, Dalian Medical University, Liaoning Provincial Core Lab of Glycobiology and Glycoengineering, Dalian, 116044,People's Republic of China
yxmsnow@126.com

Lewis Y (LeY) is a carbohydrate tumor-asssociated antigen. The majority of cancer cells derived from epithelial tissue express LeY type difucosylated oligosaccharide. Fucosyltransferase IV (FUT4) is an essential enzyme that catalyzes the synthesis of LeY oligosaccharide. In our previous study, we found that FUT4 was associated with the cell proliferation. However, despite the important role of FUT4 in cancer proliferation and apoptosis, little is known about the mechanisms of its transcription regulation. In this study, we investigated the transcriptional regulation of human FUT4 in human breast cancer. We compared the transcriptional regulation of the human FUT4 gene in human breast cancer cells (MCF-7 and MDA-MB-231) by promoter/luciferase analyses. Using a series of promoter deletion constructs, we indentified that a potential regulatory site that is located between 0.8 and 1.6 kb of the FUT4 promoter. As shown by EMSA and ChIP analysis, HSF1 and SP1 were required for promoter activity. In addition, we explored the role of HSF1 and SP1 on cell proliferation. We found that ERK1/2 MAPK and PI3K/Akt signaling pathways regulate the expression of FUT4, which play a role in the cell proliferation, *via* HSF1 and SP1. These results suggest that FUT4 is a target gene for HSF1 and SP1 that is required for cell cycle progression in breast cancer epithelial cells.

Key word: Fucosyltransferase IV; breast cancer; HSF1; Sp1; transccription regualtion

305: Effects of Cell Surface α2,3-Sialic Acid on Osteogenesis

Lan Xu, Zhi Jiang, Shiliang Wu; Department of Biochemistry and Molecular Biology School of Medicine, Soochow University, Suzhou, 215007.

A cell culture model of osteoblast differentiation was applied in our study of the effect of sialic acid on the osteogenesis by using the pre-osteoblast of MC3T3-E1 subclone 14 cells. Following the treatment of different concentrations of α2,3-neuraminidase, which specifically removed the α2,3-sialic acid from cell surface, a significant decrease of α2,3-sialic acid was detected with fluorescein isothiocyanate (FITC)-labeled *Maackia amurensis* lectin (MAL-II) by flow cytometry analysis. von Kossa staining showed that the bone mineralization decreased in MC3T3-E1 subclone 14 cells after the treatment of α2,3-neuraminidase for 2 weeks. However α2,3-neuraminidase did not affect the formation of osteoblasts in MC3T3-E1 subclone 14 cells, which was demonstrated by positive alkaline phosphatase (ALP)-staining. Characteristic biological markers and osteoblast-like cell-related factors of osteoblastic cells were also examined. Both RT-PCR and Western blot analysis demonstrated that the expression of bone sialoprotein (BSP), osteoprotegerin (OPG), and vitamin D receptor (VDR) were significantly decreased when α2,3-sialic acid expression decreased on the cell surface, while the expression of osteocalcin (OC) and osteopontin (OPN) remained unchanged. We propose a hypothesis that α2,3-sialic acid affects bone mineralization but not osteogenic differentiation.

306: Regulation of the cell surface expression level of AMPA-type glutamate receptor by N-linked glycans

Jyoji Morise, Yusuke Takeuchi, Shogo Oka; Human Health Sci., Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan shogo@hs.med.kyoto-u.ac.jp

AMPA-type glutamate receptors (AMPAR) are hetero or homotetrameric complexes composed of various combinations of four subunits (GluA1-4). AMPAR mediate most of the fast excitatory synaptic transmission in the nervous system. Their cell surface expression level and abundance in postsynaptic region modulate synaptic plasticity. So far, we found that a unique trisaccharide (HSO_3-3GlcAβ1-3Galβ1-4GlcNAc), named human natural killer-1 (HNK-1) carbohydrate specifically expressed on N-linked glycans of GluA2, regulated cell surface expression level of AMPAR and spine maturation process. Since the particular carbohydrate possesses such functions to AMPAR, it is speculated that other N-linked glycans expressed on AMPAR also have a potential to regulate functions of AMPAR. In this study, to elucidate the role of N-glycans on GluA1 and GluA2 (major subunits of AMPAR), we generated a series of GluA1 and GluA2 mutants of which Asn residue in the consensus sequence (N-X-S/T) was mutated to Ser, and investigated cell surface expression levels of these mutants by using a cell surface biotinylation assay. As a result, cell surface expression levels of GluA1N63S, N363S and GluA2N370S were decreased compared with those of wild-type (WT) GluA1 and GluA2, and these mutants were mainly distributed in ER. The cell surface expression level of GluA1N363S was increased by co-expression of GluA2WT but not GluA1N63S whereas that of GluA2WT was unchanged regardless of co-expression of GluA1 mutants, suggesting that N-linked glycans at N63 and N363 on GluA1 had different roles in trafficking from ER to cell surface. In contrast, co-expression of GluA1WT had little effect on the cell surface expression level of GluA2N370S but rather GluA1WT was likely to accumulate in ER, suggesting that the trafficking of GluA1 was controlled by N-linked glycan at N370 on GluA2. These results show that the cell surface expression level of AMPAR was regulated by N-linked glycans in a different manner.

307: Synthesis and antiviral activity of amino-glycoglycerolipids

Jun zhang, Chunxia Li, Huashi Guan; Key Laboratory of Marine Drugs, Ministry of Education, School of medicine and pharmacy, Ocean University of China, Qingdao 266003, P. R. China
lchunxia@ouc.edu.cn

Various Glycoglycerolipids have been isolated from algae, which have shown many bioactivities, such as tumor growth and DNA polymerase inhibition, fatty acid synthase inhibiton, glucose-lowing effect and anti-inflammatory action *et al.* Aminoglycoglycerolipid, 1,2-dipalmitoyl-3-(*N*-palmitoyl-6′-amino- 6′-deoxy-α-D-glucosyl)-*sn*-glycerol, which was isolated from an algal species showed high activity against the enzyme Myt1-kinase. Due to the unique structure and activity, we had synthesized its 22 analogues by altering the length of acyl chains and glycoside residues for bioactivity screening.

All the glycoglycerolipids were evaluated for anti-influenza A virus (IAV) activity by the cytopathic effects (CPE) inhibition assay. Compared with ribavirin, some compounds showed better protection from virus-induced cell death. The results indicated that the glycosyl residue and long acyl chain played critical roles in improving the inhibition of IAV multiplication in MDCK cells. Further structure–activity relationship study is underway.

308: T synthase Glycosylates and Activates c-MET in Hepatocellular Carcinoma Cells

Min-Chuan Huang[1], Chiung-Hui Liu[1], Yao-Ming Wu[2], John Huang[2]; [1]Graduate Institute of Anatomy and Cell Biology, National Taiwan University College of Medicine, Taipei 100, Taiwan, [2]Department of Surgery, National Taiwan University Hospital, Taipei 100, Taiwan
mchuang@ntu.edu.tw

Altered glycosylation has been recognized as a hallmark of cancer. T synthase, also named core 1 β1,3-galactosyltransferase (C1GALT1), controls the formation of mucin-type O-glycans. Although these carbohydrates play crucial roles in a variety of cancers, the expression and function of T synthase in hepatocellular carcinoma (HCC) have never been studied. Here we showed that T synthase mRNA and protein were frequently overexpressed in HCC tumors compared with non-tumor liver tissues. T synthase expression significantly correlated with increased histologic grade and metastasis of HCC. Knockdown of T synthase suppressed HCC cell growth and caused G1 arrest of cell cycle *in vitro* and decreased tumor growth *in vivo*. Conversely, overexpression of T synthase in Sk-Hep1 cells enhanced cell growth *in vitro* and *in vivo*. Interestingly, we found that T synthase regulated hepatocyte growth factor (HGF)-mediated phosphorylation of c-MET in HCC cells, and the expression level of phospho-c-MET was significantly associated with that of T synthase in primary HCC tissues. Mechanistic investigation showed that c-MET was decorated with O-glycans revealed by *Vicia villosa* agglutinin (VVA) and peanut agglutinin (PNA) binding. Moreover, T synthase modified the *O*-glycosylation and HGF-induced dimerization and activation of c-MET. Together, our results suggest that T synthase is frequently overexpressed in hepatocellular carcinoma and activates HGF signaling *via* modulation of O-glycosylation and dimerization of c-MET. These findings provide novel insights into the role of O-glycosylation in the pathogenesis of HCC.

309: Dietary of Chitooligosaccharides Supplementation Enhances the Reproductive Performance of Sows

Lun X. Wang[1], Yu G. Du[1*]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, PR China;

[Objective] The swine breeding efficiency in China is relatively low compared with that from other developed country such as America. The aim of this study was to investigate the effect of dietary chitooligosaccharides (**COS**) on the reproductive performance of sows. [Method] The pregnant sows (Landrace×Large White Sows) after mating were assigned randomly into four groups in corn-soybean-based diets with or without COS, including COS group, Jerusalem artichoke powder (JAP) group, Several polysaccharides mixed (SPM) group (containing COS),and the Control group (basal diet). [Result] SPM supplement improved ($P<0.05$) total piglets born and born alive compared with that in Control group (12.3±0.5/10.5±0.6, 11.7±0.4/9.8±0.5). COS supplement improved one more piglets born and born alive compared with Control group (11.7±0.5/10.5±0.6, 11.0±0.4/9.8±0.5), although there was no significant difference between them. Meanwhile, both COS supplement and SPM supplement improved ($P<0.05$) born litter weight compared with Control group (17.5±0.8/18.4±0.6/14.0±0.9). In addition, COS group improved ($P<0.05$) born individual weight compared with Control group (1.7±0.05/1.5±0.04). [Conclusion] The results indicated that dietary COS can markedly improve the reproductive capacity of sows.

310: Human recombinant Intelectin-1 expressed in a heart capillary endothelial cell line displays unique carbohydrate binding specificity

Jin Kyu Lee, Jonathan Viola, Kiara Swanier and J. Michael Pierce; Department of Biochemistry and Molecular Biology and Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30605
jinkyu@uga.edu

Since Intelectins (Omnetins) were first identified as homologs of the *Xenopus laevis* oocyte cortical granule lectin (XL35), members of the family of intelectins have been reported from many eukaryotes, including ascidians, fishes, frogs and humans. In addition to its function in formation of the fertilization, which serves as a block to sperm and microbes, intelectins have been studied as a mediator during pathogen surveillance. The infection by several pathogens induces a dramatic increase in the expression of Intelectin transcripts in intestinal and bronchial epithelial cells, mediated by IL-13. Reports have suggested that very low levels of recombinant int-1 could be secreted from transfected cells. Since we have shown that Int-1 is constitutively expressed in human heart endothelial cells (and not in most types of endothelial cells), we attempted to express cDNAs of human Int-1 and -2 in stably transfected H5V cells, a mouse heart capillary endothelial cultured line, to produce significant levels of secreted proteins. The recombinantly expressed Int-1 was secreted into the media. MS analysis determined that Int-1 was not a GPI-anchored protein in H5V cells. There have been some reports that Int-1 binds specifically to galactofuranose coupled to agarose; however, H5V-secreted, recombinant Int-1 bound to native agarose resin and was eluted by EDTA, similar to our report for XL35, but also by 100 mM ribose. The eluted protein fraction showed a single band in silver staining after SDS-PAGE resulting in a yield of X mg of purified Int-1/Y ml of starting culture media. Glycan binding array analysis by Core H of the CFG showed binding of Int-1 was to the synthetic glycan: GlcNAcβ1-4Galβ1-4GlcNAcβ-Sp8, which has not been reported in humans. The binding specificity for XL35 on the same array was clearly Galα1-3GalNAc, by contrast. Although Int-1 binds tightly to agarose and with low affinity to the synthetic glycan above in a calcium-dependent manner, a high affinity glycan ligand in humans or human pathogens has not yet been identified.

311: GOLPH3 regulates integrin-mediated cell migration *via* up-regulation of sialylation of β1 integrin

Tomoya Isaji, Wei Gu, Tomohiko Fukuda and Jianguo Gu; Division of Regulatory Glycobiology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai Miyagi, 981-8558, Japan
tfukuda@tohoku-pharm.ac.jpisaji@tohoku-pharm.ac.jp

Recently, a Golgi protein, GOLPH3 was identified as a new oncogene that is commonly amplified in human cancers. An ortholog of GOLPH3, VPS74p has been reported to be essential for glycosyltransferase activation of yeast. To investigate whether the expression of GOLPH3 was involved in the cell migration and N-glycosylation in mammalian cells, we performed a loss-of-functional study. Cell migration on fibronectin or laminin was suppressed in GOLPH3 knockdown (KD) cells, and the suppression was restored by re-introduction of GOLPH3 gene. The cell migration was abolished in the presence of neutralized anti-β1 integrin antibody. Interestingly, N-glycosylation status of β1 integrin obtained from KD cells was apparently different from those in control cells, while it was normalized in the restored cells. HPLC and LC/MS analysis showed that the sialylation level of N-glycans was specifically decreased in KD cells, and the aberrant N-glycosylation was significantly rescued in the restored cells. To explore the specific effect on sialylation by GOLPH3 expression, we examined the interactions between a glycosyltransferase and GOLPH3, and found GOPLH3 specifically associated with α2,3-sialyltransferase IV, but not β1,4-galactosyltransferase I. Furthermore, the cytoplasmic tail of α2,3-sialyltransferase IV was shown to be important for the association. Taken together, these results suggest that GOLPH3 regulates N-glycosylation status of β1integrin and it's biological functions, which may give a new insight for the functions of GOLPH3 in cancer.

