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Up-regulated PI3K signalling pathway in basal-like breast carcinomas

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Targeting specific signalling pathways is a challenge for breast cancer patient management. Basal-like carcinomas, which represent at least 15% of breast tumours, are strongly associated with poor survival and have no targeted therapy available. Therefore, there is an urgent need to identify druggable targets for these specific tumours. In order to discover such targets, we are searching for deregulated signalling pathways in human basal-like carcinomas. In this study, we have investigated specifically the oncogenic PI3K pathway in thirteen basal-like carcinomas, and compared it to the one, known to be activated, of a control series composed of eleven hormonal receptor negative- and grade III- matched HER2+ carcinomas. Both tumour populations were characterized by immunohistochemistry and gene expression analysis. Using reverse phase protein microarray and Western-blotting, PI3K signalling pathway was found to be up-regulated in basal-like carcinomas as shown with the activation of downstream molecules such as Akt and mTOR. Most importantly, basal-like carcinomas expressed significantly lower levels of the tumour suppressor PTEN that correlated negatively in a significant manner with Akt activity. Similarly to human biopsies, human basal-like cell lines exhibited an activation of the PI3K signalling pathway and a low/lack expression of PTEN. Altogether, our data provide insights into the molecular pathogenesis of basal-like carcinomas and implicate the PTEN-dependent up-regulated Akt signalling pathway as a potential therapeutic target for the management of patients with these poor prognosis breast tumours.

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Molecular mechanisms of cellular senescence

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Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. It is unclear whether DDR activation and oncogene-induced senescence (OIS) are causally linked. Here we show that the expression of an activated oncogene (H-RasV12) in normal human cells, results in a permanent cell cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates OIS and promotes cell transformation. DDR and OIS are established after a hyper-replicative phase occurring immediately after oncogene expression. Senescent cells arrest with partly replicated DNA and with DNA replication origins having fired multiple times. In vivo DNA labelling and molecular DNA combing reveal that oncogene activation leads to augmented numbers of active replicons and to alterations in DNA replication fork progression.Therefore OIS results from the enforcement of a DDR triggered by oncogene-induced DNA hyper-replication. Senescence is also associated with a global heterochromatinization of nuclear DNA. These senescence associated heterochromatic foci (SAHFs) are enriched in histone H3 di-tri methylated on lysine 9 (H3K9m) and HP1 proteins and High mobility group A (HMGA) proteins are also known to be essential structural components of SAHFs. Our most recent results on the interplay between DDR activation and oncogene-induced heterochromatinization will be presented.

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JunB: a novel key player in mitochondria-mediated apoptosis

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Cellular stress conditions that perturb the function of the endoplasmic reticulum (ER) lead to an accumulation of unfolded and/or misfolded proteins in the ER resulting in ER stress and subsequent induction of a network of signaling pathways summarized as unfolded protein response (UPR). ER stress and UPR activation has been observed in many human diseases including cancer, diabetes and late onset neurodegenerative diseases. We investigated whether JunB, a subunit of the AP-1 transcription factor that mediates gene regulation in response to a plethora of extracellular factors and stress stimuli, is implicated in the UPR and ER stress-mediated apoptosis. Although ablation of JunB in MEFs results in cellular features reminiscent of an intrinsic UPR, junB$^{-/-}$ cells were resistant to ER stress-mediated apoptosis. Cytochrome c release as well as activation of effector caspases was completely impaired in junB$^{-/-}$ MEFs. Furthermore, in the kidneys of conditional JunB knock out mice, apoptosis induced by in vivo application of tunicamycin was suppressed as compared to kidneys of littermates expressing JunB. Currently, analyses are undertaken to identify direct JunB targets implicated in the apoptosis resistance phenotype. Our data collectively demonstrate that JunB is a critical regulator of the mitochondrial pathway in ER stress-mediated apoptosis.

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Investigating the role of serum and glucocorticoid inducible kinase 3 in promoting cell transformation

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The Serum and Glucocorticoid Inducible Kinase (SGK) family consists of three distinct but highly homologous isoforms. All three isoforms share substantial homology with AKT and are similarly activated by PI3-K in a PDK1-dependent manner. An initial screen of SGK isoform expression in a panel of tumour cell lines detected an increase in SGK3 levels specifically in ovarian tumour cells, suggesting a possible role for SGK3 in ovarian tumourigenesis. Hence, we have initiated studies to further elucidate the role of SGK3 in cell transformation through the utilization of immortalized human ovarian surface epithelial cells and a genetically defined human primary fibroblast cell model transformed through the ectopic expression of hTERT, large –T antigen, small-t antigen and H-ras which together are sufficient to induce tumourigenesis [1]. We have stably over-expressed a constitutively active form of SGK3 in cells containing various combinations of these genetic elements and conducted phenotypic analyses using well characterised markers of cell transformation to assess the ability of SGK3 to interact with or substitute for factors. Initial studies have demonstrated that SGK3 is able to promote cell growth shown through an increase in ribosome biogenesis and cell size. In addition, studies into the role of SGK3 in cell survival have suggested that SGK3 is able to partially phenocopy H-ras and confer resistance to cytotoxic drugs. This data suggests a potential role for SGK3 in promoting cell transformation. We are extending these initial results to define the ability of constitutively active SGK3 to drive tumorigenesis in murine xenograft models and a transgenic model of ovarian cancer.

1. Hahn, W.C., et al., Creation of human tumour cells with defined genetic elements. Nature, 1999. 400(6743): p. 464-8.

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Survivin and Aurora B kinase, two interesting targets for cancer therapy

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During mitosis, anaphase onset is the more crucial event. The Chromosomal Passenger protein Complex (CPC) has emerged as a major actor of this mitotic checkpoint, involved both in chromosome segregation as well as in spindle tension. The CPC is expressed specifically in mitosis and several of its protein members including Survivin and Aurora B are reported to be over-expressed in many human tumours including primary colon tumours and breast cancer. CPC is thus an attractive target for cancer therapy.The Chromosomal passenger complex is composed of five proteins. These mitotic proteins share a peculiar localisation: on the whole chromatin at mitosis onset, at the inner centromere in metaphase, on the central spindle during anaphase and at the mid-body during cytokinesis.Studying ex vivo the dynamic of the passenger proteins we found that these proteins are static except Survivin, a member of the IAP family. Survivin is highly mobile on centromere and its mobility depends on Aurora kinase activity. We have studied different mutants of Survivin, in a context of pseudogenetic, and we have identified the critical phosphorylated residue for mobility. We proposed a model in which Survivin by dynamic and transitory interactions with CPC maintains the spindle checkpoint on. Moreover the phosphoSurvivin was found to be dominant-negative in cytokinesis. Survivin is thus peculiar among passenger proteins and may be proposed as a possible target for cancer therapy.Among the complex Aurora kinase is the unique enzymatic member, we are thus currently looking for Aurora kinase inhibitors. By automated screening of a chemical library we found a new family of molecules that inhibited Aurora kinase in vitro. The best hits prevented the phosphorylation of Histone H3, inactivated the spindle checkpoint, and inhibited the growth of colon HCT116 multicellular spheroids. These molecules may thus be proposed

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