

Fig 1. We hypothesize that reduced mitochondrial function caused by mtDNA variations, such as mtDNA depletion, enhances the radiation response as a consequence of reduced ATP production and increased cellular ROS exposure. Oxidative phosphorylation complexes that are encoded by the mtDNA are indicated by the orange stars. Through disruption of these complexes and thereby interfering with free radical and ATP production, radiation responses could potentially be altered. Lightning signs indicate processes that are also involved in radiotherapy.

Material and Methods: Three cell lines were depleted from their mtDNA by ethidium bromide. BEAS-2B immortalized bronchial epithelial, A549 lung adenocarcinoma and 143B osteosarcoma cell lines and their mtDNA depleted counterparts (p0) were metabolically characterized using the XF96 Seahorse. Changes in radiosensitivity were assessed by clonogenic survival (0, 2, 4, 6 and 8Gy). ROS production (by dihydrorhodamine FACS analysis), ATP (Cell-TiterGlo Luminescent cell viability test) and glutathione levels (in cell lysate) as well as γ H2AX immunostainings were assessed 24 hours post irradiation.

Results: mtDNA depletion resulted in a significant ($p < 0.05$) decreased proliferation ($64 \pm 7\%$) for all cell lines. Compared to their respective controls, increased clonogenic survival was observed for the BEAS-2B p0 cells ($p = 0.004$) after irradiation, while both tumor p0 lines were more radiation sensitive ($p = 0.013$), mainly at higher irradiation doses. ROS formation at baseline (0Gy) was similar ($p = 0.878$) for BEAS-2B parental and p0, while reduced for A549 and 143B p0 ($p = 0.021$) cells, compared to their parental counterparts. 24 hours after irradiation ROS levels were significantly ($p < 0.05$) increased for all parental cell lines, while levels for the p0 cells remained equal. Glutathione levels were lower for the A549 and 143B p0 cell lines compared to the parental lines under any experimental condition but no changes were found for the BEAS-2B cells. In agreement, increased residual DNA damage was observed upon mtDNA depletion for A549 and 143B cells. Depletion of mtDNA reduced cellular ATP levels only for the BEAS-2B cell line ($p = 0.046$), but not for the A549 and 143B cell lines in high glucose culture medium.

Conclusion: The observed differences in dependence on mitochondrial function for radioresponsiveness appear to be associated with the balance in ROS levels and the antioxidant status of the cells. Currently, the levels of MnSOD and GPX1 and the effect of ROS scavenging on radiotherapy response are investigated in our lab.

PO-0997

Interferon response genes in breast cancer resistance to endocrine treatment and radiotherapy

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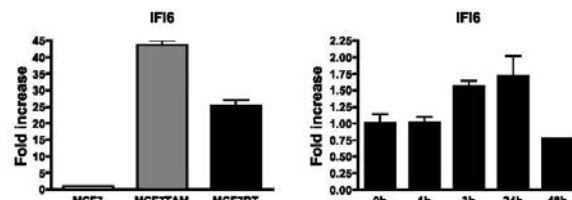
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Purpose or Objective: We have previously shown that lysosome-associated membrane protein-3 (LAMP3), a protein involved in the unfolded protein response pathway, is involved in resistance to both endocrine (tamoxifen) treatment and radiotherapy in breast cancer patients. We have created subclones of the MCF7 breast cancer cell line that are resistant to either treatment. In these subclones, we investigated common mechanisms between tamoxifen- and radioresistance, and the possible role of LAMP3 therein.

Material and Methods: The estrogen receptor positive breast cancer cell line MCF7 was grown to tamoxifen resistance (MCF7TAM) by culturing with gradually increasing concentrations of 4-OH-tamoxifen up to $10 \mu\text{M}$. Additionally, MCF7 cells were exposed to multiple fractions of 2 or 4 Gy irradiation, adding up to a total dose of at least 50 Gy (MCF7RT). Changes in expression profiles in MCF7TAM and MCF7RT cells compared to parental MCF7 cells were investigated by RNA sequencing. Pathway analysis software was used to find pathways involved in tamoxifen- and radioresistance. QPCR was used to confirm the RNA sequencing data, and to investigate the changes in genes of interest after tamoxifen treatment and irradiation. The role of LAMP3 in these treatment resistance pathways is being elucidated by performing LAMP3 gene silencing by siRNA and CRISPR-Cas mediated gene knockout.

