Results: An inverse correlation was found between the spontaneous level of yH2AX foci and the frequency of micronuclei after irradiation ($R=0.37$, $p=0.025$). After gene expression analysis with microarrays, several genes were identified whose differential expression could be associated with an efficiency of DNA repair and radiation sensitivity. XRR1 gene with unknown functions, recently associated with radiosensitivity in tumor lines, was down-regulated both before and after irradiation in radioresistant group. Furthermore, in unirradiated samples of radiosensitive individuals thrombospondin gene (THBS1), well-known radiosensitizer, was down-regulated. However, several genes were significantly up-regulated, including HERC2, important player in the assembly of DNA repair foci, and histone genes (H1, H2A, H4). After irradiation, several DNA repair genes (WHSC1, POLN, ERCC5, DCLRE1C) were significantly up-regulated, but EIF2A and PNPLA5 genes, involved in apoptosis and autophagy, were down-regulated in radioresistant group. This is consistent with low levels of apoptosis and increased proliferation in lymphocytes of these individuals.

Conclusion: The obtained results indicate that spontaneous yH2AX foci activate DNA damage response in human somatic cells and provide opportunities to clarify the role of the expression of identified genes in the formation of chromosomal aberrations in human cells after exposure to radiation.

EP-2066 Phospholipase Cε as a biomarker of prostate cancer radioresistance
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3Technische Universität Dresden and Helmholtz-Zentrum Dresden-Rossendorf, OncoRay-National Center for Radiation Research in Oncology- Faculty of Medicine and University Hospital Carl Gustav Carus, Dresden, Germany
4Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiation Oncology, Dresden, Germany
5German Cancer Consortium DKTK, DKTK, Dresden, Germany

Purpose or Objective: Radiotherapy is a curative treatment option in prostate cancer. Nevertheless, many men with prostate cancer develop recurrence of their disease. Identification of the predictive biomarkers and signaling mechanisms indicative of tumor cell radioresistance bears promise to improve cancer treatment. In our study we show that Phospholipase C epsilon (PLCε) might contribute to prostate cancer radioresistance.

Material and Methods: Gene expression profiling of prostate cancer cells and their radioresistant derivatives, western blot analysis to assess PLCε expression in the parental and radioresistant cells and in cell cultures after irradiation, radiobiological cell survival analysis of the cells with genetic modulation of PLCε expression by siRNA or cDNA transfection as well as chemical inhibition of PLCε activity, fluorescent microscopy to analyze co-expression of PLCε with other markers of radioresistance. Normal 0 21 false false false EN-US X-NONE X-NONE

Results: The results of gene expression analysis, which were validated by western blotting revealed significant upregulation of PLCε in prostate cancer radioresistant cells that can also be seen after irradiation of the parental cells with a single dose of 4 Gy. Radiobiological survival assays demonstrated that siRNA induced knockdown of PLCε activity by Edelfosine leads to prostate cancer cell radiosensitization. In contrast, overexpression of PLCε in cells transfected with plasmid DNA results to an increase in cell radioresistance. Microscopic analysis revealed a high expression level of β-catenin in prostate cancer cells overexpressing PLCε.

Conclusion: These results indicate that PLCε plays a role in prostate cancer radioresistance that can be mediated through activation of the WNT/β-catenin signaling pathway.

EP-2067 The adhesion of tumor cells to endothelial cells is increased by photon irradiation
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Purpose or Objective: In general the prognosis for cancer patients is poor even though only 10% die from the primary tumor. The majority of the deceases are due to metastasis. Given the fact, that more than 70% of cancer patients receive radiotherapy it seems important to clarify if radiation is involved in initial steps of the metastatic cascade - despite of innumerable clinical studies that confirm no enhanced risk of metastasis after radiotherapy. In this project we investigated whether the irradiation with photons increases the adhesion of cultured tumor cells (TC) to a layer of endothelial cells (EC) macroscopically and whether this might be caused by the induction of adhesion proteins.

Material and Methods: The experiments were performed with glioblastoma (U87, U373) and breast cancer cell lines (MDA-MB-231, MCF7), and with primary HUVEC cells. The cells were irradiated with 0, 0.5, 2, 4, or 8 Gy. Adhesion of TC to EC, both irradiated or not, was determined with 2 different methods: the VybrantTM cell adhesion assay and the ibidi pump system that allows to mimic the physiological blood stream in the vasculature. In addition, the expression of the adhesion-related proteins E-selectin, VCAM1, ICAM1, N-cadherin, integrin β1, and PECAM1, 4h after irradiation with 4 Gy, was analyzed by qRT-PCR and by Western blotting.

Results: Irradiation increased significantly the adhesion of TC to EC. With glioblastoma cells the highest increase of about 40% was observed when both cell types were irradiated. In contrast, with breast cancer cells the highest effect of about 25% was obtained for irradiated TC in combination with non-irradiated EC. Analysis of the expression patterns in all cell types revealed a significant increase of adhesion proteins after irradiation in more than 80% of the experimental data sets.

Conclusion: We assume that the irradiation of tumor cells as well as of endothelial cells with photons might enhance adhesive interactions of these cells and thereby might promote the first steps of metastasis. Since clinical studies reveal no enhanced risk of metastasis due to irradiation we speculate that the therapeutic effect of radiotherapy might be additionally enhanced when the induced stickiness could be blocked effectively.

