

Abstracts

radiation delivery have increased the importance of bladder preserving strategies using radiotherapy, while also improving patient's quality of life. However, the outcome of radiotherapy depends on the radiosensitivity of both the patient and his/her tumour. Recent developments in radiobiology have highlighted the importance of the assessment of radiation-induced DNA double-strand breaks (DSB) repair kinetics in predicting radiosensitivity of both normal and cancerous tissues. Indeed, after irradiation, several proteins (ATM, γ H2AX, etc.) involved in the signalling and repair of DNA DSB relocalize as nuclear foci. The purpose of this study is to characterise the radiosensitivity of bladder cell lines, based on their capacity to repair radio-induced DNA damage.

Material and methods Four human bladder cell lines were used in this study: T24, UM-UC3, RT4 and SVHUC. Clonogenic assay was performed to study cell survival after irradiation: cells were irradiated with doses ranging from 0 to 10 Gy and their capacity to form colonies was assessed. Immunofluorescent analysis using anti-pATM and anti- γ H2AX antibodies was then performed to assess DNA DSB signalling and repair kinetics after a 2Gy irradiation. Finally, we performed the three-dimensional (3D) sphere formation assay to assess the effect of irradiation on cancer/stem progenitor cells. Irradiated cells with doses ranging from 0 to 10 Gy were embedded in Matrigel and their capacity to form spheres was assessed.

Results and discussions RT4 was found to have the highest survival rate after irradiation, followed by T24, UM-UC3 and SVHUC according to the clonogenic assay results. Immunofluorescence results were consistent with cell survival as RT4 showed the fastest DSB recognition and repair kinetics while SVHUC had a much slower rate. Furthermore, the capacity of cells to recognise radio-induced DNA damage was found to have the best correlation with their survival. Lastly, the sphere formation ability of the different cell lines showed a differential response to increasing irradiation doses.

Conclusion We were able to radiobiologically characterise 4 human bladder cell lines by assessing their survival and capacity to repair radio-induced DNA damage. Moreover, we showed the differential effects of radiation on cancer stem/progenitor cells. The results highlighted the importance of DNA DSB signalling through the ATM protein and its role in cell survival after irradiation.

PO-128 MICRORNA-449A ENHANCES THE RADIOSENSITIVITY OF PROSTATE CANCER CELLS

J Si*, M Aihong, Z Hong. *Institute of Modern Physics- Chinese Academy of Sciences, Department of Radiation Medicine, Lanzhou, China*

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Introduction MicroRNAs (miRNAs) are evolutionarily conserved, endogenous, small noncoding RNAs that regulate the stability and translation of target mRNA by primarily binding to the 3' -UTR18. MiRNAs have been reported to be involved in DNA damage response induced by ionising radiation (IR). c-Myc is reduced when cells treated with IR or other DNA damaging agents. However, it is unknown whether miRNAs participate in c-Myc downregulation in response to IR. MicroRNA-449a is deregulated in various types of cancers, including prostate cancer.

Material and methods The human prostate cancer lines, LNCaP, PC-3 and DU-145 cells in exponential growth were

irradiated by x-rays at room temperature. Overexpressing miR-499a and miR-con was generated by plasmid transduction using GV214. MiR-449a antagomir (anti-miR-449a) and non-targeting sequence were synthesised. Transfection was performed with Trans IT –2020 Transfection Reagent. The cell viability assay was carried out basis on thiazolyl blue tetrazolium blue (MTT). Cell proliferation was analysed using Cell Counting Kit-8(CCK-8). Cell cycle analysis was acquired using FACS Calibur flow cytometer. MiRNAs and mRNA expression were determined using quantitative RT-PCR analysis. Protein expression were analysed by western blotting.

Results and discussions In the present study, we found that miR-449a was upregulated and c-Myc was downregulated in response to IR in LNCaP cells. Overexpression of miR-449a or knockdown of c-Myc promoted the sensitivity of LNCaP cells to IR. By establishing c-Myc as a direct target of miR-449a, we revealed that miR-449a enhanced radiosensitivity by repressing c-Myc expression in LNCaP cells. Moreover, we showed that miR-449a enhanced radiation-induced G2/M phase arrest by directly downregulating c-Myc, which controlled the Cdc2/CyclinB1 cell cycle signal by modulating Cdc25A. In summary, our study reported here demonstrated that miR-449a enhanced the sensitivity of LNCaP cells to IR by directly targeting c-Myc, which controlled the Cdc2/cyclinB1 cell cycle signal by modulating Cdc25A/Rb/E2F pathway. Furthermore, we found that both miR-449a and c-Myc responded to irradiation and either overexpression of miR-449a or knockdown of c-Myc sensitised LNCaP cells to irradiation.

