In conclusion, these results demonstrate that ZEB-1 directly inhibits SEMA3F expression in lung cancer cells. Together with its effects on E-Cadherin, these data indicate that ZEB-1 plays a critical role in the pathogenesis or progression of this disease.

351 Poster

Polymorphic microRNA binding sites within candidate genes are associated with the risk of colorectal cancer

D. Landi[1], F. Gemignani[1], A. Naccarati[2], B. Pardini[2], P. Vodicka[2], A. Försti[3], K. Hemminki[3], F. Canzian[3], S. Landi[1]
[1]University of Pisa, Department of Biology, Pisa, Italy; [2] Institute of Experimental Medicine Academy of Science of Czech Republic, Department of Molecular Biology of Cancer, Videnska, Czech Republic; [3] German Cancer Research Center (DKFZ), Genomic Epidemiology Group, Heidelberg, Germany

Introduction: The individual risk to develop colorectal cancer (CRC) is hypothesized to be modulated, at least in part, by common polymorphisms within specific candidate genes. They are involved in the carcinogenic processes, through the regulation of the cell growth, differentiation, apoptosis, and the maintenance of genome stability. Recent evidences indicate that small non-coding RNA molecules, called micro-RNAs (miRNAs), bind to the 3'UTRs of mRNAs and interfere with their translation, thereby regulating cell growth, differentiation, apoptosis, and tumorigenesis. Therefore, we hypothesized that polymorphic miRNA binding sites at the 3'UTRs of cancer candidate genes could modulate the individual risk of cancer.

Materials and Methods: To confirm our hypothesis, we selected 129 genes that, according to published data and various online resources (e.g. BioCarta and KEGG pathways; http://cgap.nci.nih.gov/Pathways) are candidate genes for CRC. Fifty-one genes are involved in inflammatory processes, 37 belong to synthesis of prostaglandins and thromboxanes, 16 genes are connected with obesity and insulin resistance, and 25 genes are involved in early and late stage of this type of tumour. We identified putative microRNAs binding sites by means of specialized algorithms (PicTar, DianaMicroT, miRBase, miRanda, TargetScan, and microInspector). Then, we found 79 SNPs within the putative binding sites for their ability to affect or impair the binding with the miRNA, by assessing the variation of ΔG (Gibbs free energy) (defined as $\Delta\Delta G$) comparing the "wild-type" and their correspondent variant alleles. Considering the validation status of the SNPs and their frequencies (MAF>0.10), we found at least 15 candidate polymorphisms of biological relevance that could be investigated by performing case-control association studies on a series of samples from Czech Republic.

Results: We found statistically significant associations between risk of CRC and variant alleles of CD86 (OR=2.74 95%CI=1.24-6.04, for the variant homozygotes) and INSR genes (OR=1.94; 95%CI=1.03-3.66, for the variant homozygotes).

Conclusion: This study suggests that SNPs in miRNA binding sites may be important in the modulation of the individual risk of cancer and encouraged to undertake future works. Moreover, since the genotyping allows the screening of a relatively large number of polymorphisms in short time, the proposed study suggested also a way to restrict the number of miRNA targets to be actually experimented using time-consuming molecular biology techniques.

352 Poster

HIF-1alpha is a novel target of the SWI/SNF chromatin remodelling complex

S. Rocha[1], N.S. Kenneth[1], P. van Uden[1], S. Mudie[1]
[1]College of Life Sciences, Wellcome Trust Centre for Gene Regulation and Expression, Dundee, United Kingdom

Background: Hypoxia inducible factor (HIF-1) is a master regulator of the transcriptional responses to hypoxic stress. The majority of HIF-1alpha control happens at the protein level and mRNA changes in response to hypoxia are not readily observed. The SWI/SNF chromatin remodelling complex is important for activation and repression of transcription, and acts by modulating chromatin structure. Despite the importance of this complex, only a few direct targets have been identified.

Methods: Using mRNA, Western Blot and promoter analysis we have investigated how chromatin remodelling complexes regulate HIF-1alpha.

Results: We demonstrate that the HIF-1alpha is a direct target of SWI/SNF. SWI/SNF components are found associated with the HIF-1alpha promoter and their depletion results in a reduction of HIF-1alpha expression and its ability to transactivate target genes. Importantly, depletion of BAF57 (a conserved subunits of SWI/SNF) results in reduced recruitment of other SWI/SNF components as well as impaired polymerase II recruitment.

Conclusions: These results reveal a previously uncharacterized dependence of HIF-1alpha on the SWI/SNF complex, demonstrating a new level of control over the HIF-1alpha system. In addition, these studies identify BAF57, as the main targeting subunit of SWI/SNF to the HIF-1alpha promoter.

