the combination of radiotherapy and DAO, in primary cultures from glioblastoma.

Material and Methods: We have used primary cultures and stabilized cell lines from patients with glioblastoma. Recombinant DAO carrying the C-terminal domain of the major lytic amidase (CLyTA) specific for binding to choline was immobilized to magnetic nanoparticles having a magnetite core covered with Diethylaminoethyl (DEAE) cellulose. Primary cultures were irradiated at 7 and 15 Gy. After irradiation, cultures were treated in the absence or in the presence of DAO (free or immobilized in nanoparticles) and D-alanine (enzyme substrate). After irradiation, cells were harvested and cell cycle distribution was determined by flow cytometry.

Results: We have demonstrated in primary cultures from glioblastoma, that the treatment with DAO after irradiation, potentiated dramatically the effect of the radiation alone, increasing especially the percentage of cells in the sub-G1 phase, an indicator of cell death. Some representative results are included in the attached file. DAO immobilized in magnetic nanoparticles is more effective than free enzyme, since DAO is more stable at 37°C immobilized in nanoparticles.

Conclusion: The combination of radiotherapy and enzymatic therapy with DAO based on the nanotechnology, induce an increase in cell death when it is compared with radiotherapy alone.

EP-2033
Combining Hedgehog inhibition with metformin to induce radiosensitization in prostate cancer cells
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Purpose or Objective: There are several indications that the Hedgehog (Hh) pathway could be a potential target for radiosensitization. Furthermore, a link between Hh signaling and the cellular energy metabolism has been described recently, more specific at the level of AMP-activated protein kinase (AMPK). Activation of AMPK, in turn, has also been shown to result in radiosensitization. Therefore, it seems worthwhile to explore whether the combination of Hh signaling inhibitors and AMPK activators such as metformin could further increase the response to radiotherapy. This combination strategy is being tested in prostate cancer (PCa) cells, as there is increasing evidence that the Hh pathway plays an important role in the development as well as progression to more advanced disease stages of PCa.

Material and Methods: Three PCa cell lines (PC3, DU145, 22Rv1) were treated for 72h with the SMO inhibitor GDC-0449 (1µM, 10µM) or GLI1/2 inhibitor GANT61 (1µM, 10µM), with or without metformin (5mM). The effects on cell survival, proliferation and radiation sensitivity were investigated by means of Sulforhodamine B (SRB) assays, Bromodeoxyuridine (BrdU) assays and colony assays. The effects on gene and protein expression (qR-PCR/Western blotting), cell cycle distribution (flow cytometry, PI staining) and DNA repair (flow cytometry, yHAX2) were also examined, both in the absence and presence of irradiation (4Gy).

Results: GDC-0449 on its own did not significantly affect cell proliferation, survival or radiation sensitivity of any of the PCa cell lines tested. Treatment with 10µM GANT61 on the other hand did result in a significant reduction of cell survival in all cell lines and induced radiosensitization in the 22Rv1 cells (DEF(SF0.5)=1.39±0.11, p<0.002) (Fig 1A). The latter could be ascribed to the drug’s effect on apoptosis (Fig 1B). Similar results as for GANT61 were observed after metformin monotherapy (DEF(SF0.5)=1.36±0.08, p<0.012). Moreover, metformin induced a significant downregulation of GLI1, both at the gene and protein expression level. While the combination of metformin and GDC-0449 resulted in no additional effects, addition of metformin to GANT61 further enhanced the radiosensitization effects as induced by single agent treatment in the 22Rv1 cells.

Conclusion: The GLI1/2 inhibitor GANT61 as well as metformin induced radiosensitization in the 22Rv1 PCa cells. The combination of both agents further enhanced the response to radiotherapy, indicating that this might be a more powerful radiosensitization strategy as compared to either agent alone. Investigations are currently ongoing to explore the underlying working mechanisms.

EP-2034
Targeting hypoxic cancer cells by inhibition of checkpoint kinases ATR and CHK1
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Purpose or Objective: The checkpoint kinases ATR and CHK1 are considered promising targets for cancer treatment due to their roles in regulation of the S and G2 checkpoints and in the repair of DNA double strand breaks through homologous recombination. Interestingly, severe levels of hypoxia (<0.1% O2) have been shown to activate ATR/CHK1 signaling, which could likely make hypoxic cancer cells sensitive to inhibitors of these kinases. The aim of this project is to explore whether inhibition of ATR or CHK1 could be used to selectively target hypoxic cancer cells, both in combination with ionizing radiation and on its own.

Material and Methods: Cancer cell lines U2OS, HCT116, H460, A549 and H1975 were treated with inhibitors of ATR (VE821, VE822) or Chk1 (AZD7762, UCN01) in the absence and presence of hypoxia (InVivo2 hypoxia chamber) and X-ray-irradiation. Cells were analyzed by flow cytometry, immunoblotting and clonogenic survival assays.

Results: We previously measured clonogenic survival, cell cycle distribution and activation of DNA damage signaling pathways in U2OS and HCT116 cancer cells at different oxygen concentrations (21%, 0.2% and 0.0% O2) in combination with the CHK1 inhibitors UCN-01 and AZD7762 and ionizing radiation. We found that hypoxia alone did not alter the sensitivity to CHK1 inhibitors, but inhibition of CHK1 after reoxygenation following periods of extreme hypoxia (0.0% O2) did result in decreased clonogenic survival and an increased fraction of γ-H2AX positive cells. Hypoxic cells were also found to be radiosensitized at least to the same extent as normoxic cells by CHK1 inhibition. Currently we are performing similar studies in lung cancer cell lines H460, A549 and H1975 treated with the ATR inhibitors VE821 and VE822. We have found that the number of γ-H2AX positive cells after ATR inhibition was higher in cells incubated at hypoxia (0.0% O2, 20h) compared to normoxia (21% O2). The ATR inhibitors also abrogated the radiation-induced G2 checkpoint. Clonogenic survival assays are ongoing.

Conclusion: These studies help determine the potential of using inhibitors of ATR and CHK1 to eradicate radioresistant hypoxic cancer cells.