

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

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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/cm²) 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 reveal 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 proteasomal 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.

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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 ultracentrifugation, 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 PDC61. 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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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.

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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