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.

Materials and Methods: The analysis was performed with a T7-phage-based peptide phage display library that was specifically constructed to detect posttranslational modifications of peptides. For this study we focused on the identification of peptides recognized by proteolytic enzyme activities. 10^9 different substrate peptides were screened against a complex proteome present in cellular lysates derived from untreated and irradiated cells (SW480, 10Gy). In a subtractive screening procedure a differential activity pattern could be monitored and substrates for treatment-specific activities could be distinguished from substrates for background activities. The method was developed and applied in a methodological and discovery-oriented approach.
Results: Radiation-specific substrate peptides were isolated in a clinically relevant radioresistant cell system (SW480). Multiple peptide sequences were selected that are specifically recognized and cleaved by treatment-dependent enzyme activities. A specific recognition sequence was identified to be part of the human nucleoporin protein (Nup50) relevant for controlled nuclear protein shuttling. Nup50 was further investigated in vivo in response to ionizing radiation.
Conclusions: We present a novel technique for the identification of treatment-induced posttranslational peptide modifications in tumor or normal tissue cells. This procedure represents a complementary tool for genome-wide screening approaches. The identified peptides, their specificity and biological counterparts suggest that intact nuclear shuttling and compartimentalization processes co-determine the cellular radiosensitivity.

89 POSTER

Gene expression profiling of cancer associated fibroblasts

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Fibroblasts located in the vicinity of tumor cells, often termed cancer associated fibroblasts (CAFs), contribute to the microenvironment that is important for cancer cell development, growth, invasion and metastatic progression. Hitherto, the defining features of CAFs and their specific differences to their normal counterparts are only incompletely understood. Therefore, based on a laser capture microdissection and microarray-based approach, the aim of this study was to comprehensively characterize CAFs in their *in vivo* environment to identify novel potential targets for anti-cancer therapy. Fresh frozen samples of basal cell carcinoma and normal skin from three different patients were used for laser microdissection of CAFs and normal fibroblasts (NFs). The RNA was extracted, amplified (~200.000 fold) and labeled with Cy3 or Cy5 modified nucleotides. Labeled RNA from CAFs and NFs (500–4000 cells) from each patient was competitively hybridized on cDNA microarrays in replicates. Results were confirmed using quantitative real time PCR. The analysis yielded three gene lists with up-regulated (patient 1: 608, patient 2: 49, patient 3: 228 genes; >1.7, t-test $p<0.05$) and down-regulated genes (patient 1: 552, patient 2: 84, patient 3: 75 genes; <0.57, t-test $p<0.05$). In the group of down-regulated genes only few genes coincide (patient 1 and 2: 14, patient 1 and 3: 5; patient 2 and 3: 3 genes). In contrast, among the up-regulated genes a significant number of genes were overlapping between the different patients (patient 1 and 2: 24, patient 1 and 3: 56; patient 2 and 3: 12 genes). Most of these genes were involved in matrix remodeling and cell-cell or cell-matrix interaction (i.e. SPARC, galectin-2, galgranulin-A, laminin alpha 2) but also in growth regulation (i.e. Grap-2, VAV3) and angiogenesis (i.e. angiopoietin-like-2). The gene lists provide a valuable tool to select genes for further studies of their functional relevance. This approach will be expanded to other solid tumors to identify additional novel CAF-specific genes, crucial for stroma-tumor interaction, which will be exploited for use in novel anti-tumor strategies.

90 POSTER

AG879, a tyrphostin c-erbB-2 inhibitor, leads to transcriptional repression of cyclin D1 in pancreas cancer cells through a p27Kip1 independent pathway

