Conclusion: Nitroglycerin causes a significant decrease in the hypoxic fraction and hypoxic volume of a majority of hypoxic non-small cell lung cancer tumours and metastatic lymph nodes. This promising result encourages further investigation of nitroglycerin as a sensitizing agent in a selected population. CT-based perfusion studies and lab experiments (data not shown) suggest this effect is mediated by an inhibition of mitochondrial respiration rather than a vascular effect.

OC-0130
Biomarker-based hypoxia-adapted radiochemotherapy: preclinical study in HPV+/- H&N cancer xenografts
E. Firat1, G. Niedermann1, A. Zaffaroni1
1Universtaet Klinikum Carl Gustav Carus- Onco Ray, Radiation Oncology, Dresden, Germany

Purpose or Objective: Previous experiments using tumour material from this trial indicate that hypoxic tumours predominantly benefit from nimorazole, supporting a predictive value for hypoxia assessment. This has not been previously evaluated for radiochemotherapy (RCTx) which represents the current standard of care in locally advanced head and neck cancer. The hypothesis of the ongoing study is that the microenvironmental parameters are also predictive for response to hypoxic cell sensitization with nimorazole in combination with RCTx.

Material and Methods: We studied 8 different human HPV-negative and -positive HNSCC in a nude mice xenograft model. Irradiation was performed with 30 fractions (fx) in six weeks combined with weekly cisplatin (3 mg/kg i. p.). Nimorazole (0.3 mg/g i. p.) was applied before each irradiation and was started with the first fx or after 10 fx. Effect of nimorazole was quantified as LC 120/180 days after irradiation. For histological evaluation tumour excised unirradiated or after 10 fx with and without nimorazole. Using quantitative image analysis, microenvironmental parameter such pimonidazole hypoxic volume (pHV), relative vascular area (RVA) and perfused fraction of vessels (PF) were determined.

Results: The data of the cell lines show pronounced heterogeneity in the effect of nimorazole on local tumour control after fractionated radiochemotherapy. Nimorazole significantly improved local tumour control in four of the eight tumours. In the two responder models FA-Du and SAS, nimorazole was equally effective when given from start of radiotherapy or after 10 fx. The treatment with both, RCTx and the application of nimorazole and cisplatin were well tolerated by the animals. Furthermore, pHV was significantly reduced after 10 fx RCT with and without nimorazole in all four responder models in contrast to the non-responders.

Conclusion: Apparently, the decrease of pHV after the first fractions of RCTx has potential as a predictive biomarker for LC for combination of RCTx with nimorazole and should therefore be further evaluated in experimental FMISO analysis and also in clinical trials using hypoxic cell radiosensitisation during RCTx.

OC-0131
miR-875-5p enhances radiation response of prostate cancer cells via EGFR suppression
R. El Bezawy1, D. Cominetti1, P. Gandellini1, R. Valdagni2, N. Zaffaroni1
1Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Experimental Oncology and Molecular Medicine, Milan, Italy
2Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Radiation Oncology, Milan, Italy

Purpose or Objective: There is increasing interest in defining a functional association between miRNAs, endogenous small non-coding RNA molecules that negatively regulate gene expression, and tumor radiation response, with the aim of rationally designing miRNA-based strategies to increase patient radiosensitivity. In this study, we investigated for the first time the ability of miR-875-5p, a miRNA the role of which in human cancer has not been so far investigated, to enhance the radiation response of prostate cancer (PCA) cells.

Material and Methods: The search for miR-875-5p targets relevant to radiation response was carried out by prediction algorithms and confirmed by the luciferase assay. miR-875-5p reconstitution by miRNA mimics in PCa cell lines (DU145 and PC-3) was used to elucidate its biological role. Radiation response in miRNA-reconstituted and control cells was assessed by clonogenic assay, immunofluorescence-based detection of nuclear γ-H2AX foci and single-cell electrophoresis comet assay.

