accepted in clinical routine. In spite of the increasing body of high-throughput generated data, molecular tools that can help better and earlier diagnosis and set the basis for a future individualized treatment are still under development.

Recently, we described Annexin A10 (ANXA10) as one of the markers included in a gene expression signature in non-muscle-invasive bladder tumours. This signature predicted both presence of concomitant CIS and progression to muscle-invasive cancer [1].

Annexins carry out biological and physiological processes including anti-coagulation, endocytosis, exocytosis, immune suppression, differentiation, tissue growth and are consistently differentially expressed in neoplasia. ANXA10 down-regulation has been correlated with poor prognosis in both hepatocellular carcinoma and gastric carcinoma [2,3].

Material and Methods: In this study, we aimed to investigate the prognostic value of ANXA10 in both non-muscle-invasive and muscle-invasive bladder cancer by immunostaining; and the function of ANXA10 following ANXA10-siRNA knock-down in bladder cancer cell lines using proliferation and wound healing techniques.

Results: Low ANXA10 nuclear staining was an independent marker for progression to muscle-invasive cancer in multivariate analysis (hazard ratio = 0.38, $P = 0.001$). In addition, low ANXA10 immunostaining in localized muscle-invasive bladder cancer ($n = 97$) was associated with development of metastatic disease ($P < 0.0000$) and short-term survival ($P < 0.0000$). The combination of ANXA10 and p53 immunostaining significantly improved the prognostic value in both non-muscle-invasive and muscle-invasive cancers.

Furthermore, ANXA10 down-regulation resulted in increased cell proliferation and migration.

Conclusions: ANXA10 can be considered an independent prognostic factor for progression to muscle-invasive disease, and for development of metastatic disease in patients with muscle-invasive bladder cancer. The combination of ANXA10 expression with other potential molecular markers as e.g. p53 and RB resulted in strong predictive models of outcome. ANXA10 may identify patients with high risk of metastatic disease that may be candidates for neo-adjuvant chemotherapy.

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140 p8 (Candidate Of Metastasis 1) drives ER-stress/autophagy/apoptosis axis induced by the synthetic cannabinoid WIN in HCC cells

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Background: Today, evidence is emerging for the role of autophagy in the regulation of life and death of tumour cells and its relationship with ER-stress signaling. Our previous results demonstrated that hepatoma HepG2 cells are sensitive to apoptotic effects induced by WIN, a synthetic cannabinoid, which acts through a mechanism involving the reduction in the levels of some survival factors and the activation of pro-apoptotic ones. Since WIN effects were observed after 36−48 hours of treatment, we investigated the possible activation of ER-stress and autophagic process in the first hours of WIN-treatment focusing our attention on p8, a factor whose expression is up-regulated in response to cannabinoid-mediated stress.

Material and Methods: ER-stress- and autophagy-related proteins were studied by RT-PCR and western blotting analysis. The autophagic morphology was estimated by MDC staining and immunofluorescence. Gene silencing was performed using small interfering RNA against p8.

Results: WIN induced ER-stress activating a pathway involving p8-CHOP-TRB3 proteins and increased the expression of the ER chaperone GRP78 which could mediate the transfer of the proapoptotic protein PAR-4 on plasma membrane. Our results indicate that WIN induced the increase in phospho-PAR-4(Thr163) level and the decrease of the pro-survival protein phospho-AKT which is responsible for an inactivating phosphorylation of PAR-4 in Ser249. Moreover, after 16 h of treatment, WIN induced the appearance of autophagic vacuoles and the increase in the lipidated form of LC3 (LC3-II) which is associated with the autophagosomal membrane. The study of beclin-1 revealed a non-canonical beclin-1 independent autophagy. To evaluate the role of p8 as an activator of death pathway we carried out experiments using specific siRNA (sip8). After p8 silencing, either the markers of ER-stress (CHOP, TRB3 and GRP78) as well as those of autophagic process (LC3-II and vacuoles formation) were significantly reduced with respect to the levels observed in WIN-treated non transfected cells.

