Metabolic Modulation of Androgen-Receptor Signaling Inhibitor Mediated Radiosensitization

Qianhua Feng, Kenichi Kudo, Maria Isabel Chosco, Chun Li, Ziyi Huang, Matthew Wong, Jeshu Mohan, Jing Huang, Leslie Wenning, Sarah Shuck, Yun Rose Li

City of Hope National Medical Center, Beckman Research Institute, Duarte, CA, 94158

Background: Prostate cancer (PCa) is the second most common cancer in men in the United States. Radiotherapy in combination with androgen blockade is one of two main forms of curative intent therapeutic options in men with high risk, locally advanced and/or recurrent PCa after surgery. Recently, metabolic intervention interventions, such as glycemic control, have been proposed as a strategy to enhance radiosensitivity through metabolic modulation. Our lab has demonstrated that fasting based interventions may protect gastrointestinal tissue from RT-mediated toxicity in preclinical PCa models. Here, we investigate the effects of hyperglycemia on radiotoxicity and AR-blockade mediated radiosensitization in both androgen-dependent (LNCaP) and independent (PC-3) prostate cancer cell lines.

Materials/Methods: LNCaP and PC-3 cells were grown in complete RPMI 1640 medium supplemented with 10% feral bovine serum, 1% penicillin/streptomycin. Cells cultured in same completed media with final 25mM glucose and 5mM glucose 48 hours before experiment are referred as high glucose and low glucose conditions, respectively. Cell viability was measured by MTS assay 48, 72, and 96 hours after 8Gy radiation exposure radiation with 48 hours HG and LG pre-treatment. Radiation induced DNA damage and repair were detected by Comet Assay after RT for 0, 2, and 4 hours with 2Gy/4Gy dose treatment. Radiation induced apoptosis was measured by flow cytometry using Annexin V-FITC and propidium iodide 24, 48, and 72 hours after 2Gy/4Gy RT. The level of ROS in mitochondrial and cellular after radiation exposure were examined by flow cytometry using MitoSOX Red and CellROX.

Results: We found that LNCaP exhibits marked radiosensitivity in response to LG, with 50 to 60% reduction in cell viability at or after 48h post-RT. Further, co-treatment with enzalutamide (10uM), an androgen-receptor signaling inhibitor (ARSI), synergistically reduces cell survival with 15% cell viability post-RT. Although PC-3 cells were radiosensitized by LG treatment, as expected, there was no synergistic effect with the combination of LG and ARSI. LNCaP in LG shows 2.7-fold higher γH2AX, a biomarker of DNA damage and repair, as quantified by flow cytometry at 2h post-RT compared to HG. Apoptosis assay with Annexin V and propidium demonstrates the elevated apoptotic and necrotic responses of LNCaP under LG, with 2-fold higher levels of early and late apoptotic cells and 1.7-fold higher level of necrotic cells. The effect of glycemic control is less clear in PC-3. Radiation induced mitochondrial and cytoplasmic oxidative stress response was also significantly increased in LNCaP cells.

Conclusions: Our results suggest LG conditions exacerbate RT-induced cytotoxicity, DNA damage, apoptosis and necrosis, in androgen-dependent PCa. The findings from our study underscore the potential of glycemic control as a synergistic strategy to enhance the therapeutic index of RT in PCa. Studies examining the impact of glycemic control and fasting on prostate tumor radiosensitivity are ongoing.

Funding: Yun Rose Li is supported by the NIH Early Independence DP5 Award and Sarah Shuck is supported by a NIH R21 award. Isabel Chosco is supported by a NIDDK T32 pre-doctoral training grant. Kenichi Kudo is supported by institutional CIRM postdoctoral fellowship.

Conflicts of Interest Disclosure Statement: The author has no conflict of interest.