Material and Methods: We studied the autophagic properties of human PC-3 prostate and MDA-MB-231 breast cancer models of acquired, stable resistance to metronomic cyclophosphamide therapy, compared to their parental counterparts. We also analyzed the anti-tumour effects of metronomic versus conventional cyclophosphamide ± chloroquine (autophagy inhibitor) therapy on PC-3 xenografts. Furthermore, we compared the *in vivo* growth properties of paired autophagy-competent and autophagy-deficient (i.e., beclin1 haploinsufficient) baby mouse kidney epithelial cells treated with either metronomic or conventional cyclophosphamide.
Results: LC-3 Western blotting and acridine orange flow cytometry of parental PC-3 and MDA-MB-231 revealed strong autophagy induction under conditions of metabolic stress mimicking the microenvironment in tumours undergoing metronomic cyclophosphamide therapy (i.e., hypoxia, low pH and reduced nutrients). In contrast, the autophagic response was reduced in a number of metronomic cyclophosphamide resistant PC-3 and MDA-MB-231 variants. Chloroquine impaired the response of PC-3 xenografts to metronomic cyclophosphamide. Similarly, the impact of metronomic cyclophosphamide was reduced in autophagy-deficient versus autophagy-competent baby mouse kidney epithelial cell allografts. In contrast, both pharmacological and genetic autophagy deficiency enhanced the antitumour effects of conventional cyclophosphamide.
Conclusions: Our studies suggest that impaired autophagy contributes to resistance to metronomic cyclophosphamide chemotherapy, and possibly to other forms of antiangiogenic or chronic anticancer therapies. In other words, chronic metabolic stress associated with metronomic chemotherapy may favor cell death promoting autophagy effects. In contrast, cell survival promoting autophagy effects prevail during acute cellular stress due to conventional chemotherapy. Thus, the impact of autophagy modulators in clinical development may vary dramatically depending on the nature of concomitant anticancer therapies.

1116 POSTER

The Influence of the Combined Treatment With Vadimezan (ASa 404) and Taxol on the Growth of U251 Glioblastoma Xenografts

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Background: One of the most important biological characteristics of Glioblastoma multiforme (GBM) is high vascular density and targeting the vasculature in this tumour could be an attractive therapeutic strategy. Vadimezan (ASA 404, DMXAA) belongs to the class of small molecule vascular disrupting agents (VDA) that cause disruption of established tumour vessels and subsequent tumour hemorrhagic necrosis. Selective antivasculogenic effects of ASA 404 are mediated by intratumoral induction of several cytokines including tumour necrosis factor-α (TNF-α), granulocyte-colony-stimulating factor (G-CSF), interleukin 6 (IL-6) and macrophage inflammatory protein 1α (MIP-1α). Preclinical studies have demonstrated that ASA 404 acts synergistically with Taxanes, which at lower concentrations inhibit angiogenesis. In this study, we investigated if treatment of mice bearing U251 human glioblastoma xenografts with ASA 404 and taxol may be synergistic. Therapy response was evaluated also by FDG-PET imaging.
Material and Methods: 1.5×10^6 U251 cells were inoculated s.c. into the right hind limb of NMRI-Foxn1^{nu} athymic female nude mice. Animals were randomly assigned in 4 groups (7−9 animals/group) for treatment: control, taxol, ASA 404 and ASA 404 plus taxol. The animals received either a single dose of taxol (10 mg/kg), ASA 404 (27.5 mg/kg), or taxol (10 mg/kg) plus ASA 404 (27.5 mg/kg) administered i.p.; ASA 404 was administred 24 hours after the treatment with taxol. 4 hours after treatment with ASA 404 (28 hours after treatment with taxol) FDG-PET scans were performed.
Results: The treatment with taxol did not affect the tumour growth in comparison to untreated controls. The treatment of animals with single dose ASA 404 alone or in combination with taxol caused a significant decrease in tumour volume. The combined treatment did not decrease the growth of the xenografts significantly more than ASA 404 alone. The final tumour weights were: control = 764±168 mg, taxol = 651±148 mg, ASA 404 = 283±127 mg, ASA 404 + taxol = 180±56 mg. FDG-PET imaging corelated with tumour response. SUV values were: control = 1.21±0.39, taxol = 1.14±0.19, ASA 404 = 0.36±0.08 and ASA 404 + taxol = 0.50±0.14.
Conclusion: The treatment with ASA 404 alone or in combination with taxol showed antitumoral effects in our glioblastoma model probably through destruction of blood vessels. The implications for the anticancer effect of this compound warrant further preclinical studies.

