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ABSTRACTS

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Nitric oxide-dependent mitochondrial DNA overproliferation and deletion in the context of Alzheimer disease and cancer

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Mitochondrial dysfunction and free radical-induced oxidative damage play a role in the pathogenesis of neurodegeneration such as Alzheimer disease (AD), tumors, and their growth and metastasis and during cellular and tissue aging. We have demonstrated ultrastructural localization of the NOS enzyme isoforms and immunoreactivity, together with biochemical and cytological detection of mitochondrial enzymes and their activity, along with mtDNA deletions in AD as well as in human metastatic liver tumors and tumors derived from colorectal cancer, as well as select brain cancers (glioblastoma multiform). Exploration of the role of nitric oxide (NO)-dependent overproliferation of mitochondrial DNA and/or deletions as well as mitochondrial enzyme activity during tumor growth and metastases provide important insights into the biochemical perturbation on mitochondrial DNA integrity and aging. Therefore, most likely these deficits can compromise vital ATP-dependent cellular operations (detoxification, system repair, DNA replication, protein degradation and osmotic balance) that to contribute to the Warburg hypothesis and a switch for some tumors and/or AD brain to undergo glycolysis to meet energy demands. These findings are would lead to the formulation of alternatives to cancer detection and treatment as mitochondrial cell constituents become a focus of new diagnostics for early detection of neurodegeneration and tumors that can be used as new treatment strategies for AD as well as a majority of the tumors.

Gamma-glutamyl di-peptides and chronic inflammation

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Because of recent great scientific evidences of molecular actions of many food bioactive peptides, they might have a role in supporting to reduce the risk of many chronic diseases. Our research group has been exploring new identification of dietary intervention to prevent chronic inflammation and a therapeutic target. We also investigate the mode of action behind these food bioactive peptides and body interactions. We introduce one of our recent successful peptide based interventions on chronic inflammation. Inflammatory Bowel Diseases (IBD)-ulcerative colitis and Crohn's disease- are chronic gastrointestinal inflammatory diseases of unknown etiology. Conventional therapies for the treatment of IBD have demonstrated limited efficacy and potential toxicity; therefore, there is a need for novel therapies that can safely and effectively treat IBD. The extracellular calcium-sensing receptor (CaSR) is the first identified G protein-coupled receptor. Recently, we identified that γ-glutamyl cysteine and γ-glutamyl valine (γ-EV) (γ-EC and γ-EV) as novel positive allosteric modulator of CaSR through the allosteric binding. We also demonstrated that activation of CaSR by γ-EC or γ-EV could suppress TNF-α-induced inflammatory responses in intestinal epithelial cells (IECs), and reduced intestinal inflammation in a mouse model of colitis. The CaSR-mediated anti-inflammatory effects of the peptides were abrogated in β-arrestin2 knockout IECs, and involvement of β-arrestin2 was found to inhibit the TNF-α-dependent pro-inflammatory signaling cascade via cross-talk with the TNF-α receptor (TNFR). Further detailed analysis using gene ontology (GO) analysis clarified the role of CaSR plays as a therapeutic target for chronic inflammation.

Towards the production of GSH-sulfur compound conjugates for therapeutic applications

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Natural organosulfur compounds (OSCs) from *Allium sativum* L. have both antioxidant and chemoprevention properties, and are able to suppress the proliferation of tumor cells in vitro through the induction of apoptosis. Although studies are necessary to identify all their molecular targets to clarify the biochemical mechanism of action, OSCs represent potential ideal anticancer agents, either alone or in association with other antitumor drugs. Water- and oil-soluble allyl sulfur compounds show different properties and capability to inhibit the proliferation of tumour cells. Moreover, previous studies have demonstrated that allyl sulfur compounds are able to interact with enzymes involved in the detoxification system, supporting the hypothesis that these enzymes and the proteins involved in the cellular redox homeostasis are preferential targets of these compounds. In this study, we have optimized a new protocol for the extraction of water- and oil- soluble compounds from *A. sativum* L. and the production of GSH-OSC-conjugates. The effects of the water-soluble fractions on the proliferation of human T lymphoblastoid cell line HuT 78 and on the thiosulfate-cyanide sulfurtransferase (TST) activity were analyzed. Further, the hydrogen sulfide (H_2S) production was also detected in order to identify new slowly releasing hydrogen sulfide donors and opening the way to the production of GSH-OSC-conjugates for therapeutic applications.

