the combination induced a good inhibition of CCF-STTG1's cell growth but was inactive on U87MG cells. Microarray gene profiling and apoptosis pathway proteins' assessments will also be presented to further characterize genes or pathways involved in the synergistic effect of Smac-083 TNFα-induced apoptosis in glioblastoma cell lines.

Reference(s)

[1] Li, L. *et al* (2004) *Science* 305(5689): 1471−4.
[2] DeAngelis L.M. (2001) *N Engl J Med* 344(2):114−23.
[3] Seneci, PF. *et al* (2009) *Bioorg Med Chem* 17(16):5834−56.

747 Human arm protein lost in epithelial cancers, on chromosome X 1 gene is transcriptionally regulated by CREB and Wnt/beta-catenin signaling

A. Takeda[1]. [1]*International University of Health and Welfare Hospital, Surgical Oncology, Tochigi, Japan*

Background: The aberrant activation of the Wnt signaling is a key process in colorectal tumourigenesis. The canonical Wnt signaling controls transcription of target genes via b-catenin and T cell factor/lymphoid enhancer factor family transcription factor complex. *Arm protein lost in epithelial cancers, on chromosome X 1 (ALEX1)* is a novel member of the Armadillo family which has two Armadillo repeats as opposed to more than six repeats in the classical Armadillo family members. The regulatory mechanism of *ALEX1* gene in normal and cancer cells remain largely unknown. Here we examined cis-regulatory elements and trans-acting factors involved in the transcriptional regulation of *ALEX1* gene.

Material and Methods: Human colon cancer cell lines (HCT116 and SW480) and pancreatic cancer cell line (PANC-1) were used in this project. The putative promoter region of the human *ALEX1* gene from −1933 to +487 was amplified by PCR and subcloned into the pCR-Blunt II-TOPO plasmid. The luciferase reporter plasmids driven by deleted mutant types of *ALEX1* promoter were generated by the same PCR-based method. The luciferase reporter assay was performed using the Dual-Glo Luciferase Assay System. PANC-1 cells were transfected with the ON-TARGETplus Non-Targeting Control siRNA or siRNAs targeting CREB using DharmaFECT1 siRNA. Chromatin immunoprecipitation was carried out with Dynabeads Protein G and polyclonal anti-CREB antibody. Indirect immunofluorescence for b-catenin was performed with Rhodamine-conjugated anti-mouse IgG antibody, whose images were obtained using a Axiovert 200M fluorescent microscope.

Results: Site-directed mutations of a cyclic AMP response element (CRE) and an E-box impaired the basal activity of human *ALEX1* promoter in colorectal and pancreatic cancer cell lines. Moreover, overexpression of CRE-binding protein (CREB) increased the *ALEX1* promoter activity in these cell lines, whereas knockdown of *CREB* expression decreased the expression level of *ALEX1* mRNA. Interestingly, luciferase reporter analysis and quantitative real-time RT-PCR demonstrated that the *ALEX1* promoter was upregulated in a CRE-dependent manner by continuous activation of Wnt/b-catenin signaling induced by a glycogen synthase kinase-3 inhibitor and overexpression of b-catenin.

Conclusions: These results indicate that the CRE and E-box sites are essential cis-regulatory elements for the *ALEX1* promoter activity, and the *ALEX1* expression is regulated by CREB and Wnt/b-catenin signaling.

748 Regulation of expression of the VE-statin/egfl7 gene in endothelial cells: a critical role for ETS and GATA factors

F. Soncin[1], A. Le Bras[1], C. Samson[1], M. Trentini[1], B. Caetano[1], E. Lelièvre[1], V. Mattot[1], S. Guichard[2], F. Beermann[2]. [1]*Institut de Biologie de Lille, CNRS UMR8161, Lille, France,* [2]*Swiss Institute for Experimental Cancer Research, Centre de Phénotypage Génomique, Lausanne, Switzerland*

The *VE-statin/egfl7* gene is specifically expressed in endothelial cells during development and in the adult [1]. We studied here the regulatory mechanisms that control this specific expression *in vitro* and *in vivo*.