312: Large protein complexes retained in the ER are dislocated by non-COPII vesicles and degraded by selective autophagy

Valerie Le Fourn[1,4,*], Sujin Park[2,*], Insook Jang[3,*], Katarina Gaplovska-Kysela[1,5], Bruno Guhl[1], Yangsin Lee[2], Jin Won Cho[2,3], Christian Zuber[1], Jürgen Roth[1,2]; [1]Division of Cell and Molecular Pathology, Department of Pathology, University of Zurich, CH-8091 Zurich, Switzerland, [2]Department of Integrated OMICS for Biomedical Science, WCU Program, Yonsei University Graduate School, Seoul 120-749, Korea, [3]Department of Systems Biology, Yonsei University, Seoul 120-749, Korea, [4]Present address: Selexis SA, CH-1228 Plan-les-Ouates/Geneva, Switzerland, [5]Present address: Department of Genetics, Comenius University, SR-84215 Bratislava, Slovak Republic
* These authors contributed equally to this study.
jurgen.roth@yonsei.ac.kr, Tel:82-2-2123-7673, Fax:82-2-312-5657

Multisubunit protein complexes are assembled in the endoplasmic reticulum (ER). Existing pools of single subunits

and assembly intermediates ensure the efficient and rapid formation of complete complexes. While being kinetically beneficial, surplus components must be eliminated to prevent potentially harmful accumulation in the ER. Surplus single chains are cleared by the ubiquitin–proteasome system. However, the fate of not secreted assembly intermediates of multisubunit proteins remains elusive. Here we show by high-resolution double-label confocal immunofluorescence and immunogold electron microscopy that naturally occurring surplus fibrinogen Aα–γ assembly intermediates in HepG2 cells are dislocated together with EDEM1 from the ER to the cytoplasm in ER-derived vesicles not corresponding to COPII-coated vesicles originating from the transitional ER. This route corresponds to the novel ER exit path we have previously identified for EDEM1 (Zuber et al. Proc Natl Acad Sci USA 104:4407–4412, 2007). In the cytoplasm, detergent-insoluble aggregates of fibrinogen Aα–γ dimers develop that are targeted by the selective autophagy cargo receptors p62/SQSTM1 and NBR1. These aggregates are degraded by selective autophagy as directly demonstrated by high-resolution microscopy as well as biochemical analysis and inhibition of autophagy by siRNA and kinase inhibitors. Our findings demonstrate that different pathways exist in parallel for ER-to-cytoplasm dislocation and subsequent proteolytic degradation of large luminal protein complexes and of surplus luminal single-chain proteins. This implies that ER-associated protein degradation (ERAD) has a broader function in ER proteostasis and is not limited to the elimination of misfolded glycoproteins.

313: Selective autophagy receptors are involved in degradation of EDEM1

Insook Jang, Sujin Park, Jin Won Cho and Jürgen Roth; Department of Biology, Yonsei University, Seoul, 120-749, South Korea
o-glcnac@yonsei.ac.kr

Misfolded and mis-assembled glycoproteins are retained in the endoplasmic reticulum (ER) where they are exposed to the protein folding machinery and protein quality control. The UPR (Unfoled Protein Response) is activated in response to an accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum. Eventually, misfolded and mis-assembled glycoproteins are targeted for degradation by a process called ER-associated protein degradation (ERAD). EDEM1 is an ERAD component that recognizes misfolded luminal glycoproteins and is routing them for dislocation to the cytosol. This is classically followed by their degradation. Although EDEM1 was initially proposed to be lectin–like and to react with $Man_8GlcNAc_2$ oligosaccharides of glycoproteins, its complex mode of interaction with substrates has become clear only recently. However, still very little is known about the turnover and degradation mechanism of EDEM1 and how this relates to the fate of its substrates. We already reported that EDEM1 becomes rapidly degraded and that this occurs by basal autophagy. Here, we provide detailed insight into the mechanism by which EDEM1 becomes degraded. After its dislocation to the cytosol, EDEM1 is apparently making complexes with the selective autophagy receptors p62, NBR1 and Alfy. We observed co-distribution of EDEM1 and selective autophagy receptors by double or triple confocal laser scanning immunofluorescence. By quantifying the relationship of EDEM1 and the selective autophagy receptors as visualized by confocal laser scanning immunofluorescence, dramatical changes were observed in HepG2 cells following inhibition of autophagy by wortmannin treatment. These changes were fully reversible upon wortmannin wash-out. In addition, we observed its ubiquitination after dislocation to the cytosol. This demonstrates that the ERAD component EDEM1 itself undergoes ERAD involving selective autophagy.

Acknowledgment
This work was supported by the World Class University program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (R31-2008-000-10086-0) and a grant from the National Research Foundation of Korea by the Ministry of Education, Science and Technology (2010-0027736). I.J. is a fellowship awardee of the Brain Korea 21 program.

314: GnT-V attenuates TGF-β-induced EMT and metastatic potential in lung cancer

Na Li[1], Haineng Xu[4], Xijun Liu[1], Kun Fan[1,2,3], Jingjing Qi[1,2,3], Liying Wang[1,2,3], Zengxia Li[1,2,3], Xiliang Zha[1,2,3];
[1] Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai,200032, China, [2]Key Laboratory of Glycoconjugate Research, Ministry of Health, Shanghai 200032, China, [3]Key Laboratory of Molecular Medicine, Ministry of Education, Shanghai, 200032,China, [4]China Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031,China
lizengxia@gmail.com; xlzha@shmu.edu

N-acetylglucosaminyltransferase V (GnT-V), a key enzyme catalyzing β1, 6-N-acetylglucosamine branching on asparagine-linked oligosaccharides of cell proteins, is overexpressed and as a metastasis-promoting oncoprotein in invasion/metastatic colon and breast

cancers. However, here, we found that GnT-V expression levels were strongly reduced during EMT of human alveolar basal epithelial cells, in small cell lung cancers and non-small cell lung cancers (NSCLC) except squamous cell carcinomas. Further study showed that suppression of β1-6GlcNAc glycosylation by swainsonine and knockdown of GnT-V expression by stably transfected with GnT-V shRNA in A549 cell line advanced EMT-like changes, cell-ECM adhesion, cell migration and invasion potential induced by TGF-β, whereas additional expression of GnT-V reduced TGF-β-induced-EMT, invasion and metastasis. Furthermore, we found that down regulation of GnT-V expression promoted metastasis formation by enhancing TGF-β/Smad signaling, actin reorganization and TGF-β/FAK signaling, which facilitates an EMT-like switch, cells spreading, cellular adhesion to extracellular matrix proteins, thereby allowing efficient cell migration and invasion of metastatic lung cancer cells. Taken together, our findings reveal a novel mechanism of GnT-V as a suppressor of both EMT and metastasis in human lung cancer cells *via* TGF-β/Smad, TGF-β/FAK signalings inhibition that suppresses lung cancer metastasis.

315: Glycan Profiling and Quantitation of Cancer Cell Exosomes and their Origin Cell Lines for Biomarker Discovery

Seunghyup Jeong[1], Serenus Hua[1], Do-Young Choi[2], Pyong-Gon Moon[3], Rudolf Grimm[1,4], Kwang Pyo Kim[2], Moon Chang Baek[3], and Hyun Joo An[1*]; [1]Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 305-764, Korea, [2]Department of molecular biotechnology, Konkuk University, Seoul 143-701, Korea, [3]School of Medicine, Kyungpook National University, Daegu 700-842, Korea, [4]Agilent technologies, Santa Clara, California, USA
shjeong0512@gmail.com

Exosomes (circulating microvesicles, extracellular microvesicles, or microparticles) are small membrane-enclosed vesicles in body fluids such as blood, urine and ascites that are secreted by various cell types, including tumor cells. These vesicles play an important role as mediators in extracellular communication. They are composed of phospholipid bilayer membrane, cellular proteins, DNA, and RNA derived from their origin cells. It is also known that exosomes are involved in tumor metastasis, angiogenesis, and antitumor immunity in cancer cells. These biological functions are probably due to the glycosylation on their membrane proteins. Thus, the study of glycosylation of exosomes will be another potential source of new biomarker. However, there is a little study about the glycosylation of exosomes. Here, we targeted and analyzed N-glycans of exosomes derived from five cancer cell lines (A549, PC9, PC9/ZD, MCF-7, and MDA-MB231) using nano-LC/MS. We also have compared glycans of exosomes with glycans on their origin cell membrane to examine glycosylation correlation between origin cells and exosomes on cancer cell lines. Additionally, we have compared anti-cancer drug resistant cell line PC9/ZD (Gefitinib resistant) and untreated PC9 in terms of origin cells and exosomes glycan profiling *via* isomer separation. Exosomes and cell membranes were prepared from three lung cancer cell lines: A549, PC9, and PC9/ZD. And two breast cancer cell lines: MCF-7 and MDA-MB231. These two types of samples were isolated by ultracentrifugation. Glycans were directly released by PNGase F, and then enriched by graphitized carbon solid phase extraction. Nano-LC/chip Q-TOF MS was used for overall glycan profiling and quantitation. We successfully release and profile N-glycans from exosomes. Origin cells and exosomes of lung cancer contain high-mannose glycans in abundance. Although, exosomes have less high-mannose glycans compared with origin cells. Both origin cells and exosomes, isomer separation of sialylated glycans are different between PC9 and PC9/ZD. On breast cancer exosomes, two different metastatic samples represent quite different profiling. This is the first study of comprehensive glycan profiling of exosomes using mass spectrometry.

316: Isolation and Identification of Hyaluronan-degrading Bacterial Strain and Anti-obesity Effects of Low-molecular Weight Hyaluronan

Chang Won Lee[1†], Joo Woong Park[2†], Woon Seop Shin[3], Doo Jin Choi[1], Jisun Lee[1], Seul Lee[1], Ji Won Choi[1], Young Hwan Kim[2], and Yong II Park[1*]; [1]Department of Biotechnology, The Catholic University of Korea, Bucheon, Gyeonggi-do 420-743, Korea, [2]Biostream Technologies, Yongin-si, Gyeonggi-do 446-930, Korea, [3]The Kwandong University of Korea, Gangneung-si, Gangwon-do 210-701, Korea
yongil382@catholic.ac.kr

Hyaluronan (HA), also called hyaluronic acid or hyaluronate, is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. Hyaluronan, a core component of the extracellular matrix, comprises a repeating disaccharide of *N*-acetylglucosamine and *D*-glucuronic acid. A marine bacterial strain that showed confluent growth on a minimal medium containing of hyaluronan as the sole carbon source was isolated and identified based on the 16S rDNA sequence analysis as a strain of *Vibrio splindidus,* and thus named *Vibrio splindidus* BST-398.

Low-molecular weight HA (LMW-HA) was produced from the high-molecular weight HA (HMW-HA, 1,922 kDa) after the reaction with a portion of culture filtrate as a crude enzyme preparation for 4 h at 30 °C. The molecular weight of the reaction product (LMW-HA) was estimated to be approximately 43.5 kDa by size exclusion HPLC (SEC). The anti-adipogenic effect of LMW-HA was evaluated using 3T3-L1 preadipocyte cells. Both LMW-HA and HMW-HA did not show any detectable level of cytotoxicity on 3T3-L1 preadipocyte cells at up to 200 μg/ml. Previously, we showed that LMW-HA causes a significant reduction in intracellular lipid drops by 53 % when measured by Oil Red O staining while the HMW-HA showed no effect. The mRNA expression of PPAR-γ and aP2 in 3T3-L1 preadipocyte cells maintained in adipocyte-induction media with 200 μg/ml LMW-HA significantly and dose-dependently decreased by 56 % and 55 %, respectively, whereas HMW-HA treatment resulted in only negligible effect (5 % and 5 % reduction, respectively). PPAR-γ and aP2 are known as the major adipocyte differentiation specific markers. In the present study, we attempted to further confirm these observations by measuring intracellular triglyceride (TG) levels and the levels of differentiation marker proteins like PPAR-γ and aP2. When measured by TG Colorimetric Assay Kit (sigma), the TG level in LMW-HA treated cells significantly, dose-dependently decreased by 53 % at 200 μg/ml. Western blot analysis also showed dose-dependent decreases in the protein levels of PPAR-γ and aP2 in LMW-HA treated cells. Taken collectively, these results clearly suggested that the LMW-HA may have an anti-obesity effect by inhibition of the differentiation of preadipocyte cells whereas the higher molecular weight HA (HMW-HA) have no anti-obesity effect.

