their EVH1 domains. Using a Yeast two-Hybrid approach, we identified NBR1 (Neighbour of BRCA1 gene 1 protein), a multi-domain scaffolding protein, as a specific binding partner of Spred-2 EVH1 domain. We show that NBR1 forms vesicular structures in vivo, which are exclusively positive for late endosomal-lysosomal markers. Spred-2 associates and colocalises with NBR1 in vivo, and in an EVH1 dependent manner. Furthermore, down regulation of signalling by Spred-2 is dependent on its association with NBR1, and results in targeting of the activated receptors to lysosomes. Overall, our findings suggest that, via interacting with NBR1, Spreds inhibit signalling by altering the endosomal trafficking of signalling receptors towards the lysosomal degradation pathway.

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The characterisation of PKB isoform specific signalling

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The PKB signalling pathway plays an important role in controlling multiple cellular processes, including cell survival, growth, proliferation, angiogenesis, and glucose metabolism, which when deregulated are considered to be hallmarks of cancer. Therefore, understanding how PKB is regulated is crucial for understanding the mechanisms of malignant transformation. The PKB family consists of 3 structurally similar isoforms, PKBα, β and γ that exhibit both common and unique functions. For example, single PKB isoform knockout mice display very different phenotypes indicative of specific functions (1-3). Conversely, double knockout mice exhibit a far more severe phenotypes suggesting there is also some functional redundancy (4). Strikingly, the deregulation of specific isoforms has been identified in distinct cancers. In order to understand the pleiotropic role of this kinase in normal and transformed cells it is critical to determine how the 3 isoforms differ in their regulation and downstream signalling. Such information might provide new drug targets for the treatment of isoform specific PKB cancers.

To determine the biochemical differences between the PKB isoforms, the kinetics of phosphorylation of peptide and protein substrates by purified GST-tagged isoforms were compared. To delineate differences in their downstream signalling, individual PKB isoforms were knocked down in HEK293 cells using isoform specific siRNAs. Western blot analysis was then used to screen for isoform specific substrates using either the phospho-PKB substrate antibody, or using phospho-antibodies towards known PKB effectors.

Purified PKBγ is more than 5 times more active than PKBα towards both peptide and protein substrates (5). These differences were also reflected in differential phosphorylation of the key regulatory sites within the catalytic (Thr308) and hydrophobic (Ser473) domains of each isoform. In fact our data suggests that phosphorylation at Thr308 rather than Ser473 dictates PKB activity levels. Similarities and differences in signalling between the PKB isoforms were also observed. All 3 isoforms signal to the ribosomal protein S6, however only PKBα and β signal to 4EBP1. Additionally, PKBα and γ were shown to signal to WNK1, whereas PKBβ did not. It will now be important to determine whether these differences in PKB signalling result in differential regulation of specific cellular processes.

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152 Poster

Sox9 regulates homeostasis of the intestinal epithelium through dual interactions with the canonical Wnt pathway

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Background: The HMG-box transcription factor Sox9 is expressed in the intestinal epithelium under the control of the Wnt/beta-catenin/Tcf4 pathway, which regulates multiple aspects of intestinal epithelium homeostasis. Activating mutations in the Wnt pathway trigger tumorigenesis. In vitro, Sox9 is required for the Wnt-dependant repression of a set of differentiation genes, and retro-inhibits the activity of the beta-catenin/Tcf4 complex.

Materials and methods: Here, we generated animals with an intestinal epithelium-specific deletion of Sox9.

Results: This results in an altered differentiation throughout the intestinal epithelium, with ablation of Paneth cells and depletion of the goblet cell lineage. In the colon, the morphology of the epithelium was severely altered and crypt hyperplasia/dysplasia occurred, with upregulation of key Wnt pathway target genes such as c-Myc and Cyclin-D1.

Conclusion: This indicates a critical role of Sox9 in regulating intestinal epithelium homeostasis, both as a transcriptional target and a regulator of the Wnt signalling pathway.

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MUC1 is a target of hypoxia-inducible factor transcription factor in renal clear carcinomatous cells

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Background: Renal clear cell carcinoma (RCC) represents 75% of renal malignancies in the adult. The von Hippel-Lindau (VHL) is a critical suppressor of renal oncogenesis. The VHL gene product is part of a ubiquitin ligase complex that targets the alpha-subunits of the heterodimeric transcription factor hypoxia-inducible factor (HIF) for proteasomal degradation, when oxygen is available. Accumulation of HIF upon loss of VHL (mutation, hypoxia) is crucial for the development of RCC. Moreover, the transmembrane MUC1 mucin is frequently overexpressed in RCC and the level of its expression is associated with the Fuhrman grade and with tumour progression. The overexpression and membrane delocalization of MUC1 is also associated with a worse prognosis and a shorter survival. In this work, our aim was to identify molecular mechanisms that could be responsible for the altered pattern of expression of MUC1 in RCC. Materials and methods: We have studied MUC1 expression and regulation under hypoxic condition (i-e HIF-1alpha accumulation) in ACHN renal carcinomatous cell line and HK-2 normal proximal tubular renal cells. We used transfection techniques, siRNA approaches and pharmalogical inhibitors; mRNA and protein levels were determined by RT-PCR and western blot, respectively. Results: We showed that, under hypoxic condition, (i) MUC1 is overexpressed at the transcriptional, mRNA and protein levels, (ii) this regulation involves HIF-1 alpha transcription factor and NF-KappaB and PI3K signaling pathways and (iii) HK-2 and ACHN invasiveness is dramatically increased. Conclusion: These findings indicate (i) that MUC1 is a target of both transcription factors and signaling pathways induced in hypoxia and (ii) suggest that MUC1 is directly involved in renal carcinogenesis.

