Material and Methods: Tumour tissue slices from colorectal cancer and breast cancer patients were prepared and cultured according to Indivumed's standard operating protocols. For antibody diffusion assays tissue slices were incubated with different commercially available antibodies followed by immunofluorescence staining and microscopy. Functional effects of Trastuzumab treatment were examined in breast cancer cell lines (BT474 and MCF-7 cells) and breast cancer tissue slices using the expression level of pAkt as readout, shown by western blotting. Her-2 expression levels of cells and tissue slices were analyzed by immunohistochemistry.
Results: The antibody diffusion assays showed a time-dependent penetration of antibodies into and through 400 μm thick cultured tissue slices within 24 hours. Treatment of breast cancer cell lines and breast cancer tissue slices with different concentrations of Trastuzumab revealed a dose-dependent reduction of pAkt only in Her-2 positive cells and tissues. Thus, drug effects of therapeutic antibody Trastuzumab could be demonstrated in secondary cell lines and verified in organoid cultures.
Conclusions: Overall the data revealed that antibodies diffuse into 400 μm thick cultured tissue slices reaching their target within 24 hours. Furthermore, we demonstrated that functional drug effects of therapeutic antibodies could be validated in organoid cultures. Therefore, the preclinical model based on cultured cancer tissue slices developed by Indivumed is suitable to examine not only the effects of classical chemotherapeutics, as we have shown recently, but also of larger molecules, such as antibodies. Thus, this model representing the natural tumour environment is a promising and important tool to prioritise drugs, support dose finding and to individualise therapy.

1162 POSTER

Frequency of Mitochondrial Point Mutations and Deletions in Late Stage Colorectal Cancer Patients

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Objective & Background: Defects in mitochondrial function have been proposed to contribute to progression of cancer. This dysfunction may result from impairment in cellular respiration, forcing the cell to revert to glycolysis for energy. This switch causes cells to become undifferentiated and cancerous. This study examined the frequency of random mitochondrial mutations and deletions in late stage colorectal cancer tumours and their corresponding normal tissue. This analysis was performed using a novel random mutation capture assay which has the sensitivity to detect one mutation in one hundred million base pairs.
Methods: Mitochondrial DNA was extracted from 20 patients with late stage colon cancer and adjacent normal tissue. This DNA was and digested with TAQ 1α for 10 hours replacing the enzyme every hour. QPCR was performed across the TAQ1α restriction sites. The primer sequences used for identifying random mitochondrial mutations are 5′-ACAGTTTATGTAGCTTACCTCC-3′ and 5′-TTGCTGCGTGCTTGATGCTTGT-3′. The primer sequences used to determine mitochondrial DNA copy number are 5′-ACAGTTTATGTAGCTTACC TCC-3′ and 5′-TTGCTGCGTGCTTGATGCTTGT-3′. PCR was performed to assess single nucleotide polymorphisms. We will assess mutation frequency of TAQ1 restriction site (TCGA) located in the gene encoding the 12S rRNA subunit (bp 634–637). By flanking multiple TAQ1 restriction sites with a primer pair, the detection prevalence of the RMC assay can be skewed towards DNA deletions. As a result, the expected frequency of a PCR product due to mtDNA point mutations drops exponentially to 1×10^{-15}. In contrast, mtDNA deletions occur at a higher rate, and hence, every mutation detected with these primer pairs will be a deletion.
Results: A statistically significant increase in the frequency of deletions was detected in normal versus tumour tissue ($p = 0.021$), however levels of point mutations did not differ between tumour and normal.
Conclusions: The lower rate of deletions seen in tumour tissue versus adjacent normal may be explained by the Warburg theory where anaerobic metabolism is predominant in cancer. In contrast to normal cells, which generate energy by the oxidative phosporylation, tumours and cancer cells generate energy through glycolysis which could result in lower levels of mitochondrial deletions in tumours.

