

as a new lead for the development of Aurora kinase inhibitors and is currently under investigation in vivo.

139 **$\alpha_5\beta_1$ integrin-emanating signals remodel nuclear architecture through the activation of ERK1/2 and p38 α MAPKs during invasive cell growth** Poster

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Invasive cell growth is a physiological process executed by stem and progenitor cells during embryonic development and postnatal organ regeneration. Growing evidence indicates that this program is usurped by cancer cells, resulting in metastasis. To this regard, the integrin receptors are deeply involved in the invasive capacity of progenitor and cancer cells. Here, we assessed the role of β_1 integrins-emanating signals in the genomic events that occur during the invasive growth of MLP29 murine hepatic progenitor and Hep16 Hepatocellular carcinoma (HCC) cells. Cytometric and immunoblot analysis of integrin expression in MLP29 cells showed high levels of β_1 integrins (fibronectin-FN and collagen IV-COL IV receptors) and low levels of α_v and β_3 integrins (vitronectin-VN receptors). In concordance, MLP29 cells presented high adhesion to FN and COL IV, and low adhesion to laminin (LMN) and vitronectin (VN). By contrast, HCC cells exhibited opposite adhesive properties, which agrees with their high levels of α_v integrins and low levels of β_1 integrins. Since $\alpha_5\beta_1$ is the main FN receptor in hepatocytes, we used a functional blocking antibody against the $\alpha_5\beta_1$ integrin to investigate MLP29 cells invasion and growth. We detected marked cell spreading and actin cytoskeleton reorganization, and this was associated with activation of the ERK1/2 and p38 α MAPKs cell signalling pathways. At the nuclear level, 3D-analysis of centromere distribution in interphase nuclei, showed that the average number of chromocenters per nucleus increased significantly by the functional blockade of $\alpha_5\beta_1$, or decreased by $\alpha_5\beta_1$ activation upon attachment to FN. Interestingly, these $\alpha_5\beta_1$ -induced alterations were abolished by pharmacological inhibition of ERK 1/2 (U0126) and p38 MAPK (SB239063). In Hep16 HCC cells, inhibition of the constitutively hyper-activated ERK 1/2 and p38 MAPKs also induced centromere reorganization. In line with these findings, gene expression analysis by microarray technology revealed that $\alpha_5\beta_1$ blocking induced the differential expression of a significant amount of genes involved in the nuclear structure and nucleic acid binding. Furthermore, these $\alpha_5\beta_1$ -mediated signals drastically increased the acetylation status of Histone H3 at lys 9/14. Collectively, these results suggest that invasive cell growth in hepatic progenitor cells involves a remodelling of the nuclear architecture regulated, at least in part, by the $\alpha_5\beta_1$ integrin-mediated activation of the ERK1/2 and p38 α MAPKs. This may be also applied to HCC cells presenting a hyper-activation of major pro-survival cell transduction cascades, which may be accountable for the high invasive capacity.

140 **Steroidogenic factor-1 gene dose and adrenocortical tumors** Poster

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Adrenocortical tumor (ACT) in children is a rare form of neoplasm but its incidence is higher in southern Brazil than in the rest of the world. In that region, it is almost invariably found associated with a specific germline TP53 mutation (R337H) and loss of heterozygosity in the other allele. We have shown an increased copy number of the steroidogenic factor 1 (SF-1; NR5A1) gene associated with its overexpression in the majority of childhood ACT compared with normal age-matched adrenal gland. Steroidogenic Factor-1 (SF-1/Ad4BP; NR5A1), a transcription factor belonging to the nuclear receptor superfamily, has a pivotal role for adrenogenadal development in humans and mice.

Using an integrated approach comprising human tumor adrenocortical cell cultures, gene expression profiling and transgenic mice analysis, we have defined the role for SF-1 dosage in adrenocortical tumors development.

We show that SF-1 overexpression increases human adrenocortical cell proliferation through opposing effects on cell cycle and apoptosis by using an inducible cellular system. This effect is dependent on an intact SF-1 transcriptional activity. Gene expression profiling showed that an increased

SF-1 dosage regulates transcripts involved in steroid metabolism, cell cycle, apoptosis, and cell adhesion to the extracellular matrix. Consistent with these results, increased SF-1 levels selectively modulate the steroid secretion profile of adrenocortical cells, reducing cortisol and aldosterone production and maintaining DHEA-S secretion. We identified a novel pro-apoptotic factor for adrenocortical cells, NOV/CCN3, whose levels are significantly reduced by SF-1 overexpression in human adrenocortical cells and are also reduced in primary adrenal tumors. In mice, increased SF-1 dosage produces adrenocortical hyperplasia and formation of tumors which originate from the subcapsular region of the adrenal cortex. These tumors express gonadal markers and activated Stat3.

