increases during progression, the potential role of GGT as candidate target for antineoplastic treatments is suggested. The development of GGT inhibitors of pharmacological significance would likely enrich the therapeutic spectrum with an additional tool, to be exploited in selected situations. Supported by FIRB 2001 and AIRC 2001–03 funds.

81 POSTER

Synergistic effects of Apo-2L/TRAIL and ionizing irradiation in human tumor cell lines without relevant damage of normal tissue cells

P. Marini, A. Schmid, H. Faltin, V. Jendrossek, W. Budach, C. Belka. *University of Tuebingen, Radation Oncology, Tuebingen, Germany*

Background: An outstanding feature of tumour necrosis factor related apoptosis inducing ligand (Apo-2L/TRAIL) is the pronounced tumour cell specificity. Thus APO-2L/TRAIL is now tested in clinical settings as new anti-cancer agent. Up to now, little is known about the effects of a combined therapy using Apo-2L/TRAIL and ionising radiation. In order to examine the efficacy of a combined treatment several malignant cell lines and diverse normal tissue systems were treated with various combinations of APO-2L/TRAIL and radiation.
Material and Methods: Colo 205 and HCT-15 (colorectal carcinoma), NCI H460 (lung adenocarcinoma), MDA MB231 (breast cancer), two squamous cell carcinoma cell lines (FaDu and SCC-4) as well as normal tissue cell system derived from prostate, mammal, renal and bronchial epithelia, fibroblasts and hepatocytes were treated with a combination of Apo-2L/TRAIL and irradiation. Apoptosis was quantified by fluorescence microscopy after Hoechst-staining. The degree of interaction was evaluated by isobologramm-analysis. Regulation of the surface expression of the APO-2L/TRAIL receptors R1/DR4 and R2/DR5 was determined by flow cytometry (Quantibrite^{TM}).
Results: The combination of APO-2L/TRAIL and radiation was associated with pronounced additive effects on apoptosis induction in tumour cell systems when APO-2L/TRAIL and radiation were applied simultaneously. In contrast a striking synergy occurred when APO-2L/TRAIL was added 14 hours after irradiation in all cell lines except the NCI H460 cells. Ionising radiation triggered an upregulation of DR5 in most cell systems. However, no straight correlation with the induction of synergistic cell death was observed. In contrast, the combined treatment of normal tissue cell systems was not associated with additive or synergistic effects regarding apoptosis induction.
Conclusion: Preirradiation sensitises several tumour cell systems towards APO-2L/TRAIL induced apoptosis. The concurrent application is less effective. Regardless of any preirradiation APO-2L/TRAIL did not induce apoptosis in any of the tested normal cell systems. Thus, the in vitro data do not suggest any increased toxicity of the combined treatment. Although DR5 is clearly upregulated in response to irradation this mechanism might not represent an exclusive regulatory mechanism responsible for the observed synergistic effects.

82 POSTER

MS-275, a potent orally active inhibitor of histone deacetylases is highly active in experimental tumor models of melanoma and prostate cancer

H. Hess-Stumpp[1], J. Hoffmann[1], A. Schott[2]. [1]*Schering AG, Experimental Oncology, Berlin, Germany;* [2]*Schering AG/Berlex, SBU Specialized Therapeutics, Montville, USA*

Background: Histone deacetylases (HDACs) are a family of enzymes that are involved in the epigenetic regulation of gene expression. HDACs keep histones in a hypoacetylated, positively charged state that tightly binds the negatively charged phosphate backbone of DNA, so preventing gene transcription. Transcription factor complexes must get access to the DNA to allow gene expression, which is normally achieved by histone acetyl-transferases, the natural counterparts of HDACs. The balance between transcriptional activity and gene silencing is often disturbed in tumors and the inhibition of HDACs may activate tumor suppressor genes such as the cell cycle inhibitor p21$^{WAF1/CIP1}$. HDAC inhibition may be antiproliferative, and induce differentiation and/or apoptosis. There is growing experimental evidence for this hypothesis and a number of HDAC inhibitors are currently in phase **I/II** clinical trials. Here, we summarize experimental results obtained with MS-275, an orally active synthetic pyridylcarbamate, in a number of melanoma and prostate cancer tumor models.
Material and Methods: Melanoma (A375, SK-Mel28, B16F10) and prostate carcinoma (DU145, PC3) cell lines were grown as xenografts in nude mice. After establishment, tumors were treated with MS-275 daily p.o. Tumor area and body weight was determined during treatment, and final tumor weight after sacrifice used to calculate the tumor/control ratio (T/C).
Results: MS-275 showed a dose-dependent efficacy in almost all experiments. Lower doses (5 and 10 mg/kg) revealed a slight response whereas higher doses (25 and 50 mg/kg) showed a marked antitumor efficacy. The highest dose of MS-275 (50 mg/kg) showed a very high efficacy in the SK-Mel28 model (T/C 0.1, i.e. 90% inhibition). A transient decrease in body weight was noted at higher doses, but this recovered within a few days without disrupting treatment. At lower doses the compound was very well tolerated. MS-275 exhibited a higher efficacy in the SK-Mel28 model compared with dacarbacine. These data support preliminary results from an ongoing phase **I** clinical trial with MS-275. Nine patients with melanoma have been treated so far, with one patient showing a partial response for 78 weeks and is still on treatment, and 5 patients having disease stabilization for $\leqslant$38 weeks. MS-275 exhibited a marked antitumor efficacy in the prostate carcinoma models, where even the lower doses of 5 and 10 mg/kg showed a significant effect in the DU145 model.
Conclusion: These results indicate that MS-275 exhibited a marked, and in most cases dose-dependent, antitumor efficacy. These data are in agreement with preliminary findings from a phase **I** clinical trial where the majority of pretreated melanoma patients showed disease stabilization. Thus, highly chemotherapeutic resistant tumors such as melanoma and prostate carcinoma may be suitable indications for phase **II** clinical trials with MS-275.