A specific expression in endothelial cells is not observed with *VE-statin/egfl7*'s closest neighbor genes *notch1* and *agpat2* on chromosome 2. Further, the acetylation state of histones around the two *VE-statin/egfl7* transcription start sites shows that the chromatin is opened in endothelial cells, not in fibroblasts at the *VE-statin/egfl7* locus. Two regions are important for the endothelial-specific expression of the gene; a −8409/−7688 enhancer and the −252/+38 region located ahead of the exon-1b transcription start site. The latter contains important ETS- and GATA-binding sites which are crucial for expression of the gene in endothelial cells. Analysis of expression, chromatin immunoprecipitation and RNA interference of endogenous transcription factors showed that Erg and GATA-2 are, by far, the most highly expressed in endothelial cells and that these two factors directly control expression of *VE-statin/egfl7*.

This first detailed analysis of the mechanisms that govern the expression of the *VE-statin/egfl7* gene in endothelial cells pinpoints the specific importance of Erg and GATA-2 factors in the regulation of genes in these cells.

Reference(s)

[1] Soncin F et al EMBO J. 2003 Nov 3;22(21):5700−11.

749 C6orf69, a PKCe interacting BTB-containing protein, is a novel Cullin3 binding partner

D. Garczarczyk[1], J. Hofmann[1]. [1]*Innsbruck Medical University, Biocenter Division of Medical Biochemistry, Innsbruck, Austria*

Protein kinase C (PKC) family members are key signaling molecules involved in many diverse cellular functions such as proliferation, differentiation, survival and death.

Among PKC isoenzymes, novel PKCε has been reported to act as an oncogene, contributing to malignancy by enhancing cell proliferation or by inhibiting cell death. PKCε seems to be involved also in mechanisms related to tumour cell invasion and metastasis, however its exact function is not known yet. By gene array approach, we identified few novel genes regulated by overexpression of constitutively active PKCε (PKCεA/E). One of the down-modulated genes, termed C6orf69, encodes a 47 kDa protein containing a single BTB/POZ domain.

C6orf69 gene is ubiquitously expressed. C6orf69 proteins were up regulated in some cancers (breast) compared to normal counterparts, suggesting a participation of C6orf69 in pathogenesis of certain tumours.

C6orf69 contains BTB domain usually involved in protein-protein interactions and implicated in the stability and dynamics of actin filaments. C6orf69 has been shown to co-immunoprecipitate with PKCε and bind to actin. Recently, BTB-domain containing proteins have been reported to regulate protein stability by recruiting proteins to the Cullin3 (Cul3) ubiquitin ligase leading to their degradation *via* ubiquitin/proteasome. Using co-immunoprecipitation method we could demonstrate that C6orf69 is interacting specifically with Cul3. We also addressed whether C6orf69 could be ubiquitylated, based on the fact that other BTB/POZ adaptor proteins have been shown to be substrates of E3 ligases. Our *in vivo* ubiquitylation assay demonstrated that C6orf69-CFP protein is efficiently polyubiquitylated and that inhibition of 26S proteasome by MG132 accumulated both unmodified C6orf69 as well polyubiquitylated C6orf69. Considering that C6orf69 binds to Cul3 and to PKCε, we are currently investigating if PKCε is ubiquitylated through the C6orf69/Cul3-based ubiquitylation system.

Analysis of the C6orf69 polypeptide showed that there are putative PKC phosphorylation sites. However, C6orf69 seems not to be a direct substrate of PKCε or other PKCs as demonstrated by *in vitro* kinase assay. Part of our current work is focused on determining which kinase (s) is responsible for C6orf69 phosphorylation, fuctional consequences of this PTM as well as determination of the precise position of the phosphorylated residues.

This work was supported by grant from the Austrian Science Fund (No. P19878).