Conclusions: These findings demonstrate that ER-stress and autophagic activation are early events in WIN-induced apoptosis of HCC cells. In particular, ER-stress-related protein p8 seems to have a key role in triggering the WIN-dependent ER-stress/autophagy/apoptosis cascade in HCC cells. Moreover, the modulation of pAKT/pPAR4 balance contributes to these events.

141 Methylation profiling in non-small cell lung cancer: clinical implications

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Background: Lung cancer is one of the most common cancer malignancies worldwide and, according to the WHO, is the leading cause of cancer death in men and second leading cause in women. Lung cancer is unique among human solid cancers in that a single environmental factor, tobacco smoke, is believed to promote sequential changes in target cells that lead to carcinogenesis. As yet, no routine screening method that enables early detection exists, and this is a key factor in the high mortality rate of this disease. Imaging and cytology-based screening strategies have been employed for early detection, and while some are sensitive, none have been demonstrated to reduce lung cancer mortality. DNA methylation has emerged as a highly promising biomarker and is being actively studied in multiple cancers. In this work, methylation of 1505 CpG loci associated with 803 cancer-related genes were studied in forty six primary non-small lung carcinomas.

Material and Methods: Forty six primary non-small cell lung carcinomas (NSCLCs) and their corresponding control tissue samples were obtained from patients who underwent potentially curative surgery between 2000 and 2005, at San Carlos Hospital in Madrid, Spain. Illumina GoldenGate Methylation® bead array was processed according to manufacturer's protocol. Illumina BeadStudio Methylation Software was use for data analysis.

Results: Sample classification based on CpG methylation profile showed a trend towards clustering tumour versus non-tumour samples. Global hypermethylation (more than 20% of the CpG islands methylated) was associated to a worse prognosis in stage IIIA NSCLCs. In a gen-by-gene comparison of CpG methylation, twelve genes showed correlations with histological type and five with differentiation grade. More interestingly, hypermethylation of genes *CALCA* and *MMP-2* were statistically associated to a worse clinical evolution of patients), whereas hypermethylation of *RASSF1* resulted a protective variable in relation to patient prognosis. These results were independent to TNM tumour stage, as demonstrated by a Cox multivariate analysis ($P = 0.06$, $RR = 2.64$; $P = 0.03$, $RR = 2.96$; $P = 0.023$; $RR = 0.53$, respectively).

Conclusions: Global hypermethylation of a wide panel of genes may be useful as a biomarker to predict prognosis in IIIA TNM stage NSCLC. Moreover, hypermethylation in *CALCA*, *MMP-2* and *RASSF1* emerged as prognostic indicators in I-IIIA TNM stage NSCLCs, independently of tumour stage.

142 Differential expression profiles for senescence and cell death pathways in non small cell lung and colorectal tumours showing telomere shortening

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Background: Differences in how pathways of senescence and cell death operate between Non Small Cell Lung Cancer (NSCLC) and Colorectal Cancer (CRC) could explain the different clinical outcome that shortening telomere reflects, as previous results from our group showed. Our aim in this work consists of investigating whether a differential expression of factors related to these pathways could determinate differential patient outcome conferred by telomere status in NSCLC and CRC.

Material and Methods: We analyzed 36 NSCLCs, 44 CRCs, and their corresponding control tissues, obtained from patients who had undergone potentially curative surgery. Telomere function was evaluated by determining telomerase activity and telomere length. Differential expression of factors related to senescence and cell death pathways was evaluated using microarrays containing a total of 113 oligonucleotide sequences corresponding to genes from these pathways. Also, using microarrays, we investigated expression profiles of 113 genes representative of 6 biological pathways involved in transformation and tumourigenesis. We tested our results by Real Time Quantitative PCR (RT-Q-PCR).