† These two authors contributed equally to this work.

317: The Identification of Nucleocytoplasmic Shuttling Mechnism of O-GlcNAcase and Its PTMs

Yeon Jung Kim[1], **Jin Won Cho**[1, 2]; [1]Department of Biology, Yonsei University, Seoul, 120-749, Korea, [2]Department of integrated OMICS for Biomedical Science, WCU Program of Graduate School,Yonsei University, Seoul, 120-749, Korea
scientist@yonsei.ac.kr

O-GlcNAcase (OGA) is the enzyme which catalyzes the removal of *O*-GlcNAc moiety on its substrates. This enzyme has two isoforms, full OGA and variant OGA that has alternative stop codon within intron which is not spliced-out. As a result, variant OGA lacks C-terminal HAT (histone acetyl transferase) domain and it contains additional 15 amino acids. People believed that full OGA exists both in cytosol and nucleus, primarily to the cytoplasm. There were two opposite reports about the subcellular localization of OGA isoforms by different research groups. The first group figured out that full OGA localizes in cytoplasm, whereas variant OGA exists within nucleus in 2001. However, recently the other group reported that full OGA localized diffusely throughout the nucleus and cytoplasm, whereas variant OGA resides with lipid droplets in cytosol. These two finding motivates our laboratory to commence this study as to nucleocytoplasmic shuttling regulation of two isoforms.

We also focus on the various post-translational modifications on OGAs. Full OGA should weigh 103 kDa, however, it weighs approximately 130 kDa. So we speculate that various modifications might be related to OGA's subcellular distribution as well.

To verify the nucleocytoplasmic regulation, we first overexpressed FLAG tagged-OGA isoforms in HeLa cells to trace the subcellular localization of them. Then we made three C-terminal domain deletion mutants to know the critical domain for its localization. And we also check modification occurred on OGAs by using immune-precipitation and mass spectrometry. Consequentially we can get schematic drawing as to OGA's nucleocytoplasmic shuttling, and we expect that this research makes it easy to approach *O*-GlcNAc modification.

Acknowledgment
This work was supported by the World Class University program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (R31-2008-000-10086-0) and a grant from the National Research Foundation of Korea by the Ministry of Education, Science and Technology (2010-0027736).

318: The Connection between Increased *O*-GlcNAcylation by stortage of Glucose and Cancer Metastasis

Eunah Kim[1], **Jin Won Cho**[1,2]; [1]Department of Integrated OMICS for Biomedical Sciences, Graduate School, Yonsei University, Seoul 120-749, Korea, [2]Department of Biology, Yonsei University, Seoul 120-749, Korea
bioniceunah@hanmail.net, Tel:82-2-2123-7673

Most cancer cells need more glucose than normal cell. because they produce energy by fermentation, even though they exist in the presence of oxygen. Cancer cell can escape a poor environment like hypoxia and undernutrition condition. Considering this fact, It is deducible that hypoglycemic

condition affect cancer metastasis. But the research about cancer metastasis has been limited to hypoxia condition until now. Interestingly, it was reported that *O*-GlcNAc modification on protein was significantly increased under hypoglycemic condition.

O-GlcNAcylation is one of the post-translational protein modification and it appears to be involved in many different cellular activities. And it was thought that *O*-GlcNAcylation is also related with EMT(Epithelial–mesenchymal transition).

In our study, we want to examine whether hypoglycemic condition can induce cancer metastasis and it is related to increased *O*-GlcNAc modification or not. So we focused on EMT to visualize cancer metastasis. We found that the amount of several EMT marker were changed under hypoglycemic condition in cancer cell. And one of them was *O*-GlcNAcylated.

These findings tell us that hypoglycemic condition can be closely connected with cancer metastasis *via* *O*-GlcNAclation. So we suggest possibilities that hypoglycemic condition induced EMT in cancer cell and it related with increased *O*-GlcNAc modification.

Acknowledgment
This work was Supported by the World Class University program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (R31-2008-000-10086-0) and a grant from the National Research Foundation of Korea by the Ministry of Education, Science and Technology (2010-0027736).

319: Structural and functional analysis of the Alg1 glycosyltransferase involved in N-glycosylation of endoplasmic reticulum

Sha Xu [1,2], Sheng-Tao Li[1,2], Xiao-Dong Gao [1,2*]; [1]School of Biotechnology, Key Laboratory of Glycobiology and Biotechnology, Ministry of Education, Wuxi 214122, China, [2]State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China
xus1984@gmail.com, xdgao@jiangnan.edu.cn

ALG1 encodes an essential beta-1,4-mannosyltransferas that adds the first mannose moiety to the growing dolichal-linked oligosaccharide (DLO) precursor on the cytosolic face of the endoplasmic reticulum (ER). Alg1 protein physically interacts with Alg2 and Alg11 manosyltransferases, which together catalyze the five mannose additions on the ER cytosolic face. To further understanding of how these mannosyltransferases function, we have undertaken a structural and functional study on eukaryotic Alg1 proteins. The *Saccharomyces cerevisiae* Alg1p contains 449 amino acids (51.9 kDa) with a hydrophobic region near the N-terminal. Sequence analysis reveals its structural homology to the heterodimeric Alg13/14 UDP-GlcNAc transferase, which produces $GlcNAc_2$-PP-dolichol intermediate before the reaction of adding first mannose by Alg1. Guided by the structural information of Alg1p, our molecular biological studies demonstrated that N-terminal hydrophobic region of Alg1 works as a transmembrane domain and is required for its ER membrane localization. Deletion of this domain clearly stabilized the Alg1 protein indicating its possibility to works as a target for protein degradation. Like the Alg13/14 complex, scAlg1p possesses a second membrane association site, which contributes to an integral interaction with ER membrane. Interestingly, human Alg1p seems lack such domain, might be due to the evolutional necessary. In the presentation, we also will discuss the protein regions of Alg1p required for the interaction with other mannosyltransferases.

* Corresponding Author

320: Nuclear localization of *O*-GlcNAc transferase is regulated by its own *O*-GlcNAc modification

Hyeon Gyu Seo, Ryum Joo Hwan and Jin Won Cho; Department of Systems Biology, Yonsei University, Seodaemun-gu, 120-749, Seoul, Korea
hyeongyuseo@gmail.com

It has been reported that one of the downstream molecules generated from glucose is uridine diphosphate-N-acetly glucosamine(UDP-GlcNAc) *via* the hexoamine biopsynthetic pathway (HBP). The dynamic cycle of addition and removal of *O*-linked-N-acetylglucosamine (*O*-GlcNAc) to Ser/Thr residues is involved in regulating nuclear and cytoplasmic proteins. Nucleocytoplasmic *O*-GlcNAc transferase (ncOGT) adds a single GlcNAc onto hydroxyl groups of serine and threonine residues. Interestingly, *O*-GlcNAc glycosylation occurs in ncOGT itself as well and several putative sites have been reported mainly within TPR domain. However, the mechanism by which nuclear translocation of *O*-GlcNAc transferase is not clear. Here, we identified specific nuclear localization signals (NLS) in *O*-GlcNAc transferase that is required for nuclear transport. The three amino acid domain in NLS inserts a non-diffusible protein to the nucleus autonomously. Also, we show that ncOGT binds importin α proteins, and the association between importin α proteins and ncOGT is interfered by *O*-

GlcNAcylation on TPR domain. This ongoing effort would give us clear understanding of the key enzyme of *O*-GlcNAc metabolism.

321: Functional relationship between ATP synthase beta subunit and its O-GlcNAc modification

Joo-hwan Ryum[1], Jin-won Cho[1]; [1]Glycobiology Laboratory, Yonsei Univ., Seoul, 120-749, Republic of Korea
chojw311@yonsei.ac.kr

O-GlcNAcylation is the addition of β-N-acetylglucosamine(O-GlcNAc) to serine or threonine residues of nuclear and cytoplasmic proteins. It is highly abundant on myriad proteins, and cycles on proteins with a timescale similar to protein phosphorylation, and has surprisingly extensive cross talk with phosphorylation, where is serves as a nutrient/stress sensor to modulate signaling, transcription, and cytoskeletal functions. Abnormal amounts of O-GlcNAcylation underlie the etiology of insulin resistance and glucose toxicity in diabetes, and this type of modification plays a direct role in neurodegenerative disease. Many oncogenic proteins and tumor suppressor proteins are also regulated by O-GlcNAcylation.

Mitochondrial membrane ATP synthase(F_1F_0 ATP synthase complex) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. Subunits α and β form the catalytic core in F_1, and rotation of the central stalk agaist the surrounding α(3)β(3) subunits leads to hydrolysis of ATP in three separate catalytic sites on the β subunits. It is also known that ATP synthase β has several phosphorylation site, and phosphorylation affects its complex formation, and catalytic activity.

To verify *O*-GlcNAc modifications in *Drosophila* SL2 cell, We performed immunoblotting with CTD 110.6 antibody. *O*-GlcNAcylated proteins in *Drosophila* SL2 cell were analyzed using two-dimensional gel electrophoresis and MALDI-TOF-MS, and ATP synthase β subunit was identified as a novel *O*-GlcNAcylated protein in *Drosophila* SL2 cell. In order to check the *O*-GlcNAcylation of ATP synthase β, immunoblotting was performed with antibody of this protein after SWGA lectin precipitation. Also, immunoblotting was performed with ATP synthase β antibody and CTD 110.6 antibody after immunoprecipitation with CTD 110.6 antibody and ATP synthase β antibody, respectively. ATP synthase β subunit is encoded in the nucleus, synthesized in the cytosol and finally transported into the mitochondria. Interestingly, we found that the *O*-GlcNAcylation of ATP synthase β subunit is increased by nucleocytoplasmic *O*-GlcNAc transferase(ncOGT). Hence we will concentrate on demonstrating how the ATP synthase β is modified with *O*-GlcNAc and what the functional roles of *O*-GlcNAcylation on ATP synthase β are.

322: A sialidase exists on the surface of PNA-nonreactive thymocytes and remove sialic acid specifically from probably CD5

Shigeko Kijimoto-Ochiai[1,2], Toshiaki Koda[2]; [1]Life Space COSMOS, Sapporo, 060-0003 Japan, [2]Graduate School of Life Science and Creative research Institution, Hokkaido University, Sapporo 001-0021, Japan
sko@cris.hokudai.ac.jp

In the thymus, T-cell progenitors progress through a series of developmental stages from double-negative, double-positive to single-positive T-cells. Mouse thymocytes that were unadsorbed on a PNA-coated dish (PNA^{unad} T) include mainly sialylated mature (single positive) T cells and some poorly glycosylated immature (double negative) T cells, while double-positive immature cells adsorbed on the dish. We prepared PNA^{unad} T and analyzed the cells by flow cytometory after staining with PNA-FITC. When the cells were incubated in PBS alone, the cells were stained more intensively with PNA-FITC than before incubation, suggesting that some sialic acids were removed from the cells during incubation. Thus, PNA^{unad} T includes cells having a sialidase on their surface. We therefore tried to identify the glycoprotein from which sialic acids were removed. We found two bands by Western-blot analysis, around 62 and 70 kDa, that were stained more strongly by PNA after incubating the PNA^{unad} T in PBS alone than those without incubation, while we detected two new bands, 97 and 100 kDa, after incubating the cells with a soluble cytosolic fraction from the thymus. We conclude that sialic acids on PNA^{unad} T were removed by a sialidase existing on the cell surface during incubation, not by a soluble sialidase released from dead cells. After removal of sialic acids from the cell surface by exogenous sialidase, apoptotic cell death was observed by FITC-annexin histogram analysis. We also showed that the 62- and 70-kDa bands corresponded to the bands detected with anti-CD5 antibody. CD5 is a cell surface glycoprotein expressed on T and B cells. In the thymus, CD5 is expressed in double-positive and single positive thymocytes and reported to tuning the interaction of the TCR and self-peptide:MHC complex. Really if the 62- and 70-kDa bands are CD5, this sialidase on the cell surface seems to have an important role in the thymus. [Acknowledgement] This study was started at Institute of Immunology when S.K-O. was there and supported technically by Dr. C. Iwabuchi and Ms. T. Matsumoto-Mizuno for flow cytometory analysis.

323: During cell culturing the signal transduction in cells is affected by the decrease of glucose concentration in the media

Ho-Joong Seo[1], Hanbyeol Kim[2], Ji Young Yoon[1] and Jin Won Cho; [1]Department of Integrated OMICS for Biomedical Sciences, Graduate School, Yonsei University, Seoul 120-749, Korea, [2]Department of Biology, Yonsei University, Seoul 120-149, Korea
existstar@hanmail.net

The only glycosylation to be found in both nucleus and cytosol, O-linked beta-Nacetylglucosamine(O-GlcNAc) is one of the post-translational modifications. UDP-GlcNAc, the sugar donor of O-GlcNAc, is mainly synthesized from glucose by Hexosamine Biosynthetic Pathway, which is sensitive to glucose concentration. We confirmed through our study, that a decrease of glucose in media can be observed during cell culturing. Along with this change, O-GlcNAcylation level also appeared to decrease. A comparison was made between a glucose-concentration-maintained sample and a non-maintained sample, and a difference in insulin sensitivity, indicated by phosphor-Akt, was observed. GSK-3beta, a downstream target protein, was also found to be less phosphorylated under glucose maintained condition meaning the level of activated Akt decreased. These findings are evidences supporting the occurrence of the crosstalk between O-GlcNAcylation and O-phosphorylation on Akt. From these results we can also hypothesize that the signal transduction can be influences by the natural change of glucose concentration in the media during cell culturing.

