irradiated CAFs may modulate signalling pathways influencing proliferation, survival and radioresistance.

1165 POSTER

Radiation Sensitization of Tumour Cells Induced by Shear Stress-Roles of Integrin Beta-1 and FAK

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Background: Interstitial flow in and around tumour tissue not only has particular importance in delivering anticancer agents to tumour tissue, but also affects the microenvironment to modulate tumour cell growth and metastasis. We investigated the roles of flow-induced shear stress in modulating radiosensitivity in two colon cancer cell lines and the underlying mechanisms.
Materials and Methods: T84 and SW480 colon cancer cells were trypsinized and seeded onto glass slides (75×38 mm) pre-coated with fibronectin (10 μg/ml). A parallel-plate flow chamber system was used to impose fluid shear stress. Irradiation was delivered using 160kV RS 2000 X-ray irradiator (Rad Source Technologies, Inc.). Cell proliferation, apoptosis and colony assay were measured after various combinations between shear stress and radiation. Cell cycle analysis and immunoblots of integrin β1/FAK/Akt signal molecules were evaluated. The combination effect of shear stress was reversed by neutralizing integrin β1 or using FAK overexpressed cell lines.
Results: In both cell lines, incubation under shear stress (12 dynes/cm^2) for 24 hours enhanced radiation induced cytotoxicity. Protein expression of integrin β1 was moderately while FAK was significantly suppressed. FAK down-regulation was mainly due to ubiquitin-dependent proteasomal pathway but not transcriptional suppression. The amount of ILK, GSK3β was not affected. Using FAK overexpressed cell lines, we demonstrated that shear stress enhanced colon cancer cell radiosensitivity by regulating FAK expression. On the other hand, incubation under shear stress for 3 hours did not revealed radiosensitizing effect in both cell lines. Using integrin β1 neutralizing antibody, we suppressed FAK/Akt activation by 3-hr shear stress and enhanced radiation related cytotoxicity in both colon cancer cell lines.
Conclusions: Shear stress of 24 hours provides radio-sensitization to colon cancer cell through proteosomal degradation of FAK via integrin β1. Our findings provide insights into the mechanism by which shear stress modulates colon cancer cell cytotoxicity in response to radiation. The results impact rationale combination between radiation and strategy in modulating tumour interstitial fluid pressure.

1166 POSTER

Triple-negative Breast Cancer Cells May Transfer Phenotypic Characteristics via Exosomes

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Background: Exosomes are membrane-bound 30−90 nm-sized vesicles which are naturally released into the extracellular environment. Here we investigated if exosomes are secreted from triple-negative breast cancer (TNBC) cells and, subsequently, if such exosomes may be involved in cell-to-cell communication.
Materials and Methods: Using a combination of filtration and ultra-centrifugation, exosomes were isolated from medium conditioned (CM) by the TNBC cell lines Hs578T; its highly-invasive syngenic variant, Hs578T(i)$_8$; and MDA-MB-231. To investigate potential clinical relevance of observations arising from our cell lines, exosomes were also isolated from serum procured from TNBC patients and matched controls (n = 18). Western blotting and electron microscopy were used to assess exosomes; confocal microscopy verified exosomes uptake into secondary cells (SKBR3); transfer of phenotypic characteristics was evaluated using proliferation assays; wound-healing migratory assays; and invasion through ECM-coated transwells.
Results: Successful isolation of exosomes from TNBC cell lines' CM and serum specimens was verified by Western blot analysis for TSG101 and PDC6I. The quantities of exosomes secreted from the Hs578T versus Hs578T(i)$_8$ did not differ significantly (p = 0.460). However, equal quantities of exosomes from these populations conferred very different effects on secondary cells. Specifically, while Hs578T exosomes did not increase the proliferation of SKBR3 cells (proliferation = 1.13±0.06 fold) compared to proliferation in the absence of exosomes, exosomes from the more motile and highly-invasive Hs578T(i)$_8$ cells induced a significant (p = 0.003) increase in SKBR3 proliferation rate (1.73±0.15 fold). Additionally, Hs578T(i)$_8$ exosomes (but not Hs578T exosomes) induced invasion of SKBR3 cells through extracellular matrix (mean increase=18%). This transfer of information is further supported by MDA-MB-231-derived exosomes also stimulating a significant (p = 0.001) increased invasion of SKBR3 cells (mean increase=24%). Furthermore, although the quantities of exosomes circulating in serum were found not to differ significantly (p = 0.307) between TNBC and controls, in all but one comparison pair, exosomes from TNBC sera -compared to control exosomes- substantially increased SKBR3 invasion (mean increase = 15%; p = 0.041).
Conclusions: This data suggests that exosomes released from TNBC cells and subsequently isolated from their CM, as well as serum exosomes from TNBC patients, can be taken up by secondary cells and may be involved in cell-to-cell communication, transferring certain phenotypic characteristics between cells.
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1167 POSTER