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The essential role of BRAF and KRAS mutations in colorectal serrated adenocarcinoma

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Backround. The serrated pathway has recently emerged as an important alternative route to colorectal cancer development. This pathway originates from serrated polyps and culminates in serrated adenocarcinoma, which we have recently shown to possess distinctive morphologic and genetic features. Serrated polyps are known to bear high frequencies of KRAS and BRAF mutations and DNA microsatellite instability (MSI). Since these alterations are frequently observed in sporadic colorectal cancers, it has been suggested that up to 20 % of colorectal cancers might evolve via the serrated pathway. The frequency of KRAS and BRAF mutations in serrated adenocarcinoma is not yet known and the link between serrated polyps with mutations either in KRAS or BRAF and serrated adenocarcinoma has not yet been established. Our study aimed to clarify the molecular pathogenesis of this pathway and to find out the possible importance of KRAS and BRAF.

Materials and methods. 37 serrated adenocarcinomas and 24 conventional adenocarcinomas matched for gender, grade, Dukes´ stage and location were analyzed for the oncogenic mutations of KRAS c12/13 and BRAF V600E. Mutational analysis was performed by using direct sequencing of the genomic PCR products. MSI of the cases was classified as stable (MSS), low level (MSI-L) or high level (MSI-H) using NIH consensus markers.

Results. A total of 61 cases were included in the mutational analysis. In serrated adenocarcinomas BRAF mutations were present in 32.4% (12/37) and KRAS mutations in 43.2% (16/37). In conventional carcinomas KRAS mutations were present in 33.3 % (8/24), but BRAF mutations were not observed ($p = 0.002$).

MSI analysis was successful in 30/37 serrated adenocarcinomas and in 24/24 conventional carcinomas. Cases with mutated KRAS did not exhibit concurrent MSI-H ($p = 0.002$), whereas 33.3 % of serrated cancers with BRAFV600E were MSI-H.

Conclusions. This is the first study to document a distinct association of KRAS and BRAF mutations with serrated adenocarcinoma. Both KRAS

and BRAF mutations have frequently been observed in serrated polyps. BRAF has been suggested to yield more potential to malignant progression. This study shows that KRAS mutations are equally important in the development of serrated adenocarcinoma.

Sporadic MSI-H cancers have been suggested to evolve via the serrated route. This suggestion has been drawn from the high frequency of MSI-H in serrated adenomas. In the present study MSI-H was observed in both serrated and conventional cancers, but only in serrated cancers it was associated with BRAF mutations.

The 75.7% frequency of KRAS and BRAF mutations in the serrated adenocarcinomas further supports the idea that the oncogenic activation of either of these genes is essential for the development of serrated cancer. As BRAF mutations were completely absent in the conventional cancers, it is likely that sporadic colorectal cancers representing with mutated BRAF originate via the serrated pathway.

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The thyroid hormone receptor b1 act as a potent suppressor of tumor invasiveness and metastasis

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Thyroid hormone receptors (TRs) play multiple roles in normal proliferation and homeostasis, whereas aberrant TR activity results in endocrine and neoplastic diseases. Although TRs are ubiquitously expressed in normal tissues, reduced TR expression, as well as alterations in TR genes, are common events in cancer. In order to study the role of TRb1 in tumorigenesis, invasiveness and metastasis formation, and since altered TRs are found in breast and liver cancer, we re-expressed the TRb1 isoform in breast cancer and hepatocarcinoma human cell lines. Expression of several angiogenic, epithelial and mesenchymal markers was analyzed in tumors formed by breast cancer cells inoculated orthotopically into the fat mammary pad and by hepatocarcinoma cells injected heterotopically into the flanks of nude mice. In order to study the role of TRb1 in metastasis development, cells were injected into the mice tail vein. The invasive capacity of the cells in culture, as well as the expression of genes and the activity of signaling pathways involved in tumor invasiveness and metastasis formation was also examined.

