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.