353 Poster

Convergent mechanisms that activate MYB transcription in colon and breast cancer which provide a therapeutic opportunity to target metastatic disease

R.G. Ramsay[1]
[1]Peter MacCallum Cancer Centre, Research, Melbourne Victoria, Australia

Background: MYB is over-expressed in the majority of colo-rectal cancers1 (CRC) and ERalpha positive breast cancers. Activation of MYB transcription occurs at the earliest stages of adenoma formation in the colon and progressively increases in primary and finally metastatic adenocarcinoma. In CRC mutations that affect the transcriptional elongation of the gene are frequent and have been functionally validated2. Conversely in breast cancer mutations in this sequence are rare but estradiol induces ERalpha mediated induction of MYB transcription obviating the need for the mutations inherent in CRC3. Materials and Methods: Experimental mouse models have bee used to identify the interplay between c-Myb and the adenomatous polyposis coli gene in synergistically driving the c-Myc gene expression and that c-Myb over-expression increases in metastatic CRC and mammary cancer. With this in mind a DNA fusion vaccine has been devised to generate a c-Myb specific immune response to potentially treat these two common cancers. This has been achieved even though c-Myb, like many tumor antigens, is weakly immunogenic as it is a "self" antigen and thus subject to tolerance. To break tolerance, a DNA fusion vaccine was generated comprising wild-type c-Myb cDNA flanked by two potent Th epitopes derived from tetanus toxin. Vaccination was performed targeting a highly aggressive, weakly immunogenic, subcutaneous, syngeneic, colon adenocarcinoma cell line MC38 which highly expresses c-Myb. Results: Prophylactic intravenous vaccination significantly suppressed tumor growth, through the induction of c-Myb specific anti-tumor immunity for which the tetanus epitopes were essential. Vaccination generated anti-tumor immunity mediated by both CD4+ and CD8+ T cells and increased infiltration of immune effector cells at the tumor site. Importantly, no evidence of autoimmune pathology in endogenous c-Myb expressing tissues was detected 4. Conclusions: These data highlight the role of MYB in 2 common epithelial cancers and establish c-Myb as a viable antigen for immune targeting and serve to provide proof of principle for the continuing development of DNA vaccines targeting c-Myb. As c-Myb is expressed at its highest in metastatic CRC we propose that a vaccine against c-Myb may have a place in patients post-surgery and adjuvant therapy.

1. Biroccio, A, et al (2001) c-Myb and Bcl-x Over expression Predicts Poor Prognosis in Colorectal Cancer. Amer. J. Pathol. 158: 1289

2. Hugo, H., et al (2006) Mutations in the MYB intron 1 regulatory sequence increase transcription in colon cancer. Genes, Chromosomes and Cancer 45:1143

3. Drabsch, Y., et al (2007) Mechanism of and Requirement for Estrogen-Regulated MYB Expression in Estrogen-Receptor Positive Breast Cancer Cells. PNAS. 104; 13762

4. Williams, B., et al (2008) Induction of T cell mediated immunity targeting the nuclear oncogenes c-Myb by DNA vaccination in a mouse model of colon cancer. Cancer Immunol. Immunotherapy (Accepted).

354 Poster

Identification of proteins implicated in Kit receptor signalling

A. Chaix[1], P. Dubreuil[1], P. de Sepulveda[1]
[1]Institut Paoli Calmette, Marseille, France

The receptor tyrosine kinase Kit is required for the development of germ cells, melanoblasts, interstitial cells of Cajal, erythroblasts and mast cells. Gain-of-function mutations of Kit are found in human proliferative pathologies such as mastocytosis, gastrointestinal stromal tumour (GIST), acute myeloid leukaemia (AML) of the CBF class or testicular germ cell tumours. Different kinds of mutations lead to ligand-independent activation of the receptor. The substitution of the aspartate 816 in the kinase domain occurs in 80% of the cases of mastocytosis. Substitutions or deletions in the regulatory juxtamembrane domain also induce constitutive receptor activation and subsequent cellular transformation.

Following stimulation by its ligand, Kit undergoes transphosphorylation on tyrosine residues, thus creating docking sites for signalling molecules. The JM domain of Kit contains 6 tyrosines of which Y568 and Y570 are autophosphorylation sites. We work with HMC-1 cells (a human mastocytoma cell line carrying the mutation D816V) as a model of Kit

dependent mast cell transformation. In theses cells, we have identified interaction partners of phosphotyrosine 568 and 570 using affinity pull down assays with synthetic phosphopeptides followed by mass spectrometry identification. Identification of ten partners (SHP-1, SHP-2, SHIP, Stat5, Grb2, CrkL, PLCγ1, PLCγ2, Fes and Syk) was confirmed by western blotting and the in cellulo relevance of these interactions was emphasised by co-imunoprecipitation with Kit in HMC-1 cells.