1117 POSTER

Overcoming the Acquired Resistance to Afatinib (BIBW2992) in HCC827, a Non-small Cell Lung Cancer Cell Line

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Understanding of the pharmacological responses to drug treatment in cancer cells is essential for discovery and development of novel anti-cancer therapies. In this study, drug resistant cell lines, HCC827-BR1 and HCC827-BR2, were developed by treatment of HCC827 cells with escalating concentration of afatinib (BIBW2992). The CC_{50} of BIBW2992 in HCC827 ranges from 2 to 10 nM while the CC_{50}s of BIBW2992 in HCC827-BR1 and HCC827-BR2 are approximately 10 uM. Gene expression analysis revealed that the epithelial-mesenchymal transition (EMT) may be involved in resistance to BIBW2992. The drug-resistant cells are more invasive as evaluated under in vitro assays. Results from this study have also identified that the drug-resistance cells are more sensitive to another kinase inhibitor; indicating that an oncogenic shift has occurred. When this drug is combined with BIBW2992 in treatment of HCC827 cells, much less colonies survived compared to cells treated by BIBW2992 alone. The clinical ramifications of these observations will be discussed.

1118 POSTER

Doxorubicin and Taxol Induce Apoptosis in Breast Cancer Cells by Activating Foxo3a Transcription Factor

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Background: Foxo3a is a member of the forkhead box O class of transcription factors (foxO), which are key regulators of apoptosis, cell cycle arrest and cell division. Foxo3a phosphorylation by kinases, such as Akt, leads to its nuclear exclusion, cytoplasmic accumulation and subsequent degradation. Foxo3a inactivation has been associated with tumorigenesis and poor survival in breast cancer, what turns this protein into a possible target for anticancer drugs. In this study, we aimed to investigate the role and regulation of Foxo3a in response to taxol and doxorubicin (doxo) treatment in breast cancer cells.
Materials and Methods: The human breast carcinoma cell lines MCF7 and MDA-MB-231 were exposed to clinically relevant concentrations of taxol and doxo and citotoxicity was assessed by the MTT assay. Cell morphological changes were microscopicaly photographed. Western blot and Annexin V/PI by flow cytometry were used to detect caspases activation and apoptosis, respectively.
Results: After taxol exposure for 24h, there was an 85% cell viability inhibition, which was also observed after doxo treatment for 72h ($p < 0.01$), showing that both drugs display high toxicity against breast cancer cells. Morphological analysis of non-adherent cells revealed that the drugs induced 65% of cell death ($p < 0.05$), indicating that citotoxicity was not resulted from cell proliferation inhibition. Taxol and doxo could effectively induce apoptosis, as detected by the Annexin-V/PI method and caspases-3, -7 and -9 activations. Western blot analysis showed that there was a 16 and 12-maximum fold increase in Foxo3a levels after treatment with taxol and doxo, respectively. However, the Real Time PCR analysis of mRNA Foxo3a expression in cells exposed to the drugs showed that Foxo3a levels were not increased, indicating that Foxo3a expression is not transcriptionally activated. This finding suggests that taxol and doxo may induce cellular mechanisms which prevent Foxo3a degradation. Data analysis was done using the t student test and a $p < 0.05$ was considered statistically significant.
Conclusion: The association between the increase in Foxo3a expression and cells sensitivity indicates that this transcription factor may act mediating taxol and doxo-induced apoptosis. These data point Foxo3a as a cellular target for anticancer drugs in breast cancer cells.
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1119 POSTER