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Variations in the metabolism of amino acids by bacteria derived from different compartment of the pig small intestine

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Bacteria in the pig intestine can actively metabolize amino acids (AA). However, little research has been focused on the variation in the AA metabolism by bacteria from different niches. This study compared the metabolism of AA by microorganisms derived from the lumen and epithelial wall of the pig small intestine, aiming to test the hypothesis that the metabolic profile of AA by gut microbes was niche specific. Samples from the digesta, gut wall washes and gut wall of the jejunum and ileum were used as inocula. Anaerobic media containing selected AAwere used and cultured for 24 h. The 24 h culture was served as inocula for the following 30 times of subcultures. Results showed that 60–95 % of glutamate, glutamine, lysine and arginine were utilized by luminal bacteria after 24 h. For the firmly attached bacteria in jejunum, AA except glutamine were constantly released into the culture for the first 12 h and were shift to utilization after that. For the loosely-attached bacteria, release of AA dominated in jejunum while catabolism of AA dominated in ileum. During subculture, utilization of AA from each compartment

decreased and the amount of AA used after the 24 h incubation was lower than 50 %. Analysis of the microbial community revealed that the diversity of luminal bacteria community was similar to that of firmly-attached bacteria, while significantly higher than that of loosely-attached bacteria. The above findings provide basic knowledge for improving the utilization and function of dietary AA in the intestine of both humans and animals. This work was supported by the National Key Basic Research Program of China (2013CB1273003).

An atomic-resolution view into protein-membrane interactions

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Myelin is a tightly packed, ordered, multilayered membrane around selected neuronal axons. Myelin carries a set of specific proteins, thought to mediate myelin membrane stacking and stabilization. Most myelin proteins are involved in neurological diseases, but the current knowledge about their structure–function relationships is limited. P2 is a fatty acid binding protein expressed in peripheral nerve myelin, where it may be involved in bilayer stacking and lipid transport. P2 binds to phospholipid membranes through its positively charged surface and a hydrophobic tip. The structure of human P2 at an ultrahigh resolution allows for detailed analyses of the structure, including the full organization of an internal hydrogen bonding network. The orientation of the bound fatty acid is linked to the protonation states of two coordinating arginine residues. An anion-binding site in the portal region may be relevant for membrane interactions and conformational changes. When bound to membrane multilayers, P2 has a preferred orientation. In P2-membrane complexes, both the protein and lipid phases are stabilized. The repeat distance in membrane multilayers indicates a single layer of P2 between membranes, and simulations show the formation of a double bilayer in the presence of P2. In cultured cells, wild-type P2 induces membrane domain formation, while a mutant does not. The results show how P2 can interact with two membranes simultaneously, while going through conformational changes.

Microarray analysis of placental gene expression in gilts receiving dietary supplementation with L-arginine between Days 14 and 25 of gestation

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We previously reported that dietary supplementation with 0.8 % L-arginine between Days 14 and 25 of gestation improved embryonic survival and growth in gilts. The present study involved microarray analysis of placental gene expression to explore the underlying mechanisms. Gilts were checked daily for estrus with boars in the morning and bred at onset of the second estrus and 12 h later (the time of breeding = d 0 of gestation). Between d 14 and 25 of gestation, 15 gilts/treatment were housed individually and fed twice daily 1 kg of a corn- and soybean meal-based diet supplemented with 0.0, 0.4, or 0.8 % L-arginine. All diets were made isonitrogenous by addition of L-alanine. At d 25 of gestation, gilts were hysterectomized to obtain conceptuses. All snap-frozen placental samples were stored at −80 °C until extraction of total RNA. Total RNA (400 ng) was reverse-transcribed to cDNA. T7 RNA polymerase-driven RNA synthesis was used for the preparation and labeling of cRNA with Cy3 or Cy5 dye. The labeled cRNA probes were purified with the RNeasy Mini Kit (Qiagen Inc., Valencia, CA). Purified cRNA was quantified with the NanoDrop 1000, and 825 ng for each were hybridized on a 44 K Agilent porcine gene expression microarray (Agilent, Santa Clara, CA). This array includes 43,803 probes which were prepared using gene sources from RefSeq, UniGene, and TIGR. The slide format was printed using the Agilent's 60-mer SurePrint technology. The false discovery rate (Q value) was calculated for each *P* value using the R program. Genes were annotated by basic local alignment search tool (BLAST) in the database of the national center for biotechnology information (NCBI) and the institute for genomic research (TIGR). The database for annotation, visualization and integrated discovery (DAVID) version 6.7 was used to generate specific functional annotations of biological processes for the differentially expressed genes. Microarray results from 22 expressed sequence tags (ESTs) were verified using quantitative RT-PCR. One hundred and forty-six ESTs were up-regulated and 429 ESTs were down-regulated by dietary supplementation with 0.8 % arginine between d 14 and 25 of gestation. Functional analysis by the DAVID program revealed alterations in placental expression of genes in response to dietary supplementation with 0.8 % L-arginine. These genes are known to play important roles in fatty acid biosynthesis, as well as insulin, transforming growth factor beta, and notch signaling pathways. (Supported by USDA-NRI and NSFC).

