MGMT gene in matched tumour tissue samples from patients with head and neck cancer.
Material and Methods: Methylation was analyzed in primary tumours and healthy tissue from 37 patients. DNA was isolated from the tumour samples by conventional phenol chloroform extraction. Methylation of the genes was analyzed by Methylation-Specific Multiplex Ligation Dependent Probe Amplification (MS-MLPA) using 32 different probes. Different regions of the genes were analyzed by using 21 probes. 11 probes which did not have recognition sites for the HhaI enzyme were used as reference probes. The PCR products were analysed by capillary electrophoresis using the ABI 310 genetic analyzer. Two samples from each patient were compared to each other. The signals were normalized by dividing each peak area to the area of the reference probes. A ratio higher than %20 was considered as methylation-positive.
Results: In 12 (%55) patients more than one gene was methylated while 10 (%45) patients displayed only one metyhlated gene. Methylation was not observed in the repair genes in 15 patients. The most frequently methylated gene was the MGMT gene (%43) followed by the MSH6 (%21) and MLH1 (%19) genes. The MGMT gene was also freuently methylated at more than one site.
Conclusion: Our results indicate that methylation of the mismatch repair genes is a frequent event in head and neck cancer and may play a role in the development of the diesase.

592 PI3K cooperates with TGFβ in the regulation of the TGFβ malignant autocrine loop in glioblastoma

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Human glioblastoma (GBM) is one of the most aggressive and recalcitrant human tumours and is virtually not curable. GBM presents a high TGFβ-Smad activity that confers poor prognosis. High expression of TGFβ2 in GBM is responsible for the increased activity of the TGFβ-Smad pathway. This increased secretion of TGFβ2 is caused by a malignant autocrine loop through which TGFβ induces its own expression. In this work we aimed to study the molecular mechanisms implicated in this malignant autocrine loop. Specifically we studied how TGFβ regulates the expression of TGFβ2 in GBM. Using GBM cell lines and GBM patient samples we have identified a new crosstalk between the PI3K and TGFβ signaling pathway at the level of TGFβ2 secretion. We demonstrate that hiperactivation of PI3K signaling increases TGFβ mediated expression of TGFβ2. These results have been confirmed in human GBM specimens. At the moment we are looking for the transcriptional complex that mediates this process. This work provides new molecular targets to restore normal TGFβ function as new therapeutic strategies against this disease.

593 Stressor effect of zoledronic acide in rabbit heart tissue

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Background: Treatment with a bisphosphonate was linked with a significantly increased risk for atrial fibrillation (AF) in a few studies. Once-yearly infusions of intravenous zoledronic acid (ZA) was also significantly increased serious AF in postmenopausal women with osteoporosis. In this study, in order to investigate of zoledronic acid on oxidative stress and antioxidant effect of rabbit in the heart tissue.
Material and Methods: In the study, 7 rabbits on the 100 mcg/kg given daily basis Zoledronic acid (ZA) group, control group (7 rabbits) was fed 28 days ad.lib at the same time. The MDA levels in the tissue of both groups were examined using Uchiyama and Mihara methods (1978). The method is based on the production of the pink compound producing maximum absorbance at 535 nm as a result of thiobarbutyric acid's reaction with MDA. The GSH level was examined using the Ellman method (Fairbanks and Klee, 1986). The level of NO was measured by reading the maximum absorbance at 545 nm after cadmium reduction of nitrate to nitrite (Cortas and Waked, 1990). All tissue were examined histopathologically. The data are presented in mean values and standard deviations. Normality test was done with Shapiro–Wilk method. Independent samples t-test was used for the statistical analysis. $P < 0.05$ was considered statistically significant.
Results: Our findings, ZA group MDA and NO levels were found statistically significantly higher when compared to control group ($P < 0.0001$), GSH levels were found to be lower in ZA group when compared to control as statistically significant ($P < 0.0001$).
Conclusions As a result, the rabbit heart tissue Zoledronic acid, induced oxidative stress, reduces antioxidant levels were observed. Regarding the safe use of these agents, further studies with antioxidant supplements are needed.