750 Normal breast tissue from cancer patients is different in expression of Bmi-1 and Mel-18 compared to breast tissue from non cancer patients

M. Riis[1], T. Luders[2], V. Kristensen[2], I. Bukholm[1]. [1]*Akershus University Hospital, Surgery, Nordbyhagen, Norway*, [2]*University of Oslo, Institute of Clinical Epidemiology and Molecular Biology (Epi-Gen), Nordbyhagen, Norway*

Background: Polycomb Group (PcG) proteins are epigenetic silencers involved in maintaining cellular identity, and their deregulation can result in cancer. Mel-18 and Bmi-1 are both members of PcG. Bmi-1 was initially considered an oncogene, although recent studies have suggested that Bmi-1 overexpression is associated with good outcome in breast cancer. Mel-18 is considered a tumour suppressor gene. There are different mechanisms to this tumour suppressiveness. Both Mel-18 and Bmi-1 have been studied in tumour tissue, but to our knowledge it has not been studied in normal breast epithelium. Our study compares the expression of the two genes in normal breast epithelium of cancer patients and compares this to the level of expression in breast epithelium of healthy women.

Material and Method: We studied a total of 71, 23 of which we have normal tissue from viscinity of the tumour. In addition we had 6 fibroadenomas, 2 DCIC, and 12 reduction mammoplasties. The tissue samples were stored in RNAlater, RNA was isolated and microarray performed to achieve a molecular profile. These two genes were then studied more closely first on mRNA expression level and later on protein expression level. using immunohistochemistry.

Results: Bmi-1 mRNA is significantly up regulated in normal breast tissue in breast cancer patients compared to normal breast tissue from noncancerous patients, while the mRNA expression of Mel-18 was found to be lower in normal breast from patients operated for breast cancer compared to breast tissue from mammoplasty. mRNA expression of these two genes was inversely correlated. When protein expression of these two genes was evaluated, we observed that most of the epithelial cells were positive for Bmi-1 both groups of tissue samples, although the expression intensity was stronger in normal tissue from cancer patients compared to mammoplasty tissue samples. Protein expression of Mel-18 showed stronger intensity in tissue samples from mammoplasty compared to normal breast tissue from patients operated for breast cancer.

Conclusion: Bmi-1 mRNA expression is increased and Mel-18 mRNA expression is decreased in normal breast tissue of cancer patients as compared to normal breast tissue in women having had reduction mammoplasties.

751 Correlation of copy number, gene and protein expression for breast cancer related genes and proteins

S. Myhre[1], B. Hennessy[2], J. Alsner[3], J. Overgaard[3], G. Mills[4], T. Sørlie[1], A.L. Børresen-Dale[1]. [1]*Institute for Cancer Research Oslo, University Hospital Radiumhospitalet and Institute for Clinical Faculty of Medicine University of Oslo, Department of Genetics, Oslo, Norway,* [2]*Beaumont Hospital, The Royal College of Physicians of Ireland, Dublin, Ireland,* [3]*Aarhus University Hospital, Department of Experimental Clinical Oncology, Aarhus C, Denmark,* [4]*M. D. Anderson Cancer Center The University of Texas, Department of Systems Biology, Houston, USA*