83 POSTER

Antitumor activities of MGCD0103, a novel isotype-selective histone deacetylase inhibitor

Z. Li, N. Zhou, M. Fournel, G. Rahil, J. Wang, D. Delorme, O. Moradei, R. MacLeod, J. Besterman. *MethylGene Inc., Montreal, Canada*

Targeting Histone deacetylases (HDACs) is a new approach in human cancer therapy in recent years. Several HDAC inhibitors had been advanced into human clinical trials. We have rationally designed MGCD0103, a non-hydroxamate small molecule HDAC inhibitor, as a novel anti-cancer therapeutic. MGCD0103 selectively targets certain specific class **I** HDAC enzymes at IC50's of submicromolar concentrations *in vitro* and induces hyperacetylation of histones in cultured human cancer cells. MGCD0103, but not its inactive analog, selectively and potently inhibits proliferation of human cancer but not normal cells. It causes G2/M cell cycle block and induces apoptosis in human cancer cells in a dose-dependent manner. By using cDNA expression array analysis of human cancer cells treated with either MGCD0103 or other HDAC inhibitors in clinical development, we found MGCD0103 regulates transcription of a smaller subset of downstream genes, reflecting its inhibitory specificity. *In vivo*, MGCD0103 significantly inhibits growth of human tumors in various xenograft models in nude mice in a dose-dependent manner with minimal toxicity. In correlation with its antitumor activities, MGCD0103 induces hyperacetylation of both white blood cells and tumors in tested animals. We conclude that MGCD0103 appears to have a favorable therapeutic index *in vivo*. MGCD0103 is now under investigation in Phase **I** clinical trials.

84 POSTER

Regulation of the oncogenic x-protein of hepatitis B by cellular chaperones

X. Yuan, W. Xu, L. Neckers. *National Cancer Institute, Urologic and Oncology Branch, Rockville, Maryland, USA*

HBx, the x-protein of the Hepatitis B virus, has been shown in cells and animal models to promote the development of hepatocellular carcinoma, probably due to its transactivating function. It is thus of great interest to understand the regulation of HBx in the host cell. Here we describe a novel mechanism by which HBx is regulated by cellular chaperones, and discuss its pathophysiological implications. HBx was previously shown to interact with XAP-2, an immunophilin that can serve as a co-chaperone for Hsp90 or Hsp70, implicating these chaperones in the regulation of HBx. To determine the functional role of Hsp90, we treated the hepatoma cell line HepG2 transiently expressing HBx with the antibiotic geldanamycin (GA), an inhibitor of Hsp90. GA induces the degradation of diverse Hsp90 client proteins. To our surprise, instead of reducing HBx levels, GA treatment increased the expression of HBx in HepG2 cells. Interestingly, differential lysis and western blotting indicated that the increase occurred mainly in the cytosol. In contrast, the nuclear fraction showed a modest decrease in HBx level. These observations were confirmed by immunofluorescence experiments which showed increased appearance of HBx in the cytosol of GA-treated cells. These data suggest that Hsp90 is involved in cellular distribution of HBx. Given that the major effect of HBx occurs in the nucleus, one may be able to inhibit HBx function by targeting its interaction with Hsp90, thereby inhibiting HBx nuclear entry. We also observed that GA induced HBx binding to Hsp70, and that a dominant negative CHIP protein, a co-chaperone of Hsp70, demonstrated GA-like effects on HBx expression. Taken together, these data suggest that cooperation between