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Internalization of iron nanoparticles by macrophages for the improvement of glioma treatment

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Purpose or Objective: An alternative approach for the improvement of radiotherapy consists in increasing differentially the radiation dose between tumors and healthy tissues using nanoparticles (NPs) that have been beforehand internalized into the tumor. These high-Z NPs can be photo-activated by monochromatic synchrotron X-rays, leading to a local dose enhancement delivered to the neighboring tumor cells. This enhancement is due to secondary and Auger electrons expelled from the NPs by the radiations. In order to carry the NPs into the tumor center, macrophages are currently under study for their phagocytosis and diapedesis abilities. In this study we characterized J774A.1 macrophages' internalization kinetics and subcellular distribution of iron NPs and compared them to the internalization abilities of the F98 glioblastoma cell line.

Material and Methods: Three aspects of internalization were examined: first, the *location of internalized NPs* in J774A.1 macrophages and F98 glioblastoma cells following a 24h incubation with iron NPs (0.3 mg/mL in the cell culture medium) was determined by optical microscopy after cell slicing. Subsequently, the *iron intake* after a 24h incubation with NPs (0.3 mg/mL and 0.06 mg/mL in the cell culture medium) was characterized for the two types of cells using ICP-MS. Finally, the *internalization dynamics* were studied by live phase-contrast microscopy imagining for 11 hours and by absorbance measurements for 24 hours using a plate reader.

Results: F98 tumor cells and J774A.1 macrophages are both able to endocytose NPs: we measured ~61±10 pg of internalized iron per macrophage compared with ~33±5 pg per F98 cell (initial iron concentration: 0.3 mg/mL in culture medium). F98 internalizing NPs for 10 hours showed stress signs during the first minutes after the NPs injection, but behaved like F98 control cells during the rest of the experiment. Finally, we determined that the internalization kinetics for J774A.1 had a typical saturation time of one hour.

Conclusion: Macrophages seem to be promising vectors for NPs, being able to endocytose and retain in their cytoplasm larger quantities of NPs than tumor cells. Our following studies will attempt to shed light on their other potential abilities as "Trojan Horses".

EP-2036

A flow cytometry-based screen for compounds that increase S-phase damage after Wee1 inhibition

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Purpose or Objective: Inhibitors of Wee1 are in clinical trials for cancer treatment in combination with radiation or chemo-therapy. The antitumor effects have largely been attributed to their role in G2 checkpoint abrogation. However, our previous work has shown that Wee1-inhibition also causes DNA damage in S phase. To understand mechanisms behind the S-phase damage and to identify promising combination treatments, we initiated a flow cytometry-based screen for compounds that increase S-phase damage when combined with the Wee1-inhibitor MK1775.

Material and Methods: The screen was performed in 384-well plates by using a pipetting robot and a flow cytometer equipped with a plate loader. REH leukemia suspension cells were treated with the LOPAC 1280 and Selleck Cambridge cancer 384 compound libraries in the presence and absence of the Wee1 inhibitor MK1775 (4h, 400nM), stained with the DNA-stain Hoechst and the DNA damage marker yH2AX, and analyzed by flow cytometry using the FlowJo software. In addition to drugs present in the compound libraries, three additional Chk1-inhibitors (LY60638, MK8776 and UCN01) were included in subsequent validation experiments.

Results: The Chk1 inhibitor AZD7762 was among the top hits of 1664 tested compounds, giving synergistically increased S-phase damage when combined with MK1775. Similar effects were found with with three other Chk1-inhibitors. In addition, the screen identified several expected negative and positive regulators of the S phase damage, such as inhibitors of Cyclin-Dependent-Kinase (CDK) and Topoisomerase, and some unexpected hits such as Dasatinib.

Conclusion: This study helps understanding how Wee1-inhibition causes S-phase damage, and will likely identify combinations of MK1775 and drugs relevant for future clinical studies. These drug combinations may also be useful to apply together with radiation therapy to eliminate radioresistant S-phase cells.