Results: The MCF7TAM cells were completely resistant to treatment with $10 \mu\text{M}$ 4-OH-tamoxifen. Remarkably, these cells had also become resistant to irradiation, with a surviving fraction at 4 Gy (SF4) of 19.7%, compared to 8.3% for the parental MCF7 cells. MCF7RT cells were less sensitive to irradiation with a SF4 of 9.6% compared to 3.9% for the parental cells. RNA sequencing of MCF7TAM and MCF7RT cells revealed an increase of genes involved in the antiviral response, including classic interferon response genes such as IFI6 (shown in figure, left), IFI27, STAT1, OAS1 and DDX60. These genes were increased in parental cells following 4 Gy irradiation (figure, right) or tamoxifen treatment as well.



Conclusion: MCF7 cells resistant to tamoxifen treatment are also less sensitive to irradiation, suggesting a common mechanism in the resistance to these diverse types of treatment. Using an unbiased approach, we here show that interferon response genes are increased in both MCF7TAM and MCF7RT cells. Interestingly, others have shown LAMP3 to be a regulator for this pathway. We are currently investigating the role of LAMP3 in our treatment resistant breast cancer clones.

PO-0998

The Robo1-receptor is involved in the migration of irradiated glioblastoma cells

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Purpose or Objective: The brain tumor glioblastoma multiforme (GBM) is highly malignant with a very short OS due to rapid recurrences adjacent to the primary tumor. Even radio-chemotherapy extends the survival only for a few months. In this project we tested whether or not the Slit2/Robo1 axon guidance system might be involved in the migration of metastatic GBM cells and whether irradiation with photons might modify this putative effect.

Material and Methods: The experiments were performed with 2 human GBM cell lines (U87 and U373) and in parallel after irradiation with 0.5, 2, or 8 Gy photons. The motility/migration of the cells was analyzed by time-laps videography. Travelling cells were tracked and the parameters accumulated distance and Euclidean distance were determined. The expression of Slit2, Robo1, and FAK (focal adhesion kinase) was tested by Western blot and qRT-PCR. In addition, the cells were transfected either with a Robo1 expression-vector or with a siRNA construct and analyzed similarly.

Results: Irradiation with low doses enhanced the motility of the cells. Slit2 as well as Robo1 was extremely low expressed in the cell line with higher motility (U87). Irradiation reduced the expression even more. On the other hand, a stable overexpression of Robo1 decreased significantly the migration of the cells and suppressed the increase in motility observed after irradiation. In contrast, the siRNA mediated knockdown of Robo1 increased the migratory potential of the cells. The analysis of FAK, a key player in cellular migration, revealed a decreased expression in Robo1-overexpressing cells.

Conclusion: Our data indicate a role for Robo1 in the migration of malignant GBM cells. The expression of Robo1 reduced the migration of these cells and was also able to impede the increase in motility observed after irradiation with photons.

Poster: Radiobiology track: Radiobiology of protons and heavy ions

PO-0999

Reduced side effects by proton minibeam radiotherapy in a mouse ear model

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Purpose or Objective: Proton minibeam radiotherapy aims to minimize normal tissue damage in the entrance channel while keeping tumor control through a homogeneous tumor dose due to channel widening with increasing track length. Side effects of proton minibeam irradiation were examined in an in-vivo mouse model to account for immune system, vasculature and higher complexity. Here, we report on our comparative study of minibeam and broad beam irradiation in the ear of Balb/c mice, to prove this hypothesis of reduced adverse effects in normal tissue.