Our studies reveal the critical role of SF-1 gene dosage for adrenocortical tumorigenesis and constitute a rationale for the development of drugs targeting SF-1 transcriptional activity for ACT therapy.

141 **NPM-ALK modulates the p53 tumour suppressor pathway in a JNK and PI 3-Kinase dependent manner: MDM-2 is a potential therapeutic target for the treatment of ALK-expressing malignancies** Poster

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Anaplastic large cell lymphoma (ALCL) is in the majority of cases a paediatric disease of a T- or null-cell phenotype and is characterised by the presence of the t(2;5)(p23;q35) or variant translocations involving the ALK gene on chromosome 2. This chromosomal translocation generates the Nucleophosmin-Anaplastic Lymphoma Kinase (NPM-ALK) fusion protein, a hyperactive kinase with transforming properties. The p53 tumour suppressor gene is rarely mutated in ALK-expressing ALCL, perhaps one reason why this disease has a good prognosis. However, the mechanism controlling p53 activity in ALCL has not been fully elucidated. We show in patient-derived ALCL cell lines and NPM-ALK transformed BaF3 cells that NPM-ALK induces post-translational modification of the p53 antagonist MDM2, leading to inactivation of the p53 tumour suppressor pathway. Furthermore, we demonstrate that the PI 3-Kinase-Akt pathway downstream of NPM-ALK is responsible for this activity. It therefore follows that inactivation of MDM2 with the specific inhibitor nutlin-3 results in a decrease in proliferation and subsequently apoptosis of NPM-ALK-expressing ALCL cells, a response that is enhanced when cells are exposed to nutlin-3 in conjunction with the PI 3-Kinase inhibitor LY294003. We also demonstrate that NPM-ALK activates JNK by phosphorylation in turn leading to JNK-mediated sequestration and degradation of p53. This activity can be attenuated following administration of a specific JNK inhibitor. We conclude that NPM-ALK regulates the activity of the p53 tumour suppressor pathway via sequestration by JNK and MDM2 leading to its degradation. MDM2 antagonists in combination with JNK/PI 3-Kinase inhibitors may therefore be potential targets for the treatment of ALK-expressing malignancies such as ALCL.

142 **PKC theta increases phosphorylation and stability of the Fra-1 protein in invasive breast cancer cell lines** Poster

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In contrast to cells expressing estrogen receptor α (ER+), the most invasive ER- breast cancer cell lines express high constitutive AP-1 binding activity mainly due to high concentration of Fra-1, a member of the FOS family. Fra-1, which induces the expression of genes implicated in breast cancer progression, enhances in vitro proliferation and invasiveness of these cells. These results led us to investigate the molecular mechanisms responsible for high expression of Fra-1 in the most invasive cells.

The effect of PKC θ on Fra-1 expression and phosphorylation was evaluated by transient transfection of constitutively active and dominant negative PKC θ mutants in ER+ MCF7 cells and ER- Hs578T human breast cancer cells, respectively. Implication of ERK1/2 and/or ERK5 in this regulation was determined by the use of the MAPK inhibitors U0126 and PD98059 and of Fra-1 proteins mutated on S252 and S265 whose phosphorylation by ERK1/2 prevents Fra-1 degradation by the proteasome.

Results show that PKC θ , whose expression can be detected in ER- but not ER+ cells, increases Fra-1 expression. Ectopic expression of a constitutively active PKC θ mutant in MCF7 cells increases Fra-1 level and Fra-1 phosphorylation as observed by the appearance of low migrating bands. Moreover, introduction of a dominant negative PKC θ mutant in

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 PKC θ on Fra-1 half-life. In addition, PKC θ increases ERK1/2 activation in MCF7 cells. However, whereas MAPK inhibitors inhibit Fra-1 up-regulation by PKC θ , ERK1/2 is unlikely implicated in Fra-1 stabilization by PKC θ . Indeed, constitutively active PKC θ 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 PKC θ 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 PKC θ are now under investigation.

We therefore propose that high PKC θ 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 assembly 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. Firstly, 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 a screen in order to isolate novel fission yeast genes required for chromosome segregation. We have characterized the phenotype of cells carrying random 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 these 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 Pes1-Bop1 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 ($\log_2 > 0.5$, $p > 10^{-3}$) when compared to normal mucosa. In contrast, only 14 % of all genes analyzed (54000 probes) are upregulated with $\log_2 > 0.5$.

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 tumorigenesis 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