APC/C beyond the cell cycle regulation. We propose that the acute response to proteotoxic stress is delicately modulated by adjusting the abundance of promoter-bound HSF2. This spatiotemporal regulation is facilitated by recruitment of Cdc20 to the *Hsp70* promoter and subsequent degradation of HSF2, suggesting that APC/C^{Cdc20} actively participates in the heat shock response.

668 Vitamin D receptor and colon cancer: effect of the Snail family of transcription factors

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Background: 1alpha,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and a number of less calcemic analogs are in clinical trials as anticancer agents against colon cancer and other neoplasias based on their antiproliferative, prodifferentiation, pro-apoptotic and antimetastatic activity in cultured cells and experimental animal models. Most, if not all, $1,25(OH)_2D_3$ actions are mediated by vitamin D receptor (VDR). Thus, VDR expression is the major determinant of cell responsiveness to $1,25(OH)_2D_3$. VDR is expressed in normal colon epithelial cells and in some colon cancer cells. However, VDR expression is lost during colon cancer progression, possibly causing unresponsiveness to $1,25(OH)_2D_3$.
Material and Methods: We ectopically expressed Snail1 or Snail2 in human colon cancer cells to analyze the effect of these transcription factors on VDR RNA and protein expression and $1,25(OH)_2D_3$ action. In addition, we study VDR, Snail1 and Snail2 RNA expression using quantitative-RT-PCR in one hundred human colon cancer samples and their normal counterparts.
Results: The transcription factors Snail1 and Snail2 repress VDR expression and block $1,25(OH)_2D_3$ action in human colon cancer cells. By contrast, other inducers of epithelial-to-mesenchymal transition such as Twist1, Zeb1, Zeb2 and E47 did not affect VDR levels. Snail1 and Snail2 have a strong additive effect and cooperate to repress VDR expression. In addition, we found that Snail1 and/or Snail2 overexpression in human colon tumours correlates with VDR downregulation. Accordingly with data from cultured cells, the strongest VDR repression was found in those colon tumours that overexpress both transcription factors.
Conclusions: Our results suggest that Snail1 and Snail2 are probably responsible for VDR downregulation and $1,25(OH)_2D_3$ unresponsiveness in advanced colon cancer. Our data indicate that patients with high levels of these transcription factors will be poor responders to therapy with $1,25(OH)_2D_3$ or its analogs, and may contribute to generate more rational protocols for the clinical use of these compounds.
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669 Quantitative expression analysis of nine ETS transcription factors and of the MYC and PTEN genes in a consecutive series of 200 prostate carcinomas

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Background: Genomic rearrangements involving the androgen regulated gene TMPRSS2 and several members of the ETS family of transcription factors are early events in prostate carcinogenesis and gain of MYC and loss of PTEN have been associated with disease progression. We aimed to evaluate whether ETS genomic changes and expression correlate with MYC and PTEN expression using a consecutive series of 200 prostatectomy specimens.
Material and Methods: We used TaqMan Low Density Arrays (TLDAs) to simultaneously assess the expression levels of nine ETS transcription factors, MYC, PTEN and the common fusion between TMPRSS2 exon 1 and ERG exon 4. The panel of ETS transcription factors was chosen according to either the chromosomal localization or the involvement in genomic rearrangements in different cancer models and included ERG, ETV1, ETV4, ETV5, ELK4, FLI1, FEV, ETV6 and ETS2. Whenever necessary, the presence of a genomic rearrangement was assessed by FISH analysis on the correspondent paraffin-embedded sections using dual color or tricolor probe combinations.
Results: The TMPRSS2-ERG transcript was found in 104 cases ($Ct \leqslant 30$). Four samples that were negative for the fusion between TMPRSS2 exon 1 and ERG exon 4 showed high expression of ERG. FISH analysis using a tricolour probe flanking ERG and the 5′ region of TMPRSS2 revealed that two of these cases are also TMPRSS2-ERG rearranged (expressing a different TMPRSS2-ERG transcript), whereas in the other two cases ERG is rearranged with a different 5′ partner. Outlier expression was found for ETV1 in 16 cases (8%), for ETV4 in two (1%) and for ETV5 in one case. FISH analysis with BAC probes is being used to identify the 5′ fusion partners. No outlier expression was found for FLI1, FEV, ETV6 or ETS2. Correlation analysis between TMPRSS2-ERG and MYC expression shows a week positive association ($r_s = 0.197$, $p < 0.01$), while correlation of TMPRSS2-ERG with PTEN expression shows a week negative association ($r_s = -0.167$, $p < 0.02$).
Conclusions: Assessment of gene expression proved to be an efficient approach to identify prostate cancers with ETS rearrangements. We confirm that the pattern of ETS fusion genes in prostate carcinomas is heterogeneous and show that the TMPRSS2-ERG rearrangement is associated with MYC overexpression and PTEN downregulation.

