roles on cell growth and expression of differentiation. Thyroid cells express 3 of the 21 Cx gene family members: Cx32, Cx43 and Cx26. Using genetically-modified mice, we found that Cx32 acts as a down regulator of growth of normal thyroid. In this study, we examined the impact of the inactivation or the over-expression of the Cx32 gene on oncogene-driven growth and tumorogenesis of the gland. Cx32-KO mice or mice over-expressing Cx32 in the thyroid (Cx32-T+) were crossed with transgenic mice expressing, selectively in the thyroid, either the E7 oncogene (from human papilloma virus) or the Ret/PTC3 oncogene. The Ret/PTC3 (RP3) oncogene derives from a chromosomal rearrangement leading to a fusion of the 3'-part of RET gene (encoding a tyrosine kinase) and the 5'-part of ELE1 gene. Mice expressing E7 or RP3 oncogene develop thyroid hyperplasia and tumors. At 2 months of age, E7 and RP3 mice exhibited i) a 6 to 8-fold increase in thyroid weight as compared to normal mice, ii) an increase in expression levels of thyroid-specific genes, PAX8, TITF1, FOXE1, NIS and TPO and iii) histological signs of tumorogenesis (follicles with an abnormal shape and papillary structures). At 5 months, there was a further rise in thyroid size (up to 180 mg in E7 versus 3 mg in wild-type mice) but a decreased expression of thyroid genes indicating thyroid de-differentiation. As previously reported, the thyroid size of Cx32-KO and Cx32-T+ mice was similar to and about 30% smaller than that of wild-type mice, respectively. Thyroid parameters (size of the gland, histology, gene expression) were neither different in Cx32-T+/E7 and E7 mice nor in Cx32-T+/RP3 and RP3 mice. This was unexpectedly due to oncogene-induced block of Cx32 over-expression. Interestingly, mice depleted in Cx32 and expressing either E7 (Cx32-KO/E7) or RP3 (Cx32-KO/RP3) showed a reduced thyroid mass (by 40 %) as compared to E7 or RP3 mice but no difference in thyroid histology or differentiation status. In conclusion, we show that thyroid hyperplasia and tumorogenesis induced by E7 or RP3 was reduced in the absence of Cx32. Thus, Cx32 which exerts a negative control on thyroid growth regulated by thyrotropin and cAMP cascade, would be a positive operator of thyroid growth triggered by oncogenes acting through other signalling cascades including MAPK cascade.

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Characterisation of an intestinal neoplasm modifier locus in Apc Min mice

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ApcMin mice have provided examples of loci modifying adenoma numbers in the intestines of inbred strains (Modifier of Min 1 and 2; Mom). Because of unknown variation introduced by a single founding male mouse, our Min stock was not on a pure C57BL/6J background and exhibited several polymorphic loci, including a region on chromosome 18 distal to Apc. Through selective breeding for homozygosity for distal chromosome 18 markers, six recombinant lines that presented with limited intra-line variation in adenoma numbers were established. One line (V) showed a particularly severe phenotype (mean adenoma number ± SEM, 370 ± 21) compared with the other lines that recorded significantly lower means (3- to 5-fold; $P < 10–3$, t test). A modifying locus for this phenotype was mapped to proximal chromosome 18. We discuss here several experiments aiming to characterise this tumorigenesis modifier, which is termed Mom3. Taking into consideration the possibility of the existence of a modifier gene and of potential structural variation in the region, we have sequenced a panel of candidate genes and performed array comparative genomic hybridisation. In addition, the novel role of a microRNA in mediating the variation in polyp burden between the 2 lines is described, with complimenting functional analyses from cell line work detailed.

