from the cell lines, and expression of HIF-1-α and HIF-2α was analyzed by Western blotting. Small interfering RNAs (siRNAs) were used to downregulate HIF-1α and HIF-2α. Activity of the PI3k/Akt/mTOR pathway was examined by western blotting. The effect of PHD3 on cell proliferation was evaluated by using transfectants of PHD3-specific siRNA and a PHD3-expressing plasmid.
Results: SMKT-R2 and SMKT-R3 had stable overexpression of PHD3 and HIF-1α/2α. On the other hand, PHD3 expression was induced in the nonconfluent state without accumulation of HIF proteins in Caki-1, whereas ACHN did not have PHD3 expression under normoxia. In Caki-1, in the nonconfluent state, the PI3K/Akt/mTOR pathway was activated, and inhibition of the pathway with LY294002 reduced PHD3 expression. Even in HIF-1α/2α double-knockdown Caki-1, activation of the PI3k/Akt/mTOR pathway induced overexpression of PHD3. In addition, PHD3 siRNA promoted cell proliferation compared with control siRNA in Caki-1 ($p < 0.0001$) without induction of HIF protein expression. Even in VHL-mutant SMKT-R2 and SMKT-R3, PHD3 siRNA showed the same effect ($p < 0.05$ and $p < 0.01$, respectively). On the other hand, PHD3-expressing plasmid transfection into ACHN reduced cell proliferation compared with empty vector transfection ($p < 0.005$).
Conclusions: We demonstrated that PHD3 expression could be induced in VHL-intact RCCs under normoxia by activation of the PI3K/Akt/mTOR pathway, independently of HIF-1α and HIF-2α. In addition, we also found that PHD3 had an anti-proliferative function that was independent of HIF and VHL gene status in RCCs.

677 Growth suppression activity of tensin2 in human hepatocellular carcinoma is dependent on PTEN and SH2 domains

Y.S. Yeung[1], E.Y.T. Tse[1], F.C.F. Ko[1], L.K. Chan[1], K.M.F. Sze[1], I.O.L. Ng[1], J.W.P. Yam[1]. [1]*The University of Hong Kong, Pathology, Pokfulam, Hong Kong*

Background: Tensin is a group of focal adhesion proteins that serves as a link between cytoskeleton and signal transduction. Dysregulation of tensin members have been revealed in various human cancers, including hepatocellular carcinoma (HCC). Our previous study has shown that tensin2 exerted pronounced cell death in HCC cells and was downregulated in 41% of human HCCs. In this study, we functionally characterized the role of tensin2 in HCC.
Materials and Methods: Functional characterization of tensin2 was studied by stable overexpression or knockdown of tensin2 in HCC cells with lentiviral delivery system. The proliferation rate, migration and invasion ability of the stable clones were monitored by growth curve, transwell assay and matrigel invasion assay, respectively. The *in vivo* effect of tensin2 in HCC tumour formation was studied in nude mice. Tensin2 knockdown stable clones were subcutaneously injected into nude mice and tumour growth was monitored for 4 weeks. Tensin2 deletion mutants were expressed in HCC cells and their apoptotic inducing activities were analyzed by flow cytometry and TUNEL assay.
Results: Tensin2 overexpression stable clones displayed lower proliferation rate, decreased anchorage-independent growth, inhibited motility and invasiveness when compared with the vector control. Conversely, stable knockdown clones of tensin2 showed higher proliferation rate, increased motility and invasiveness. Enhanced tumour formation in nude mice was also observed in stable knockdown clones. Transient expression of tensin2 induced significant suppression in colony formation of HCC cells. However, the suppression effect was lost in tensin2 mutants with either PTEN or SH2 domain deleted. TUNEL assay revealed that the number of apoptotic cells was inversely correlated with the number of HCC colonies formed.
Conclusions: Our study showed that tensin2 plays a negative regulatory role in HCC development and revealed the biological significance of the PTEN and SH2 domains in the growth suppression activity of tensin2.

678 Generation of novel cancer mouse models for protocadherin-10 and protocadherin-11

I. Kahr[1], U. Fuchs[1], K. Staes[1], F. van Roy[1]. [1]*VIB & Ghent University, Department for Molecular Biomedical Research, Ghent, Belgium*

