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

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$\alpha_5\beta_1$ integrin-emanating signals remodel nuclear architecture through the activation of ERK1/2 and p38a MAPKs during invasive cell growth

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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 αv 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 p38α 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 acethylation 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 p38α 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.

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Steroidogenic factor-1 gene dose and adrenocortical tumors

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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 adrenogonadal 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.

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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

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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.

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

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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 PKCθ on Fra-1 expression and phosphorylation was evaluated by transient transfection of constitutively active and dominant negative PKCθ 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 UO126 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 PKCθ, whose expression can be detected in ER- but not ER+ cells, increases Fra-1 expression. Ectopic expression of a constitutively active PKCθ 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 PKCθ mutant in