Conclusion These findings highlighted an unrecognised mechanism of miR-449a-mediated c-Myc regulation in response to IR, which provides a support for the combination of ionising radiation with miRNAs regulation as a therapeutic strategy for patients with prostate cancer.

PO-129 IN VITRO RADIOSENSITIVITY AND REPAIR KINETICS OF PBMCS FROM PROSTATE CANCER PATIENTS AND HEALTHY DONORS EVALUATED BY COMET ASSAY

¹Z Zizak*, ²B Janovic, ¹I Besu Zizak, ²M Vujcic, ³Z Vujcic, ⁴V Stankovic, ¹I Matic, ^{4,5}M Nikitovic, ¹T Stanojkovic. *¹Institute of Oncology and Radiology of Serbia, Department of Experimental Oncology, Belgrade, Serbia; ²Institute of Chemistry- Technology- and Metallurgy, Department of Chemistry, Belgrade, Serbia; ³Faculty of Chemistry- University of Belgrade, Department of Biochemistry, Belgrade, Serbia; ⁴Institute of Oncology and Radiology of Serbia, Department of Radiotherapy, Belgrade, Serbia; ⁵School of Medicine- University of Belgrade- Serbia, Department of Radiology, Belgrade, Serbia*

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Introduction A high cellular radiosensitivity is connected with a risk for development of severe side effects after radiotherapy. In this study we have attempted to find a correlation between the initial radiosensitivity of *in vitro* irradiated peripheral blood mononuclear cells (PBMC) of prostate cancer patients and the adverse side effects of radiotherapy.

Material and methods PBMCS isolated from 7 prostate cancer patients, before the onset of radiotherapy and 3 healthy men were exposed *in vitro* to 2 Gy of x-ray radiation (Varian, 6 MeV). Following irradiation cell death was measured using the Annexin V/PI assay. DNA repair kinetics (0 and 24 hour after irradiation) was performed using the comet assay, and the results were expressed as% of tail DNA. Acute toxicity were graded according to the EORTC radiation morbidity scoring scale.

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* PBMCs 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.

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PO-130

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

¹WC Lee*, ¹S Jin, ^{1,2,3,4}N Cordes. ¹Technische Universität Dresden- Dresden- 01307-Germany and Helmholtz-Zentrum Dresden – Rossendorf- Dresden- 01328, OncoRay – National Center for Radiation Research in Oncology- Faculty of Medicine and University Hospital Carl Gustav Carus, Dresden, G; ²Technische Universität Dresden, Department of Radiation Oncology- University Hospital Carl Gustav Carus, Dresden, Germany; ³Germany and Germany German Cancer Research Center DKFZ, German Cancer Consortium DKTK-Dresden- 01307- Dresden, Heidelberg, Germany; ⁴Institute of Radiooncology-OncoRay, Helmholtz-Zentrum Dresden – Rossendorf, Dresden, Germany

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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 (3DHTesiS) in PDAC cell cultures (established and patient-derived (PDC)) grown in laminin-rich extracellular matrix (IrECM). 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 antimycin A or oligomycin pre-treatment.

Conclusion Our findings, generated in 3D IrECM 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-131

REPROGRAMMING OF CANCER CELL METABOLISM BY ADAPTATION TO CHRONIC CYCLING SEVERE HYPOXIA INCREASES RADIATION RESISTANCE

¹J Matschke*, ²T Shlomi, ³J Hlouschek, ⁴L Klein-Hitpass, ⁴V Jendrossek. ¹Institute of Cell Biology Cancer Research, University of Duisburg-Essen- University Hospital Essen, Essen, Germany; ²Department of Computer Science and Biology, Lokey Center for Life Science and Engineering, Haifa, Israel; ³Institute of Cell Biology Cancer Research, University Duisburg-Essen- University Hospital Essen, Essen, Germany; ⁴Institute of Cell Biology Cancer Research, University of Duisburg-Essen- University Hospital Essen, Essen, Germany

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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* 201625:89–107; 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 central metabolism. Targeting of induced metabolic alterations disturbed redox homeostasis, altered