Background: The development of the microarray technology has made it possible to measure multiple entities (eg. genes, probes) simultaneously. For example, spotted DNA probes on microarrays can be utilized to measure gene copy number covering the complete genome. Similarly, whole genome arrays can be utilized to measure the expression of all transcribed mRNA in a sample. At the protein level, the reverse phase protein array (RPPA) can be used to measure and quantify the amount of a specific protein in lysates from multiple samples simultaneously. There are many potential mechanisms of regulation between copy number of a certain genomic region, to the final expressed protein. Methylation, microRNAs, post-translational modification etc. influence the relationship between the copy number level and the expression of the corresponding proteins.
Material and Method: In this study, 267 fresh frozen tumour samples were obtained from high risk breast cancer patients enrolled in the Danish Breast Cancer Cohort 82bc trials and utilized to look at the cis-correlation between copy number, gene expression and protein expression in a panel of proteins selected for their involvement in cancer and the PI3K/AKT-pathway. The Agilent 244 K Comparative Genomic Hybridisation (aCGH) array was utilized for copy number measurements, the Applied Biosystem Whole Genome Array for mRNA expression and classification into molecular subtypes of breast cancer, and the RPPA for protein expression. For the three platforms high quality data for 194, 196 and 210 unique samples was obtained, respectively. The intersection of no. of samples successfully analyzed differed between the three comparisons: copy number–gene expression (154 samples), gene expression–protein expression (164 samples) and copy number–protein expression (161 samples). Spearman correlation was estimated for each measured gene and protein expression across the samples in the three comparisons, and a positive correlation cut-off was set to >0.3. The cut-off reflects significant correlation after Bonferroni-adjustment of the p-values.
Results: We were able to identify entities with a high correlation between all three comparisons (e.g. ERBB2, RPS6KB1, PDPK1). Some genes showed high correlation between copy number and gene expression, (eg. PARP1, RPS6, RB1) but low correlation to the expressed protein, while others showed high correlation between gene and protein expression but low correlation to copy number (e.g. CAV1, CCNB1, ESR1). Finally, some entities showed low correlation across all three platforms (e.g. STK11, FRAP1, SRC).
Conclusion: The correlations for each gene and protein across the three levels of measurements can be utilized to propose drivers of regulation of gene and protein expression, and the correlations vary between different molecular subtypes of breast cancer.

752 Gene expression profile of spontaneously immortalised T lymphocytes

M. Chechlinska[1], A. Pfeifer[2], M. Zajdel[1], A. Swiercz[1], M. Oczko-Wojciechowska[2], M. Jarzab[3], B. Jarzab[2], K. Goryca[4], L. Wyrwicz[4], J.K. Siwicki[1]. [1]*Cancer Centre and Institute of Oncology, Department of Immunology, Warsaw, Poland,* [2]*Cancer Centre and Institute of Oncology, Department of Nuclear Medicine and Endocrine Oncology, Gliwice, Poland,* [3]*Cancer Centre and Institute of Oncology, Department of Tumour Biology and Clinical Oncology, Gliwice, Poland,* [4]*Cancer Centre and Institute of Oncology, Department of Bioinformatics and Systems Biology, Warsaw, Poland*

Background: Immortalisation is a critical step in carcinogenesis. The majority of studies on cell immortalisation have employed models of fibroblasts or epithelial cells, immortalised by virus-transformation or gene-transfection. These procedures introduce unspecific alterations, which make the results obtained in such model systems difficult to interpret. Data on the immortalization of T cells are scarce and inconsistent. Here we used our in vitro model of spontaneously immortalized human T cells to gain insights into the molecular changes responsible for the acquisition of an unlimited growth potential.
Materials and Methods: Three spontaneously immortalised, IL-2-dependent T cell lines, their matched primary lymphoblasts and other primary, IL-2-dependent T lymphoblasts were assessed for gene expression, with the use of the Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays.
Results: Approximately 1300 upregulated and 1100 downregulated geneprobes were found to discriminate between immortalised and primary lymphocytes, the former group comprised GAS1, TP63, IGFBP3, SIX1, IL8 and SYK and the latter – KLF12, BIM, FGL2 and IRF8. Pathway analyses revealed altered cell activation circuits, including T-cell receptor signalling pathway and MAPK pathways, cytoskeletal genes, interferon- and insulin growth factor-related pathways. Among the most significantly changed chromosomal regions there were 2p13−25 and 3q26−29. Significant alterations were also found within the target pathways regulated by 85 transcription factors, including SOX9, YY1, MYC, E2F, AP2, NFAT and NF**K**B.
Conclusions: (1) Immortalisation of T cells involves an immense change in gene expression. This points at the complexity of the processes that drive an unlimited proliferation potential. (2) Overexpression and down-regulation of both tumour suppressor genes and oncogenes, suggest that their actual roles are relative and may depend on the biological context. (3) Spontaneously immortalised T lymphocytes overlap with other immortalisation models in the altered cell activation pathways, but lack the significant changes in the oxidative stress pathway. (4) Chromosome regions 2p13−25 and 3q26−29 of the spontaneously immortalised T lymphocytes host many overexpressed genes. Since amplification of these regions is typical for many cancers, spontaneously immortalised T cells may provide a relevant model to study molecular alterations in these malignancies.

