established in BEL7402 and HLE using a lentiviral-based short-hairpin knock-down approach. For each cell line, two stable ILK knock-down (shILK) clones and one stable non-target control (shCTL) were established. To functionally characterize ILK in HCC, the knock-down stable clones were subjected to various functional assays including cell proliferation assay, soft agar colony formation assay, cell migration assay, wound-healing assay and cell invasion assay. *In vivo* tumourigenicity of BEL7402 ILK knock-down stable clones was assessed by subcutaneous injection of the cells into nude mice.
Results: Western blotting revealed a higher ILK protein expression in HCC cell lines than in normal liver cell line. In the physiological context, qPCR analysis showed that ILK was over-expressed in 36.9% (21/57) of HCC tissues when compared to the corresponding non-tumourous livers. The overall ILK expression level was significantly higher in tumourous tissues ($P = 0.005$), with a stepwise increase of expression along tumour stage. Functional characterization of ILK in HCC using the two ILK stable knock-down cell lines showed a reduction in the rate of cell proliferation, migration, invasion and anchorage-independent growth. Knock-down of ILK in BEL7402 also suppressed tumour formation in nude mice, thus decreasing the *in vivo* tumourigenicity of HCC cells. To probe the underlying mechanism, AKT activity was evaluated in the shILK clones. Western blotting analysis showed a decrease in phospho-AKT(Ser473) level upon ILK silencing.
Conclusion: Our study suggests that ILK plays a role in the progression of HCC via the activation of the PKB/AKT pathway.

728 The TGFbeta co-receptor endoglin modulates the expression and transforming potential of H-Ras

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Background: Endoglin is a transforming growth factor-β (TGF-β) co-receptor that acts as a suppressor of malignancy during mouse skin carcinogenesis. H-Ras plays a crucial role in this model, by modulating tumour initiation and progression. We have studied the effects of endoglin on the expression of H-Ras in transformed keratinocytes.
Material and Methods: Epidermal mouse cell lines derived from chemically-induced skin carcinomas were used. H-Ras expression and promoter regulation was determined by RT-PCR, Western-blot and reporter assays. MAPK pathway was studied using specific antibodies and phospho-antibodies in western-blot assays. Foci formation assays were performed in mouse NIH3T3 fibroblasts transfected with $H\text{-}Ras^{Q61K}$ or $H\text{-}Ras^{G12V}$.
Results: TGF-β_1 increases the expression of H-Ras. The TGF-β_1-induced *H-Ras* promoter transactivation was Smad-independent, however it is necessary the activation of the TGF-b type I receptor ALK5 and the Ras-mitogen-activated protein kinase (MAPK) pathway. Endoglin attenuated stimulation by TGF-β_1 of both MAPK signalling activity and H-Ras gene expression. Furthermore, endoglin inhibited basal MAPK activity in transformed epidermal cells containing an H-Ras oncogene, as found by analyzing the levels of phospho-ERK1/2. Endoglin inhibited ERK phosphorylation without affecting MEK or Ras activity by an unknown mechanism strongly dependent on the endoglin extracellular domain. Finally, endoglin was able to inhibit the transforming capacity of $H\text{-}Ras^{Q61K}$ and $H\text{-}Ras^{G12V}$ oncogenes in a NIH3T3 focus formation assay.
Conclusions: The ability to interfere with the expression and oncogenic potential of H-Ras provides a new face of the suppressor role exhibited by endoglin in H-Ras-driven carcinogenesis.

729 The impact of hypoxia on differential expression of neurotensin receptors (NTR) in colorectal and prostate carcinoma cells

