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

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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

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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

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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

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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

Hsp90 and Hsp70 is required for HBx activity, and that this process can be interdicted at multiple points of the chaperone cycle.

85 POSTER

Progenitor cells derived from human bone marrow contribute to the growth of breast cancer cells

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Solid tumors consist of vasculature and stroma that support the growth of cancer cells. Blood vessels within tumors can arise in part through the recruitment of endothelial precursor cells (EPC) from hematopoietic progenitors in the bone marrow. Mesenchymal stem cells (MSC) can also be isolated from human bone marrow. We compared gene and protein expression in human EPC vs. MSC, and investigated the effect of these immature, partially differentiated cells on the growth of MDA-MB-231 breast cancer cells. EPC were derived from a select population of bone marrow cells expressing CD133 and CD34 that were stimulated with VEGF, bFGF, and heparin. MSC were obtained from the culture of whole bone marrow cells under nonstimulatory conditions. Both methods of bone marrow culture resulted in the establishment of adherent cell lines. EPC and MSC long SAGE (serial analysis of gene expression) libraries were constructed and profiles revealed a 38% overlap of genes between EPC and MSC libraries, indicating moderate similarity between the cell populations. Sixteen TEMs (tumor endothelial markers) were present that were also detected in SAGE libraries prepared from brain, breast, and colon tumors. Protein expression of molecular markers was determined by flow cytometry. Markers expressed by both EPC and MSC include CD105 (endoglin), P1H12, and CD90 (Thy-1). Differences include two adhesion molecules, VCAM and ICAM-1, that were absent or expressed at low levels by EPC but present in MSC. The ability of EPC and MSC to support cancer cell growth was evaluated in a co-culture assay involving clusters of MDA-MB-231 breast cancer cells embedded in collagen. EPC and MSC are each added in suspension to the wells and by day 2, invasion of the cancer clusters by EPC or MSC is evident. Subsequently, the MDA-MB-231 cancer clusters sprout and grow outwards at a greater rate when EPC or MSC are present compared to cancer clusters cultured in isolation. In vivo, co-injection of EPC or MSC enhanced the growth of subcutaneous MDA-MB-231 tumors by two-fold; it is likely that this effect may be observed also with other cell types. These results indicate that while EPC and MSC exhibit differences at the level of gene and protein expression, they share an ability to promote cancer cell growth and may play an important role in supporting the development of tumors.

86 POSTER

Preclinical pharmacokinetics (PK) and CNS distribution of RTA 203, a novel mammalian vacuolar H+-ATPase inhibitor

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RTA 203, an analog of salicylihalamide A, is a member of a new class of mammalian-specific V-ATPase inhibitors called the benzolactone enamides. This new class is unique in chemical structure and specificity when compared to the first class of V-ATPase inhibitors called macrocyclic lactones or plecomacrolides, which include the bafilomycins and concanamycins. While the concanamycins demonstrate excellent *in vitro* activity, further development was halted due to a poor toxicity profile and difficult synthesis. In contrast, RTA 203 is 100 times less toxic than the concanamycins (I.P. LD_{90} RTA 203 100 mg/kg vs. concanamycin A 1 mg/kg) and it can be reliably synthesized. RTA 203 has demonstrated excellent activity in cell lines exhibiting the MDR phenotype including NIH/ADR-RES (breast), HCT-15 (colon), and CAKI-1 (kidney), and preliminary data suggests RTA 203 increases the sensitivity of A549 lung cancer cells to irradiation. One untoward effect of RTA 203 is dose-dependent seizure activity observed, to some extent, across a dynamic dose range of 5 to 100 mg/kg in a murine model. To determine the preclinical PK, CNS distribution, and relationship between RTA 203 plasma-CNS drug concentrations and seizure activity male CD1 mice were administered RTA 203 as a 5 mg/kg IV bolus via the tail vein (0.1 mL;). Blood and tissue samples were collected (5 animals/timepoint) over a span of 5 minutes to 48 hours following drug administration, processed and stored at −80ºC until analysis. RTA 203 was extracted from plasma samples by solid phase extraction and from tissues using tissue disruption followed by liquid-liquid extraction. Plasma samples were analyzed by HPLC/UV (LLOQ=12.5 ng/mL) or LC/MS/MS in ES-mode (LLOQ=0.5 ng/mL). Tissue samples were analyzed using HPLC/UV (LLOQ=5 ng/g) with tissue concentrations expressed as ng/g of tissue. PK parameters were determined by fitting a two-compartment linear model to the mean measured plasma concentration-time data. Bolus IV administration produced mild seizures, occurring within seconds of dosing, followed by recovery within 20 minutes. RTA 203 rapidly distributed to all tissues with a measured peak CNS concentration of 212.7±46.8 ng/g at 5 min (25% of the simultaneously measured plasma concentration) coinciding temporally with the seizures observed post-injection. Drug could be measured in plasma and CNS for up to 5 hrs following dosing, with CNS concentrations paralleling plasma concentrations from 45 mins to 4 hrs. Seizure activity ceased 20 minutes after injection when plasma concentrations declined to <100 ng/ml. Mean C_{max}, V_{ss}, $t_{1/2,\ \beta}$, and Cl_p were 1438.7±4.2 ng/mL, 7.3 L/kg, 0.66 hrs, and 17.0 L/hr/kg, respectively. These parameters are now being used to develop continuous infusion dosing models designed to target specific steady-state plasma concentrations in an effort to eliminate CNS toxicity and improve efficacy.