753 Epigenetic regulation of miR-196b expression in gastric cancer

W. Lin[1], K.W. Tsai[1], H.W. Kao[1]. [1]*Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan*

Background: MicroRNAs (miRNAs) are short noncoding RNAs that play important roles in cellular processes and disease pathogenesis via the control of specific targeted gene expression. The miR-196s miRNA is encoded at three paralogous loci in three HOX clusters and acts as an oncogenic miRNA in cancer progression. The aims of this study were to investigate the mechanisms that underlie the expression and regulation of these three paralogous transcripts of miR-196.
Material and Methods: MiR-196s expression levels in several human cell lines were assessed by real-time PCR approach. The methylation status of miR-196s promoter was analyzed using bisulfite restriction assay, methylation-specific PCR, and bisulfite sequencing in different cells. Luciferase assay studies were carried out to assess the promoter activity of miR-196b promoter in human cell lines.
Results: The methylation status correlated well with miR-196b expression in different cell lines. Treatment with the demethylating drug 5-Aza-dC reactivated miR-196b transcription in methylation-silenced cells. Using in vitro methylation approach, we further provided evidences that promoter hypermethylation tightly repressed miR-196b transcriptional activation in human cancer cell lines. Interestingly, we first demonstrated that the expression of miR-196b was significantly elevated in gastric cancer and that hypomethylation status of miR-196b CpG islands were frequently observed in primary gastric tumours.
Conclusions: Our findings aid in the understanding of miR-196s regulation showing that abnormal DNA hypomethylation to induce overexpression of miR-196b in gastric cancer.

754 DGKa, by regulating atypical PKC, is a key transducer of SDF1a-induced invasive behaviour in breast cancer cells

A. Graziani[1], M. Gaggianesi[1], P.E. Porporato[1], E. Rainero[1], C. Cianflone[1], P. Ontano[2], A. Pighini[1], F. Chianale[1], G. Chiorino[2], G. Baldanzi[1]. [1]*University of Piemonte Orientale, Clinical and Experimental Medicine, Novara, Italy,* [2]*Fondo Edo Tempia, Laboratory of Cancer Pharmacogenomics, Biella, Italy*

Background: Invasive epithelial carcinomashave acquired mesenchymal phenotype, enabling egression from primary tumour site and ability to invade and grow in different tissues, giving rise to distal metastasis. The invasive phenotype, include the ability to produce membrane protrusion in extracellular matrix (ECM) and the ability to degrade it. Chemokines, such as SDF1a, acting through their GPCRs, contribute to the invasive phenotype, sustaining in vitro and in vivo metastatic growth. We previously showed that Src-mediated activation of Diacylglycerol kinase alpha (DGKa) by HGF and VEGF is required for cell scatter and for migration and angiogenesis in epithelial and endothelial cells, respectively. More recently we showed that DGKa, by generating PA, recruits atypical PKCs (aPKCs) in a complex with RhoGDI and Rac, thereby defining the site of Rac activation and ruffle formation (Chianale et al. PNAS 2010). In this study we investigated the role of DGKα in SDF1a-induced invasive and migratory phenpotype of MDA-MB-231 cells.
Material and Methods: Experiments have been carried out in serum starved MDA-MB-231 cells, treated or transfected as indicated and stimulated in presence of SDF1α (50 ng/ml).