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.

143 Poster

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.

144 Poster

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.

145 Poster

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

146 Poster

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