324: Self-Evolving Oxidative Stress With Identifiable Pre- and Postmitochondrial Phases in PC12 Cells

Ganlin Zhang[1,2], Xiaoxin Zhu[3], Morin Christophe[1], Ping Li[4], Xiaomin Wang[2], Guowang Yang[2], Dulce Papy-Garcia[1]; [1]Laboratoire CRRET, EAC CNRS 7149, Faculté des Sciences et Technologie, Université Paris Est Créteil, France, [2]Beijing Hospital of Traditional Chinese Medicine, affiliated with Capital Medical University, Beijing, People's Republic of China, [3]Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, People's Republic of China, [4]Beijing Institute of Chinese Medicine, Beijing, People's Republic of China

Abstract: During the neurodegenerative process in several brain diseases, oxidative stress is known to play important roles in disease severity and evolution. Although early events of stress, such as increased lipid peroxidation and decreased superoxide dismutase, are known to characterize early onsets of these diseases, little is known about the events that participate in maintaining the chronic evolving phase influencing the disease pro- gression in neurons. Here, we used differentiated PC12 cells to identify premitochondrial and postmitochondrial events occurring during the oxidative stress cascade leading to apoptosis. Our data indicate that an acute and strong oxidative impulse (500 lM H2O2, 30 min) can induce, in this model, a 24-hr self-evolving stress, which advances from a premitochondrial phase characterized by lysosomes and cathepsin B and D translocations to cytosol and early mitochondrial membrane hyperpolarization. This phase lasts for about 5 hr and is followed by a postmitochondrial phase distinguished by mitochondrial membrane depolarization, reactive oxygen species increase, caspase-9 and caspase-3 activations, and apoptosis. Inhibition of cathepsins B and D suggests that cells can be protected at the premitochondrial phase of stress evolution and that new cathepsins regulators, such as glycosaminoglycans mimetics, can be considered as new therapeutic prototypes for neurodegeneration. Insofar as early oxidative stress markers have been related to the early onset of neurodegeneration, strategies protecting cells at the premitochondrial phase of oxidative stress may have important therapeutic applications.

Key words: neurodegeneration; lysosome; cathepsin; apoptosis; glycosaminoglycans

325: HBV infection downregulates miR-122 contributes to Sorafenib resistance by targeting GALNT10 in hepatocellular carcinoma

Qian Wu[1], Jiejie Xu[1,2,*], Jianxin Gu[1]; [1]Department of Biochemistry and Molecular Biology, [2]Key Laboratory of Medical Molecular Virology, Ministry of Education and Health, Shanghai Medical College, Fudan University, Shanghai, China
jjxufdu@fudan.edu.cn

The pathological relevance and significance of microRNAs (miRNAs) in hepatocarcinogenesis have attracted much attention in recent years; however little is known about the underlying molecular mechanisms through which miRNAs are involved in the development and progression of hepatocellular carcinoma (HCC). In this study, we demonstrated that miR-122 is frequently down-regulated in HCC and that its expression is further suppressed by hepatitis B virus X protein (HBx). Furthermore, the reduced expression of miR-122 could be rescued by over-expression of hepatocyte nuclear factor 4α (HNF4α). GALNT10 (N- acetylgalactosamine transferase-10) was identified as the direct and functional target of miR-122 with integrated bioinformatics analysis and messenger RNA array assay. This regulation was further confirmed by luciferase reporter assays. In addition, our

results, for the first time, showed that GALNT10 was frequently increased in HCC by way of immunohistochemical staining assays. The increased expression of the GALNT10 significantly inhibited, whereas knockdown of the GALNT10 markedly enhanced sorafenib induced cell apoptosis and proliferation inhibition in HBx transfected hepatomca cells. Conclusion: The newly identified miR-122/GALNT10 axis elucidates the molecular mechanism of HBV-related HCC sorafenib resistance and represents a new potential therapeutic target for HCC treatment.

326: HBV promotes migration and invasion of hepatoma cells through miR-9 mediated down-regulation of GALNT4

Yidong Liu[1], Jiejie Xu[1,2*], Jianxin Gu[1]; [1]Department of Biochemistry and Molecular Biology, [2]Key Laboratory of Medical Molecular Virology, Ministry of Education and Health, Shanghai Medical College, Fudan University, Shanghai, China.
jjxufdu@fudan.edu.cn

Recent evidence indicates that aberrant O-glycosylation may play a critical role during tumorigenesis including the development of hepatocellular carcinoma (HCC). Polypeptide GalNAc-transferases are a number of enzymes which are responsible for the initiation of mucin-type O-linked protein glycosylation, in which N-acetylgalactosamine is transferred to serine and threonine amino acid residues. Here, we found that HBV down-regulates GALNT4 expression significantly in HCC cells. And over-expression of GALNT4 *in vitro* can attenuate HBV driving migration and invasion of HCC cells. Further studies showed that HBV activates NF-κB to up-regulate miR-9, which directly targets the 3′UTR of GALNT4 mRNA to promote its degradation, thus the deregulation of GALNT4 mediating O-glycosylation induces malignant behavior of HCC cells. Moreover, we found an inverse correlation between GALNT4 and HBV infection in HCC clinical specimens. Taken together, this study reveals a novel regulatory circuit adopted by HBV, which may contribute in part to migration and invasion of HCC cells. Hence, strategies that blockade this circuit might be developed to eliminate the metastatic potential of HCC cells conferred by HBV.

327: Galectin-9 affects phagocytosis in THP-1 cells

Chi-Shan Li[1], Dan Hsu[1,2], Fu-Tong Liu[1,2], Huan-Yuan Chen[1,2]; [1]Institute of Biomedical Sciences, Academia Sinica,Taiwan, [2]Department of Dermatology, University of California, Davis, School of Medicine, Sacramento, CA, USA
chishan@ibms.sinica.edu.tw

Galectin-9 (Gal9), a member of the beta-galactoside-binding animal lectin family, is involved in various cellular biological events, including tumor cell adhesion, metastasis, and modulation of various immune responses. Gal9 is expressed in antigen presenting cells (APCs) including macrophages and dendritic cells. Since the main functions of APCs are antigen recognition, uptake and presentation to T cells, it is possible that Gal9 affects the biological functions of APCs. By immunoblotting and *immunofluorescence staining,* we found that Gal9 is expressed in the cytoplasm of THP-1, a human monocytic leukemia cell line. With treatment of phorbol esters, *THP-1* cells could differentiate into macrophage-like cells. By using fluorescein-labeled ovalbumin and dextran, the ability of endocytosis was decreased in *Gal9 knocking down* (Gal9KD) THP-1 derived macrophages compared to control cells. Moreover, Fc receptor-mediated phagocytosis of opsonised sheep red blood cells was also reduced in Gal9KD THP-1 derived macrophages compared to control cells. *There were no differences of IL6 and IL1-β levels between control and Gal9KD THP-1 cells* when treating cells *with 1 μg/ml LPS for 24 h.* Since it has been demonstrated that Gal9 is one of the components of phagosomes in human dendritic cells, the intracellular Gal9 may play an essential role for antigen uptake in human APCs.

328: Role of Galectins in Lung Cancer Progression

Po-Cheng Chiang[1], Dan Hsu[1, 2], Fu-Tong Liu[1, 2], Huan-Yuan Chen[1, 2]; [1]Institute of Biomedical Sciences, Academia Sinica, Taiwan, [2]Department of Dermatology, University of California, Davis, School of Medicine, Sacramento, CA, USA
cpc0325@ibms.sinica.edu.tw

Non small cell lung cancer (NSCLC) is one of the most common cancers and the leading cause of cancer-related death in many countries around the world. A large body of experimental and clinical work supports the view that epidermal growth factor receptor (EGFR) is a relevant target for lung cancer therapy. Somatic mutations within the tyrosine kinase domain of *EGFR* are the most reliable predictors of response to *EGFR* tyrosine kinase inhibitors (TKI), such as Iressa (Gefitnib), in patients with NSCLC. Deletions in exon 19 and a point mutation L858R in exon 21 account for over 90 % of all sensitizing mutations and result in the constitutive activation of EGFR that promote cell proliferation and survival. Galectins are a family of evolutionary-conserved carbohydrate-binding proteins. They are distributed widely in organisms and have been implicated in many essential functions including development, differentiation, cell-cell adhesion, cell-matrix interaction, growth regulation

and apoptosis. Several members of the galectin family have also been shown to be involved in cancer progression and metastasis. Galectin-1 and -3 can mediate neoplastic transformation and cell cycle progression by regulating ras activity and cell-cycle regulators expression. Our Q-PCR data indicate that galectin-1, -3 and -7 are expressed in lung cancer cells with distinct EGFR status. Galectin-1 has been reported to be an important factor to promote lung cancer progression and chemoresistance. However, role of galectin-3 and -7 are still unclear in lung cancer. In our preliminary data, knockdown of galectin-3 reduced cellular ability of migration, proliferation, colony formation and anchorage-independent cell growth in several lung cancer cells. Overexpression of galectin-7 reduced cell proliferation, invasion and migration in PC9 cells. Interestingly, overexpression of galectin-7 led to Iressa resistance in both CL100 and PC9 cells. Comparing to parental PC9 cells, higher galectin-7 level was observed in PC9-IR, an acquired resistant cell, suggesting the correlation between galectin-7 and Iressa sensitivity. Taken together, galectin-3 and -7 may play an important role in mediating lung cancer progression and resistance to TKIs therapy in lung cancer.

329: Wnt/β-catenin up-regulates hFUT8 expression in hepatocarcinoma cell SMMC-7721

Nanyang Li♣ [1], Lijun Zhang♣ [1], Linhua Liu [1], Shujing Wang[1], Jianhui Fan[1], Jianing Zhang [1]*; [1] Department of Biochemistry, Institute of Glycobiology, Dalian Medical University, Dalian 116044, Liaoning Province, China
♣*These authors contributed equally to this work.*
**Correspondence to: Jianing Zhang*
jnzhang@dlmedu.edu.cn

Wnt signaling plays important role in determining cell fate during embryogenesis and maintaining the tissue homeostasis after birth. β-catenin is a key component of Wnt signaling and mediates canonical Wnt pathway. The protein glycosylaiton is widely exit of the organism and is closely related to the disease. Fucosylation is one of the glycosylation and is catalyzed by Fucosyltransferase. Fucosyltransferase 8 (FUT8) is only one enzyme to catalyze α1, 6-fucosylation in mammals. Up-regulation of FUT8 has been observed in several malignant cancers including liver, ovarian, thyroid, and colorectal cancers. However, the pathological role and the regulatory mechanism of FUT8 in cancers remain largely unknown. In this study, we report that FUT8 is up-regulated by Wnt signaling activator LiCl and overexpressed β-catenin in hepatocarcinoma cell line SMMC-7721. In contrast, knocking down β-catenin can down-regulates expression levels of FUT8 in SMMC-7721. And then, Bioinformatic analysis predicted that *Fut8* promoter region contains TCF/LEF binding sites. So, we constructed *hFut8* gene reporter vector that contain *hFut8* region from −1910 to +329. Reporter assay showed that transcriptional activity of *hFut8* gene is significantly increased by Wnt-1, β-catenin in SMMC-7721. Taken together, these data suggest that Wnt/β-catenin signaling can up-regulates hFUT8 expression in human hepatocarsinoma cell SMMC-7721. This work was supported by grants from the Major State Basic Research Development program of China (2012CB822103), and National Natural Science Foundation of China (31170774, 31000372 and 31000618).

330: Shedding of GPI-anchored proteins by a GPI-cleaving enzyme

Morihisa Fujita[1], Gun-Hee Lee[1], Yoshiko Murakami[1], Yusuke Maeda[1], and Taroh Kinoshita[1],[1] Research Institute for Microbial Diseases, Suita, Osaka, 565-0871, Japan
morihisa@biken.osaka-u.ac.jp

Glycosylphosphatidylinositol (GPI) anchoring of proteins is a conserved post-translational modification in eukaryotes. It has been suggested that GPI-anchors act as functional molecules regulating trafficking and membrane localization of the modified proteins. One of the characteristic features of GPI-anchored proteins is that these proteins are releasable from the cell membrane by cleaving GPI moieties. There are several GPI-cleaving enzymes. Here, we identified a novel GPI-cleaving enzyme. PGAP3 is required for the removal of an unsaturated fatty acid from GPI lipid at the Golgi apparatus, which is essential for GPI fatty acid remodeling. By homology to PGAP3 that belongs to a transmembrane hydrolase superfamily, an uncharacterized protein, designated PGAP6a, was found in the superfamily. PGAP6a is a transmembrane protein conserved among metazoa and was mainly localized at the cell surface. In PGAP6a overexpressing cells, surface expressions of several GPI-anchored proteins including CD55, CD59 and Sca-1 were significantly decreased. These results suggest that PGAP6a is a novel GPI-cleaving enzyme at the cell surface.

