and clinical parameters (such as bone metastases, pain and quality of life), circulating bone remodeling (skeletal) parameters (alkaline phosphatase, C- and N-terminal fragments of type I collagen, osteocalcin, Vitamin D), inflammatory (IL-8 and TNF-alpha) and metabolic parameters (BMI, serum cholesterol and triglycerides).
Materials and Methods: From April 2010 to August 2010, we enrolled 33 metastatic cancer patients with tumors at different sites (M/F: 16/17, mean age 66 years): 17 patients with bone metastases, 16 with metastases not involving bone. Comparison between groups (controls vs cancer patients and cancer patients with vs without bone metastases) was performed by two-sided Student's t test. Correlation between OPN/SPARC and the other variables was performed by Spearman's correlation analysis.
Results: OPN and SPARC in cancer patients were significantly higher compared to controls but did not differ between patients with or without bone metastases. OPN showed a positive significant correlation with C and N terminal fragments of type I collagen ($r = 0.390$ and $r = 0.410$, $p = 0.024$ for both), IL-8 ($r = 0.390$, $p = 0.034$) and a negative significant correlation with quality of life ($r = -0.400$, $p = 0.025$) and BMI ($r = -0.300$, $p = 0.046$). SPARC showed a positive significant correlation with BMI ($r = 0,360$, $p = 0.049$). Moreover, patients with 3 month survival (613.7 ± 229.2 ng/ml versus 195.8 ± 165 ng/ml, $p < 0.001$).
Conclusion: The results of the present study show that high OPN levels are associated with poor survival in advanced cancer patients. Further studies are warranted to assess the role of OPN and SPARC to both monitor the effects of antineoplastic regimens and to assess them as potential targets of new treatment strategies.

PP 78

Role of mucin3 gene in intrinsic resistance to oxaliplatin

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Background: Although representing a milestone in the treatment of colorectal cancer (CRC) little is known about the mechanisms responsible for intrinsic resistance to oxaliplatin (OXA). We previously identified a set of genes whose expression was associated with intrinsic resistance to OXA in a panel of 14 CRC human cell lines. The aim of this work was to validate these findings in order to typify genes that could play a role as predictive markers of response to OXA-based treatment in CRC patients.
Materials and Methods: Candidate genes selected in a previous microarray analysis were validated by qRTPCR with specific Taqman® Assays in 14 CRC cell lines comparing resistant ($R = IC50 > 1\ \mu M$) versus sensitive ($S = IC50 < 1\ \mu M$) groups. MTT assay was used to establish OXA, SN38, cisplatin and 5-fluorouracil sensitivity profiles. Genes showing statistically significant differences between both OXA sensitivity groups (U-Mann–Whitney) were considered as positively validated. MUC3A gene methylation status was analyzed by bisulphite treatment and PCR and correlated with mRNA expression results.
Results: Expression levels of 6 (MUC3, ABHD3, ERN1, JARID2, MPP6, TNFSF13) out of 16 genes were analysed by qRTPCR. Only Mucin3 (MUC3A and B) was positively validated so that as compared with S cells, R cells expressed very low levels of MUC3 A and B (MUC3A mean 4.89 ± 2.91 and $0.97 \pm 1.11$for S and R cells respectively, $p = 0.01$; MUC3B mean 8.52 ± 5.49 and 1.47 ± 1.76 $p = 0.008$). These data correlated with that obtained from the microarray (Rho-Spearman: $R=0.7$ $p = 0.006$). This feature was OXA-specific since we did not observe cross-resistance with other drugs. We only observed a slightly correlation with gene methylation status in those cells with the lowest and highest values of MUC3A expression. In a small sample ($N = 22$) of paraffin-embebbed tumors from CRC patients treated with first line OXA-based chemotherapy MUC3A and B overexpression was associated with response to treatment (86.7% of responders had expression levels >33% percentile; Fisher's $p = 0.12$; OR 3.75).
Conclusion: MUC3 A and B gene down-regulation is associated with intrinsic resistance to OXA both in vitro and in CRC patients. We could not demonstrate that regulation of MUC3A expression was dependent on methylation status. Although MUC3A and B are potential predictive markers of OXA response in CRC patients, functional studies are being conducted in order to elucidate the molecular mechanisms linking them with OXA intrinsic resistance and their potential role in CRC treatment selection.