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The poor prognosis of pancreatic cancer with the current traditional modalities makes molecular targeted therapies a high priority. c-erbB-2 provides cell survival and proliferative signals through Cyclin D1. Our hypothesis is that inhibition of cyclin D1 expression will induce cell cycle arrest and apoptosis of pancreatic carcinoma. We investigated in a human pancreatic cell line L3.5sL (SL) that has a constitutively active c-erbB-2, the effects of c-erbB-2 inhibition through two different interventions: 1) degradation of its mRNA (siRNA) to prevent protein expression and 2) blockade of its catalytic activity in its tyrosine kinase domain (AG879, tyrphostin small molecule). AG879 treatment induced G1 cell cycle arrest and apoptosis. Treatment with siRNA also inhibited proliferation. The effect of these two approaches on $p27^{Kip1}$ expression was distinctly different. siRNA treatment decreased expression of c-erbB-2 and increased $p27^{Kip1}$. However, AG879 at the IC50 dose did not alter expression of c-erbB-2 or $p27^{Kip1}$ but repressed transcription of cyclin D1 mRNA 50% as measured by RT-PCR assay and cyclin D1 promoter activity fourfold as measured by luciferase promoter assay. Furthermore, AG879 had no effect on proteasome activity, excluding degradation as a plausible pathway. Since the cyclin D1 promoter has STAT3 and STAT5 binding sites, we assessed whether these STATs were involved in regulating Cyclin D1. STAT5 was not detectable in SL cells but STAT3 was. STAT3 function requires phosphorylation on Y^{705} and S^{727}. Y^{705} pSTAT3 was decreased 30% after AG879 treatment but S^{727} pSTAT3 was completely inhibited at 3 hr after AG8790 treatment. Mobility shift assays confirmed that STAT3 binding to the cyclin D1 promoter was decreased beginning 3 hr after AG879 treatment. We propose that AG879 at doses that do not decrease c-erbB-2 protein expression but induce G1 cell cycle arrest and apoptosis inhibits S^{727} STAT3 phosphorylation and that this is a critical step in repressing cyclin D1 transcription. In contrast, lowering expression of c-erbB-2 protein causes cell cycle arrest through upregulation of $p27^{Kip1}$ Further elucidation of the mechanism involved in regulating S727 STAT3 phosphorylation may provide insights into novel therapies for pancreas cancer.

91 POSTER

17-allylamino-17-demethoxygeldanamycin overcomes trail resistance in colon cancer cell lines

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Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is a promising candidate for treatment of cancer, but its cytotoxicity is limited in some cell lines. The mechanisms of this resistance have not been fully elucidated, but both AKT and NF-κB pathways have been shown to modulate cytotoxic responses. We studied the effect of combination of TRAIL and the hsp90 inhibitor 17-AAG, which we have shown to enhance the cytotoxicity of oxaliplatin in colon cancer cell lines through inhibition of NF-κB. In a series of 9 colon cancer cell lines IC50 values for a 72-hour exposure to TRAIL ranged from 30 to 3000 ng/ml. Cytotoxicity assays demonstrated additivity or synergism of the TRAIL/17-AAG combination in all cell lines, with combination indices at IC50 ranging from 0.65 to 1. The sensitizing effect of 17-AAG was greater in the TRAIL-resistant cell lines. 17-AAG enhanced TRAIL-induced activation of caspase 3 in all cell lines tested. In TRAIL-resistant cell lines, the combination of 17-AAG and TRAIL resulted in activation of either extrinsic or intrinsic apoptotic pathways in a cell line-specific manner. In the RKO cell line AKT inhibition was associated with activation of the mitochondrial apoptotic pathway, while in HT29 cells inhibition of NF-κB was permissive of caspase-8 dependent apoptosis. In both cell lines the combination resulted in down-regulation of X-linked inhibitor of apoptosis protein (XIAP), which may facilitate the activation of effector caspases. The ability of 17-AAG to target multiple putative determinants of TRAIL sensitivity warrants their further investigation in combination.

92 POSTER

A phase I, pharmacological and biological study of Sarasar® (lonafarnib, SCH 66336), cisplatin and gemcitabine in patients with advanced solid tumors

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Sarasar (lonafarnib, SCH 66336) is a novel, oral tricyclic farnesyl transferase inhibitor (FTI) with broad anti-tumor activity in human xenograft models. Preclinical studies have shown that Sarasar is synergistic with cisplatin (C) and additive with gemcitabine (G). The purpose of this study is to determine the dose-limiting toxicity (DLT), maximum tolerated dose (MTD), and biologic and pharmacokinetic behavior of the combination of Sarasar (75–125 mg BID daily), C (75–100 mg/m2 IV day 1), and G (750–1000 mg/m2 IV weekly d1, 8 or d1, 8, 15) given every 3–4 weeks. The study was amended to investigate an every three-week schedule and to delay institution of Sarasar until day 8 of course 1, in order to better differentiate the emetogenic effects of Sarasar and C. To date, 21 pts (median age 53, range 37–72; median PS 1) have received a total of 40