Results: Our results indicated that 75% and 72.7% of NSCLCs and CRCs showed telomerase activity. The median telomere length was 4.15 Kb in NSCLCs and 3.8 Kb in CRCs. Microarray data indicated that NSCLCs significantly overexpressed a group of genes related to senescence and cell death pathways: *BNIP3*, *NDRG1*, *DAPK1*, *AATF*, *GADD45A* and *SHC1*, after comparing NSCLCs and CRCs with telomere attrition. *EGFR* was high and significantly overexpressed in lung tumours as compared with CRCs. Expression data from arrays were confirmed investigating gene expression by RT-Q-PCR. For NSCLCs, RT-Q-PCR analysis showed that expression levels of genes such as *BNIP3, EGFR, AATF* and *NDRG1* did not change with telomere status, however genes such as *p53, p16, DAPK1, GADD45A* and *SHC1* showed a significant overexpression in the group of tumours in which telomere shortening was not 20% higher than in corresponding non tumour tissues.

Conclusion: Our data suggest a differential impact for senescence and cell death pathways in CRC and NSCLC, in relation to telomere function.

143 Expression and clinical significance of the Kv3.4 potassium channel subunit during the development and progression of head and neck squamous cell carcinomas

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Background: Increasing evidences indicate that ion channels are involved in tumour cell biology and the concept of ion channels as membrane therapeutic targets and diagnostic/prognostic biomarkers is attracting growing interest. Dysregulation of the voltage-gated potassium channel Kv3.4 has been linked to a human neuromuscular disease, periodic paralysis and Alzheimer's. In addition, increased Kv3.4 mRNA expression has been reported in oral and oesophageal squamous cell carcinomas. This prompted us to investigate the expression pattern and clinical significance of the Kv3.4 channel subunit in the development and progression of HNSCC.

Material and Methods: Kv3.4 mRNA levels were determined by real-time RT-PCR in both HNSCC tissue specimens and derived cell lines. Kv3.4 protein expression was evaluated by immunohistochemistry in paraffin-embedded tissue specimens from 84 patients with laryngeal/pharyngeal squamous cell carcinomas and 67 patients with laryngeal dysplasias. Molecular alterations were correlated with clinicopathological parameters and patient outcome.

Results: Increased Kv3.4 mRNA levels were found in 15 (54%) out of 28 tumours, compared to the corresponding normal epithelia and varied mRNA levels were detected in 12 HNSCC-derived cell lines. Increased Kv3.4 protein expression was observed in 34 (40%) of 84 carcinomas and also at early stages of HNSCC tumourigenesis. Thus, 35 (52%) of 67 laryngeal lesions displayed Kv3.4-positive staining in the dysplastic areas, whereas both stromal cells and normal adjacent epithelia exhibited negligible expression. No significant correlations were found between Kv3.4-positive expression in HNSCC and clinical data, however Kv3.4 tended to diminish in advanced-stage tumours. Interestingly, patients carrying Kv3.4-positive dysplasias experienced a significantly higher laryngeal cancer incidence than did those with negative lesions ($p = 0.0209$). In addition, functional studies using HNSCC cells revealed that Kv3.4 blockade by siRNA leads to the inhibition of cell proliferation via selective G2/M cell cycle arrest without affecting apoptosis.

Conclusions: These data demonstrate for the first time that Kv3.4 expression is frequently increased during HNSCC tumourigenesis and significantly correlated with a higher cancer risk. Our findings support a role for Kv3.4 in malignant transformation and provide original evidence for the potential clinical utility of Kv3.4 expression as a biomarker for cancer risk assessment.

144 UGT-expression in breast tissue from healthy women is associated with mammographic density

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Introduction: Mammographic density (MD) is one of the strongest risk factors for breast cancer and confers a four to six fold risk elevation of developing breast cancer, even after adjustment for other known breast cancer risk factors. The relative content of adipose, connective and epithelial tissue in the female breast determines the MD which in turn is assessed from film screen mammograms. Today, little is known about the biologic correlates of MD.