Results: EGFR was predicted by 6 different algorithms and confirmed by luciferase assay as a direct target of miR-875-5p. Given the role of EGFR in determining tumor cell resistance to ionizing radiation by promoting epithelial-to-mesenchymal transition (EMT) and enhancing DNA-dependent protein kinase activity and DNA damage repair, we assessed whether miR-875-5p reconstitution in PCa cells was able to counteract EGFR-mediated radio-resistance. Indeed, miRNA ectopic expression significantly increased the sensitivity of both DU145 and PC-3 cell lines to radiation, as indicated by changes in cell morphology, marked cytoskeleton architecture rearrangements, reduced migration ability and increased mRNA and protein levels of E-cadherin and β-catenin, the two most important molecular players in the EMT process.

Conclusion: Overall, results from this study support the clinical interest in developing a novel therapeutic approach for PCa based on miR-875-5p reconstitution to increase response to radiotherapy.

OC-0132
FoxO proteins and non-functional p53 determine stemness and radiosensitivity of GBM-stem cells
F. Firl1, G. Niedermann1
1Uniklinik Freiburg, Dept. of Radiation Oncology, Freiburg, Germany

Purpose or Objective: Dual inhibitors of PI3K and mTOR do not efficiently radiosensitize glioblastoma multiforme stem cells (GBM-SC). However, p53-proficient GBM-SCs are more...
Radioresistance of glioblastoma stem-like cells is associated with replication stress. GBM-SCs and that non-functional p53 can maintain these crucial for functional stemness and survival in p53-proficient differentiation upon combination treatment with gamma-IR and dual PI3K/mTOR inhibitors.

Material and Methods: Patient-derived GBM-SCs were cultured under stem cell culture conditions. Western blot was used for protein expression analyses. Sphere formation served as a surrogate assay for self-renewal and cell death was assessed flow cytometrically. Lentiviral RNA-knockdowns or overexpression of p53 and FoxO proteins were employed for molecular studies. ChIP assay was used to assess binding of FoxO transcription factors to the regulatory region of the sox2 gene.

Results: p53-proficient GBM-SCs lost stem cell markers and self-renewal ability and underwent differentiation a few days after the combination treatment with γIR and a PI3K/mTOR inhibitor (PI-103 or NVP-BEZ235); expression of FoxO proteins was also lost. In contrast, stem cell markers and FoxO proteins were not lost anymore upon p53 shRNA knockdown or in p53-deficient GBM-SCs. FoxO1/3 knockdown also caused reduced sphere formation and cell survival after the combination treatment in p53-proficient but not in p53-deficient GBM-SCs. Furthermore, FoxO1 and FoxO3 were found to bind to the sox2 regulatory region in GBM-SCs, and combined FoxO1/3 deletion abolished Sox2 expression which was confirmed with a novel synthetic FoxO1 inhibitor. Finally, FoxO overexpression prevented GBM-SC differentiation by combination treatment with gamma-IR and dual PI3K/mTOR inhibitors.

Conclusion: Our results suggest that FoxO proteins are crucial for functional stemness and survival in p53-proficient GBM-SCs and that non-functional p53 can maintain these functions instead.

OC-0133
Radioresistance of glioblastoma stem-like cells is associated with replication stress
R. Carruthers, S. Ahmed, D. Biasoli, K. Strathdee, E. Hammond, A. Chalmers
1Beatson West of Scotland Cancer Centre, Clinical Oncology, Glasgow, United Kingdom
2University of Glasgow, Institute of Cancer Sciences, Glasgow, United Kingdom
3University of Oxford, Oxford Institute of Radiation Oncology, Oxford, United Kingdom
4University of Glasgow, Institute of Cancer Sciences, Glasgow, United Kingdom

Purpose or Objective: Tumour recurrence in glioblastoma (GBM) patients is inevitable despite multi-modality treatment with surgery, radiotherapy and chemotherapy. Tumour recurrence is thought to be driven by a small population of glioblastoma stem-like cells (GSCs) that are resistant to conventional therapies. DNA damage response (DDR) signalling has been shown to be up-regulated in GSCs and implicated in radioresistance and treatment failure. However the cause of enhanced DDR signalling in GSCs and its contribution to radiation resistance and tumour recurrence is not well understood. The objectives of this study were to investigate the underlying cause of DDR upregulation and treatment resistance in GSCs and to identify novel therapeutic targets.