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DNA methylation profiles in colorectal cancers of Lynch-syndrome patients

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Lynch syndrome is an inherited disease that manifests as carcinomas forming predominantly in the colorectum. Cancer development is initiated by a DNA mismatch repair (MMR) malfunction that is caused by germline mutations in MMR genes, mainly MLH1 and MSH2. A significant hallmark of repair defects is a high level of instability in microsatellites (MSI-H). In many sporadic colorectal cancers this MSI phenotype is caused by an epigenetic event, MLH1 promoter hypermethylation. Unstable sporadic cancers are characterized by inactivation of many tumour suppressor genes by extensive promoter methylation (known as the CpG island methylation phenotype, or CIMP). To investigate the possible role of epigenetic alterations in causing MMR deficiency and thereby hereditary cancers we evaluated the MLH1 specific and global hypermethylation in colorectal tumours of Lynch-syndrome patients.

To analyze the methylation status of the MLH1 promoter, methylation-specific PCR (MSP) and genomic sequencing of bisulfite-modified DNA were performed in 22 Lynch-syndrome patients, plus one individual with sporadic MSI+ tumour and 10 patients who suffered from microsatellite stable (MSS) colorectal cancer. Ten healthy persons were used as controls. Global hypermethylation level in all samples was evaluated by MSP using four informative MINT markers (1, 2, 12 and 31).

Out of 22 Lynch-syndrome colon cancers evaluated by MSP, 14 (63.6%) demonstrated various levels of MLH1 methylation in distal region from the transcriptional start site. Ten patients had an absence of MLH1 protein expression and/or MLH1 germline mutation, and in three patients an absence of MSH2 protein expression or MSH2 germline mutation were found. Methylated CpG sites in both the distal and proximal regions were found in tumour samples of 4 (18.2%) patients regardless of whether they had germline alterations in their MLH1 or MSH2 genes. Moreover, only 7/18 (38.9%) of the patients with positive MSP methylation findings were confirmed by sequencing. In addition, similar methylation patterns in MLH1 promoter were observed in five MSS cancers, where the normal function of DNA mismatch repair was expected. None of 22 Lynch-syndrome patients had CIMP in their tumours. Our results do not indicate a relevant association between the methylation patterns and MLH1 transcription silencing in tumours of Lynch-syndrome patients.

In summary, more detailed analyses of the MLH1 promoter and additional study of global hypermethylation documented that epigenetic events are redundant in Lynch-syndrome aetiology, in contrast to the widespread DNA methylation that is observed in sporadic unstable colorectal tumours. These methylation-profile differences can lead to more effective molecular diagnosis of Lynch syndrome.

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Expression of mammary derived growth inhibitor (MDGI) results in phenotypic reversal in breast cancer

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MDGI (also known as FAPB-3/H-FABP) is a small cytosolic protein, which has been suggested in some studies to function as a tumor suppressor in breast cancer. However, no mechanism of action has been described thus far. We demonstrate that MDGI is lost in cultured cells but is expressed in normal breast epithelium and a subset of breast cancers in vivo. Interestingly, reconstitution of MDGI expression results in reduced proliferation and partial phenotypic reversion of breast cancer cells specifically in three-dimensional (3D) ECM. Concomitantly, re-expression of MDGI in breast cancer cells results in a dramatic re-localization of EGFR to an intracellular compartment where the receptor remains active and is not degraded. Thus, cells expressing MDGI exhibit alterations in EGFR trafficking resulting in increased intracellular EGFR. Taken together, these results suggest that MDGI regulates proliferation and cell morphology in EGFR over-expressing breast cancer cells via altering EGFR function in cells cultured in 3D basement membrane cultures.

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SPRED redirects activated receptors to lysosomes via scaffolding protein NBR1

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Spreds (Sprouty Related protein with EVH1 Domain) comprise a conserved family of signalling inhibitors which act downstream of a variety of mitogenic signals such as EGF, FGF, and cytokines. While sharing a Cys-rich C-terminal SPRY domain with Sprouty proteins, Spreds further contain a central KBD (Kit Binding Domain), and an N-terminal EVH1 (Ena/VASP Homology 1) domain, the later being pivotal for their function. However, the molecular mechanism underlying Spreds inhibitory activity has remained largely undefined. Given their functional importance, and since EVH1 domains are known protein-protein interaction modules, we hypothesized that an as yet unidentified critical partner of Spreds might be interacting with

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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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