Material and Methods: At the ion microprobe SNAKE, 20 MeV protons were administered to the right ear of 2-3 months old, female Balb/c mice, using an average dose of 60 Gy in a field of 7.2 x 7.2 mm² in the central part of the ear, in two irradiation modes, homogeneous and minibeam. The 4 x 4 minibeam of 180 x 180 µm² size were set in a distance of 1.8 mm, resulting in a dose of 6000 Gy in the channels, but with negligible dose in between. Inflammatory response, i.e. ear swelling and skin reactions were monitored for 90 days following irradiation, as well as genetic damage and release of inflammatory proteins.

Results: No ear swelling or other skin reaction was detected after the minibeam irradiations, while significant ear swelling (up to 4-fold), erythema and desquamation (crust formation) developed in homogeneously irradiated ears 3-4 weeks after irradiation. Loss of hair follicles was only detected in the homogeneously irradiated fields after 4-5 weeks.

Conclusion: Our results prove that proton minibeam radiotherapy leads to reduced side effects compared to conventional broad beam irradiation and could become an option in clinical proton and/or heavy ion therapy. Supported by the DFG Cluster of Excellence: Munich-Centre for Advanced Photonics.

PO-1000

Effect of X-rays and carbon ions on cell survival and expression of Hh pathway genes in cancer cells

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Purpose or Objective: Metastasis is an important cause of mortality in cancer patients and evidence shows that irradiation could actually increase the formation of metastasizing cells. An important pathway implicated in the process of metastasis is the Hedgehog (Hh) signaling pathway. Recent studies demonstrated that activation of this pathway can lead to radioresistance. So far, the impact of high-LET radiation on the Hh pathway is still unknown. In the present study the impact of different radiation qualities (e.g. X-rays and carbon ions) on Hh gene expression was investigated in prostate cancer cells (PC3) and medulloblastoma cells (DAOY).

Material and Methods: *In vitro* models used for prostate cancer and medulloblastoma were PC3 and DAOY, respectively. Colony survival assays were performed to analyze the effect of radiation on cell survival. The impact of radiation on the expression of the different Hh signaling pathway components (SHH, PTCH, SMO, GLI1, GLI2, GLI3 and SUFU) was investigated by means of RT-qPCR. Experiments with X-rays were performed at SCK-CEN (Mol, Belgium) whereas carbon ion irradiation (LET = 33.7 KeV/µm) experiments were performed at the Grand Accélérateur National d'Ions Lourds (GANIL) (Caen, France).

Results: Colony survival assays showed that DAOY cells were more radioresistant than PC3 cells (respectively D10=5.3 Gy and D10=4.2 Gy). Evaluation of the Hh signaling pathway showed that basal gene expression is present in both PC3 and DAOY, although very low. However, basal gene expression of the Hh components differed between both cell lines. Moreover, the more radioresistant cell line DAOY had higher expression levels of Gli1 compared to the PC3 cells. Preliminary RT-qPCR results show that different radiation qualities induce different changes in the expression of the Hh signaling components.

Conclusion: In conclusion, radiation exposure can induce changes in the Hh pathway. Future experiments will address whether modulation of the Hh pathway also affects the radio-responsiveness of cancer cells.

Poster: RTT track: Strategies for treatment planning

PO-1001

Dosimetric impact of flattening filter and flattening filter-free beams on IMRT planning of NSCLC

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Purpose or Objective: This retrospective study aimed to compare and determine the potential dosimetric benefits of intensity-modulated radiotherapy (IMRT) treatment plans with (FF) and without flattening filter (FFF) as well as to explore the dosimetric differences in 6MV FFF and 10MV FFF plans for non-small-cell lung carcinoma (NSCLC).

Material and Methods: Ten cases of CT data were selected from NSCLC patients. 4 sets of 5-field-IMRT plans were computed with FFF beams (X6FFF, X10FFF) and flattened beams (X6FF, X10FF) with the prescription of total 60Gy in 30 fractions. Planning constraints were based on the Radiation