Sox2. However, very little is known about Sox2 regulators. It is well-established that mir-21 has an anti-apoptotic effect in various cancer cells.
Conclusions: The Sox2 suppressing effect of mir-21 suggests a hitherto unknown novel pathway. These findings could be implicated in anti-glioma therapy. Targeting mir-21 would not only lead to increased apoptosis, as has previously been demonstrated by several investigators, but also to decreased expression of a transcription factor which is required for the maintenance of stemness.

672 Dissecting the protective role of vitamin D3 on colon cancer: new targets from the protein degradation machinery

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Background: Colorectal cancer (CRC) is one of the most common human neoplasias. Epidemiological and preclinical studies have shown that 1alpha,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the most active metabolite of vitamin D_3, has wide but not fully understood antitumour activity. Transcriptomic analyses of $1,25(OH)_2D_3$ action in human CRC cells have revealed a number of genes encoding proteases, protease inhibitors and members of the ubiquitin-proteasome system as $1,25(OH)_2D_3$ candidate target genes. One of these genes is *CST5*, which encodes cystatin D, an inhibitor of several cysteine proteases of the cathepsin family.
Material and Methods: Several human colon cancer cell lines as well as human normal and tumour tissue samples were used. Ectopic *CST5* expression was performed by stable transfection of human cDNA. *CST5* silencing was done by viral transduction of shRNA. Protein expression was determined by Western blot, immunofluorescence and immunohistochemistry. RNA levels were measured by quantitative RT-PCR.
Results: $1,25(OH)_2D_3$ increases *CST5* RNA and protein levels in human CRC cells. In cells lacking endogenous expression, ectopic cystatin D inhibited cell proliferation, migration and anchorage-independent growth. Additionally, cystatin D repressed the epithelial-mesenchymal transition inducers *SNAI2, ZEB1* and *ZEB2,* and, conversely, induced E-cadherin and other adhesion proteins. Furthermore, ectopic cystatin D expression blunted xenograft tumour growth in immunodeficient mice. *CST5* knockdown using shRNA abrogated the antiproliferative effect of $1,25(OH)_2D_3$, and attenuated E-cadherin expression. In human CRC tumours, we found a strong correlation between the expression of VDR and that of cystatin D. Moreover, the loss of cystatin D correlated with poor tumour differentiation. In addition, quantitative RT-PCR analyses have validated additional proteases and protease inhibitors as $1,25(OH)_2D_3$ target genes.
Conclusions: Our results show that *CST5* acts as a tumour suppressor gene with unpredicted effects that may contribute to the antitumour action of $1,25(OH)_2D_3$. Moreover, the large number of genes regulated by $1,25(OH)_2D_3$ that are related with the protein degradation machinery suggests a role of $1,25(OH)_2D_3$ regulating protein integrity and stability. Thus, the gene regulatory action of $1,25(OH)_2D_3$ may be exerted by a dual, transcriptional and post-translational regulation of its target genes.
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673 Withdrawn

