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

M.J. Larriba[1], F. Pereira[1], A. Barbáchano[1], H.G. Pálmer[1], F. Bonilla[2], A. Muñoz[1]. [1]*"Alberto Sols" Biomedical Research Center, Cancer Biology, Madrid, Spain,* [2]*Hospital Universitario Puerta de Hierro Majadahonda, Medical Oncology, Majadahonda, Spain*

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.
The work in authors' laboratories is supported by the Ministerio de Ciencia e Innovación of Spain (SAF2007–60341, ISCIII-RETIC RD06/0020/0009 and RD06/0020/0020) and Comunidad de Madrid (S-GEN-0266/2006).

669 Quantitative expression analysis of nine ETS transcription factors and of the MYC and PTEN genes in a consecutive series of 200 prostate carcinomas

P. Paulo[1], J.D. Barros-Silva[1], F.R. Ribeiro[1], J.R. Carvalho[1], B. Mesquita[1], C. Jerónimo[1], R. Henrique[2], R.I. Skotheim[3], R.A. Lothe[3], M.R. Teixeira[1]. [1]*Portuguese Oncology Institute Francisco Gentil, Genetics, Porto, Portugal,* [2]*Portuguese Oncology Institute Francisco Gentil, Pathology, Porto, Portugal,* [3]*Institute for Cancer Research – The Norwegian Radium Hospital, Cancer Prevention, Oslo, Norway*

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

T. Barøy[1], D. Hopmeier[1], S.H. Kresse[1], O. Myklebost[1], L.A. Meza-Zepeda[1]. [1]*The Norwegian Radium Hospital, Department of Tumour Biology, Oslo, Norway*

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

J. Põlajeva[1], P. Roswall[2], F. Johansson-Swartling[3], U. Singh[1], B. Westermark[1]. [1]*The Rudbeck Laboratory, Department of Genetics and Pathology, Uppsala, Sweden,* [2]*Karolinska Institutet, MBB, Stockholm, Sweden,* [3]*University of California at San Francisco, Department of Neurology, San Francisco, USA*

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

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

S. Alvarez-Díaz[1], N. Valle[1], J.M. Freije[2], A. Astudillo[3], C. López-Otín[2], A. Muñoz[1]. [1]*Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC-UAM, Madrid, Spain,* [2]*Instituto Universitario de Oncología, Universidad de Oviedo, Oviedo, Spain,* [3]*Instituto Universitario de Oncología, Hospital Universitario Central de Asturias, Oviedo, Spain*

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.
The work in authors' laboratories is supported by the Ministerio de Ciencia e Innovación of Spain (SAF2007–60341, ISCIII-RETIC RD06/0020/0009 and RD06/0020/0020) and Comunidad de Madrid (S-GEN-0266/2006).

673 Withdrawn

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

A. Thollet[1], J. Vendrell[1], L. Payen[1], S. Ghayad[1], S. Ben Larbi[1], C. Collins[2], M. Villedieu[1], P. Cohen[1]. [1]*INSERM U590, Faculté de Pharmacie, Lyon, France,* [2]*Vancouver Prostate Centre, Department of Urology, Vancouver, Canada*

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

C. Cavalcante Bitu[1], M.F.S.S. Destro[1], K.G. Zecchin[2], E. Graner[1], M.A. Lopes[1], L.P. Kowalski[3], R.D. Coletta[1]. [1]*State University of Campinas (UNICAMP), Department of Oral Diagnosis School of Dentistry, Piracicaba, Brazil,* [2]*State University of Campinas (UNICAMP), Department of Clinical Pathology School of Medicine, Campinas, Brazil,* [3]*A.C. Camargo Cancer Hospital, Department of Head and Neck Surgery and Otorhinolaryngology, Sao Paulo, Brazil*

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

T. Tanaka[1], T. Torigoe[2], Y. Hirohashi[2], H. Kitamura[1], N. Masumori[1], T. Tsukamoto[1], N. Sato[2]. [1]*Sapporo Medical University, Department of Urology, Sapporo, Japan,* [2]*Sapporo Medical University, 1st Department of Pathology, Sapporo, Japan*

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