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Background: Recent studies showed increased expression of neurotensin receptors (NTR), particularly, NTR1 and NTR3, in various tumours, thus NTR is assumed a potential target for tumour imaging and therapy. However, the knowledge about the quantitative expression of NTR on mRNA and protein level, e.g., under hypoxic conditions is limited. The aim of this study was to develop a quantitative method for determination of absolute NTR mRNA amount in tumour and non-tumour cells and tissues. For method evaluation the NTR mRNA amounts in human colorectal (HT-29) and prostate (PC3) carcinoma cell lines under normoxic and hypoxic conditions *in vitro* were compared.
Material and Methods: A novel real-time RT-PCR method using an external standard was established. The elongation factor 1 alpha (EF1α) gene served as housekeeping gene and glucose transporter protein type 1 gene (GLUT1) was used as indicator for cellular hypoxic regulation effects. The derived standard curves allow for calculation of the number of specific mRNA molecules normalized to 1000 molecules of EF1α. Acute and chronic experimental hypoxia was induced by cultivation of cells at an oxygen concentration of 0.6% for 4 to 72 hours.
Results: Both HT-29 cells and PC3 cells show high mRNA expression of NTR1 in normoxia. In acute hypoxia (till 24 hours) the expression level of NTR1 did not change. However, under conditions of chronic hypoxia in HT-29 cells, at the latest after 48 hours, the NTR1 mRNA expression was significantly decreased. In contrast, the NTR1 mRNA in PC3 cells remained at a high level also in hypoxia. The mRNA level of NTR3 was about 5 orders of magnitude lower than NTR1 in both cell lines. Expression of NTR3 in both cell lines showed no significant differences during hypoxia, with a tendency to increase.
Conclusion: A novel standardizable and reproducible quantitative method for measurement of NTR mRNA in cancer cells was established. The use of NTR1 as a target for imaging or therapy strongly depends on tumour cell type and tumour hypoxia. Ongoing investigations will compare quantitative mRNA expression with data on functional expression of NTR, e.g., protein synthesis and radioligand interaction, in human samples and rodent tumour (xenograft) models.

730 MicroRNA-based, p53 dependent post-transcriptional circuits: mechanisms, targets and inter-individual variation

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The tumour suppressor p53 is a sequence specific transcription factor that regulates the expression of many target genes linked, among others, to the control of cell cycle, apoptosis, angiogenesis and DNA repair. Recent studies identified direct p53 regulation of miRNAs and related regulatory circuits.
Using bioinformatics approaches, we identified an additional group of candidate miRNAs for direct p53 transcriptional control. Furthermore, some of those miRNAs can be predicted to target mRNAs in genes relevant to p53-mediated responses. Notably, we found examples of miRNA seed binding sequences at target 3′UTRs that contain SNPs predicted to modulate miRNA binding. Our work aims at the validation of p53-mediated control of the newly predicted miRNA genes and related circuitries that would provide additional negative and/or positive feedback loops for p53 regulation. To validate p53-responsiveness of 13 miRNA promoters not previously described to be under control of this family of transcription factors, we initially evaluated the potential for wild type p53, p63 and p73 to transactivate the predicted p53 response elements (REs) in those miRNA promoters. For these experiments we developed in the model system *S. cerevisiae* a panel of isogenic reporter strains harboring the chosen p53 REs upstream of the firefly luciferase reporter gene. 9 REs (including miR10b, 23b, 106a, 151, 191, 198, 202, 221, 320) were responsive to p53 of which 7 were also inducible by p63 or p73, even though to a lower extent. Moreover, we developed RT-qPCR and ChIP assays in human cell lines where p53 proteins could be ectopically expressed or induced by genotoxic stress. In general, results confirmed p53-dependent transcriptional regulation of the studied miRNAs, although cell line differences were observed. To establish miRNA targeting of selected mRNAs and the functional impact of SNPs at the miRNA binding sites we developed 3′UTRs reporter constructs differing for the SNP status or with mutagenized miR binding sites. We also measured allele imbalance at the endogenous gene level by quantitative RT-PCR analysis in cell lines heterozygous for the SNPs and relative protein levels by western blot to evaluate the impact of the SNP allele as well as of p53-dependent or -independent miR modulation. Specific examples of p53-directed post-transcriptional circuits will be presented.

731 Chemical induction of mitotic slippage by proteolytic degradation of spindle assembly checkpoint proteins

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Background: Chemicals were recently identified that induce cells arrested at mitosis by antimitotic agents to undergo mitotic slippage and enter interphase without chromosome segregation (Riffell *et al.*, Cell Cycle 8(18): 3025−38 (2009)), resulting in DNA endoreduplication and cell death through apoptosis; this project examines the mechanism whereby the mitotic slippage inducers SU6656 and geraldol force cells to escape mitotic arrest.
Materials and Methods: T98G glioblastoma cells were arrested in mitosis by exposure to paclitaxel or vinblastine and induced to undergo mitotic slippage by incubation with SU6656 or geraldol in the absence or presence of protease