Electronic Poster: Radiobiology track: Tumour biology and microenvironment

EP-2037

Radiation-induced abscopal effect in normoxic and hypoxic conditions in lung adenocarcinoma

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Purpose or Objective: Many experimental evidences proved the existence of radiation-induced abscopal effect (RIAE), a phenomenon of non-targeted radiobiological effect which is rarely, unintentionally induced in vivo, mostly with high doses per fraction. We explored different biological, biochemical and physical factors on which the type and intensity of RIAE could depend and whose manipulation could lead to induction of strong, clinically applicable RIAE. Also, the radio-sensitizing potential of abscopal signals (AS) and the status of RIAE in hypoxia (H) were examined. After observation of AS transmission by tumor cells exposed to H, which were able to affect proliferation of normoxic (N) and H cells, irradiated as well as unirradiated, we introduce a new scientific term:"Hypoxia-induced abscopal effect" (HIAE).

Material and Methods: A549 and H460 lung cancer cells were incubated in H (Oxygen<2%) or N for 3 days and then irradiated (2 or 10Gy) or not. After 24h, unirradiated H (HCM) or N (NCM) conditioned media (CM) and irradiated H (HRCM) or N (NRCM) CM were collected. H-resistant (HR) clones A549/HR and H460/HR were generated by 3 weeks-exposure of cells to H. 2 identical sets of unirradiated N cells and HR clones were exposed to HCM, NCM, HRCM or NRCM and only 1 set was irradiated (2Gy) to evaluate the radio-sensitizing potential of AS. Cell growth was monitored using real time cell electronic sensing system. Cell survival was assessed by colony forming assay. Levels of basic fibroblast growth factor (GF)(bFGF), placental GF (PlGF), Soluble fms-like tyrosine kinase (sFlt-1) and vascular endothelial GF (VEGF) were assessed in CM.

Results: AS released by cells exposed only to H were active affecting proliferation and radio-sensibility of N cells and HR clones. Those effects depended on cell histotype, respiratory status of cell-inducers and cell-recipients of AS (N vs. H) and duration of cell-exposure to H (24 vs. 72h). Depending on time-exposure to H, HIAE promoted both increased and reduced proliferation. The type and intensity of RIAE depended on dose and notably changed if AS were transmitted by N or H irradiated cells (Tab.1, Fig.1).

Treatments	A549	A549/HR	H460	H460/HR
N-RCM (2Gy) vs. H-RCM (2Gy)	↓ (60%) sFLT-1, PIGF	↑ (20%)	↑ (25%) bFGF	↓ (20%)
N-RCM (10Gy) vs. H-RCM (10Gy)	↓ (40%) sFLT-1, PIGF	↑ (15%)	No diff sFLT-1, PIGF, VEGF	↓ (30%)
N-RCM (2Gy)+2Gy vs. H-RCM (2Gy)+2Gy	↓ (70%)	↓ (20%)	↑ (20%)	↓ (80%)
N-RCM (10Gy)+2Gy vs. H-RCM (10Gy)+2Gy	↓ (50%)	↓ (7%)	No diff	↓
N-CM+2Gy vs. H-CM+2Gy	↓ (65%)	↓ (20%)	↑ (25%)	↓

Table 1: Comparison of radiation-induced abscopal effects in normoxic and hypoxic conditions on proliferation of A549, A549/HR, H460, and H460/HR cells

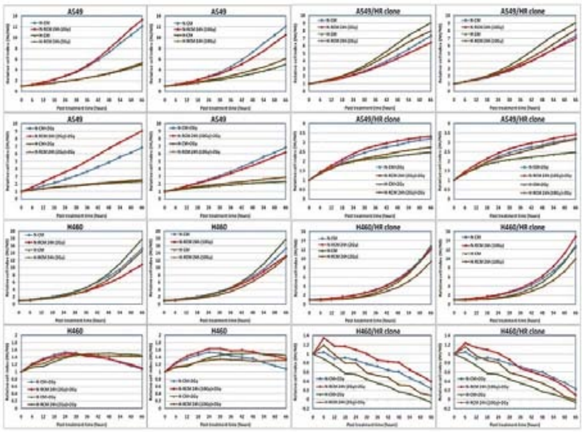

Figure 1: Comparison of radiation-induced abscopal effects in normoxic and hypoxic conditions on proliferation of A549, A549/HR, H460, and H460/HR cells

In H460 RIAE caused radio-resistance, a phenomenon similar to adaptive response but in this case acquired via AS by cells that have never been irradiated. Manipulating the respiratory ambient of cell-receivers of AS the effects of both RIAE and HIAE on proliferation and radio-sensibility changed significantly. The comparative analysis of GF levels with cell proliferation and survival showed a correlation between anti-proliferative sFLT-1 and almost all CM types for both cell lines.