In an attempt to confirm the binding specificity of these partners to the dityrosine motif, we have mutated both tyrosines to phenylalanine and introduced the mutant receptor in Ba/F3 cells. Some partners described still coimmunoprecipitate with Kit in the cells stably transfected with the mutant receptor Kit Y568F-Y570F-D816V, suggesting a redundancy of binding sites. However, this dityrosine motif has shown to be the only docking site for the tyrosine kinase Syk, as coimmunoprecipitation is lost with mutant receptor.

We are now addressing the function of this complex using RNA interference. We have successfully silenced Syk expression in HMC-1 cells and we are now focussing on the functional and biochemical consequences of Syk depletion. In the context of Kit oncogenic activation, we aim to define whether the kinase Syk is implicated in proliferation, in adhesion to fibronectine, in migration towards SCF in a Boyden chamber or in degranulation.

355 Poster

Cell-cell communications in a breast cancer senescence model

L. Maccoux[1], M. Hermes[2], M. Mariani[1], A. Manz[1], J. Franzke[1], J. Hengstler[2], J. West[1]
[1]Institute for Analytical Sciences, Department of Miniaturisation, Dortmund, Germany; [2] Leibniz Research Centre for Working Environment and Human Factors, Department of Toxicology, Dortmund, Germany

BACKGROUND: The tyrosine kinase receptor ERBB2 is a key receptor in the development of breast cancer and demonstrates an aggressive ability to transform cells via mitogenic and anti-apoptotic signals. However, recent evidence suggests premature senescence of cells undergoing mammary tumourgenesis through the ERBB2 pathway is a type of anti-carcinogenic programme. The over expression of ERBB2 induces the up-regulation of cell cycle inhibitors triggering senescence in breast cancer cells. Intracellular and intercellular communication plays a prominent role in the cell cycle and in particular cell cycles in cancer. Direct cell-cell contact through the formation of gap junctions is of major interest in investigating senescence of tumour cells. Over the past decade, microdevices have become increasingly popular tools for addressing key questions in the life sciences. We have developed a cell-cell contact microdevice to enable contact modes of communication between breast cancer cells to be characterised.

MATERIALS AND METHODS: Doxycycline induces senescence in the breast cancer cell line MCF7 carrying the ERBB2 variant vector NeuT (MCF7/NeuT). Cells were cultured with and without doxycycline on custom made individual cell culture plates. Once doxycycline had induced senescence these cells were interfaced with untreated and non-senescent cells using the contact microdevice. The distance between the cell culture plates, in both lateral and vertical dimensions, was less than 10μm to allow for sufficient cell-cell interaction.

RESULTS: The uninduced MCF7/NeuT cells proliferated and were in contact with the senescent MCF7/NeuT cells. Senescence was observed in induced cells when the NeuT gene was switched on by doxycycline. This was confirmed by multiple cell protrusions, granular structures and flattened cell morphology as well as cell cycle arrest. Also, the cyclin-dependent kinase inhibitor p21 was shown to be up-regulated and centralised to the nucleus. The knock-down of p21 abolished cell cycle arrest allowing for tumourgenesis to progress.

CONCLUSIONS: In these first contact experiments we have demonstrated the potential for the cell-cell contact microdevice to be used to bring two different cell types into contact for short periods (1 min to 4 hours). Presently, we are studying the effect of such a transient contact between senescent and native MCF7 cells to determine the possibility of a 'kiss of senescence'.

356 Poster

Cell type-specific methylation of the SNCG and S100A4 genes and their relation to expression changes in urothelial cancer

