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

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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.

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

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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

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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

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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;

c.−67G>C) and examined their impact on the $p16^{INK4a}$ 5′UTR activity using two luciferase-based reporter vectors, pGL3-basic and pGL3-promoter, that differ in basal transcription level and that were transfected into the melanoma-derived WM266−4 and in the breast cancer-derived MCF7 cells. The $p16^{INK4a}$ 5′UTR variants cloned into the pGL3-promoter plasmid type were also tested in two additional p16-null, melanoma-derived cell lines G361 and SK-Mel-5. Luciferase activity and mRNA levels were quantified to assess the impact of the mutations both at transcriptional and post-transcriptional levels. The wild type 5′UTR sequence, containing a reported SNP (c.−33G>C) and a known melanoma-predisposing mutation (c.−34G>T), were included as controls. Results revealed that the variants at −21 and −34 severely reduced the reporter activity. The variants at −56 and at −25&−180 exhibited a milder impact, while results with c.−67G>C were dependent on the plasmid type. Quantification of the luciferase mRNA indicated that the effects of the variants were mainly post-transcriptional. Using a bicistronic dual-luciferase reporter plasmid, we confirmed that c.−21C>T and c.−34G>T had a severe negative impact in both cell lines. We also applied a polysomal profiling technique to samples heterozygous for the 5′UTR variants, including patient-derived lymphoblasts and the analysis of allelic imbalance indicated that in addition to the c.−21C>T variant, the c.−56T>G and c.−67G>C variants also reduced mRNA translation efficiency. Overall, our results suggest that the c.−21C>T sequence variant negatively impact on $p16^{INK4a}$ 5′UTR activity, acting mainly at a post-transcriptional level, and can thus be of clinical significance in the melanoma proneness. We propose that these variants should be considered as potential mutations.

656 MiniSOX9, a dominant-negative isoform of the transcription factor SOX9 in colon tumour cells

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Background: Inherited and acquired changes in pre-mRNA splicing have been demonstrated to play a significant role in human diseases, especially in cancer. Splice variants, found predominantly in tumours, have a clear diagnostic value and may provide potential drug targets. Deciphering the mechanisms underlying aberrant splicing in cancer may provide crucial insight to our understanding of malignant transformation. The transcription factor SOX9 is over-expressed in tumours of many origins and particularly in colon (Blache et *al*, 2004 and Lu et *al*, 2008). However, the anti-proliferative activity of SOX9 (Bastide et *al*, 2007) as well as its direct stimulation of the tumour suppressor CEACAM1 (Zalzali et *al*, 2008) and repression of the carcinoembryonic antigen (Jay et *al*, 2005) clearly indicate that SOX9 acts as a tumour suppressor. Therefore, its anti-oncogenic activity is inhibited in colon tumour expressing SOX9. This assumption led to the discovery of MiniSOX9, a truncated version of the transcription factor SOX9.
Material and Methods/Results: MiniSOX9 results from the retention of the second intron of the SOX9 gene, leading to a protein devoid of the transactivation domain. MiniSOX9 behaves as a SOX9 dominant-negative inhibitor and stimulates the canonical Wnt pathway. We showed that MiniSOX9 is strongly expressed in intestinal tumours of APCdelta14 mice. In addition, MiniSOX9 is present at high levels in human colon cancer samples whereas it is almost undetectable in the surrounding healthy tissues.
Conclusions: Our data point to an unexpected oncogenic activity produced from the *SOX9* locus and gives a new perspective on its role in colon cancer.

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657 Protein kinase C and epidermal growth factor receptor signalling mediate growth stimulation by neurotensin in the colon carcinoma cell line HCT116