670 Characterisation of LSAMP, a novel candidate tumour suppressor gene in osteosarcomas

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Background: Osteosarcomas are the most common primary malignant tumours of bone. The tumours are highly aggressive and show complex genomic aberrations. We have recently identified a small frequently deleted region in 3q13.31in osteosarcoma tumours and cell lines. This region contains the limbic system-associated membrane protein (*LSAMP),* which has previously been reported to be a candidate tumour suppressor gene in other cancer types. Interestingly, our data shows that low expression of *LSAMP* is statistically correlated with shorter patient survival. We are further investigating the potential use of *LSAMP* as a biomarker for osteosarcomas, as well as its role in osteosarcoma development.
Material and Methods: The gene copy number and expression level of *LSAMP* are being investigated in a larger panel of osteosarcomas using qRT-PCR. The promoter methylation status will be further investigated using bisulfite sequencing, and the protein level will be analysed using immunohistochemistry on tissue microarrays and Western blotting. The expression of LSAMP protein will be restored in cell lines showing deletion and no expression in order to identify transcriptional and phenotypic changes, using microarray expression profiling and cell assays.
Results: We are currently analysing the gene copy number and expression level of *LSAMP* in a larger panel of osteosarcoma tumours. The results will be correlated with different clinical variables, including patient survival, in order to elucidate the potential use of *LSAMP* as a biomarker for osteosarcomas.
In addition, we have examined the expression level of other genes and non-coding RNAs located in the small deleted region, identifying two other genes and one non-coding RNA that may be additional candidate targets for this deletion. The expression level of these genes will be examined in a larger panel of osteosarcomas as well.
We have identified a number of osteosarcoma cell lines showing deletion and no expression of *LSAMP*, which will be used to identify transcriptional and phenotypic changes when expression of LSAMP protein is restored. Currently, we are making constructs in order to over-express LSAMP and the three other candidate targets in these cell lines.
Conclusion: We have previously identified *LSAMP* as a novel candidate tumour suppressor gene in osteosarcomas. Further studies are being done in order to elucidate the potential use of *LSAMP* as a biomarker for osteosarcomas, as well as its role in osteosarcoma development.

671 The role of retrovirally-tagged microRNAs in glioma development

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Background: The importance of non-coding RNAs in cancer has become evident in recent years. A previous screen for brain tumour genes in a PDGF-driven mouse model identified retroviral integrations close to microRNAs, which suggests that they have a role in glioma development.
Materials and Methods: The expression of three of the identified microRNAs was evaluated with a stem-loop real-time TaqMan PCR and Northern blotting. Potential target genes of the microRNAs were estimated using bioinformatic tools.
Results: The expression of mature mir-21 was increased in mouse glioma cell lines, compared to normal adult brain. The expression of mature mir-29a and mir-29b was decreased in the same set of samples indicating a tumour-suppressive role of the mir-29 family. One of the potential targets of mir-21 according to bioinformatic prediction was Sox2. This transcription factor is known to be essential in maintenance of self-renewal of embryonic stem cells and has been implicated to have a role in cancer initiating cells. Intriguingly, our results indicate that levels of Sox2 are decreased upon siRNA treatment of glioma cells as determined by Western blot. This finding suggests that Sox2 is positively regulated by mir-21 and that the direct target of mir-21 is upstream of