331: Research on effect of expression of β1,4-GalT-I by estrogen and progesterone and correlation with EGFR signaling pathway

Ying Xia[1], Yunpeng Xie, Cheng Zhang, Ting Gong, lin Zhang, Ying Kong *; [1]Department of Biochemistry, Dalian Medical University. Dalian, Liaoning Province. People's Republic of China.
kongying@dlmedu.edu.cn

β1,4-Galactosyltransferase-I(β1,4-GalT-I) gene codes two proteins, long form and short form: The short and some long form play the role in glycosyltransferase, which mainly transfer galactose from UDP-galactoside to GlcNAc, and form Galβl,4-GlcNAc; The other parts of the long form β1,4-GalT-I,which distribute on cell membrane surface, play a crucial role as a cell adhesion molecule through recognizing and adhering other extracellular matrix and galactose of cell surface glycoprotein and glycolipid. Epidermal growth factor receptor(EGFR) is a multi-function transmembrane protein, which widely distributes on cell membrane surface of each human body tissues. EGFR has protein tyrosine kinase(PTK) activity which is a important receptor. After binding to its ligand, EGFR starts intracellular signal transduction through the cytoplasm adapter protein and enzyme cascade reaction, then regulates nuclear transcription factor kappa B (NF-κB) gene. The enabled NF-κB regulates intercellular adhesion molecule-I(ICAM-1) transcription through MAPK/PKC and other signal pathway, sequentially controls the blastocyst implantation. In this study, we demonstrated the female progestational hormone induced the β1,4-GalT-I and Galβl,4-GlcNAc up-regulation in RL95-2 cells; Under the effect of progesterone, the expression of β1,4-GalT-I was positively correlated with EGFR related pathways; Inhibition of β1,4-GalT-I increased EGFR protein. In addition, female progestational hormone promoted the adhesion of blastocystscells *in vitro* in β1,4-GalT-I-dependent manner. β1,4-GalT-I,which was regulated by progesterone, induced embryo implantation *via* EGFR related signal path.

(This work was supported by National Natural Scientific Grant No.30970646,PR China)

332: The Synergistic Inhibitory Effect of Tetraspanin CD82 and Gangliosides on Cell Migration through EGFR or cMet-activated Pl3K/Akt signal pathway

Ying Li[1#], Xiaohua Huang[1, 2#], Jianing Zhang[1], Keli Ma[1]*; [1] Department of Biochemistry and Molecular Biology, Dalian Medical University, Dalian 116044, China;[2] Department of Clinical Biochemistry, College of Laboratory Medicine, Dalian Medical University, Dalian 116044, China
[1]*Corresponding author: makeli666@yahoo.com.cn
[#] *These authors contributed equally to this work*

It has been proposed that the metastasis suppressor CD82/KAI-1,which is a member of the tetraspanin superfamily, exerts its biological activity by associated with glycosphingolipids (GSLs); however, its mechanism has not been fully elucidated. The present study aimed to investigate the synergistic inhibitory effect of Tetraspanin CD82 and gangliosides on cell migration and its correlation with activation of EGFR and cMet in Hepa1-6 cell lines whose motility and migration *in vitro* can be stimulated by both EGF and HGF. We found that (i) When infection with the CD82 gene, decreased migration ability was observed in Hepa1-6 cells that were induced by EGF and HGF. Transfection of the CD82 gene to Hepa1-6 inhibited EGF-stimulated phosphorylationof EGFR at tyr1173 and contributed to the attenuation of EGFR; while, ectopic expression of CD82 in Hepa1-6 inhibited HGF-stimulated tyrosine phosphorylation of cMet at tyr1313 and tyr1365 without affecting the expression of cMet. These inhibitory effects were enhanced when CD82 is in conjunction with GM3 or particularly with GM2/GM3. (ii) Reduction of CD82 expression by RNA interference and depletion of glycosphingolipids with P4 together, significantly enhanced cell motility and increased expression of EGFR and activation of EGFR at tyr1173 stimulated by EGF; however, increased cell motility and activation of cMet at tyr1313 and tyr1365 stimulated by HGF, were not only due to the effect of decreased CD82 alone but also on account of the effect of increased GM3. Furthermore, CD82 selectively attenuated EGFR and cMet signaling *via* phosphatidylinositol 3-kinase/Akt,but had no affect on the activity of MAPK signaling pathway. These results suggest that the different effects of CD82 and synergistic effects with GM3 and GM2/GM3 on the phosphorylation and expression of EGFR and cMet may be associated with a mechanism by which CD82 exerts inhibitory actions on the motility and migration of Hepa1-6 cells induced by EGF and HGF *in vitro*.

Poster Session II-Physiology & Signalling

333: α1, 6-Fucosylation bidirectionally regulates TGF-β/activin-mediated signaling

Wei Gu, Tomohiko Fukuda, Tomoya Isaji, Hirokazu Hashimoto, Yuqin Wang and Jianguo Gu; Division of Regulatory Glycobiology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai Miyagi, 981-8558, Japan
jgu@tohoku-pharm.ac.jp

It is well known that α1,6-fucosyltransferase (Fut8) and its products, α1,6-fucosylated N-glycans, are highly expressed in brain tissue. Recently, we reported that *Fut8*-knockout mice exhibited multiple behavioral abnormalities with a schizophrenia-like phenotype, suggesting that α1,6-fucosylation plays important roles in the brain and neuron system. In the present study, we screened several neural cell lines, and found that PC12 cells express the highest levels of

α1,6-fucosylation. The knockdown (KD) of Fut8 promoted a significant enhancement of neurite formation and induction of neurofilament expression. Surprisingly, the levels of phospho-Smad2 were greatly increased in the KD cells. Finally, we found that the activin-mediated signal pathway was essential for these changes in KD cells. Exogenous activin, not transforming growth factor beta (TGF-β), induced neurite outgrowth and phospho-Smad2. In addition, the α1,6-fucosylation level on the activin receptor 2A was greatly decreased in KD cells, whiles the total expression level was unchanged, suggesting that α1,6-fucosylation negatively regulated activin-mediated signaling. Furthermore, inhibition of activin receptor-mediated signaling or restoration of Fut8 expression rescued cell morphology and phospho-Smad2 levels, which were enhanced in KD cells. Considering the fact that α1, 6-fucosylation is important for TGF-β-mediated signaling, the results of this study strongly suggest that Fut8 plays a dual role in TGF-β/activin-mediated signaling.

334: The effect of an alginic acid from *Sargassum fusiforme* on NF-κB signaling pathway

Qifei Cong, Xinyan Ni, Qun Dong, Kan Ding; Glycobiology and Glycochemistry Lab. Shanghai Institutes of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
qdong@mail.shcnc.ac.cn (Q. Dong), kding@mail.shcnc.ac.cn (K. Ding)

Sodium alginate is a natural polysaccharide abundant in various brown sea weeds, which is used in pharmaceutical industry as a suspending agent, a disintegrant, or a tablet binder. Alginate is a linear polysaccharide containing 1,4-linked β-D-mannuronic acid (M) and 1,4-linked α-L-guluronic acid (G) in various proportions dependent on the algal species and growth conditions, arranged in either homogeneous or heterogeneous block patterns. As an effort to find immunoregulatory polysaccharides, we try to obtain the polysaccharide fractions and evaluate their NF-κB activation or inhibition activity. An alginic acid polysaccharide, 04S2P, was isolated from *Sargassum fusiforme*, by boiling water extraction, DEAE-Cellulose anion-exchange chromatography, and Sephacryl S-300 HR gel permeation chromatography, as well as by acid precipitation. The average molecular weight of 04S2P, as determined by high performance gel permeation chromatography, was 29.2 kDa. The sugar compositions, as characterized by high performance liquid chromatography method of pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP), were mannuronic acid and guluronic acid. The molar ratio of mannuronic acid to guluronic acid was estimated to be 7.5: 1.0, according to integral of anomeric proton resonances in ^{1}H NMR. According to HSQC and HMBC analysis, the mannuronic acid is found to be β-1,4-linked, while guluronic acid is α-1,4-linked, as in common alginate. The nuclear kappa B (NF-κB) pathway plays a key role in the signaling transduction in many pathological events, particularly in the innate immune response. To investigate the activity and mechanism of action of the algal polysaccharides on immune system, the effect of 04S2P on NF-κB signaling pathway was tested, and the result showed that the activation rate was close to 52 %. However, 04S2P displayed no significant activation after the sample was treated with Affi-Prep polymyxin matrix to remove endotoxin. In comparison, commercial alginate with a lower molar ratio of M/G (1.9:1) demonstrated an obvious inhibition to NF-kB even after endotoxin was removed. Therefore, the M/G molar ratio, glycosyl sequence, and the contamination of endotoxin in alginic acid all probably contribute to its activity on NF-κB signaling pathway.

335: Structure characterization of a glucuronoxylan isolated from *Cassia obtusifolia* seeds and its effect on NF-κB signaling pathway

Qifei Cong, Mingsheng Shang, Xinyan Ni, Qun Dong, Kan Ding; Glycobiology and Glycochemistry Lab. Shanghai Institutes of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
qdong@mail.shcnc.ac.cn (Q. Dong), kding@mail.shcnc.ac.cn (K. Ding)

Xylan is one of the major hemicelluloses, which generally consists of a backbone of 1, 4-linked β-D-xylose units, in most cases, with α-L-arabinfuranosl and 4-O-methyl-α-D-glucuronopyranosyl residues occasionally attached to O-2/O-3 as side groups. In this study, a heteroxylan, designated as COB1B1S2, was isolated from the alkaline extract of the whole seeds of *C. obtusifolia*, by ethanol precipitation, DEAE-cellulose anion-exchange chromatography, and Sephacryl S-300 gel permeation chromatography. Using chemical and spectroscopic methods, the polysaccharide was elucidated to be a glucuronoxylan. Its average molecular weight was estimated to be 70.4 kDa. The polysaccharide, as determined by HPLC after pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP), contains xylose, glucuronic acid, in the molar ratio of 7.8: 1.0. Combined with methylation analysis and NMR spectroscopy, COB1B1S2 was elucidated to contain a poly-(1→4)-β-D-xylose backbone, with α-D-glucuronosyl residues as branches, substituted exclusively at O-2 position. Interestingly, no 4-O-methyl-D-glucuronic acid was identified, which is reported to be present in most heteroxylans, and this characterizes COB1B1S2 as a novel

glucuronoxylan different from those reported previously. As one of the most important nuclear factors, NF-κB takes part in the signaling transduction in the innate immune system. To investigate the biological activity of the xylans on immune system, the effect of COB1B1S2 on NF-κB signaling pathway was tested, and the result showed that the activation rate was close to 77 %. Furthermore, the polysaccharide remains to exhibit an activation rate of 40 % even after it was treated with Affi-Prep polymyxin matrix to remove possible endotoxin contamination. In conclusion, a novel glucuronoxylan was isolated from *Cassia obtusifolia* seeds, and it was shown to be capable of activating NF-κB signaling pathway, indicating a potential immunostimulatory activity.

336: Mice lacking fucosyltransferase 8 exhibit abnormal behavioral abnormalities associated with a schizophrenia-like phenotype

Tomohiko Fukuda, Wei Gu, Tomoya Isaji, Naoyuki Taniguchi, and Jianguo Gu; Division of Regulatory Glycobiology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai Miyagi, 981-8558, Japan, Disease Glycomics Team, RIKEN, Advanced Science Institute, Wako 351-0198, Japan
tfukuda@tohoku-pharm.ac.jp

α1,6-Fucosyltransferase (Fut8) catalyzes the transfer of a fucose residue from GDP-fucose to position 6 of the innermost GlcNAc residue to form α1,6-fucose in hybrid and complex N-linked oligosaccharides of glycoproteins. α1,6-Fucosylated glycoproteins are abundantly present in brain tissue. We investigated the physiological functions of α1,6-fucose in brain tissue by using Fut8-deficient (Fut8KO) mice. We examined Fut8KO mice using a combination of neurological and behavioral tests. Fut8KO mice exhibited multiple behavioral abnormalities consistent with a schizophrenia-like phenotype. Fut8KO mice displayed increased locomotion compared with wild-type and heterozygous mice. In particular, Fut8KO mice showed strenuous hopping behavior in a novel environment. Novelty-induced hyperactivity has been viewed as a preclinical model of the positive symptoms of schizophrenia and of psychomotor agitation in particular. Working memory performance was impaired in Fut8KO mice as evidenced by the Y-maze tests. Deficits in social interaction are hallmarks of schizophrenia. Furthermore, Fut8KO mice showed prepulse inhibition (PPI) deficiency, which had been reported in schizophrenia patient as well as mouse models. These results suggest that reduced expression of Fut8 is a plausible cause of schizophrenia and related disorders. Because locomotor hyperactivity is commonly associated with increased monoamine tone, the effect of Fut8 deficiency on monoamine turnover was determined by HPLC. The levels of serotonin metabolites were significantly decreased in both the striatum and nucleus accumbens of the Fut8KO mice. Likewise, treatment with haloperidol, which is an antipsychotic drug that antagonizes dopaminergic and serotonergic receptors significantly reduced hopping behaviors. Based on these results, we hypothesize that the balance between dopaminergic and serotonergic signaling might be disrupted in the Fut8KO mice.

