Hs578T cells decreases Fra-1 concentration and this inhibition is totally reversed by the proteasome inhibitor MG132. Chase experiments using cyclohexymide, and protein synthesis inhibitor, suggest an effect of PKCθ on Fra-1 half-life. In addition, PKCθ increases ERK1/2 activation in MCF7 cells. However, whereas MAPK inhibitors inhibit Fra-1 up-regulation by PKCθ, ERK1/2 is unlikely implicated in Fra-1 stabilization by PKCθ. Indeed, constitutively active PKCθ increases the half-life of a Fra-1 mutant in which S252 and S265 are changed in alanine preventing phosphorylation by ERK1/2. Conversely, a dominant negative PKCθ decreases expression of Fra-1 protein when the 2 serines are replaced by aspartic acid miming phosphorylation. The hypothesis of an implication of ERK5, which has been also reported to stabilize the Fra-1 protein in other cells, and the possibility of a direct effect of PKCθ are now under investigation.

We therefore propose that high PKCθ expression level in ER- cells could be at least in part responsible for the aberrant Fra-1 expression observed in these cells leading to the maintenance and/or acquisition of the aggressive phenotype.

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Survival to genotoxic stress in the presence of chromosome instability in fission yeast

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During mitosis sister chromatids must be equally segregated to daughter cells in order to maintain the accurate transmission of genetic material. This requires the assembly of a bi-polar spindle in which one sister chromatid is attached to microtubules from the other pole, in a process known as chromosome bi-orientation. The attachment of sister chromatids to the mitotic spindle apparatus is controlled by the spindle assamble checkpoint (SAC) Attachment of spindle microtubules to chromosomes occurs through the kinetochore, a specialized protein structure that associates with the centromeric region of the chromosome. The fission yeast, Schizosaccharomyces pombe, is an attractive model system in which to examine the mechanisms governing the establishment of spindle bi-orientation for a number of reasons. Firsly, fission yeast centromeres closely resemble those in animal cells and, secondly, each kinetochore is bound to multiple microtubules. Thus chromosomes can become both syntelically and merotelically attached during mitosis. These configurations need to be corrected to allow equal segregation of sister chromatids at anaphase in order to conserve the euploidy (normal chromosome number) in eukaryotic cells. A dysfunctional kinetochore represents one possible source for chromosome instability (CIN) and the generation of aneuploidy. The kinetochore is a large complex of proteins and associated centromeric DNA that is responsible for mediating the segregation of sister chromatids to daughter cells via its interactions with the mitotic spindle.

In this study, we have designed an screen in order to isolate novel fission yeast genes required for chromosome segregation. We have characterized the phenotype of cells carrying ramdon mutations in the genome which are critical for chromosome stability and exhibits high rate of chromosome loss. We are investigating the mechanisms controlling correct anaphase. The genetic interaction of this mutants with SAC genes is critical for survival, and also the correct function of the genes are required for genotoxic stress recovery. The identification of the homologues of these genes in humans could provide new candidate genes that may be mutated or misregulated in human cancers. Also open the possibility of new therapies that allow to increase the sensitivity to the treatment.

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Autocrine hGH-regulated PAX5 inhibits mammary neoplastic progression

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Autocrine hGH has been demonstrated to increase cell proliferation, survival and oncogenic transformation in a human mammary carcinoma cell line. In addition, autocrine hGH is sufficient to promote oncogenic transformation of an immortalized, but otherwise normal, human mammary epithelial cell line and promote tumour formation in nude mice. We have identified paired homeobox 5 (PAX) as a gene upregulated by autocrine hGH. RT-PCR, western blot and reporter assays confirmed upregulation of PAX5 mRNA, protein expression and transcription activity by autocrine hGH in MCF-7 cells.