EP-2068 Effect of a 0.2 T magnetic field during radiation on DNA damage and repair in prostate cancer cells
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Purpose or Objective: Radiotherapy is a curative treatment option in prostate cancer. Nevertheless, many men with prostate cancer develop recurrence of their disease. Identification of the predictive biomarkers and signaling mechanisms indicative of tumor cell radioresistance bears promise to improve cancer treatment. In our study we show that Phospholipase C epsilon (PLCε) might contribute to prostate cancer radioresistance.

Material and Methods: Gene expression profiling of prostate cancer cells and their radioresistant derivatives, western blot analysis to assess PLCε expression in the parental and radioresistant cells and in cell cultures after irradiation, radiobiological cell survival analysis of the cells with genetic modulation of PLCε expression by siRNA or cDNA transfection as well as chemical inhibition of PLCε activity, fluorescent microscopy to analyze co-expression of PLCε with other markers of radioresistance. Normal 0 21 false false false EN-US X-NONE X-NONE

Results: The results of gene expression analysis, which were validated by western blotting revealed significant upregulation of PLCε in prostate cancer radioresistant cells that can also be seen after irradiation of the parental cells with a single dose of 4 Gy. Radiobiological survival assays demonstrated that siRNA induced knockdown of PLCε activity by Edelfosine leads to prostate cancer cell radiosensitization. In contrast, overexpression of PLCε in cells transfected with plasmid DNA results to an increase in cell radioresistance. Microscopic analysis revealed a high expression level of β-catenin in prostate cancer cells overexpressing PLCε.

Conclusion: These results indicate that PLCε plays a role in prostate cancer radioresistance that can be mediated through activation of the WNT/β-catenin signaling pathway.
Purpose or Objective: Real time MR-guided radiotherapy is an emerging technology. The effect of magnetic field exposure on radiosensitivity is unknown. This study aimed to determine the effect of magnetic field exposure on the repair of radiation-induced DNA double-strand breaks in human prostate cancer cells.

Material and Methods: Human PC-3 prostate cancer cells and benign prostatic hyperplasia (BPH) cells were cultured and plated into 96-well dishes and irradiated with 2 Gy of 6 MV photons on a linear accelerator. Each cell line was exposed to either 2 Gy of ionizing radiation alone (IR) or 15 minutes of 0.2 T magnetic field concurrently with 2 Gy IR (IR + B). Cells were fixed at 15 minutes or 24 hours following IR and immunostained with fluorescent-labelled antibody to γH2AX, a marker of DNA double-strand breaks. For each experimental scenario, the number of γH2AX foci per cell were determined using a Molecular Devices MetaXpress High Content Imaging Platform, for sample sizes between 3370 and 8402 cells. To classify response, radiation-induced damage was associated with cells having more than five foci.

Results: Magnetic field exposure resulted in a significantly higher percentage of PC-3 cells with five or fewer γH2AX foci at 24 hours following IR (42 vs 37 percent, p < 0.01) but had no significant effect on BPH cells (89 vs 88 percent, p = 0.26). In both cell lines, magnetic field exposure significantly reduced the percentage of cells with five or fewer γH2AX foci 15 minutes following IR (p < 0.01) (Table 1).

Table 1. Percentage of BPH and PC-3 cells with ≤ 5 γH2AX foci at 15 minutes and at 24 hours after exposure to 2 Gy of ionizing radiation alone (IR) vs 2 Gy of ionizing radiation with 15 minutes of concurrent 0.2 T magnetic field exposure (IR + B).

<table>
<thead>
<tr>
<th>Time Post-IR</th>
<th>BPH</th>
<th></th>
<th>PC-3</th>
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</tr>
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<tbody>
<tr>
<td>15 minutes</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>24 hours</td>
<td>100</td>
<td>94</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>Percentage of cells with ≤ 5 γH2AX foci</td>
<td>100</td>
<td>94</td>
<td>100</td>
<td>90</td>
</tr>
</tbody>
</table>

Conclusion: The preliminary results suggest that the presence of a magnetic field during irradiation reduces DNA damage at 24 hours post-irradiation for PC-3 human prostate cancer cells. Conversely, magnetic field exposure increased the DNA damage present 15 minutes following IR in both cell lines, suggesting a different mechanism at play, such as altered free radical flux or differences in the kinetics of the initiation of the DNA damage response. Cell viability assays, gene expression profiling and testing of other cell lines will yield important insights into the implications for real time MR-guided radiotherapy.

EP-2069
CDC73 deficiency: a syndrome with multiple tumours is predicted to show excessive radiosensitvity

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Purpose or Objective: It has previously been demonstrated that prolonged expression of the γ-H2AX DNA repair biomarker in irradiated peripheral blood lymphocytes correlated with excess toxicity from radiotherapy treatment in patients. γ-H2AX fluorescence in cells has been established as an indicator of double strand breaks, and a marker for DNA damage and repair of cells after irradiation. This case study illustrates that the peripheral blood lymphocytes of a patient with CDC73 deficiency retained γH2AX fluorescence over 24 hours to a greater degree than a patient with normal DNA repair.

Conclusion: It may be confidently predicted that this patient with CDC73 deficiency would demonstrate more vigorous radiation reactions in normal tissues for any standard dose of radiotherapy, due to a possible defect in DNA repair and this should be considered when planning his Cyberknife treatment for the carotid body paraganglioma. The exact mechanism for this will need to be considered along with current knowledge of the role of CDC73.