Secondary structure of Huntingtin amino-terminal region

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Huntington's disease is an autosomal-dominant neurodegenerative disorder caused by a polyglutamine (polyQ) expansion (>35Q) in the first exon (EX1) of huntingtin protein (Htt). mHtt protein is thought to adopt one or more toxic conformation(s) that are involved in pathogenic interactions in cells. However, the structure of mHtt is not known. Here, we present an atomic resolution structure of mHtt36Q-EX1. To facilitate crystallization, three histidine residues (3H) were introduced within the Htt36Q stretch. The Htt36Q3H region adopts α-helix, loop, b-strand hairpin conformations. Furthermore, we observed interactions between the backbone of the Htt36Q3H β-strand with the aromatic residues mimicking putative-toxic interactions with other proteins. Our findings support previous predictions that the expanded mHtt-polyQ region adopts a β-sheet structure. Detailed structural information about mHtt improves our understanding of the pathogenic mechanisms in HD and other polyQ expansion disorders and may form the basis for rational design of small molecules that target toxic conformations of disease-causing proteins.

Variable escape of HSD17B10 gene from X-chromosome inactivation

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Hydroxysteroid (17β) dehydrogenase X (HSD10) is a multifunctional protein encoded by the HSD17B10 gene at Xp11.2. In response to stress or hypoxia–ischemia its levels increased rapidly. Expression of this gene was also changed significantly in colonic mucosa of patients with ulcerative colitis. However, accurate information about its transcripts is still lacking, and additional evidence for its escape from X-chromosome inactivation remains to be secured to settle a debate (He XY, Dobkin C, Yang SY: Does the HSD17B10 gene escape from X-inactivation? Eur J Hum Genet 2011, 19: 123–124). Epigenetic analysis of the 5'-flanking region of the HSD17B10 gene showed that there is little 5-methylcytosine (<3 %) in a normal male, and that none of CpG dinucleotides in the CpG island were close to 50 % 5-methylated in females. The hypomethylation of the CpG island provided additional evidence for the variable escape of the HSD17B10 gene from X-chomosome inactivation which could influence the range of severity of HSD10 deficiency, an inherited error in isoleucine metabolism, in heterozygous females.

Analysis of the mechanism of action of tryptophan-induced protein synthesis in rat liver

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Tryptophan (Trp), an essential amino acid, has been shown to promote protein synthesis in rat liver, but the mechanism of action has yet to be elucidated. We investigated the mechanism of action of Trp-induced protein synthesis in the liver using a proteomic and metabolomic approach. Wistar rats were fasted for 18 h and divided into 3 groups. One group of rats was sacrificed without any treatment as a control. The remaining two groups of rats were administered 135 mg of L-tryptophan/100 g of body weight by oral gavage and then sacrificed 1 or 3 h after administration. Proteomic analysis revealed decreased ornithine aminotransferase (OAT) expression at 1 and 3 h after administration. OAT is a key enzyme in ornithine (Orn) catabolism. Liver and serum Orn levels were significantly increased at 3 h after administration. Moreover, the level of spermine (Spe), which is involved in cell growth, was increased in the liver at 3 h after administration. Trp administration reportedly enhances the enzymatic activity of ornithine decarboxylase (ODC), which catalyzes the decarboxylation of Orn to form putrescine and is the rate-limiting enzyme in Spe synthesis. Based on these results, we speculate that increases in the Orn level resulting from suppression of OAT expression and stimulation of ODC activity by Trp administration may lead to accelerated Spe synthesis, thereby promoting protein synthesis in the liver.