Comparison of the Impact of the Targeted Therapy Everolimus (Afinitor®) and the Chemotherapy 5-FU on Cognitive Functions and Cerebral Plasticity in an Animal Model

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Background: Cancer and treatments can induce cognitive impairments such as deficits of visual and spatial memories, and of psychomotor processing speed in patients, symptoms referred to as "chemofog". The targeted therapy Everolimus (Afinitor®), which blocks the mTOR pathway, alters cell proliferation, metabolism and neoangiogenesis. Thus, we used a validated behavioral animal model to evaluate the potential cognitive impairments induced by Everolimus and to compare its effect with the 5-fluorouracil (5-FU) chemotherapy.
Methods: Everolimus (5 mg/kg) was daily administered for two weeks and 5-FU (37 mg/kg) was injected once a week during 3 weeks in adult C57BL/6J Rj mice. Learning and memory processes were then evaluated by means of the object recognition and the Morris water maze tests. *Ex situ,* hippocampal neurogenesis and vascularization processes were investigated by immunohistochemistry in each group of mice. *In vitro*, neural stem cells (NSC) and/or endothelial cells (EC) in culture were treated with Everolimus.
Results: Everolimus slowed body weight gain from the last day of the treatment period until the end of behavioral sessions. Although 5-FU-treated mice were impaired in the cognitive flexibility-dependant task in the Morris water-maze test, and exhibited a more pronounced preference for the novel object in the object recognition test, behavioral flexibility and object recognition memory were not impaired by Everolimus. These data correlated with absence of altered neurogenesis in Everolimus-treated mice. *In vitro*, increasing concentrations of Everolimus induced a significant EC death without affecting NSC survival.
Conclusion: At short term after the end of the treatment, Everolimus did not modify mice cognitive functions evaluated by means of the hippocampal-dependent behavioral tasks. These observations differ from our studies demonstrating that chemotherapy (5-FU) led to selective long-term cognitive deficits, *i.e.* behavioral flexibility and recognition memory.

1168 POSTER

Discovery of Active New Drugs in Malignant Mesothelioma

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Background: Malignant mesothelioma (MM) is an aggressive tumour of serosal surfaces including pleura. In this study we aimed to test a patient's tumour for its individual susceptibility to emerging anticancer drugs and to discover new active drugs for treatment of MM by screening a library of compounds already approved for clinical use (Johns Hopkins Clinical Compound Library − JHCCL).
Material and Methods: A panel of 7 mesothelioma cell lines [3 ATCC cell lines (H28, H226 and MSTO211H), 2 UWA cell lines (LO68, JU77) and 2 TPCH cell lines (MM05, PF05)] was tested for chemosensitivity to 6

