Results and discussions Our data show that the in vitro irradiation of PBMCs in prostate cancer patients initially caused a significantly higher DNA damage than in the control group of healthy donors. After a repair time of 24 hour, samples from all healthy donors showed no residual DNA damage (average 2.6%) in PBMCs. If all patients are observed for 0 hour and 24 hour after in vitro irradiation then there is a significant reduction in the degree of DNA damage, from 35.5 to 30.4 tail% DNA. What is also important is that 4 patients had a very low level of DNA damage, which can be considered as normal cellular reaction to irradiation, while 3 patients had a very high level of damage. Also, in 5 out of 7 examined prostate cancer patients, there was an increase in the percentage of PBMCs in the early and late stages of apoptosis 24 hour after in vitro irradiation of their PBMCs. Most patients had mild side reactions to radiotherapy and were graded as grade 1 (per EORTC scale). Only in 1 of 7 patients observed side effects are classified as grade 2 and this is in correlation with a very high level of DNA damage (82.25% initial, versus 64.77% 24 hour after in vitro irradiation).

Conclusion Our study was insufficient to reveal the relationship between the risk of developing side effects to radiotherapy and the sensitivity of PBMCs irradiated in vitro, measured by comet assay. A larger number of patients and further studies are necessary to confirm the potential application of the comet assay.

Introduction Pancreatic ductal adenocarcinoma (PDAC) is one of the five most lethal malignancies in the world and has a 5 year relative overall survival rate of less than 5%. Thus, there is a great need for functional targeting strategies. As cell-matrix adhesion is essential for the survival, invasion and therapy resistance, we sought to identify the function of 117 focal adhesion proteins (FAP) in PDAC cell radiochemoresistance. Intriguingly, β8 integrin turned out to be one of the most potential novel targets in PDAC.

Material and methods We performed a 3D endoribonuclease-prepared siRNA (esiRNA)-based high throughput screening (3DTOtesis) in PDAC cell cultures (established and patient-derived (PDC)) grown in laminin-rich extracellular matrix (lECM). In addition to characterising β8 integrin expression, distribution and co-localization with other cellular organelles such as golgi apparatus, clonogenic survival assays were performed upon esiRNA-mediated knockdown, X-ray irradiation (6 Gy single dose) and gemcitabine. Fiji software was used to determine Pearson’s correlation coefficient, vesicle distribution and expression patterns upon irradiation or gemcitabine. An inhibitor screen was conducted to identify pathway involved in changes of β8 integrin localization upon treatment.

Results and discussions We identified a series of novel targets including β8 integrin. Without cytotoxicity, β8 integrin depletion elicited radiochemosensitization in PDAC, PDCs cell lines and reduced sphere formation and 3D invasion into collagen-I. Intriguingly, we found β8 integrin located in perinuclear area where it colocalized with the cis-Golgi matrix protein GM130. Upon irradiation and gemcitabine, β8 integrin dissociated from the perinuclear region and spread throughout the cytosol without enhanced localization to exosomes; a process abrogated by antymycin A or oligomycin pre-treatment.

Conclusion Our findings, generated in 3D lECM PDAC cell cultures, suggest β8 integrin as a novel determinant of PDAC radiochemoresistance. Moreover, β8 integrin may, although not found in the cell membrane to facilitate cell adhesion, a critical role in intracellular vesicle trafficking under stress conditions. Ongoing work will unravel the underlying mechanisms how β8 integrin is controlling cytoplasmic and nuclear survival pathways.

PO-130 β8 INTEGRIN CRITICALLY CONTRIBUTES TO PANCREATIC CANCER CELL RADIOCHEMORESISTANCE AND INTRACELLULAR VESICLE TRAFFICKING UNDER STRESS CONDITIONS

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Introduction Hypoxia-mediated resistance of solid tumours to ionising radiation is a major obstacle to successful radiotherapy. We showed previously that chronic cycling hypoxia drives the evolution of anoxia/reoxygenation-tolerant (ART) cancer cells with increased resistance to ionising radiation. Radiation resistance of ART cancer cells was associated with complex metabolic reprogramming, Matschke et al., Antioxid Redox Signal 2016(5):89–107; Matschke et al., Radiat Oncol 201611(1):75. Aim of the present study was to gain a more comprehensive understanding of the metabolic adaptation of cancer cells and to systematically explore opportunities for targeted pharmacologic intervention based on their suspected specific metabolic needs upon irradiation.

Material and methods We compared gene expression profiles of ART and control cancer cells by microarray analysis and validated genes of interest by qRT-PCR. We used LC-MS high-throughput metabolomics, metabolic flux analyses, nutrient deprivation and drugs interfering with metabolism to characterise the cellular metabolic state without/with irradiation.

Results and discussions Our microarray data indicated changes in major metabolic pathways after chronic cycling hypoxia selection. Furthermore, tolerance to severe hypoxia was associated with the formation of enlarged mitochondria in ART NCI-H460 cells. The analysis of metabolic alterations in irradiated cancer cells by LC-MS high-throughput metabolome analysis demonstrated a high and time-dependent need of irradiated cancer cells in major metabolic pathways. Targeting of induced metabolic alterations disturbed redox homeostasis, altered
mitochondrial metabolism and sensitised cancer cells to ionising radiation.