87 POSTER

Selection of pre-invasive lung cancer binding peptides using random phage display libraries

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The genetic changes that occur during the multi-step process of lung carcinogenesis can lead to mutant or altered protein expression patterns within the cells or on the cell surface membranes. The purpose of this study was to identify peptides that bind with high selectivity to pre-invasive lung cancer tissues but not their normal counterparts using random peptide phage display technology and to determine if such affinity selection method can be applied to archival materials that are formalin-fixed, paraffin-embedded, tissues that are tethered on glass slide. A 12-mer random peptide phage display library was screened against 12 pre-invasive lung cancers (severe dysplasia and carcinoma in situ (CIS)). Affinity selection starts with the normal cells used for depletion, followed by five rounds of binding to cells of interest (targets). This preliminary subtractive selection enriches for differentially expressed proteins in pre-invasive lung cancer while removing or reducing ubiquitously expressed proteins and improves the binding differential. Ten uL of the phage library, which contained 4x10e10 plaque forming unit (pfu), was added directly onto 5um sections of paraffin-embedded formalin-fixed tissue of depletory-normal bronchial epithelial cells (obtained by bronchial biopsy), and incubated for one hour at room temperature. The unbound phage were transferred onto 5um sections of paraffin-embedded formalin-fixed tissue with severe dysplasia or CIS cells (target cells) and incubated for another hour. The cells used for depletion (i.e. normal cells) as well the target cells are normally paired clinical specimens (i.e. biopsies) obtained from the same individual. Any unbound phage were washed off and the bound phage, were eluted and amplified by infection of E. Coli 2738 and used in the next round of selection and binding. Affinity selection and amplification were repeated five times to allow for enrichment of cancer specific clones, with each successive round of affinity selection performed on the next serial sections of tissues with target cells. The sequences of DNA coding inserts were determined for 25 of the single-phage from the fifth round of affinity selection. The library, which initially contained about 2.8x10e9 different sequences collapsed after five rounds of selection such that most of the recovered clones expressed consensus sequences and database (SWISS-PROT) searched for proteins matching the peptide consensus sequences using the BLAST programme showed some biologically relevant matches. In summary, these data demonstrate that this affinity selection method can be applied to archival materials that are formalin-fixed, paraffin-embedded, tissues that are tethered on glass slide. (Supported in parts by Canadian Institute of Health Research (CIHR), Cancer Research Society and a new investigator award to JYH from CIHR-BC Lung Association).

88 POSTER

Substrate phage display: screening-approach for radiation-induced posttranslational peptide modifications in a complex proteome

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Background: The identification of tumor-specific and therapy-induced processes reveals important information on the cellular phenotype and leads to potential novel targets for anticancer therapies. We present a novel screening technique to identify radiation-induced enzyme activities in complex proteomes. The method selects substrates for treatment-dependent enzyme activities regulated on the gene-, protein-expression-, and posttranslational level.