Material and Methods: A panel of primary GBM cell lines cultured under conditions to enrich for or deplete the tumour stem cell population (GSC vs bulk respectively) were utilised to investigate enhanced GSC DDR under basal conditions and after ionising radiation. Confirmatory studies were performed in cells sorted for the putative GSC marker CD133. The effects of a panel of small molecule DDR inhibitors on cell survival in GSC and bulk cells were explored.

Results: GSCs exhibited higher levels of total and activated DDR targets ATR, CHK1, ATM and PARP1 under basal conditions and were radioresistant compared to paired bulk populations. Augmented DDR in GSCs has been linked to increased reactive oxygen species levels by other authors, however we were unable to demonstrate this in our GSC cultures. Instead, we show that RPA is significantly higher in replicating GSCs and confirm by DNA fibre assays that GSCs and CD133+ cells have increased numbers of stalled replication forks, fewer new origins and slower DNA replication compared to bulk or CD133+ populations, suggesting that replication stress may be important to constitutive DDR activation seen in GSCs. Importantly, inhibition of ATR or CHK1 was cytotoxic to GSCs and when combined with PARP inhibition caused DNA double strand breaks and reduced neurosphere formation.

Conclusion: This study demonstrates that replication stress is a hallmark of GSCs. We implicite replication stress in GSCs as the driver of enhanced DDR and radioresistance in GSCs and therefore a cause of tumour recurrence in GBM. This suggests that replication stress is a GSC specific therapeutic target, and we are able to demonstrate the effectiveness of inhibitors of replication stress response in targeting this treatment resistant tumour subpopulation.

Purpose or Objective: Although prostate cancer is the most common malignancy in men, the cellular and molecular mechanisms underlying tumor progression and therapy resistance remain poorly understood. Within this study we discovered cancer stem cell (CSC)–related properties, CSC plasticity and tumor heterogeneity as a source for radiotherapy resistance. Therefore, analysis of CSC-based biomarkers might be an important predictive tool for individualized radiotherapy and treatment.

Material and Methods: Global gene expression and membrane proteomic profiling of radioresistant sublines from established prostate cancer cell lines identified novel biomarker for prostate cancer radioresistance, which were validated in NMRI nu/nu mice in vivo, with immunohistochemical analysis of tumor sections and in short-term ex vivo cultures of primary prostate cancer tissue.

Results: Within this study we found that the aldehyde dehydrogenase (ALDH) activity is a predictive marker of a radioresistant prostate cancer progenitor population with enhanced DNA repair capacity and activation of epithelial-mesenchymal transition (EMT). The activation of the WNT/B-catenin signaling pathway was identified as a key molecular mechanism, which linked CSC-related properties to radioresistance. We found that the B-catenin/TCF transcriptional complex is directly activating the ALDH1A1 gene transcription, and molecular targeting of the WNT pathway with XAV939 leads to radiosensitization. Moreover, our study revealed that irradiation causes long-term upregulation of stem cell markers and induces tumor cell reprogramming. This phenotypic plasticity is associated with genetic and epigenetic changes induced by irradiation, such as the histone H3 methylation within the promotor sequence of the ALDH1A1 gene. The inhibition of histone methylation by DZNep triggered radiosensitization by apoptosis induction in vitro and in vivo.

Conclusion: Our findings suggest that ALDH-positive CSCs contribute to tumor radioresistance, but these radioresistant...