W.A. Schulz[1], O.Y. Dokun[1]
[1]HHU, Urology, Duesseldorf, Germany

Hypomethylation of repeat DNA sequences is common in human cancers, but only a few single-copy genes have been reported to be activated by DNA hypomethylation, including SNCG and S100A4. Since both genes were suggested to be overexpressed in urothelial cancers by microarray expression studies performed in our institution, we wondered whether the increases were caused by hypomethylation. We compared expression in 13 urothelial carcinoma (UC) cell lines and cultured normal urothelial cells as well as in tumor and benign tissues, analyzed the effect of the methylation inhibitor 5-aza-2-deoxycytidine (azadC), and determined methylation patterns by bisulfite sequencing, also in fibroblasts and blood cells. Of 13 UC lines, 6 showed SNCG overexpression vs. normal cells, but 7 very low levels. Similarly, in carcinoma tissues, both increased and strongly diminished SNCG expression were evident. Treatment with azadC restored expression in UC lines with undectable mRNA levels. In these lines, the SNCG promoter was densely methylated, whereas it was unmethylated in normal urothelial cells and in UC lines with elevated gene expression. Fibroblasts, blood leukocytes and ureteral connective tissue showed partial methylation. Similarly, the SNCG was unmethylated and expressed in normal prostate epithelial cells, but densely methylated and silenced in prostate cancer cell lines. S100A4 was expressed more strongly in six UC lines than in normal cells, but was undetectable in three cell lines, in which azadC treatment increased expression. Fibroblasts displayed high S100A4 expression. Methylation of the promoter and an intronic regulatory site was accordingly lowest in connective tissue and blood cells, whereas all cells of urothelial origin showed at least partial and often heterogeneous methylation with moderate correlation to expression. No significant overall difference in S100A4 expression was found between benign and cancerous bladder tissues. Our data identify SNCG and S100A4 as new cell type-specific methylated genes. SNCG downregulation in some urothelial carcinomas is associated with hypermethylation, whereas upregulation in other cases occurs independent of methylation changes at the promoter. S100A4 methylation is likewise cell type-specific. Interestingly the gene can be expressed despite substantial methylation at its promoter, likely due to a low density of methylatable CpG-sites. Of note, our findings suggest caution in ascribing overexpression of the two genes in cancers to DNA hypomethylation, without considering changes in the cellular composition of the tumors. For instance, apparent hypomethylation and overexpression of SNCG might reflect an increased proportion of carcinoma cells, whereas apparent hypomethylation and overexpression of S100A4 might result from an increased proportion of fibroblasts.

357 Poster

EGR1 expression in breast cancer cells exposed to PPARg agonists occurs in a PPARg-independent pathway

S. Chbicheb[1], S. Salamone[2], M. Boisbrun[2], I. Grillier-Vuissoz[1], Y. Chapleur[2], S. Flament[1], S. Mazerbourg[1]
[1]Université Henri Poincaré, EA 3442, Nancy, France; [2] Université Henri Poincaré, UMR 7565 - Groupe S.U.C.R.E.S, Nancy, France

Both natural (Prostaglandin PGJ2) and synthetic ligands (Troglitazone (TGZ), Ciglitazone (CGZ), Rosiglitazone (RZG)) of PPARγ nuclear receptors inhibit the growth of several cancerous cell lines. Several studies suggest that these agonists possess PPARγ-independent effects. Indeed, Baek et al have shown that TGZ induced the expression of the early transcription factor EGR1 (Early Growth Response gene 1) followed by the expression of the pro-apoptotic Growth Differentiation Factor 15 (GDF15) in human colon cancer cells. In this report, EGR1 induction appeared to be independent of PPARγ because this event was not blocked by the PPARγ antagonist GW 9662 and not induced by other PPARγ ligands (Baek et al, 2004). Based on this study, we aim at characterizing the potential PPARγ - independent signaling pathway of PPARγ agonists in the breast cancer cell line MCF7. We are focusing on the early response of the cells involving phosphorylation pathways.

Cells were treated with PGJ2 and the synthetic ligands TGZ, CGZ, RGZ. Kinetics and dose-dependent induction of EGR1 mRNA expression were studied. EGR1 mRNA level peaked after 3 hours of incubation with 25 microM TGZ, CGZ and PGJ2 and then gradually decreased. RGZ did not show this effect. The increase in EGR1 protein level in MCF7 cells was observed by immunofluorescence. In contrast to the study in colon cancer cells, most of the PPARγ ligands induced EGR1 expression in MCF7 but did not lead to the increase in GDF15 mRNA level.

EGR1 induction by PPARγ ligands still occurred in cells co-treated with PPARγ antagonists (GW9662, T007) suggesting that PPARγ receptors were not involved in the early response. This was further confirmed by the induction of EGR1 mRNA using the non-PPARγ ligand, derived from TGZ, delta2-TGZ. Moreover, MEK/ERK inhibitors (PD098059, U0126) abolished the EGR1 mRNA induction by TGZ, CGZ and PGJ2. Furthermore, 5 min TGZ-treatment of the cells induced the phosphorylation of ERK1/2.

Overall, these results demonstrate that natural and synthetic PPARγ agonists, with the exception of RGZ, induce the activation of MAP Kinases followed by the expression of the early transcription factor EGR1. This early response is independent of PPARγ receptors and the mechanism of activation of the MAP Kinase pathway need to be elucidated. It would be interesting to determine if EGR1 induction is involved in a later PPARγ-independent event: the proteasomal degradation of the estrogen receptor alpha.