Conclusion: Our results proved that exposing of cells to H and irradiation of H cells lead to significant HIAE and RIAE, respectively, which are able to affect cell proliferation and radio-sensibility. Both phenomena depend on several factors whose manipulation is possible and leads to induction of clinically applicable RIAE.

EP-2038

Manipulation of radiation-induced bystander effect in prostate adenocarcinoma

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Purpose or Objective: Radiation-induced bystander effect (RIBE) has been described only for certain cancer types as the appearance of radiation effects in not directly irradiated cells. This study evaluated the ability of prostate adenocarcinoma (ADC) to induce RIBE exploring the factors that may affect its intensity. The idea was to produce a strong, clinically applicable RIBE, that could lead to development of innovative approaches in modern radiotherapy treatment of prostate cancer, especially for those patients with hormone-refractory ADC in which radiotherapy might have a limited role.

Material and Methods: 2 prostate ADC cell lines of different differentiation, PC-3 - hormone-resistant and DU-145 - hormone-sensitive, have been irradiated using wide range of doses (15 cGy-3000 cGy in 1 fraction) to obtain radiation-conditioned medium (RCM) which was then used to "treat" the unirradiated cells and to evaluate the cytokines level. Each sample of RCM was subjected to triple immunoassay assessment of the following cytokines: Eotaxin, Interferon-gamma, Interleukin(IL)-2, IL-4, IL-6, IL-8, IL-10, IL-12, Macrophage Inflammatory Protein-1-alpha, Tumor Necrosis Factor-alpha and Vascular Endothelial Growth Factor. Using a spectrophotometer cell growth was assessed. All comparisons were made to the negative control using paired t-tests. Significance was set at p-value < 0.05, 2-tailed test.

Results: Prostate ADC was able to induce RIBE which intensity depended on dose and tumor differentiation grade: the strongest RIBE for PC-3 was achieved with 2000 cGy and for DU-145 with only 15 cGy (Fig.1).

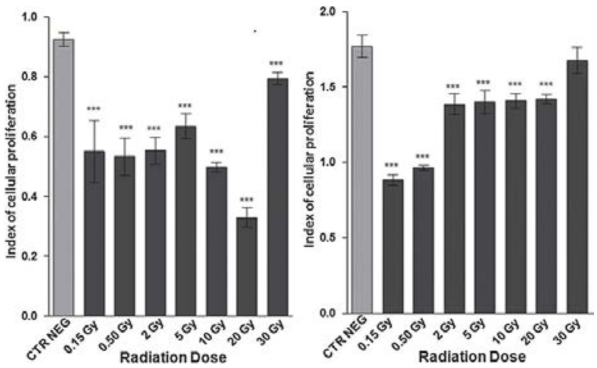

Figure 1. Bystander effect in prostate adenocarcinoma: the strongest proliferative blocking in PC-3 achieved with 20 Gy (left graph) and in DU-145 with 0.15 Gy (right graph)

For DU-145 there wasn't correlation between cytokines level and RIBE intensity while for PC-3 IL-6 correlates with strongest RIBE. The dose required to kill all cells exposed to irradiation was different for 2 cell lines: for DU-145 a lethal dose was reached with 2500 cGy, while PC-3 resisted to 3500 cGy after which tumor repopulation was observed starting 2 weeks after irradiation from just a few survived cells that have undergone particular "giant" differentiation.

Conclusion: RIBE intensity can be manipulated by modifying radiation dose and depends on differentiation grade. IL-6 correlates with strongest RIBE after exposure of PC-3 to a very high dose of radiation thus indicates its possible involvement in bystander signals transmission.

EP-2039

The impact of surgical wound fluids after IORT on the breast cancer stem cell phenotype

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Purpose or Objective: Breast cancer is the most common cancer in women. The conventional conservative treatment