PP 90

Glioma cell motility is modulated by CXCL12/CXCR4

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Background: Gliomas are brain tumours that account for more than 50% of the tumors that arise within the central nervous system. They are higly proliferative, angiogenic and locally very invasive. Despite the considerable advances in the knowledge of the mechanisms underlying the genetics, biology and clinical behavior, GBM pathogenesis is not completely understood and until now, there is not a therapeutic strategy to reduce the invasive and proliferative ability of the glioma cells. One of the therapeutic targets currently studied is the chemokine receptors. CXCR4 is the cell-surface receptor of CXCL12 also named stromal-derived stromal factor 1 and was associated to the tumorigenesis process in breast, prostate, kidney and brain. In order to understand the role of CXCL12/CXCR4 in gliomas we studied the survival and motility of glioma cells treated with AMD3100, an antagonist of the CXCR4.
Materials and Methods: We used the U-118 glioma cell line. The assays were performed in the presence and/or absence of CXCL12, and AMD3100. CXCR4 expression in glioma cells was evaluated by western blot and immunofluorescence. Cell survival was evaluated by flow cytometry. Cell migration study was performed using the scratch assay. Cytoskeleton organization was analysed using phalloidin.
Results: Our results showed that CXCR4 is expressed in the U-118 cell line. AMD3100, the CXCR4 antagonist, induces disruption of the cytoskeleton and a significant reduction of the migration and invasion ability of the glioma cells. The study of cell survival demonstrated that the inhibition of CXCR4 activity induces a decrease in cell survival.
Conclusion: Taking these results altogether it is possible to conclude that the activation of CXCR4 by CXCL12 promoted proliferation, survival, and migration of U-118 glioma cells, confirming that CXCR4/CXCL12 signaling pathway may contribute for the growth and invasive characteristics of GBM. In addition, it was demonstrated that the inhibition of the CXCR4/CXCL12 pathway by AMD3100, significantly inhibited the survival and motility of the glioma cells, indicating that the effect of AMD3100 should be confirmed in vivo in order to evaluate the potential of CXCR4 as a therapeutic target in the GBM treatment.

PP 74

A subtype representing activated ATM signalling in PTEN-null tumours

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Background: PTEN is frequently lost in cancer cells through genetic mutation or epigenetic silencing. Loss of PTEN function has been widely reported to cause up-regulation of the PI3K/AKT signalling pathway resulting in increased cell growth, proliferation and survival. More recently it has been reported that PTEN null cells demonstrate genomic instability through increased ROS and oxidative stress induced DNA damage. The aim of this study was to identify a biomarker for PTEN status in human cancers.
Materials and Methods: A metagene representing ATM activation was generated from public cell line data of AT fibroblasts treated with gamma-irradiation. This was used to perform hierarchical clustering analysis of a public DNA microarray profiling dataset with known PTEN IHC status. The metagene was validated in PTEN wildtype and null cancer cell lines.
Results: We found that PTEN null cells have elevated levels of ROS and furthermore activation of the DNA damage signalling kinase, ATM. In agreement with this, the ATM metagene signature correlated with PTEN mutation in breast cancer tumours. Scoring of PTEN wildtype and null cancer cell lines from various tissues using the metagene correlated with ATM activation and sensitivity to inhibition of ATM. Furthermore we show that inhibition of ATM caused DNA damage, cell cycle arrest and apoptosis in PTEN deficient cells suggesting a novel therapeutic strategy.
Conclusion: These observations suggest that ATM may represent a therapeutic target in PTEN deficient tumours and furthermore ATM activation may also be an important biomarker of PTEN mutation or loss.

PP 94

Polyamine Transport System (PTS) activity and hijacking in cancer cells: new option in Head and Neck tumors treatment with the polyamine-containing drug candidate F14512

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Background: The Polyamine Transport System, although not clearly identified at the molecular level in eukaryotic organisms, was found over activated in many types of cancer cells, such as leukemia, prostate, melanoma and NSCLC. Polyamines are implicated in many biological functions, and the need for polyamines in tumor cells, conveyed by the PTS, is crucial. New therapeutic strategies consist to use this transport system to deliver a cytotoxic agent specifically into cancer cells. Head and neck cancer remains the 6th common cancer with a very poor survival rate indicating the crucial need for new targeted strategies.