594 A novel form of cellular senescence induced by hyper-activation of the PI3K/Akt pathway

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In normal human cells, expression of oncogenes such as Ras and c-myc, results in p53-dependent senescence or apoptosis respectively, both of which are recognized as self-protection mechanisms against tumourigenesis. Senescence induced by oncogenic Ras follows a hyper-proliferative phase associated with accumulation of DNA damage. The DNA damage response triggers changes in global gene transcription leading to irreversible proliferation arrest. For cell transformation additional genetic alterations are required to bypass these proliferation arrest mechanisms. Enhanced activity of the PI3K/Akt pathway is detected in approximately 30% of human cancers with downstream effects on cell survival, proliferation, metabolism, cell migration and angiogenesis via effectors including GSK3b, MDM2, FOXO1/3a, TSC2 and p27. In this study we examine the senescence-like phenotype induced by hyper-activation of the PI3K/Akt pathway driven by expression of constitutively active (CA) Akt, mutant PIK3CA, or PTEN depletion in normal human fibroblasts. We have examined the accumulation of senescence markers including cell cycle inhibitors and senescence associated β-galactosidase activity, and markers of the DNA damage response. We have also investigated the additional genetic alterations required for bypass of CA-Akt induced proliferation arrest using SV40 T antigens and shRNA introduced into isogenic cell lines. Interestingly, we find that hyper-activation of the PI3K/Akt pathway results in a novel form of p53-dependent proliferation arrest that is not associated with an initial hyper-proliferative phase or DNA damage accumulation. Using chemical inhibitors, we have implicated the stress activated p38MAPK and the mTORC1 cell growth pathway as two key elements in the activation of p53 by CA-Akt. The formation of senescence-associated heterochromatic foci (SAHFs) is implicated in irreversible silencing of proliferation promoting genes. Notably, as compared to oncogenic Ras, heterochromatic reorganization was not detected upon expression of CA-Akt, which may affect the response to these cells in vivo. Given that pro-senescence therapies are being suggested as cancer prevention and treatment strategies, understanding the differences between types of oncogene-induced senescence will become increasingly important./

Monday 28 June 2010 09:45–17:30

Poster Session
Survivorship Research

595 The clinical and biological significance of the immunophenotypic assessment of CD81 in multiple myeloma clonal plasma cells

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Background: Although CD19 is typically down regulated in myelomatous plasma cells (MM-PC), we have recently shown that a minority of multiple myeloma (MM) patients (4%) express this marker at diagnosis, which correlates with adverse outcome. The CD19 expression is thought to be regulated by CD81, a tetraspanin involved in mechanisms of cell proliferation. However, phenotypic or genomic studies of CD81 expression in MM are scanty, and its potential prognostic value remains unknown.
Material and Methods: A total of newly diagnosed 36 smoldering MM (SMM) patients and 229 untreated symptomatic MM patients were included in this study, the latter group uniformly treated according to the Spanish GEM05>65y protocol. Expression of CD81 on MM-PC was assessed by multiparameter flow cytometry (MFC), staining BM samples using a four-color direct immunofluorescence technique that allowed the identification of MM-PC as well as CD81 surface expression. In a subset of patients (18 SMM and 23 MM) mRNA gene expression profiling (GEP) was performed on immunomagnetically enriched MM-PC.
Results: MFC studies detected positive staining for CD81 in MM-PC of 15/36 (42%) SMM and 90/229 (39%) MM patients. Interestingly, both SMM and MM CD81+ cases showed a higher frequency of CD19 expression on MM-PC compared to CD81− cases (13% vs. 0%, P=.08 and 7% vs. 1%, P=.01; respectively), in line with the regulatory role of CD81 over CD19. Concerning GEP analysis, we found a significantly (P=.003) lower relative expression of *CD81* mRNA in MM-PC of SMM (6.8) and MM (6.7) patients compared to normal PC (9.3), which could explain, at least in part, the absence of