674 ZNF217 confers resistance to the pro-apoptotic signals of paclitaxel

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Introduction: *ZNF217* is a candidate oncogene located at 20q13, a chromosomal region frequently amplified in breast cancers. *ZNF217* amplification correlates with shorter patient survival in breast and ovarian cancers. The first direct evidences for a potentially oncogenic function of *ZNF217* was the demonstration that transduction of mammary and ovarian cells with *ZNF217* could give rise to immortalized cells. ZNF217 is a Krüppel-like zinc finger protein that localizes to the nucleus and interacts with co-repressors and histone modifying proteins, suggesting that ZNF217 may be a part of a transcriptional repressor complex. Moreover, ZNF217 promotes cell viability in HeLa cells by interfering with the apoptotic pathway and attenuates apoptotic signals resulting from doxorubicin-induced DNA damage or from functionally compromised telomeres. Activation of the Akt pathway and overexpression of the oncogenic translation elongation factor eEF1A2 have been proposed to mediate ZNF217 tumourigenic functions, but the precise molecular mechanisms involved in ZNF217 pro-survival function are currently unknown.
Methods: In order to decipher the functional consequences of aberrant ZNF217 expression on breast cancer cell behavior: (i) we established stable MDA-MB-231 cells constitutively overexpressing the ZNF217 protein, (ii) we used two *ZNF217*-targeted siRNAs to promote the extinction of ZNF217 expression.
Results: We firstly examined the involvement of ZNF217 on cell proliferation *in vitro* and on tumour growth in mouse xenograft models. We then explored the contribution of ZNF217 in cancer therapy response to determine whether ZNF217 is able to counteract apoptotic signals other than those induced by DNA damage stimuli. Paclitaxel, a microtubule-stabilizing agents that cause cell cycle arrest and apoptosis, is recognized as an extremely active chemotherapeutic agent in the treatment of early-stage or metastatic breast cancers. We found that ZNF217 confers a paclitaxel-resistant phenotype to MDA-MB-231 breast cancer cells. To decipher the molecular mechanisms likely responsible for such phenotype, we investigated the possible involvement of ABC transporters and of the intrinsic apoptotic pathway.
Conclusion: Our results suggest that ZNF217 might play an important role in breast neoplastic progression and chemoresistance, and that clinical strategies targeting ZNF217 would be a valuable approach for the management of breast cancer.

675 Overexpression of HOXB7 homeobox gene in oral cancer induces cellular proliferation and is associated with poor prognosis

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HOX genes are master regulators of cell proliferation and cell differentiation throughout fetal development. They have been shown to be dysregulated in several malignancies such as melanomas, colon, lung, kidney, prostate cancers and also in leukemias. There are not many studies correlating the dysregulation of HOX genes in oral squamous cell carcinoma and therefore the goal of this study was to investigate the role of HOX genes in oral squamous cell carcinoma (OSCC). To achieve this we quantifyed HOX expression levels in OSCC fresh tissue samples, normal mucosal samples from these same patients and tissue samples from individuals who have not been exposed to known oral carcinogenics. Additionally, we used OSCC cell cultures (SCC-4, SCC-9, SCC-15 and SCC-25) and immortalized but not transformed keratinocytes (HaCAT). Our results show that HOXB7 was found to be upregulated in both the squamous cell carcinoma lesions and normal tissue from these patients when compared to their normal counterparts. We then decided to investigate the effects of the overexpression of HOB7 in HaCAT cells and this resulted in increased proliferation. When endogenous levels of HOXB7 were downregulated in SCC-9 cells, the proliferation decreased. In OSCC tissue samples high expression of HOXB7 and Ki67, a marker of proliferation correlate strongly with each other ($rs = 0.79$, $p < 0.006$). High immunohistochemical expression of HOXB7 was correlated with T stage ($p = 0.06$), N stage ($p = 0.07$), disease stage ($p = 0.09$) and Ki67 expression ($p = 0.01$), and patients with tumours showing high number of HOXB7-positive cells had shorter overall survival ($p = 0.08$) and shorter disease-free survival after treatment ($p = 0.10$) compared with patients with tumours exhibiting low amount of HOXB7-positive cells. Our data suggest that HOXB7 may contribute to oral carcinogenesis by increasing tumour cell proliferation, and imply that HOXB7 may be an important determinant of OSCC patient prognosis.

676 PHD3 is expressed independently of HIF protein and has a HIF-independent anti-proliferative function in renal cell carcinoma: the novel expression mechanism and function

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Background: Hypoxia-inducible factor prolyl hydroxylases (PHDs) are involved in the degradation of hypoxia-inducible factor (HIF) proteins in cooperation with von-Hippel Lindau (VHL) protein. One member of the family, PHD3, is barely detected in normal adult tissues. However, we previously found that PHD3 was frequently overexpressed in renal cell carcinomas (RCCs). The purpose of this study was to examine the expression mechanism and the function of PHD3 in RCC.
Materials and Methods: The VHL-mutant RCC cell lines SMKT-R2 and SMKT-R3, and VHL wild-type ones Caki-1 and ACHN, were used. All cells were cultured under normoxia. Total RNA was extracted from the cell lines and the expression of PHD3 was detected by RT-PCR. Cell lysates were prepared