Recently, Electrophysiologic data of hippocampal CA1 region showed the long-term potentiation was decreased in Fut8KO mice, compared with Wild-type mice. Although the detailed underlying molecular mechanisms remain unclear, the present study is the first to clearly demonstrate that α1,6-fucosylation plays an important role in the brain, and that it might be related to schizophrenia-like behaviors.

337: GnT-V expression levels regulate colon cancer stem cells and colon adenoma progression of *Apcmin*/+ mice through a canonical Wnt signaling pathway

Huabei Guo[1*], Heather Johnson[1], Tamas Nagy[2], and Michael Pierce[1*]; Department of [1] Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, and [2] Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA,30606
**Address correspondence: Department of Biochemistry and Molecular Biology and Complex Carbohydrate Research Center, 315 Riverbend Rd. University of Georgia, Athens, Georgia 30602; Tel: 1-706-542-1701; Fax: 1-706-542-1759;* hawkeye@uga.edu; hbguo@uga.edu

There is increasing evidence that a variety of cancers are initiated and maintained by a small proportion of cells called cancer stem cells (CSC). Our recent results show that deletion of GnT-V, which catalyzes a specific modification of N-glycans with β(1,6) branching, reduced the size of the compartment of CSC in the her-2 mouse model, consequently leading to a inhibited tumor onset. GnT-V expression is increased during the oncogenesis of human colorectal cancer, including adenoma formation. In the present study, the regulation of colon CSC and colon adenoma development were investigated both *in vitro* and *in vivo*. Overexpression of GnT-V increased anchorage-independent cell growth and tumor formation induced by injection of colon tumor cells into NOD/SCID mice, indicating a regulation of cell proliferation and tumorigenicity by GnT-V. Using Apcmin/+ mice with different GnT-V backgrounds, we found that knockout of GnT-V had no significant effect on intestine (colon) adenoma formation (number of adenoma/mouse), but adenoma size

was remarkably reduced, which was accompanied by increased survival of *ApcMin/+* mice with GnT-V deletion ($p<0.01$), suggesting an inhibition in the progression of colon adenoma caused by deletion of GnT-V. Increased expression level of GnT-V up-regulated the population of colon (intestine) CSC by affecting their self-renewal, and their tumorigenicity in NOD/SCID mice. Furthermore, altered nuclear translocation of β-catenin and expression of Wnt target genes were observed after aberrant expression of GnT-V, indicating the regulation of canonical Wnt/β-catenin signaling by GnT-V. These results demonstrate that GnT-V expression and its branched N-glycan products effectively modulate Wnt signaling pathway that, in turn, regulates the relative proportion of colon CSC, as well as colon tumor development, thereby providing a potential therapeutic target for the inhibition of colon carcinoma progression.

338: Study of Chitosan oligosaccharide inducing resistance to TMV virus on *Arabidopsis thaliana* and its resistance pathway

Qingshan Meng[1,2], Yin Heng[1], Duyu Guang[1*]; [1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023 China; [2]Dalian Ocean University, Dalian 116023 China
dyguang@gmail.com

Many recent researches have found chitosan oligosaccharide is an effective elicitor which can induce plant to resist against pathogen. In my paper, we use different concentrations of chitosan oligosaccharide solution to spray plant leaves. After pretreating leaves 24 h, 48 h and 72 h individually, TMV virus is injected in plant leaves and incubated for different days. Then I use western blot to detect TMV coat protein as an indicator to quantify TMV. By using this model,I have screened 50 ppm as an optimum concentration of chitosan oligosaccharide to pretreat wild type *Arabidopsis thaliana* 24 h and incubate TMV virus for 7 days in *Arabidopsis thaliana* leaves which can resist against TMV virus efficiently. By the way we also build a platform which can be used to research oligosaccharides inducing *Arabidopsis thaliana* resistance to resist against TMV virus successfully.

339: HCG to β1,4-GalTI in embryonic implantation and the influence of role and regulation mechanism analysis

Lili Chen[1], Cheng Zhang,Ting Gong, lin Zhang, Ying Kong *; [1]Department of Biochemistry and Molecular Biology, Dalian Medical University, Dalian, 116044, China.
kongying@dlmedu.edu.cn

The change of β 1,4 - GalTI expression level in endometrial cancer cells (RL95-2 cells),which can be seen as implantation period endometrial cells, as well as the adhesive ability of embryos and endometrial was discussed after the treatment of hCG.At the same time, some pathways have been investigated. Control RL95-2 cells β 1, 4 - GalTI expression, testing the corresponding matrix metalloproteinase MMPs and extracellular matrix ECM expression change, analysis and utilization of endometrial adhesion of influence, further discussed β 1, 4 - GalTI embryonic implantation and the molecular mechanism Methods: (1)Building model *in vitro* implantation in villi cancer cells (JAR cells) simulation before implantation embryo to endometrial cancer cells (RL95-2 cells) simulation before implantation endometrial; Change the endometrial environment hCG levels, western blot, RT - PCR detection technology of hCG β 1, 4 - GalTI expression influence; Immune cell chemical dyeing analysis under the action of hCG β 1, 4 - GalTI galactosyl change change; RCA - 1 Lectin - blot technical analysis under the action of hCG Gal β 1, 4 - GlcNAc glycoprotein branch form; HCG test influence related signal path EGFR protein expression level; Respectively through the fluorescence microscope and flow cytometry analysis technology RL95-2 cells and JAR intercellular adhesion influence.(2)Beta-secretase 1, 4 - GalTI gene regulation plasmid (after expression plasmid, interference plasmid), interference contrast plasmid and empty plasmid transfection respectively to RL95-2 cells, using immunofluorescence, RT - PCR and western blotting technology, immune cell chemical dyeing technology respectively the beta testing each transfection cell 1, 4 - GalTI expression and the influence of MMPs, ECM expression; Respectively through the fluorescence microscope and flow cytometry technique to observe β 1, 4 - GalTI to *in vitro* implantation model RL95-2 cells and JAR cell adhesion influence; RT - PCR detection technology of HCG β 1, 4 - GalT I and MMPs - TIMP - ECM path expression effect. Results: (1) endometrial cells β 1, 4 - GalT - I expression and hCG is dose, time - effect relationship, hCG best action conditions for 5×10-4 ug/ul, 36 h ($P<0.01$); (2) under the action of hCG obviously raised EGFR, the nf-kappa B, ICAM - 1, EGF expression ($P<0.01$); (3) hCG can promote JAR cell to RL95-2 cell adhesion effect ($P<0.01$); (4) endometrial cells transfection β 1, 4 - GalT - I gene regulation plasmid after express group can obviously increase MMP2, MMP9, and significantly cut LN, TIMP - 1, interference group can obviously cut MMP2, MMP9, and obviously increase LN, TIMP - 1 ($P<0.01$); (5) the endometrial cells transfection β 1, 4 - GalT - I gene regulation plasmid after express group can obviously improve the JAR cell to RL95-2 cell adhesion effect, interference group can make the JAR cell to RL95-2 cell adhesion effect decrease ($P<0.01$); (6) RT - PCR results show that obvious increase hCG MMP2, MMP9 expression, cut LN, TIMP - 1 expression ($P<0.01$). Conclusion: (1) hCG adjustable endometrial cells β 1, 4 - GalT - I

expression (present time, dose dependency relationship), promote the JAR cell to RL95-2 cell adhesion effect; (2) hCG adjustable endometrial cells EGFR signaling pathways related molecular expression to adjust and control the embryo implantation. (3) regulation of β 1, 4 - GalT - I express influence embryo implantation may through the MMPs, ECM related signal path, adjust then JAR cell to RL95-2 cell adhesion effect. (4) hCG adjustable endometrial cells MMPs - TIMP - ECM path expression to adjust and control the embryo implantation.

(This work was supported by National Natural Ssientific Grant No.30970646,PR China)

340: Molecular Mechanisms of Lycoris Aurea Agglutinin-induced Apoptosis and G2/M Cell Cycle Arrest in Human Lung Adenocarcinoma A549 Cells both in *in vitro* and *in vivo*

Chun-yang Li, Zheng shi, Jingchun Zhang, Yong-xi Liu, Jin-ku Bao*; School of Life Sciences, Sichuan University, Chengdu 610064, China
baojinku@scu.edu.cn

Lycoris aurea agglutinin (LAA) has been drawing rising attention due to its remarkable bioactivities. Herein, we report that LAA triggers G2/M phase cell cycle arrest *via* up-regulating p21 expression as well as down-regulating cdk1-cyclinA singling pathway, and induces apoptotic cell death through inhibiting PI3K-Akt survival pathway in human lung adenocarcinoma A549 cells. While LAA has no significant cytotoxic effect toward normal human embryonic lung fibroblast HELF cells, and moreover, LAA could amplify the anti-neoplastic effects of cisplatin toward A549 cells. Lastly, LAA also bears anti-cancer and apoptosis-inducing effects *in vivo*, and it could decrease the volume and weight of subcutaneous tumor mass obviously as well as expand lifespan of mice. These findings may provide a new perspective for elucidating the complicated molecular mechanisms of LAA-induced cancer cell growth-inhibition and death, providing a new opportunity of LAA as a potential candidate anti-neoplastic drug for future cancer therapeutics.

341: Molecular Mechanisms of Agaricus Bisporus Lectins-induced Apoptosis and G1 Cell Cycle Arrest in Human Breast Adenocarcinoma MCF-7 Cells both in *in vitro* and *in vivo*

Chun-yang Li, Yong-xi Liu, Jin-ku Bao*; School of Life Sciences, Sichuan University, Chengdu 610064, China
baojinku@scu.edu.cn

Mushrooms from Agaricus Bisporus are edible possessing tonic and medicinal attributes. Herein, in silico analyses first demonstrated that Agaricus Bisporus lectin (ABL) could bind with specific sugar-containing receptors EGFR, inferring that ABL could suppress EGFR-mediated anti-apoptotic or survival pathways, ultimately resulted in growth-inhibition and cell death. Subsequent *in vitro* investigations confirmed the simulation results, showing that ABL induced apoptotic cell death through inhibiting EGFR-Ras-Raf-PI3K-Akt survival pathway in MCF7 cells. Also, ABL triggered G1 phase cell-cycle arrest *via* down-regulating cyclinD-cdk4 singling pathway. Finally, *in vivo* anti-tumor effects of ABL were detected, ABL decreased the volume and weight of subcutaneous tumor mass obviously as well as expanded lifespan meanwhile ABL could induce apoptotic cell death in mice. Taken together, ABL isolated from Agaricus Bisporus exerts remarkable anti-tumor effects both in *in vitro* and *in vivo* with noncytotoxic fashion, making ABL as an 'ideal' anti-cancer candidate drugs in future cancer therapy.

Poster Session II-Development & Differentiation

342: The Regulatory Function of ppGalNAc-T13 in Neural Differentiation of P19 Embryonal Carcinoma Cells

Yingjiao Xu, Lei Zhang, Wenjie Pang, Wei Li, Yan Zhang; Ministry of Education Key Laboratory of Systems Biomedicine,Shanghai Center for Systems Biomedicine (SCSB), Shanghai Jiao Tong University, Shanghai, 200240, China
yanzhang2006@sjtu.edu.cn

Glycosylation plays vital regulatory roles in various biological processes such as development, immunity and neural functions. Neural cells require correct glycosylation patterns for their viability, function and differentiation. Mucin-type *O*-glycosylation is initiated by a family of UDP-GalNAc:polypeptide α-*N*-acetylgalactosaminyltransferases (ppGalNAc-Ts, EC 2.4.1.41) containing 19 members in mouse. A member of this family, ppGalNAc-T13, has been found highly and restrictively expressed in the brain and neurons. In this study, we have explored the effect of ppGalNAc-T13 on mouse neural differentiation. We found that the mRNA expression of ppGalNAc-T13 was dramatically increasing during the process of mouse brain development and the maturation of primary neuronal cultures. P19 mouse embryonal carcinoma cells can be induced by all-trans retinoic acid (ATRA) to differentiate into neural cells *in vitro*. Similarly, ppGalNAc-T13 expression was increasing from the later period of ATRA treatment to neural

differentiation stage of P19 cells, accompanying with elevated *O*-glycosylation level. Interestingly, without ATRA treatment, overexpressing ppGalNAc-T13 in P19 cells induced the formation of projection-like structures and improved the *O*-glycosylation level as well. Inhibition of ppGalNAc-T13 expression by RNA interference suppressed P19 cell growth during ATRA treatment and impeded the neurite outgrowth. Moreover, we found ppGalNAc-T13 occurred in axons of the neurons derived from P19 cells. Together, our results suggest a critical function of ppGalNAc-T13 in neuronal differentiation and neurite outgrowth by regulating the *O*-glycosylation level.