Material and Methods: Gene expression analysis using whole genome arrays was performed on breast biopsies from 79 women with no malignancy (healthy women) recruited through mammographic centres. To compare with findings in tumour samples, 64 newly diagnosed breast cancer patients were recruited. MD percentage was determined using a previously validated, computerized method on scanned mammograms. Significance analysis of microarrays (SAM) was performed to identify genes associated with MD.

Results: SAM identified 24 genes differentially expressed between high and low MD in the healthy women, including three uridine $5'$-diphospho-glucuronosyltransferase (*UGT*) genes: *UGT2B7*, *UGT2B10* and *UGT2B11*. These genes had a reduced expression in samples from breasts with high MD compared with samples from breasts with low MD and reduced expression in breast cancers compared with healthy breasts. These *UGT* genes were the only genes among the 24 differentially expressed which had a similar expression in breasts with high MD and in breast cancers. The UGT enzymes inactivate several endogenous and exogenous compounds, including sex hormones. The reduced expression in breasts with high compared with low MD was most significant in the subpopulation with higher levels of female sex hormones (premenopausal women and postmenopausal women on hormone replacement).

Conclusions: Twenty-four genes associated with MD were identified. Three UGT2B genes had reduced expression in breasts with higher MD and breast cancers compared with healthy breasts. We hypothesise that reduced expression of *UGT* genes in women exposed to female sex hormones, increase MD and that this may be associated with an increased risk of breast cancer. Validation and further analysis of these genes is ongoing.

145 Role of collagen in the anti-metastatic activity of a ruthenium-based drug

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Background: NAMI-A, namely imidazolium tetrachorido (S-dimethylsulfoxide) imidazoleruthenate (III) is an anti-metastasic drug independent of conventional cell cytotoxicity. Interactions of NAMI-A with the components of extracellular matrix, including collagen, are thought to be crucial for its anti-metastatic action.

Materials and Methods: Structural changes in collagen and cultured cancer cells treated with NAMI-A were investigated by using a combination of X-Ray absorption spectroscopy (XAS), field-emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), X-Ray photoelectron spectroscopy (XPS) and gel electrophoresis.

Results: The *XAS* results showed that the incubation of collagen with NAMI-A at pH 7.4 resulted in a significant change in coordination environment, most of the Cl^- ligands being replaced with N- and O- donor ligands of the protein. The *SEM* observation demonstrated that the Ru treatment leads to the formation of Ru clusters of 25−35 nm between collagen fibrils, where the thin fibrils exhibit an increased axial D periodicity. The secondary structure of collagen was monitored by *FTIR*. When the native form of collagen was subjected to Ru treatment, the amide I band attributed to helical protein structures increased, and that attributed to random coil formation decreased. *Gel electrophoresis* confirmed the formation of crosslinks between collagen chains in Ru-treated collagen, as well as matrix metallo-proteinase (MMP) inhibition. The *TEM* results showed that the co-culture of lung cancer cells (A549) with Ru-treated collagen resulted in the formation of 20−35 nm particles along the plasma surface of cells' invasive protrusions. *XPS* revealed that these particles contained Ru.

Conclusion: Combination of the above results points to the formation of Ru clusters deposited among collagen fibrils, as well as of Ru-induced intra- and inter-molecular cross-links. The binding of Ru to collagen leads to an increase in the structural order, but does not destroy the triple helices of collagen. These changes cause inhibition of matrix metallo-proteinase (MMP) interactions with collagen, which is expected to contribute to the anti-metastatic activity of NAMI-A.

146 Identification of novel tumour-associated autoantibody signatures in gastric cancer

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Background: Gastric cancer, despite an overall global decrease in incidence, remains the third most common malignancy in Eastern Europe, and in more than 80% of the cases it is diagnosed at late stages when therapy is ineffective. Thus, the identification and validation of novel biomarkers for the early detection of gastric cancer would contribute significantly to the decrease of gastric cancer-related morbidity and mortality.

Material and Methods: We applied the T7 phage display-based SEREX technique to identify a representative set of antigens eliciting humoral responses in gastric cancer and gastritis patients. All identified antigens