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Background: In addition to receptor tyrosine kinases, G protein-coupled receptors may also stimulate cell proliferation. The aim of the present study was to examine the signalling pathways mediating growth stimulation by neurotensin, which activates G_q-coupled receptors, in the colon carcinoma cell line HCT116.
Material and Methods: Cells were plated at a density of 50,000/cm^2 in medium containing 10% serum and cultured overnight, followed by serum starvation for 24 hours before stimulation with neurotensin. DNA synthesis was determined by pulse labelling for 3 hours with radiolabelled thymidine 12 hours after neurotensin addition. Activation of phospholipase C was determined as inositol phosphate accumulation in cells labelled with radioactive inositol for 24 hours. Phosphorylation of extracellular signal-regulated kinase (ERK), Akt, and the epidermal growth factor receptor (EGFR) was determined by Western blotting using phospho-specific antibodies.
Results: Neurotensin dose-dependently stimulated inositol phosphate accumulation and DNA synthesis. Preincubation with a protein kinase C (PKC) inhibitor, GF109203X, inhibited neurotensin-stimulated DNA synthesis. Both basal and neurotensin-stimulated DNA synthesis was reduced following preincubation with inhibitors of the EGFR tyrosine kinase (AG1478 and gefitinib), as well as by inhibitors of phosphoinositide 3-kinase (wortmannin) and the ERK kinase, MEK (PD98059). Neurotensin-stimulated ERK phosphorylation was inhibited by preincubation with the PKC inhibitor, but was not affected by inhibition of the EGFR tyrosine kinase. In contrast, neurotensin-induced phosphorylation of Akt was not affected by inhibition of PKC. Chelation of intracellular calcium, inhibition of matrix metalloproteases with GM6001, and inhibition of the EGFR with cetuximab or gefitinib suppressed neurotensin-stimulated phosphorylation of Akt.
Conclusions: The present results suggest that neurotensin stimulates ERK through PKC, while Akt phosphorylation is mediated by Ca^{2+}-dependent activation of matrix metalloproteases, which promote release of ligands that activate the EGF receptor. Furthermore, the results suggest that activation of the EGF receptor is required for neurotensin to exert an optimal growth stimulatory effect in HCT116 cells.

658 USP1-regulated FANCD2/FANCI monoubiquitination controls the DDB1-dependent degradation of phosphorylated CHK1

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Background: The maintenance of genetic stability in response to genotoxic stress and stalled replication forks depends on the timely regulated and interconnected action of cell cycle checkpoints, which delay cell cycle progression, and DNA repair mechanisms, which eliminate DNA lesions. The delayed mitotic entry of DNA damaged cells mainly depends on the ATR-mediated phosphorylation and activation of the checkpoint kinase CHK1. Previous works have identified that termination of CHK1 signalling during recovery requires SCF^{bTrCP}-dependent destruction of CHK1 mediator CLASPIN, a process impeded by the deubiquitinating enzyme ubiquitin-specific protease 7 (USP7). Here we describe a new pathway involved in turning off activated CHK1 in human cells involving the USP1 deubiquitinase.
Materials and Methods: *Cells*: For our experiment we used HeLa cells, U2OS, 293T and wild-type SV40-immortalized MRC5 fibroblasts as well as Fanconi anemia cells and their ectopically corrected counterpart. DNA damage was induced by exposure to UVC, DNA crosslinkers and replication inhibitors.
Transfections: Proteins depletion was obtained by siRNA transfection performed with Oligofectamine and experiments were carried out 48 h to 72 h later.
Proteins analysis: Chromatin fractionation, proteins extraction and analysis by Western blot were performed following standard methods.
Immunofluorescence: Two days after transfection, cells grown on glass coverslips were pre-extracted with 0.5% Triton X-100 in PBS for 5 min at RT before fixation in 4% paraformaldehyde during 15min and processed following standard procedures. Cells were examined at a magnification of x630 using a fluorescence microscope.
Results: We show that depletion of USP1 deubiquitinase decreases the amount of phosphorylated and total CHK1. We demonstrate that CHK1 inhibition in USP1-depleted cells is the mainly the consequence of high levels of monoubiquitinated FANCD2/FANCI and we establish that monoubiquitinated FANCD2/FANCI stimulates a DDB1-dependent degradation of phosphorylated CHK1.
Conclusions: Combining our observations with previously published data, we propose a model in which CHK1 is necessary to activate the DNA damage-induced FANCD2/FANCI monoubiquitination that, in turn, shuts down CHK1 activation. This pathway is likely compromised in Fanconi anemia contributing to the clinical and cellular phenotype of this rare syndrome that associates cancer predisposition and genetic instability.