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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