Results: Trop-2 was demonstrated to be overexpressed by most human cancers, but not on non-epithelial malignancies, suggesting strong selective pressure for a conserved function. Trop-2 was then demonstrated to be necessary and sufficient to stimulate cancer growth, with a linear relationship between growth rates and Trop-2 expression levels. Cell growth stimulation was shown to be conserved across cell-types and species. These findings indicated impingement on a ubiquitous downstream signal-transduction module. Trop-2 was demonstrated to bind multiple tetraspanins, triggering their growth-promoting ability *via* a feed-forward activation loop of CD9-recruited PKCa and phosphorylation of the Trop-2 cytoplasmic tail. We demonstrated that both CD9 and PKCa stimulate growth in a Trop-2-restricted manner and that these signaling structures are coordinately transported in recurrent waves to membrane ruffles and podosomes. Trop-2 induction was shown to activate the ERK pathway, to up-regulate NF-kB, and to modulate apoptotic factors, including p53 and Rb. Key members of the Trop-2 signaling pathway were shown to be coordinately upregulated in large human cancer case series, indicating functional relevance of this growth stimulatory mechanism in tumours in man.
Conclusions: These findings reveal the existence of a unique, strikingly widespread mechanism of stimulation of cancer growth. This is quantitatively driven by overexpressed, but otherwise wild-type, Trop-2 and acts upon ready-to-signal, but otherwise silent, ubiquitous signal-transduction platforms.

652 ADAM23 splicing isoforms: distinct roles on the modulation of avb3 integrin activity

F.P. Cavalher[1], E.T. Costa[1], A.A. Camargo[1]. [1]*Ludwig Institute for Cancer Research, Laboratory of Molecular Biology and Genomics, Sao Paulo SP, Brazil*

The ADAMs (a disintegrin and metalloprotease domain) constitute a family of type I transmembrane glycoproteins with a common structural organization, which includes a metalloprotease and a disintegrin domain. Because of their proteolytic and cell adhesion activity, the ADAMs are involved in both the remodelling of the extracellular matrix and the changes in cell adhesion that characterize many biological and pathological processes, such as tumour development and progression. ADAM23 exhibits the typical structure of ADAM family members; however its metalloprotease domain is inactive, suggesting that it is exclusively involved in cell adhesion [1]. More than 12 ADAMs (including ADAM23) have been shown to interact with integrins *in vitro*, modulating integrin-mediated cell migration, adhesion and proliferation [2]. The ADAM23 protein was demonstrated to interact specifically with avb3 integrin by its disintegrin domain [3]. The ADAM23 gene is frequently silenced by promoter hypermethylation in breast, gastric, pancreatic, colorectal and head and neck tumours. In breast tumours, silencing of ADAM23 gene is associated with the development of distant metastasis and a worse disease outcome [4–5]. Recently, we demonstrated that ADAM23 negatively modulates avb3 integrin activation during metastasis [5]. Knockdown of ADAM23 expression using shRNA enhanced integrin activation by 2–4 fold and increased cell migration and adhesion to classical avb3 integrin ligands. Three ADAM23 splicing isoforms have been described so far, two of them *(alpha* and *beta)* encode transmembrane domains that share 54% similarity in their aminoacid sequence, and the third one *(gama)* does not encode a transmembrane domain, suggesting to be a secreted or cytoplasmic protein [6]. Here we show that ADAM23 splicing isoforms are differentially expressed in a panel of 12 tumour cell lines derived from several tissues. Moreover, using siRNA to specifically knockdown the expression of each splicing isoform, we found that they play different roles on the modulation of avb3 activity, affecting migration and adhesion to classic avb3 ligands.

Reference(s)

[1] Sagane K et al (1998). Biochem J **334:**93–8.
[2] Arribas J et al (2006). Cancer Metastasis Rev **25:** 57–68.
[3] Call S et al (2000). Mol Biol Cell **11:** 1457–69.
[4] Costa FF et al (2004). Oncogene **23:**1481–8.
[5] Verbisck NV et al (2009). Cancer Research **69:** 5546–52.
[6] Sun YP et al (2004). Gene **325:** 171–8.

653 CK2 phosphorylation controls PRH/HHEX dependent transcriptional repression of multiple VEGF signalling genes and cell survival

P. Noy[1], A. Sawasdichai[2], K. Gaston[2], P.S. Jayaraman[1]. [1]*University of Birmingham, Immunity and Infection, Birmingham, United Kingdom,* [2]*University of Bristol, Department of Biochemistry, Bristol, United Kingdom*