Influence of Soluble CD40 Ligand on Colorectal Cancer Cells: a Flow Cytometric Study

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Background: The cell surface costimulatory molecule CD40 is a member of the Tumour Necrosis Factor Receptor family widely expressed on various

tumour types. It has been indicated as prognostic/predictive biomarker and as a potential target for therapy in human tumours (Fonsatti E. et al. Sem Oncol, 2010). The CD40 pathway regulates humoral and cellular immunity, exherts direct anti-proliferative effects on selected tumours and plays a crucial role in angiogenesis. This multifunction nature of the CD40 signalling depends on target cells, on microenvironment and on different mechanisms of stimulation (ligation or cross-linking), so that the exact mechanism for cell growth inhibition in solid tumours is not completely elucidated (Rui L. et al. Mol Biol Rep, 2011). Discordant results have been reported about the growth-inhibitory effects and the potential for inducing apoptosis of the two forms of the CD40 ligand: soluble (sCD40L) and membrane-bound.

Material and Methods: We studied three previously established colon cancer cell lines, well-characterized for CD40 expression: Colo320 (moderate expression), HCT116 and SW48 (highly positive). To investigate the growth inhibitory mechanism of the sCD40L we evaluated its effect on cell cycle phase distribution and apoptosis induction. Cell cycle analysis was performed on Propidium Iodide (PI)-stained cells and apoptosis was assessed by Annexin V-FITC/ Propidium Iodide assay, by flow cytometry (FCM).

Results: CD40 antigen was expressed on 8% of Colo320 cells and 32% and 53% of SW48 and HCT116 cells, respectively. 48 hrs after incubation with sCD40L the studied cell lines were markedly ($p < 0.005$) accumulated in G0/G1 phase with a significant ($p < 0.05$) decrease of cells in S- phase, compared to untreated cells. At the same time point after treatment no significant apoptosis was observed in all the studied cell lines.

Conclusions: In our study the inhibition of colorectal cancer cell proliferation by sCD40L was mainly due to a slowing down of the cell cycle progression while apoptosis was not involved in the growth inhibition. This finding is in contrast with recent reports on CD40 +ve colorectal cancer cells. These data should be taken into account when the CD40 pathway is utilized as a therapeutic target, in view of a possible combination of standard chemotherapy and/or antiangiogenetic therapy with antitumour immunotherapy in advanced colorectal cancer (Manzoni M. et al. Ann Oncol, 2011).

1120 POSTER

Hypermethylation of Tumour Suppressor Gene 14-3-3sigma in Serum of Sporadic Breast Cancer Patients

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Objective: Expression of 14-3-3 σ is a tumour suppressor gene induced in response to DNA damage, and has been implicated in G2/M cell cycle arrest by p53. To correlation methylation levels of promoter 14-3-3σ with association prognostic factors in breast cancer.

Material and Methods: This is a prospective study we quantified methylation levels of promoter 14-3-3σ gene in 107 women with breast cancer and 108 control subjects by Real Time QMS-PCR SYBR green and analyzed association with prognostics factor in breast cancer.

Results: Median age was 58 years (32−88); 69% were postmenopausal women. Nodal involvement N0; 63%, N1; 30%, N2; 7%), tumour size (T1; 58%, T2; 35%, T3; 4%, T4; 4%) and grade G1; 20%, G2; 37%, G3; 30%). The methylation of 14-3-3σ were 60% of sporadic breast cancer patients and were 34% of normal breast ($p = 0.0047$). The methylation of 14-3-3σ gene in serum was markedly related with T3−4 stage ($p < 0.05$),nodal positive status ($p < 0.05$) and poor outcome. With a median follow up 6 years we saw more probability of developing distance metastasis in patients with methylation 14-3-3σ ($p > 0.05$).