cytotoxic drugs (carboplatin, cisplatin, gemcitabine, fluorouracil, etoposide and doxorubicin). Three cell lines (H28, MSTO211H and LO68) were screened against 1524 JHCCL compounds. SYBR(R) Green I- fluorometric assay was used to measure compound activity.
Results: All lines were sensitive to doxorubicin and gemcitabine except MM05 and H226, which were resistant to gemcitabine. MSTO211H was chemosensitive to carboplatin and etoposide and H226 was resistant to flurouracil.
50 drugs (9 antineoplastic, 10 antheminithic, 14 antiseptic, 5 antibiotics, 3 antidote, 3 antihistaminic, 2 antihyperlipidemic, 2 antimalarial, 3 carditonic, 2 dermatologic, 2 progestogen, and other include aesthetic, antifungal, antiparkinsonian, antiprotozoal, antipsychotic, diagnostic aid, hemostatic) have been short listed after first screening with 10uM of each drug of JHCCL. Compound activity was analysed by comparison to an arbitrary point within the dynamic range defined by assay controls (e.g. representing 50% cell death). A five-log range of final concentrations from 100 uM to 1 nM was tested and IC50 was determined. The results ranged within 0.7 uM−10 uM.
Conclusions: Active compounds were identified from a panel of agents with history of clinical use. The anti-mesothelioma action of several candidates active *in vitro* at levels below PPC now requires validation in vivo or in clinical settings.
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Oral Presentations (Mon, 26 Sep, 09:00−10:55)
Drug Development

1200 ORAL

RP5237- a Novel, Selective, and Potent Inhibitor of PI3Kdelta With Therapeutic Potential in B-cell Lymphomas

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Background: Pan-PI3K inhibitors currently in development have been associated with adverse side-effects such as insulin resistance, thus necessitating the need to develop isoform specific inhibitors of PI3K. Because expression of PI3Kδ isoform is limited to blood cells, it serves as an ideal target against cancers associated with dysfunctional expansion of hematopoietic cells. Herein, we describe the biological and pharmacokinetic properties of RP5237, a novel and small molecule PI3Kδ inhibitor with scope to be further developed as a clinical candidate for B-cell lymphomas.
Material & Methods: Activity of RP5237 on individual PI3K isoforms was determined by a Homogenous Time Resolved Fluorescence assay (Millipore, Billerica, MA) with modifications. Potency of the compound on the delta isoform was further corroborated in FcεR1 induced CD63 expression studies using human whole blood and anti-IgM induced human B-cell proliferation assays. Anti-tumour efficacy of the compound was confirmed *via* cell viability and apoptosis assays besides testing for inhibition of pAkt, a downstream kinase regulating cell survival and growth. Metabolic stability of the compounds was evaluated in liver microsomes. Pharmacokinetic parameters were estimated in plasma from mice and rat.
Results: RP5237 demonstrated significant potency against PI3Kδ (**13.8 nM**) with several fold selectivity over the α (**>1000**), β (**>50**), and γ (**>9**) isoforms. Additionally, the compound inhibited B-cell proliferation (**32.2 nM**) and FcεR1 induced CD63 expression in human whole blood basophils (**48.9 nM**) indicating specificity towards the delta isoform. Viability assays demonstrated that the compound caused a dose-dependent inhibition in growth of B-cell mediated cancerous cell lines such asTHP-1, TOLEDO, HL-60, and Raji. Reduction in viability was accompanied by a reduction in pAKT along with a significant increase in apoptosis manifested by an induction of caspase-3 activity in the cell lines tested. Pharmacokinetic studies in mice and rat indicated good oral absorption with favourable peak plasma concentrations.
Conclusions: Results demonstrate the therapeutic potential of RP5237 in B-cell mediated cancers *via* the PI3Kδ pathway. *Ex vivo* studies using blood obtained from naive lymphoma patients are currently underway to determine the efficacy of the compound in different tumour sub-sects. Additionally, the compound shall be tested in mouse xenograft models of haematological malignancies.