**Conclusion** Specific metabolic requirements under stress conditions such as severe hypoxia or irradiation render cancer cells vulnerable to metabolic inhibitors alone and in combination with ionising radiation in a context and cell type-dependent manner.

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**PO-132** DISSECTING THE RADIOBIOLOGY OF TARGETED RADIONUCLIDE THERAPY REVEALS AN INTRA-TUMORAL HETEROGENEIC RESPONSE IN A PRECLINICAL IN VIVO MODEL

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**Introduction** Neuroendocrine tumours (NETs) are a relatively rare, but deadly group of cancers. Since the cells of origin are neuroendocrine, diverse tumours can arise throughout the body. Often, patients are presented with metastases, making resection as a treatment strategy alone insufficient. In the clinic, localization of NETs has been assessed by targeting the somatostatin receptor 2 (SSTR2) which is overexpressed on tumour cells, with radiolabeled somatostatin analogues. Radiolabeling of these somatostatin analogues (e.g. DOTA-(TYr3)-octreotate (DOTA-TATE)) with the DNA damaging nuclide (Lutetium-177) is currently utilised to treat the disease: peptide receptor radionuclide therapy (PRRT). PRRT with the use of 177Lu-DOTA-TATE proves effective in improving progression-free survival and quality of life. However, mortality rates are still high. To increase the efficacy of PRRT we set out to dissect the cellular response of tumour cells to radionuclide therapy, the effect on dose-limiting organs (bone marrow and kidney) and mechanisms of therapy induced DNA damage in vitro and in vivo.

**Material and methods** The SSTR2-expressing small cell lung cancer cell line NCI-H69 was treated with 177Lu-DOTA-TATE and fixed for assessment of DNA damage by means of 53 BP1 and γH2AX stainings on multiple time points. Also, BALB/c-nude mice were engrafted subcutaneously with NCI-H69 cells and were treated with 177Lu-DOTA-TATE after tumorigenesis. Mice were euthanized at different time points until 14 days post-treatment, and tumours, bone marrow and kidneys were excised for downstream analyses.

**Results and discussions** After PRRT we observed a time-dependent intensity in DNA damage signalling and subsequent repair both in vitro and in vivo. We observed the peak of DNA double-strand break repair after two days. Although NCI-H69 tumours have a homogeneous nature, we observed intra-tumoral heterogeneity in cell death and activation of the DNA damage response. Furthermore, acute, but transient damage was observed in the kidneys and bone marrow.

**Conclusion** With this investigation, we studied in detail the radiobiology of PRRT in a preclinical setting. Our results will contribute to a better understanding of PRRT effects. This might help to improve future patient treatment by finding the optimal therapeutic window for radiosensitization of the tumour without increase of side effects.

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**PO-133** INHIBITION OF HIF-1α ENHANCES RADIATION SENSITIVITY IN TUMOUR CELLS AND MEDIATES TUMOUR GROWTH DECREASE IN MICE

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**Introduction** Poor prognosis of many solid tumours is often associated with hypoxic regions and an increased level of hypoxia-inducible-factor-1α (HIF-1α). Previous findings indicate that HIF-1α expression is relevant for radiation resistance. It was shown that HIF-1α is connected to molecules involved in DNA damage repair and checkpoint control. HIF-1α has an enhanced activity in tumours after radiation treatment (IR) and also mediates low radiation sensitivity.

**Material and methods** In vitro cultured Lewis Lung Carcinoma (LLC) cells with doxycycline (Dox)-inducible knockdown (KD) of HIF-1α were used. Cells were incubated under hypoxic conditions (Hx, 1%) and irradiated with 5 Gy. Cell cycle distribution analysis was performed by PI staining and FACS. Colony formation assay, caspase-3 assay, PARP-1 cleavage and Annexin/PI staining were used to quantify apoptosis and long term survival. DNA double strand break (DSB) repair was investigated by pulse field gel electrophoresis, Western blot and immunofluorescent staining with γH2AX, 53 BP1, RAD51, BRCA1 antibodies. In vivo the induction of HIF-1α KD was performed by tamoxifen administration to HIF-1α10Cre-ER12/12 C57BL/6 mice. The LLC cells with constitutive HIF-1α targeting (shHIF) or scrambled (shscr) shRNA-expressing vectors were implanted into the flank of the mice. IR was delivered once at day 10 with a dose of 15 Gy using an X-ray source.

**Results and discussions** We demonstrated an inhibition of decrement of phosphorylated H2AX after Hx and IR in HIF-1α deficient cells. Fast repair kinetics of the cells remained unchanged, whereas the long term survival of the cells with reduced HIF-1α was decreased. Moreover, these cells displayed an increased rate of apoptosis after IR. We also observed persisted RAD51 foci after 24 hour in HIF-1α deficient cells which indicates an alteration in homologous recombination repair (HRR) of the cells. Furthermore, in vivo experiments with a HIF-1α deficient stromal cells in the mice and injected HIF-1α deficient tumours cells indicate a decrease of tumour growth.

**Conclusion** These results imply that inactivation of HIF-1α disrupts DSB repair, in particular HRR. Furthermore, they highlight the importance of the interaction of tumour and microenvironment with respect to radiation sensitivity. It was shown that inhibition of HIF-1α enhances radiation sensitivity of tumour cells which is potentially helpful for the development of novel tumour therapies.