We have observed that expression of TRb1 in hepatocarcinoma and breast cancer cells reduces tumor growth, causes partial mesenchymal to epithelial cell transition and has a striking inhibitory effect on angiogenesis, invasiveness, extravasation and metastasis formation in nude mice. These changes correlate with the reduced ability of TR-expressing cells to grow in the absence of a solid substrate and to migrate through a matrigel matrix. The underlying mechanism for these TRb1 actions appears to be the down-regulation of expression of genes required for tumorigenesis and metastasis formation and the reduced response of signaling pathways, such as MAPK and PI3K. Thus, we have found that TRb1 represses expression of genes that have been clinically correlated with metastasis formation. These genes include among others growth factor and chemokine receptors, metalloproteases, COX2 and ID1. In addition, TRb1 increases the expression of the anti-metastatic genes caspase1 and IGFBP3. Finally, the receptor blocked the proliferative response to growth and transforming growth factors by antagonizing activation of signaling pathways such as MAPK or PI3K.

These results define a novel role for TRb1 as a tumor and metastasis suppressor gene,and provides a starting point for the development of novel therapeutic strategies for the treatment of human cancer.

POSTER SESSION

General, molecular and genetic epidemiology 1

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p53PIN3 polymorphic motif involved in G-quadruplexes: effect on alternatively spliced p53 transcripts

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The tumour suppressor protein p53 is activated by several stresses to regulate proliferation, apoptosis and DNA repair. This protein corresponds to a full-length product, termed TAp53, and is encoded for by the fully spliced p53 mRNA (FSp53). Its suppressor activities are counteracted by a N-terminal truncated isoform, ?Np53, generated by an alternatively spliced mRNA retaining intron 2 (p53I2). In addition, several common polymorphisms are found in the TP53 gene including p53PIN3, a 16bp duplication in intron 3 (A1: non-duplicated allele; A2: duplicated allele), which is associated with an altered cancer risk and a reduced level of p53 mRNA. In this study, we investigated whether the p53PIN3 polymorphism may impact on the levels of p53 transcripts. We demonstrated using a reverse transcriptase elongation assay that G-quadruplex structures overlap the p53PIN3 sequence and that their topologies are dependent upon the p53PIN3 status. In A1 cells, site-directed mutagenesis and treatment with TMPyP4, a cationic porphyrin, which modulates G-quadruplex formation, showed that disruption of the G-quadruplexes favours the retention of intron 2 and thus the production of FSp53 mRNA. In A2 cells, the same experiments showed that G-quadruplexes are also involved in p53 mRNA expression, but that other mechanisms of mRNA processing are involved, suggesting a complex pattern of p53 mRNAs expression depending on p53PIN3 status. Analysis of both FSp53 and p53I2 transcripts in lymphoblastoid cells, carrying either A1 or A2 allele, revealed a large decrease of these two transcripts in A2 cells compared to A1 cells. This reduction may be explained in part by the influence of G-quadruplexes on alternative splicing of p53. The polymorphic nature of the G-quadruplexes provides both a mechanism for the regulation of the alternative splicing of intron 2 leading to Np53 isoform production and for the genetic susceptibility associated with A2 p53PIN3 allele.

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Alcohol drinking and head and neck cancer: a meta-analysis on aldehyde dehydrogenase-2 evidence a causal relationship from mendelian randomisation

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Background. Individuals homozygous for *2 variant allele of the Aldehyde dehydrogenase-2 (ALDH2) gene are unable to metabolize acetaldehyde, that prevent them from alcohol drinking, while 1*2 heterozygotes have 6-fold higher blood acetaldehyde concentration with respect to 1*1 post-alcohol consumption. If acetaldehyde is pathogenetic, 2*2 should be protected from head and neck cancer and 1*2 being at higher risk. Since this polymorphism is distributed randomly during gamete formation, its association with head and neck cancer should be unconfounded by smoking. We carried out a meta-analysis of ALDH2 and head and neck cancer association studies, and we investigated the consistency between the expected odds ratio for head and neck cancer among drinkers from the largest pooled-analysis among never smokers, and the observed odds ratio from our meta-analysis.

Methods. We searched Medline and Embase up to 31st January 2008, for all relevant studies on the association between ALDH2 polymorphism and head and neck cancer. Authors of the eligible papers were invited to provide genotype data stratified for selected covariates. Pooled odds ratio and 95%CI were calculated by random effects model. Consistency between the expected and observed odds ratio was assessed by an interaction test.

Results. Six studies were selected, with a total of 945 cases and 2917 controls. Risk of head and neck cancer was reduced among 2*2 homozygotes [OR of 0.64 (95%CI: 0.39-1.03)] relative to 1*1, and increased among heterozygotes [OR of 1.83 (95%CI: 1.21-2.77)] especially if heavy drinkers. The expected odds ratio for head and neck cancer due to alcohol intake compared with never drinkers based on the pooled-analysis was 1.40 (95%CI: 0.89-2.21) in 1*1 individuals. In our meta-analysis the odds ratio for head and neck cancer was 1.56 (95%CI: 0.97-2.56) among 1*1 homozygotes compared to 2*2 (p-value for interaction = 0.75).

Conclusion. These data support the theory that alcohol raises head and neck cancer risk through the carcinogenic action of acetaldehyde and the concordance between the expected and observed odds ratio is consistent with a causal role of alcohol in head and neck cancer aetiology.