1201 ORAL

Early Studies of the Safety, Pharmacokinetics (PK), Pharmacodynamics (PD), and Anti-tumour Activity of the Humanized Monoclonal Antibody (huMAb) Anti-EGFL7 (MEGF0444A) Alone and in Combination With Bevacizumab (Bev) With and Without Paclitaxel (Pac) in Patients (pts) With Advanced Cancer

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Background: Epidermal growth factor-like domain 7 (EGFL7) is a vascular-restricted, tumour-enriched, extracellular matrix protein that forms perivascular tracks and promotes endothelial cell adhesion and survival. Anti-EGFL7 (MEGF0444A) is a huMAb that inhibits the activity of EGFL7 and reduces vascular density and perfusion in murine tumour models. Anti-EGFL7 as a single agent (SA) has limited anti-tumour activity, but it significantly enhances the anti-tumour activity of anti-VEGF in multiple murine tumour models.
Materials and Methods: A standard 3+3 dose escalation was used to study safety, PK, PD, and anti-tumour activity of MEGF0444A in 2 serial Phase I trials. In a Phase Ia study, 30 pts were treated with SA MEGF0444A in 21-day cycles at doses ranging from 0.3 to 15 mg/kg. In a subsequent 2-arm Phase Ib study, 40 pts were enrolled. In Arm A, MEGF0444A was given at doses of 2, 5, or 10 mg/kg along with Bev at 10 mg/kg on Days 1 and 15 of each 28-day cycle; in Arm B, pts additionally received Pac (90 mg/m^2) on Days 1, 8, and 15 of each cycle. PD biomarkers including circulating progenitor cells (CPCs) and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) were assessed.
Results: In the Phase Ia trial, the highest planned dose of 15 mg/kg was reached without dose-limiting toxicities. MEGF0444A was well tolerated with no attributed Grade ⩾3 serious adverse events (AEs). There were no responses. In the Phase Ib study, the combination of MEGF0444A and Bev with or without Pac did not appear to exacerbate Bev-related AEs. Five partial and 2 minor responses were observed in multiple tumour types in the Phase Ib trial. In both studies, MEGF0444A had linear PK typical of an IgG1 huMAb. Enumeration of CPCs showed a decrease in a subset of pts within 15 days of MEGF0444A therapy. DCE-MRI results were suggestive of anti-angiogenic activity in select pts. Five mg/kg q2weeks (w) was chosen as the recommended Phase II dose.
Conclusions: MEGF0444A has favorable PK and is well tolerated as a SA and in combination with Bev and Bev/Pac. Changes in CPC levels and DCE-MRI parameters are consistent with MEGF0444A anti-angiogenic and anti-vascular activity. Study data support a Phase II dose of 5 mg/kg q2w (equivalent to a flat dose of 400 mg q2w or 600 mg q3w). Phase II trials of MEGF0444A with chemotherapy/Bev are planned.

1202 ORAL

A Phase I Study of the Potent AKT Inhibitor MK-2206 in Combination With Carboplatin and Paclitaxel, Docetaxel or Erlotinib in Patients With Advanced Solid Tumours

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Background: MK-2206 is a novel allosteric inhibitor of all 3 isoforms of AKT, which are targets implicated in malignant progression and resistance to anti-cancer therapies. *In vitro*, MK2206 demonstrated synergistic or additive anti-cancer effects when combined with C+P, D and E.
Material and Methods: Pts with advanced solid tumours, ECOG PS ⩽1 were recruited to a 3-arm phase I study of MK2206 QOD (days 1, 3, 5, 7) or Q3W with carboplatin (C) (AUC6) and paclitaxel (P) (200 mg/m^2) (Arm 1), or docetaxel (D) (60 & 75 mg/m^2) (Arm 2) or QOD (alternate days continuous) and QW with erlotinib (E) (100 and 150 mg) OD (Arm 3). The primary objectives were to determine the maximum tolerated dose (MTD) and dose limiting toxicities (DLT) of MK2206 in combination with C+P, D or E. Secondary objectives were to determine preliminary activity