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.

147 Poster

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.

148 Poster

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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149 Poster

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.

150 Poster

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