Paired domain homeodomain (PAX) genes are expressed in a distinct spatial and temporal manner during embryo development, controlling organogenesis through regulation of tissue development and cellular differentiation. We therefore investigated the role of PAX5 in mammary neoplastic progression. We established stable forced expression of PAX5 as well as siRNA mediated stable depletion of endogenous PAX5 expression in the mammary carcinoma cell line MCF-7. We demonstrated in vitro that forced expression of PAX5 in MCF-7 cells decreased total cell number, accompanied with a reduction of cell cycle progression and survival, while PAX5 siRNA mediated silencing in MCF-7 cells increased total cell number. RT-PCR and luciferase assays demonstrated that PAX5 regulates the expression of several key genes involved in cell cycle regulation, such as p53, p21, Cyclin D1, Bcl-xL and Bcl-2. We demonstrated by wound healing and migration assays that PAX5 transient forced expression in MDA-MB-231 cells reduced their motility and migration, while the depletion of endogenous PAX5 expression stimulated motility and migration of MCF-7 cells. We also demonstrated that PAX5 forced expression reduced the invasiveness of MCF-7 and MDA-MB-231 cells, while PAX5 silencing promoted the invasiveness of MCF-7 cells. Using a colony formation in soft agar assay, we also demonstrated that forced expression of PAX5 dramatically reduced MCF-7 anchorage independent cell growth. Finally, we demonstrated that forced expression of PAX5 reduces tumour growth in immunosuppressed mice. Thus our results identified PAX5 as a potential tumour suppressor gene in mammary carcinoma. Expression of PAX5 induced by autocrine hGH therefore appears to function as a negative regulator of autocrine hGH stimulated oncogenic effects.

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Upregulation of genes involved in rRNA processing in colon cancer

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Protein translation and ribosome biogenesis are essential cellular processes, and the control of these two activities is tightly regulated at different levels. Ribosome biogenesis is a very coordinated multi-step process that processes and assembles rRNA into ribosomal subunits and finally adds ribosomal proteins to constitute the mature ribosome. It is well known that single components of this machinery are deregulated in cancer. The increase of cellular growth or proliferation needs an enhanced protein content and protein translation, and has been known already reported. However, whether protein translation is a direct effect or a cause of the carcinogenic development is still a wide open question.

In colorectal cancer, the third most frequent form of cancer worldwide, the differential expression of several ribosomal proteins has been reported in neoplastic tissue. These proteins are exported from the cytoplasm to the nucleolus, where ribosome assembly takes place.

The contribution of several components of the PesI-BopI complex, involved in ribosomal biogenesis has been studied and showed that, in particular Bop1 is upregulated in colorectal cancer. This BOP1 upregulation is associated with increased gene copy number suggesting that BOP1 overexpression may be one of the main oncogenic consequences of 8q24 amplification in colorectal cancer.

Based on a microarray profiling comprising 168 colorectal samples and 10 normal mucosas using U133plus2.0 arrays we have analyzed the pattern of expression of the 170 genes that comprise Coute's (1) human ribosome biogenesis dynamics model.

Interestingly, the pattern of expression of these genes is almost identical for microsatellite stable (MSS) and microsatellite instable (MSI) samples. In both cases over 78 % of the studied genes are significantly upregulated ($\log2 > 0.5$, $p > 10\text{-}3$) when compared to normal mucosa. In contrast, only 14 % of all genes analyzed (54000 probes) are upregulated with $\log2 > 0.5$.

EXOSC5, BOP1 and RUVBL1 are the top 3 upregulated genes in MSS specimens. We have mapped the genes subject of the study into a transcriptome correlation map and found that 20% of the genes can be associated with an increased gene dosage.

This data and specially the fact that MSI samples mirror the results found for MSS samples suggest that there must be other mechanisms that contribute to alteration of ribosome biogenesis genes upregulation, besides gene copy number alterations. This general vision of the ribosome biogenesis dynamics expression profile suggests that somehow the upregulation is a coordinated multi-step process that has the potential to converge in the overproduction of matured ribosomal RNA.

(1).Couté Y, et al. Mass Spectrom Rev. 2006 25, 215-34

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Impact of Connexin32 deletion on E7 or RET/PTC3 oncogene-driven growth and tumorogenesis of the thyroid gland

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Connexins (Cx) form gap junctions (GJ) and allow cell-to-cell exchange of small molecules (<1kDa). Cx through GJ or by themselves play regulatory

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