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.

1. Chen, W. S, et al (2001) Genes Dev 15, 2203-2208
2. Easton, R. M, et al (2005) Mol Cell Biol 25, 1869-1878
3. Cho, H., et al (2001) Science 292, 1728-1731
4. Peng, X. D, (2003) Genes Dev 17, 1352-1365
5. Cristiano, B. E, et al (2006) Cancer Res 66, 11718-11725

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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