343: Characterization of an Immunologically Active Pectin from the Fruits of *Lycium ruthenicum*

Qiang Peng[1], Yuguang Du[1], [1] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China; [2] Northwest University, Xi'an, 710069, China
articles1805@gmail.com

Lycium ruthenicum Murr. belongs to the Solanaceae family and which is widely distributed in the salinized desert of Qinghai-Tibet Plateau. Its special physiological characteristics of drought-resistance and salt-resistance make it an ideal plant for preventing soil desertification and alleviating the degree of soil salinit-alkalinity. In addition, *Lycium ruthenicum* is a famous traditional Chinese herb which has been used for treatment of heart disease, irregular menstruation and menopause. In our previous studies, we have isolated and characterized the neutral polysaccharides from *Lycium ruthenicum*, which were type II arabinogalactan-proteins containing a (1→3)-linked β-D-galactopyranosyl residues main chain, substituted at O-6 of galatose by arabinosyl groups. In the present study, an immunologically active pectin, named LRGP5 (*Lycium ruthenicum* glycoconjugate polysaccharide 5), was firstly isolated from the fruits of *Lycium ruthenicum* Murr. It contained rhamnose, arabinose, xylose, galactose and galacturonic acid in the molar ratio of 1:2.2:0.5:1.2:4.7. Its molecular weight was estimated to be approximately 2.25×10^5 Da by HPGPC. The structure was elucidated using methylation analysis, partial acid hydrolysis, NMR and ESI-MS analysis. Results showed that LRGP5 consisted of a (1→4)-linked galacturonic acid backbone occasionally interrupted by (1→2)-linked rhamnose. The side chains were attached to position 4 of the rhamnose, including (1→5)-linked arabinose, (1→3)-linked galactose, (1→3, 6)-linked galactose, (1→4)-linked galacturonic acid, (1→2)-linked rhamnose and (1→2,4)-linked rhamnose, and the termini were arabinose and rhamnose. The immunological assay results demonstrated that LRGP5 could significantly promote macrophages proliferation and enhance the secretion of nitrogen monoxide *in vitro*.

344: A glycosyltransferase-related gene, *Galntl5*, is a functional molecule indispensable to mature sperm formation

Nobuyoshi Takasaki[1], Kouichi Tachibana[1], Satoshi Ogasawara[1], Hideki Matsuzaki[1], Keiji Mochida[2], Kimiko Inoue[2], Narumi Ogonuki[2], Atsuo Ogura[2], Toshiaki Noce[3], Chizuru Ito[4], Kiyotaka Toshimori[4], Hisashi Narimatsu[1], [1]Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan; [2]RIKEN BioResource Center, Tsukuba, Ibaraki 305-0074, Japan; [3]Research Center of Animal Life Science, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan; [4]Department of Anatomy and Developmental Biology, Graduate School of Medicine, Chiba University, Chuo-ku, Chiba 260-8670, Japan
E-mail: nobu-takasaki@aist.go.jp

It is important for normal fertilization that functionally mature sperm consist of canonical motility and acrosome formation. A novel glycosyltransferase-related gene, *Galntl5,* expresses exclusively in differentiating spermatids. *Galntl5* belongs to the polypeptide GalNAc-transferase (pp-GalNAc-T) gene family for its conserved glycosyltransferase domains, whereas GALNTL5 exhibits no *in vitro* glycosyltransferase activity. Moreover, in contrast to typical pp-GalNAc-Ts positioned in the Golgi apparatus, the exogenous GALNTL5 is retained within vesicles in the cytoplasm and abolishes the Golgi apparatus of cultured cells. To investigate the role of *Galntl5* in spermiogenesis, we established hetero-deficient mutant male mice, which were infertile and exhibited impaired sperm motility. In spermatozoa, the hetero-deficiency attenuated glycolytic enzymes required for motility, disrupted protein loading into acrosomes and caused aberrant localization of the ubiquitin-proteasome system. These findings suggest that GALNTL5 is a functional molecule indispensable for mature sperm formation.

Acknowledgments
This work was supported by a grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.

345: Identification of Genes Involved in Protein Glycosylation in Halophilic Archaea

Hua Lu, Cheng Jin; State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China
publuhua@126.com

Archaea express proteins that enable them to succeed in extreme habitats, which are hostile to many other organisms. Post-translational modifications may help archaeal proteins overcome the challenges presented by their surroundings. In Archaea, the cell wall consists solely of S-layer protein lattices, which are glycosylated by both *N*- and *O*-glycans. However, as compared with the glycosylation in eukaryotes and bacteria, less is known of the post-translational modification in archaea.

As S-layer glycoproteins have been widely used as reporter proteins to study post-translational modifications in archaea, in this study we identified the S-layer glycoprotein from halophilic archaeon *Haloarcula hispanica* by MALDI-TOF and confirmed its glycosylation through SDS-PAGE glycoprotein staining and chemical deglycosylation. Then glycans were released from purified S-layer glycoprotein and subjected to HPLC separation and ESI-Mass. It turned out that *H. hispanica* S-layer glcoprotein was decorated by neutral disaccharide containing a glucose and a galactose, moreover, acidic oligosaccharide containing a glucose and a unknown sulfurted or phosphated monosaccharide.

Furthermore, two putative genes that are invovled in glycosylation were identified in *H. hispanica*, namely *pmt1* and *pmt2*. RT-PCR analysis showed that both genes were actively transcripted during early log phase. Deletion of *pmt1* led to a complete loss of glycosylation on S-layer glycoprotein and reduced stability of the S-layer, while the mutant lacking *pmt2* was unable to modify S-layer glycoprotein with acidic oligosaccharide. Both *H. hispanica* mutants showed a retarded growth at high salt conditions as compare with the wild type. These results suggest that glycosylation may play an important role in maintaining intact and stable cell envelope in hypersaline surroundings and thus ensure survival of *H. hispanica* in this extreme environment. Further investigations of these two genes are undertaken.

Poster Session II-Infection & Immunity

346: CLEC-2 was involved in the phagocytosis of microglia by interacting with its ligand podoplanin

Tao Tao[1], Aiguo Shen[2]; [1]Department of Immunology, Medical Colledge, Nantong University, Nantong, 226001, P.R. China, [2]The Jiangsu Province Key Laboratory of Neuroregeneration, Nantong University, Nantong 226001, P.R. China

Inflammation occurs in many neurologic diseases and is believed to exacerbate neuronal loss. Because dead neurons may both initiate and accelerate disease progression, clearing dead neurons from extracellular spaces may be critical. It is well-kown that dead neurons are quickly removed through phagocytosis by the microglia. It is reported that CLEC-2 is a phagocytic activation receptor expressed on myeloid cells and its ligand podoplanin is expressed in apoptotic cells. In this study, we hypothesized that the process which activated microglia engulf apoptotic neurons may enhance by CLEC-2 interacting with its ligand podoplanin. We found that CLEC-2 was expressed in microglia and podoplanin was expressed in neuron.

347: Two polysaccharides from fruit bodies of *Grifola frondosa* (Fr.) S. F. Gray, induce cytokines release by dectin-1 and toll-like receptor 2 in macrophages and inhibit tumor growth *in vivo*

Jianping Fang, Ying Wang, Kan Ding*; Glycochemistry & Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Pudong, Shanghai 201203, China
kding@mail.shcnc.ac.cn

Grifola frondosa is a widely consumed edible mushroom in Asian countries. Recently, intense researches have been focused on isolation and bioactivities investigation of polysaccharides from this mushroom. However, only few polysaccharides isolated are homogeneous, and little is known about the cellular mechanism of them on the immunomodulatory activities. In this study, two homogeneous polysaccharides named GFPBW1 (300 kDa) and GFPBW2 (27.3 kDa) were purified from the fruit bodies of *Grifola frondosa*. Using various methods such as IR, NMR, methylation, monosaccharide composition analysis, partial acid hydrolysis, and Smith degradation, GFPBW1 was determined to be a β-D-(1-3)-linked glucan backbone with a single β-D-(1-6)-linked glucopyranosyl residue branched at C-6 on every third residue. GFPBW2 possesses a backbone consisting of β-D-1,3, β-D-1,4-linked glucopyranosyl residues, with branches attached to *O*-6 of β-D-1,3-linked glucopyranosyl residues.

Bioactivity study indicated that GFPBW1 and GFPBW2 were effective inducers of TNF-α and IL-6 secretion in murine resident peritoneal macrophages. Using quartz crystal microbalance (QCM) analysis, we found that GFPBW1 and GFPBW2 could bind dendritic cell-associated C-type lectin-1 (dectin-1) with an affinity constant (K_d) value of 2.18×10^{-9} M, and 1.08×10^{-7} M respectively. Meanwhile, both of them could activate Syk and enhance TNF-α production in RAW264.7 cells overexpressing wild type but not mutant

dectin-1. Furthermore, Syk/NF-κB signaling and cytokines release in resident peritoneal macrophages induced by them were significantly inhibited by a specific dectin-1 blocking reagent, laminarin. In addition, two polysaccharides also activated the transcriptional activity of NF-κB through toll-like receptor (TLR) 2 in the luciferase reporter HEK293/NF-κB cells expressing TLR2 but not TLR4 and dectin-1. Moreover, they induced antitumor activity against Sarcoma 180 allografts growth in ICR mice accompanied with increased splenic indices but not in immunodeficient nude mice. These results indicated that both GFPBW1 and GFPBW2 were effective immunomodulator and inhibited tumor growth mainly through activation of macrophages and inducing secretion of cytokines *via* dectin-1 and TLR2 receptors, and GFPBW1 showed a better effect than GFPBW2. Both of them might be promising biological response modifiers as immunotherapy tools for antitumor therapies.

348: Profiling Trypanosomatid-Pattern Recognition Receptors interaction

[1]Laura Rangel, [1]Laura Corvo, [2]Miguel Ángel Pineda, [1]Manuel Soto, [1]Pedro Bonay; [1]Centro de Biología Molecular "Severo Ochoa"-Universidad Autónoma de Madrid. Madrid 28049. Spain, [2]Institute of Infection, Immunity and Inflammation, University of Glasgow, Scotland
pbonay@cbm.uam.es

Innate immunity activation largely depends on recognition of micro-organism structures by Pattern Recognition Receptors (PRRs). PRR downstream signaling results in production of pro- and anti-inflammatory cytokines and other mediators that define the pathogenic curse of disease. Moreover, PRR engagement in antigen-presenting cells initiates the activation of adaptive immunity. C-type lectins like galectins are conserved receptors recognizing carbohydrate structures on viruses, bacteria, parasites, and fungi. C-type lectins such as DC-SIGN, langerin, and dectin-1 and galectins are expressed by dendritic cell subsets and macrophages. Pathogen recognition by those proteins triggers signaling pathways that lead to the expression of specific cytokines which subsequently instruct adaptive T helper immune responses. No systematic analysis of the interaction between PRRs and pathogens has been carried to date. Among the main human PRR systems described so far we can find the C-type lectin and galectin families. Here we present the binding profile of representative C-type lectins (langerin, intelectin-1, DC-SIGN, dectin-1 and mincle) and some galectin (1,3,4,7,8) against parasite protozoans (pathogenic and non-pathogenic) from the Kinetoplastidae family (*Trypanosoma cruzi* and *T. rangeli* as well as several *Leishmania* species) as a first step to delineate the hypothesis that differential recognition by innate immune system components may lead to differential and specific responses to each parasite and subsequent pathology. The data presented suggest that indeed there is a differential recognition profile that is able to distinguish pathogenic from non-pathogenic species inside a genus and furthermore able to discriminate tissue tropism among a similar pathogenic group.

349: The functional studies of *M. tuberculosis* Rv0431, a cell wall protein

Guoying Deng[1], Jian Kang[2], Yufang Ma[2]; [1] Department of Biochemistry and Molecular Biology, Dalian Medical University, Dlian, 116044, China; [2] Department of Microbiology, Dalian Medical University, Dlian, 116044, China
yufang_ma@hotmail.com

Once *Mycobacterium tuberculosis* is inhaled and exposed to host immune cells, the immune responses including innate immunity and adapted immunity to the pathogen occur. The macrophages are of mostly important for innate immune response because of their potent microbicidal activities. However, resting macrophages fail to harm *M. tuberculosis*, which allow *M. tuberculosis* to replicate within these cells and evade the innate immune defenses. Many proteins were found in *M. tuberculosis* cell wall, where they function to regulate the action of macrophages or play a role in bacterial escape from the host macrophage through apoptosis. Therefore, to characterize these cell wall associated proteins is critical to understanding bacterial survival and immune modulation in the host.