1163 POSTER

A Therapeutic Sphingosine 1-phosphate Antibody Inhibits Intratumoral Hypoxia and Sensitizes to Standard Chemotherapy in a Preclinical Model of Prostate Cancer

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Background: Hypoxia promotes neovascularization, metastasis, growth and resistance to treatments. The activation of HIF-1α has been identified as the master mechanism of adaptation to hypoxia. We recently identified the sphingosine kinase 1/sphingosine 1- phosphate (SphK1/S1P) pathway as a new modulator of HIF-1α activity under hypoxia in multiple cancer cell models (Ader et al, Cancer Res, 2008). S1P elicits proliferation, survival, or angiogenesis, and is believed to exert most of its actions as a ligand for a family of specific GPCRs to elicit paracrine or autocrine signaling. We have suggested that inhibiting SphK1/S1P signaling, which is up-regulated under hypoxia, may help normalizing the tumour microenvironment and increase sensitivity to chemotherapy, in the broader concept of "normalization of tumour vessels" as tumour oxygenation is known to enhance response to chemotherapy (Ader et al., Cancer Res, 2009).
Methods: Quantitation of hypoxia and angiogenesis, and treatment efficacy using an orthotopic (o.t) xenograft model of fluorescent HRPC cells.
Results: We provide *in vitro* evidence that inhibiting the S1P exogenous signaling, through pharmacological inhibition of its receptors or by taking advantage of a monoclonal antibody neutralizing S1P, blocks HIF-1α accumulation and its activity in prostate cancer cells under hypoxia. Second, using an o.t model of prostate cancer, we show that an anti-S1P antibody inhibits intratumoral hypoxia, modifies vessel architecture and improves tumour perfusion within 5 days of treatment. Third, we demonstrate that an anti-S1P strategy sensitizes to docetaxel, the 'gold standard' treatment for HRPC. A 5-day anti-S1P antibody pretreatment markedly sensitizes to docetaxel in an o.t. PC-3/GFP model established innude mice. The combination anti-S1P antibody together with docetaxel was not only accompanied by a smaller primary tumour volume compared to docetaxel alone, but also significantly reduced the occurrence and number of metastases.
Conclusions: These data establish the proof-of-concept that blocking the exogenous action of S1P reduces intratumoral hypoxia and sensitizes to chemotherapy in prostate cancer animal model.

1164 POSTER

The Impact of Ionizing Radiation on the Motility and Matrix Remodelling Properties of Carcinoma-associated Fibroblasts

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Purpose: Carcinoma-associated fibroblasts (CAFs) are key components of solid malignancies and play central roles in cancer sustainability. In this work we have investigated the invasive capacity and matrix remodelling properties of CAFs after ionizing radiation (IR).
Methods: CAFs were isolated from fresh lung tumour specimens from 14 different donors. Initially, radiation protocols were established by monitoring cellular viability at different doses of radiation. For further analyses, the migrative, invasive and adhesive capacities of CAFs were determined after a single dose of 18 Gy. Additionally, protein levels of secreted major matrix modulators represented by matrix metalloproteinases (MMPs)-1, -2, -3, -7, -8, -9, -13 and their endogenous inhibitors (TIMPs)-1, -2, -3, -4 were measured 4 to 6 days post-irradiation, as well as cell surface expression of various integrins.
Results: IR resulted in premature cellular senescence and caused moderate but significant inhibition of the proliferative, migrative and invasive capacity in CAFs. IR also promoted MMP-3 and inhibited MMP-1 appearance, whereas expression and activity of the gelatinases MMP-2 and MMP-9 were unchanged. Furthermore, the levels of TIMPs were not affected. Surface expression of integrins α2, β1, α5 was consistently enhanced.
Conclusions: Our data indicate that therapeutic doses of IR exert advantageous inhibitory effects on the proliferative, migratory and invasive capacity of lung CAFs, along with a prominent reduction of MMP-1 expression. However, the observed enhancement of MMP-3 could represent a negative outcome from radiation. Also, the altered surface expression of integrins in

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