Conclusions: Hypermethylation of the 14-3-3σ a promoter is an early and frequent event in breast neoplastic transformation, leading to the suggestion that silencing of 14-3-3σ may be an important event in tumour progression and particularly in breast carcinogenesis. Therefore, it is possible that loss of σ expression contributes to malignant transformation by impairing the G2 cell cycle checkpoint function, thus allowing an accumulation of genetic defects. Perhaps in the detection of CpG methylation of 14-3-3σ may be used for diagnostic and prognostic purposes.

1121 POSTER

The Prevalence of Histone Deacetylase (HDAC) Expression in Korean Non-small Cell Carcinoma Patients

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Background: DNA methylation and histone modification are dynamically linked in the epigenetic control of gene silencing and they play an important role in tumorigenesis. We evaluated the role of histone deacetylase (HDAC) in the development of lung cancer and the relationship between a HDAC overexpression and survivin, p16 and p53 overexpression.

Materials and Methods: We performed immunohistochemical staining for HDAC1, HDAC2, HDAC3, p16, and p53 in 129 lung cancer specimens.

Results: HDAC overexpression was detected in 51% (HDAC1 and HDAC2) and 64% (HDAC3) and it was more frequently seen in the squamous cell carcinomas than in the adenocarcinomas ($p < 0.05$). There was statistical significances between HDAC overexpression and survivin overexpression ($p < 0.05$), but not with p16 and p53 overexpressions.

Conclusions: HDAC overexpression might be involved in lung carcinogenesis, and especially in a squamous cell carcinoma, and a HDAC overexpression may be associated with survivin overexpression, however, overexpression of these genes are not related with patient survival. These results suggest that HDAC inhibitors are putative therapeutic agents in subgroup of non-small cell lung cancer patients.

1122 POSTER

Histone Deacetylase Inhibitor Potentiates Chemotherapy-induced Apoptosis in Burkitt's Lymphoma Cells

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Background: Although polychemotherapy regiments have improved the clinical outcome for Burkitt's lymphoma (BL) patients, salvage treatment of patients with refractory or recurrent disease remains very poor. Combined therapy protocols have been emerging to improve treatment strategies to circumvent responseless BL patients. Several histone deacetylase inhibitors (HDACI), which have recently entered early clinical trials, exert their anticancer activity in part through the induction of apoptosis although the precise mechanism of this induction is not known. In this study, we evaluate the cell death enhancement effect of HDACI combined with etoposide (VP-16) and cisplatin (CDDP), two of the drugs commonly used as salvage chemotherapy on BL patients.

Material and Methods: To evaluate the effect of HDACI NaB, CDDP, VP-16 on cell viability, Raji and BL41 cell lines were treated with NaB (1.0−10 mM), CDDP (1.0−30 μM), and VP-16 (0.1−10 μM). After 24 h, the viable cells were determined using the cell proliferation reagent, 3-(4.5-Dimethyl-thiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT). Apoptosis was assessed using Annexin V and PI assay on a CyAn ADP flow cytometer. Cleaved caspase-3 labeling was determined using Polyclonal phycoerythrin (PE)-conjugated anti-active caspase 3 antibody on flow cytometer. Effects on pro-apoptotic (Procaspase-9, Bim, Bax) and anti-apoptotic (Mcl-1, Bcl-2) Bcl-2 family members were analyzed by Western blotting. Drug-Interaction analysis followed the procedure developed by Fischel et al, 2005.

Results: The combination effect of NaB/VP-16 and NaB/CDDP were found to be synergistic and additive, respectively, in both the cell lines. Moreover, the apoptotic effects of the HDACI and VP-16 combined treatment were followed by upregulation of caspase-3, caspase-9, and Bim proteins, followed by Mcl-1 downregulation. However, Bim overexpression was not correlated with Bcl-2 inhibition and was accompanied by activation of Bax, a potent inductor of apoptosis.

Conclusions: We have provided strong evidence for the synergistic effects of the association of HDCI and chemotherapy in BL cells harboring p53 mutations. As HDACIs can modulate a variety of pro- and anti-apoptotic proteins, combination regimens with HDACIs should be investigated. Ultimately, these studies will hopefully improve our treatment strategies for patients with relapsed and refractory BL.

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