The proteomics studies on *M. tuberculosis* indicated that Rv0431 is a cell wall protein. WGA (wheat germ agglutinin)-affinity purification for *M. tuberculosis* glycosylated proteins showed that Rv0431 is a glycoprotein. However, the composition, structure and function of sugar chain of Rv0413 are unclear now.

In this study, we cloned Rv0431 from *M. tuberculosis* H37Rv genome and constructed mycobacterial expression vectors pVV2-Rv0431 (the histidine tag at N-terminus of Rv0431) and pVV16-Rv0431 (the histidine tag at C-terminus of Rv0431). The vectors were electroporated to *M. smegmatic* mc^2155 strain respectively. The purified Rv0431 protein will be identified by Western blot using different primary antibodies, such as *Triticum vulgaris* lectin, limulus polyphemus lectin, *etc.* We also performed Co-

IP and MS/MS to find out the interacted proteins of the host cells with Rv0431 protein.

This work was supported by the National Basic Research Program of China (2012CB518803).

350: Combined effects of DNA vaccination and Polysaccharide isolated from fruits of *Physalis alkekengi* L. var. *francheti (Mast.) Makino* enhances the protective immunity against systemic candidiasis in mice

Huimin Yang, Shuying Han, Danyang Zhao, Guohua Zhang, Guiyun Wang*; School of Life Sciences, Northeast Normal University, 5268 Renmin Street, Changchun City, Jilin Province, 130024, China
*Corresponding author. wanggy737@nenu.edu.cn

Protective efficacy against systemic candidiasis mediated by DNA vaccine alone or with polysaccharide was evaluated in this study. DNA vaccine (pD-HSP90C) used was recombinant plasmid containing epitope C (LKVIRK) from heat shock protein 90 (HSP90) of *Candida.albicans* (*C. albican*). Polysaccharide (PPSB) was isolated from the fruits of *Physalis alkekengi*, L. var. *francheti (Mast.) Makino*. ICR mice were immunized intramuscularly followed by electroporation with pD-HSP90C in the absence or presence of PPSB (20, 40, 80 μg); pcDNA3.1, PPSB, and PBS were used as controls. This finding indicated pD-HSP90C was able to elicit the protective immune responses against systemic candidiasis by inducing the production of IgG, IgG2b, IgG1, IL-2, and IL-4 in sera of mice; pD-HSP90C reduced greatly colony forming unites (CFU) of *C.albican* in the kidneys of mice, which were challenged with living *C. albican* cells after immunization with pD-HSP90C. The results also indicated that PPSB (40, 80 μg) significantly enhanced specific antibody titers IgG, IgG1, IgG2b, and concentration of IL-2 and IL-4 in sera of mice immunized with pD-HSP90C ($p<0.05$ or $p<0.01$). More importantly, it was found that the mice immunized with pD-HSP90C/PPSB-40 μg not only had fewer CFU in the kidneys than mice immunized with pD-HSP90C, but also a statistically significant higher survival rate over PBS-injected group ($p<0.05$) when the immunized mice were challenged with living *C. albican* cells. However, no statistically significant difference in survival rate was observed between pD-HSP90C-immunized group and PBS-injected group. Therefore, pD-HSP90C could be a useful candidate for the development of DNA vaccine against systemic candidiasis; PPSB can be considered as a promising adjuvant eliciting both Th1 and Th2 responses to enhance the efficacy of DNA vaccines. (The authors thank the Natural Science Foundation of China for financial support, Grant No. 30970639).

351: *Cryptococcus neoformans* mannoproteins: cloning, expression and characterization in *Pichia pastoris*

Pedro Antônio Castelo Teixeira[1], Isis Cristina Ferreira[1], Luciana Loureiro Penha[1], Diego Allonso[2], Ronaldo Mohana-Borges[2], Lucia Mendonça-Previato[1] and José Osvaldo Previato[1]; [1]Laboratório de Glicobiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil, [2]Laboratório de Genômica Estrutural, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil
pedroacteixeira@gmail.com

The oportunistic fungal pathogen *Cryptococcus neoformans* is the etiological agent of cryptococcosis, a potentially fatal disease, especially in immunocrompromised patients, as those with AIDS. Growth at 37 °C, melanin production and, mainly, the polysaccharide capsule production are the most important virulence factors of this fungus. *C. neoformans* capsular polysaccharide has three components known as glucuronoxylomannan (GXM), galactoxylomannan (GalXM) and mannoproteins (MP), comprising around 90–95 %, 5–8 % and <1 % of the capsule mass, respectively. Despite MPs are the minority capsule components, and the less studied, four of these molecules with molecular weight of 115, 98, 88 and 84 kDa were identified, and characterized as immunoreactive antigens and potential vaccine candidates against cryptococcosis. Due to the involvement of MPs in the pathogenesis of this mycosis, it is important to characterize the chemical structure and the biological function of these molecules. The first step for these studies is to obtain a good amount of MPs. Thus, the aim of our work was to clone, express and characterize MP98 and MP84 on *Pichia pastoris* yeast. A *C. neoformans* mutant strain (CAP67), deficient in GXM production, was used in this study. The c-DNA was obtained from the fungal RNA, and was used as template to PCR reactions to amplify the encoding genes of MP98 and MP84. The amplified genes were cloned into expression vectors pPICZαA, to MP98, and pPICZαB to MP84, and then transformed into *P. pastoris*. Expression tests were performed to find the colonies that most express MP98 and MP84. The two MPs had a histidine tag which allowed their purification, from the culture supernatant, by an affinity chromatography with nickel columns. Here, we demonstrated suitable protocols for *C. neoformans* MPs expression and purification.

352: Aptamers against ManLAM inhibited virulent *Mycobacterium tuberculosis* H37Rv infection in mice and rhesus monkey

Qilong Wang, Qin Pan, Min Li, Dongdong Shi, Xianru Xia, Xiao-Lian Zhang*; State Key Laboratory of Virology, Department of Immunology, Hubei Province Key Laboratory of Allergy and Immunology, Wuhan University School of Medicine, Wuhan 430071, China
zhangxiaolian@whu.edu.cn

The *Mycobacterium tuberculosis* (M.tb) surface heavily lipoglycans, mannose-capped lipoarabinomannan (ManLAM) has strong immunosuppressive effects which might be one of reasons that causes persistent infection *in vivo*. In this study, aptamer "antibody" ZXL1 which specifically bound to ManLAM of virulent M.tb H37Rv was screened out by Systematic Evolution of Ligands by EXponential enrichment (SELEX). The selected ssDNA aptamer ZXL1 demonstrated the highest binding affinity to the ManLAM of M.tb H37Rv and was measured as $8.907X10^{-8}$ M of quilibrium dissociation constant (KD) value. We found that ZXL1 significantly blocked ManLAM or H37Rv binding to mannose receptor (MR) and reversd ManLAM-induced immunosuppressive effects on DCs and T cells. More importantly, we demonstrated that single injection of ZXL1 significantly protected mice and rhesus monkeys against M.tb H37Rv infection. These results suggested that ManLAm-aptamer can be used as a new potential drugs for treatment of M.tb infection and as a TB vaccine immune-enhancing adjuvant.

353: Immunopotentiating and adjuvant effects of an α-(1→4)-glucan from *Isatis Indigotica* immunized with H1N1 influenza vaccine in mice

Junjie Shan, Qian Li, Ting Zhu, Peiyuan Jia, Hao Ma, Yuxia Wang*; Beijing Institute of Pharmacology and Toxicology, Beijing, 100850, China

The root of *Isatis Indigotica* is a traditional Chinese herbal medicine. The water-extract of the roots is widely used in clinical practice for the treatment of influenza, epidemic hepatitis *etc.* In the previous study, we investigated an α-(1→4) -linked glucan (Mw=3600 Da) from the roots and it had a significant adjuvant activity immunized with H1N1 influenza vaccine and hepatitis B virus protein in mice. In this study, we further investigated the immuno-regulation effects of the α-glucan on the cellular and humoral immune response in Balb/C mice after its co-immunization with H1N1 influenza vaccine. The results showed that the Con A-, LPS-, and H1N1-induced splenocyte proliferation and the serum H1N1-specific IgG, IgG1, IgG2a, and IgG2b antibody titers in the immunized mice were significantly enhanced by the α-glucan. The glucan also significantly promoted the production of Th1 (IFN-γ) and Th2 (IL-4) cytokines in splenocytes from the immunized mice. The results indicated that the α-(1→4)-glucan has a strong potential to increase both cellular and humoral immune responses, and that it may be a safe and efficacious adjuvant candidate suitable for a wide spectrum of prophylactic and therapeutic vaccines.

354: Matrix Metalloproteinases (MMPs) involved in degradation of glycoconjugates of extracelular matrix (ECM) during murine subcutaneous sporotrichosis

Andreia Dantas Medeiros [1], Pedro Antonio Castelo Teixeira [2], Lucia Mendonça-Previato [2], Jose Osvaldo Previato [2]and Christina Maeda Takiya [1]; [1] Laboratório Multidisciplinar de Pathologia, Instituto de Biofísica Carlos Chagas Filho (IBCCF), Universidade Federal do Rio de Janeiro (UFRJ) and [2] Laboratório de Glicobiologia, IBCCF/UFRJ
cmtakiya@gmail.com

The ECM is a complex, interdigitating network comprised of glycoconjugates such as glycosaminoglycans, collagen, fibronectin which serve as a scaffold for attachment, growth, differentiation and migration of cells. The MMPs are considered the main proteases involved in proteolytic remodeling of ECM. They participate in many physiological and pathological mechanisms and seem to be involved in the tissues' invasion by infectious agents and cancer cells.

Sporothricosis is a mycosis caused by *Sporothrix schenckii*. Most cases of sporotrichosis involve the skin, subcutaneous tissue and lymphatic vessels. However, the fungus can disseminate, especially in immunocompromised patients. But, the fungus-related mechanisms of invasion are not totally clear.

Material and Methods: C57Bl6 mice were subcutaneously inoculated into the right footpad with 5×10^6 yeasts of two *S. schenckii* strains isolated in the zoonotic sporotrichosis endemic area: Ss 5822 or Ss 5555-1 and with sterile saline (control group). Mice were euthanized at 15 days-post infection. Another group of animals were either inoculated with yeasts or saline and received daily 30 mg/Kg doxycycline subcutaneously for 4 days. Biopsies from mouse footpads were collected for histological and immunohistochemical/morphometrical analyses and also for gelatin zymography and *in situ* zymography (ZIS). Sections were stained with hematoxylin-eosin (HE), Grocott's methenamine silver (GMS) and Picrosirius. For immunohistochemistry, sections were incubated with antibodies directed against MMP-1, MMP-9, MMP-12, myeoloperoxidase and F4/80.

Results: The HE examination revealed a diffuse infiltration of inflammatory cells widely distributed in the dermis and subcutaneous tissue in infected mice. A large number of round or oval yeast-like fungal forms were evidenced with GMS. Picro-sirius staining showed that the infection by the fungus reduces collagen. Furthermore, MMP-1, MMP-9, MMP-12, myeloperoxidase and F4/80 were increased in mice infected by the fungus. MMPs were predominantly found in keratinocytes and in the inflammatory cells (macrophages, neutrophils and lymphocytes) as well as in ECM. It was also observed higher gelatinase and collagenase activities in *S. schenckii* infected mice. However, gelatin zymography and ZIS showed that doxycycline administration was able to inhibit the activity of MMPs, especially the collagenase.

Ours results suggest the participation of MMPs in the pathogenesis of sporotrichosis.

355: The effect of exopolysaccharides production on swarming motility of *Pseudomonas aeruginosa* PAO1

Shiwei Wang[1], Zhenyin Zhang[2], Di Wang[1], Hongsheng Liu[2], Luyan Ma[1*]; [1]State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China; [2] College of Life Sciences, Liaoning University, Shenyang, 110136, China

Pseudomonas aeruginosa is an ubiquitous microorganism, and a model organism for biofilms research. Exopolysaccharides are a key biofilm matrix component of many bacteria including *P. aeruginosa*, as they contribute to the overall biofilm architecture and stress resistance. Bacterial motilities also affect the biofilm architecture. However, it is not clear whether the production of exopolysaccharides can affect bacterial motilities. *P. aeruginosa* have three types of motilities, flagella-mediated swimming, Type-IV Pili (T4P)-driven twitching, and swarming that requires the production of rhamnolipids and flagella/T4P. In this study, we found that enhances of exopolysaccharides production reduced the swarming, but not swimming and twitching motility. This suggested that the effect of exopolysaccharides production on swarming motility was due to the change of rhamnolipids production. Further investigation demonstrated that overproduction of Psl or/and Pel did decrease rhamnolipids production and the reduction occurred at post-transcriptional level. Previous reports showed that synthesis of exopolysaccharides Psl and Pel competed for sugar precursors and Psl may share sugar precursors with rhamnolipids synthesis pathway. Taking together, this suggested that the synthesis of rhamnolipids competed with exopolysaccharides Psl and Pel for the sugar precursors. In summary, our results indicate the effect of exopolysaccharides production on swarming motility of *P. aeruginosa* by reduction of rhamnolipids synthesis.

Author Index