Protocadherins are transmembrane proteins that differ in various aspects from classic cadherins, and whose functions are largely unexplored. We are especially interested in two smaller protocadherin subfamilies (delta1- and delta2-protocadherins) featuring two or three conserved motifs (CM) in their cytoplasmic domains. In this study we are focusing on protocadherin-10 (PCDH10) and protocadherin-11Y (PCDH11Y), which were recently found to act either as candidate tumour suppressor or as proto-oncogene product.
The human *PCDH10* gene is frequently silenced in several carcinomas, and its ectopic expression strongly suppresses tumour cell growth, migration and invasion. Recently, a germline *Pcdh10* knockout mouse has been reported on. This mouse has a severe brain abnormality leading to death within three weeks after birth. To avoid this lethality problem we aim at ablating Pcdh10 in a tissue- and time-specific manner. First, we are establishing a model in which all isoforms of Pcdh10 can be conditionally knocked out. This mouse will then be crossed with different Cre mice as well as with various tumour mouse models to elucidate the role of Pcdh10 in important cellular processes, such as control of proliferation, migration, differentiation and programmed cell death. Second, we are generating mice for conditional knockout only of the long isoforms, in this way deleting also the conserved CM1 and CM2 sequences. The latter model will be used to explore the role of these conserved domains in various intracellular signaling pathways, including oncogenic and tumour progression pathways.
A cytoplasmic form of PCDH11Y has been implicated in Wnt signaling and in acquisition of hormone resistance by progressed prostate tumours. We are generating transgenic mice with conditional overexpression of selected *PCDH11X* and *PCDH11Y* isoforms. Of particular interest to us is the generation of a transgenic mouse conditionally expressing the cytoplasmic, human-specific PCDH11Y variant, which has been proposed to be causally related to prostate cancer progression.
All mouse models will be analyzed in detail to confirm and extend the hypothesis that these two delta-protocadherins play key roles in either stimulating or repressing tumourigenesis or tumour progression.
Research supported by the Research Foundation (FWO) – Flanders, the Foundation against Cancer, Belgium, and the Queen Elisabeth Medical Foundation (GSKE), Belgium.

679 Functional impact of cancer-associated mutations in the tumour suppressor protein ING4

A. Moreno de la Gándara[1], A. Palacios[2], J.L. Orgaz[1], B. Jimenez[1], F.J. Blanco[2], I. Palmero[1]. [1]*Instituto de Investigaciones Biomédicas CSIC-UAM, Departamento de Cáncer, Madrid, Spain,* [2]*CIC bioGUNE, Structural Biology Unit, Derio, Spain*

ING4 (Inhibitor of growth 4) is a member of the ING family of proteins, which are associated with tumour suppression processes in connection with the p53 pathway and the chromatin remodeling machinery. Alterations in ING proteins, most notably ING1 and ING4, are frequently observed in different types of human tumours. Here, we analyze the functional consequences of two point mutations in ING4 associated with human tumours (Y121N and N214D) to understand the role of this protein in tumour suppression. To this end, we use a set of cell biology, structural and biochemical assays to test the impact of these mutations. We report that the N214D mutation dramatically dampens the ability of ING4 to inhibit proliferation, anchorage independent growth or cell migration, or to sensitize to cell death. In turn, the Y121N mutant did not differ significantly from wild-type ING4 in our assays. The normal predominantly nuclear localization of ING4 was not altered by either of the mutations. We investigated the molecular basis of the defective activity of the N214D mutant. The folding and ability to bind histone marks is not significantly altered by this mutation. Rather, we find that the N214D mutant shows reduced protein stability, due to increased proteasome-mediated degradation. In summary, our data demonstrates that a point mutation of ING4 associated to human tumours leads to the loss of several essential functions of ING4 pertinent to tumour protection and highlight the importance of ING4 function to prevent tumourigenesis.

680 Epigenetic silencing of miR-203 is a disease initiation event of multiple myeloma

K.Y. Wong[1], D.Y. Jin[2], R. Liang[1], C.S. Chim[1]. [1]*The University of Hong Kong, Medicine, Hong Kong, China,* [2]*The University of Hong Kong, Biochemistry, Hong Kong, China*

Background: Epigenetic inactivation of tumour suppressor microRNAs (miRs) has been implicated in carcinogenesis.
Material and Methods: We studied the role of miR-203 promoter methylation in 8 normal marrow controls, 8 MM cell lines, 20 monoclonal gammopathy of undertermined significance (MGUS), and 123 diagnostic multiple myeloma (MM) samples by methylation-specific PCR.
Results: Promoter of miR-203 was unmethylated in normal marrow controls but homozygously methylated in 25% myeloma cell lines. Treatment of 5-Aza-2′-deoxycytidine (5-AzadC) led to promoter demethylation, re-expression, and consequent direct inhibition of a common proto-oncogene across haematological malignances. In MGUS samples, 25% patients showed miR-203 hypermethylation. In primary myeloma marrow samples, 24% patients showed miR-203 hypermethylation.
Conclusions: miR-203 hypermethylation is cancer-specific and associated with gene silencing, which can be reversed by 5-AzadC hypomethylation treatment and inhibit a novel target expression in MM. Frequent miR-203 hypermethylation consistently occurs across MGUS and MM patients, but not in normal controls, suggesting a role of miR-203 hypermethylation in the disease initiation of MM.