Background: The Proline Rich Homeodomain protein (PRH) is a repressor of transcription that regulates haematopoietic and vascular development. Protein kinase CK2 phosphorylates PRH and blocks its DNA binding activity and its ability to regulate transcription. CK2 is up-regulated in many tumours including Acute Myeloid Leukaemia (AML) and its up-regulation correlates with a poor prognosis. Vascular Endothelial Growth Factor (VEGF) is a mitogen that stimulates proliferation and survival of endothelial and haematopoietic cells, via its cell surface receptors VEGFR-1 and VEGFR-2. VEGF and the VEGF receptors are elevated in many tumours and haematopoietic malignancies. Loss of PRH expression has been shown to correlate with abnormal vascular development and elevated VEGF expression.
Material and Methods: VEGF and the VEGF receptor gene expression was studied using qPCR in K562 cells after knockdown and over-expression of PRH. Chromatin Immunoprecipitation (ChIP) and promoter reporter assays were used to analyse gene specific PRH binding. Cell growth and apoptosis were analysed using trypan blue cell staining, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, and Annexin V staining analysed by flow cytometry.
Results: We show using that the genes encoding Vegf, Vegfr-1, and Vegfr-2 are all repressed by PRH. ChIP and reporter assay data reveals that PRH binds to the promoter regions of all three of these genes. Thus we demonstrate that PRH is a direct repressor of multiple genes within a single signalling pathway. We also demonstrate that the manipulation of PRH levels directly impinges on the survival of haematopoietic cells and breast cancer cells. Moreover we show that VEGF and VEGF receptor signalling mediates the effects of PRH on cell growth. Importantly we demonstrate that CK2 can antagonise both PRH-induced cell death and transcriptional repression of these genes.
Conclusions: These findings suggest that PRH is a key regulator of multiple genes in the VEGF signalling pathway and loss of PRH transcriptional activity, through elevated CK2 activity, could play a role in tumourigenesis and leukaemogenesis.

654 The effect of p53 isoforms on p73 activity in tumour cells

A. Zoric[1], A. Horvat[1], J. Pavelic[1], N. Slade[1]. [1]*Rudjer Boskovic Insitute, Division of Molecular Medicne, Zagreb, Croatia*

Background: The p53 tumour suppressor protein is critical in the cell growth control and the maintenance of genomic stability. These activities are due, at least in part, to its ability to form homooligomers that bind to specific DNA sequences and activate transcription. Recently discovered, p73, a homologue of p53, can transcriptionally activate p53 target genes *in vivo*. It generates transactivating forms (TAp73) as well as a number of N-terminally truncated transactivation-deficient transdominant isoforms (called ΔTAp73). Recently was discovered that *p53*, like *p73*, has a second promoter P2 and undergoes alternative splicing to generate multiple isoforms that might play important roles in carcinogenesis. Since some mutant p53 form complexes with TAp73α or TAp73β it was important to find out whether p53 isoforms can do the same and potencially act as dominant-negative inhibitors of TAp73.
Materials ans methods: Human lung cancer p53 null cells H1299 were transfected using Lipofectamine 2000®. Proteins were extracted and western blot was preformed by standard methods. Coimmunoprecipitation assay was used to detect the protein complex. Apoptosis was detected using annexin-V assay by flow cytometry and fluorescent microscope, and to analyze transcriptional activity, we performed reporter assays using promoters with the p73/p53 binding site driving the luciferase reporter.
Results: All six p53 isoforms can form complex with TAp73β, while only isoforms D133p53, D133p53β and D133p53γ can form complex with TAp73α. Inhibitory interactions of two proteins in complex often lead to their stabilization. Our results have shown that only three isoforms (Δ133p53, Δ133p53β i Δ40p53) stabilize TAp73β. Furthermore, we have shown that all isoforms of p53 inhibit transcriptional activity but with different efficiency. The apoptotic acitivity of TAp73β was augmented by coexpression of p53b, but Δ133p53 and Δ133p53β inhibit its apoptotic activity most efficiently. We have determined the half lives of different p53 isoforms and have shown that p53γ isoform has the shortest while Δ133p53γ has the longest half life.
Conclusions: Defining the interactions between p53/p73 would gain insight into how the p53 isoforms modulate the functions of p73. The discovery of p53/p73 network could have a major clinical impact in prognostic use and p53 targeted drug design.

655 Functional analysis of CDKN2A/p16INK4a 5′UTR variants predisposing to melanoma

A. Bisio[1], S. Nasti[2], L. Pastorino[2], S. Gargiulo[2], J. Jordan[3], A. Provenzani[4], A. Quattrone[4], G. Bianchi-Scarrà[2], P. Ghiorzo[2], A. Inga[4]. [1]*National Institute for Cancer Research, Unit of Molecular Mutagenesis and DNA Repair, Genova, Italy,* [2]*DOBIG University of Genoa, Laboratory of Genetics of Rare Hereditary Cancers, Genova, Italy,* [3]*MIT, Center for Environmental Health Sciences, Cambridge, USA,* [4]*University of Trento, Centre for Integrative Biology, Trento, Italy*

The CDKN2A gene, located on 9p21, is the most common high penetrance susceptibility gene identified to date in melanoma families. Germline *CDKN2A* mutations are observed in 20–50% of melanoma-prone families. We identified melanoma patients that were heterozygous for non-coding germline variants in the 5′UTR of *CDKN2A* (c.–21C>T; c.–25C>T & c.–180G>A; c.–56G>T;