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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