P14 Enhanced radiosensitization by apalutamide via suppression of DNA damage response in prostate cancer

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Introduction & Objectives: External beam radiation therapy (EBRT) is the first line treatment for locally advanced prostate cancer (PCa), but tumor recurrence is frequent. Combination of EBRT with androgen-deprivation therapy (ADT) has shown to improve treatment outcome. The underlying mechanism to how the androgen receptor (AR) pathway affects EBRT efficacy is not fully uncovered. DNA damage response (DDR) pathway has been suggested to play a role, but conflicting results have been reported. Apalutamide is a next-generation AR inhibitor that fully blocks activation of the signaling pathway. This study aims to reveal the role of the AR in EBRT-induced DDR and to investigate whether apalutamide can work as a radiosensitizer for PCa.

Materials & Methods: PCa cell lines with different AR expression (PC346C, PC346C-Flu1, and LNCaP), AR negative (PC346C-DCC, DU145) and precision-cut tissue slices (300 µm) from patient-derived xenograft (PDX) tumour tissue (PC295, AR+) were treated with anti-androgen alone or combined with irradiation (IR). The effect of apalutamide on PCa cell growth and tissue viability was investigated using sulforhodamine B (SRB) and EdU/TUNEL assay, respectively. We determined DDR foci (γH2AX, 53BP1) kinetics and measured double-strand break (DSBs) by pulsed-field electrophoresis (PFGE). Cell cycle was measured by flow cytometry. AR expression in tissues was detected by immunohistochemistry. RAD51 and DNA-PKcs levels were determined by western blotting. DNA-PKcs knockdown was performed by RNA interference. Functional homologous recombination (HR) capacity was measured by Dr-GFP assay.

Results: The SRB assay showed clear additive effects when anti-androgen was combined with IR exposure in AR-expressing PCa cells. This effect was dependent on their AR-dependence, but synergism could not be proven. Interestingly, in ex vivo tumor slice culture, a clear synergistic effect was observed with the combination of apalutamide and IR. A significant increase in DSBs in the combination group reflecting slow repair of IR-induced DNA damage was observed as early as 1 day upon treatment. At this time point, IR and apalutamide treatment alone had no effect. Reduced RAD51 expression, reflecting HR DNA repair capacity, was associated with cell cycle alteration induced by ADT and apalutamide treatment in AR positive PCa cell lines. However, downregulation of DNA-PKcs and impaired non-homologous end-joining (NHEJ) in these cells contribute to radiosensitization induced by ADT and apalutamide,

Conclusions: Our data show that apalutamide radiosensitizes AR-expressing PCa by decreasing cell survival and delaying DNA repair. Mechanistic investigations demonstrated that HR is not directly regulated by AR, and that inhibition of NHEJ by blockage of AR-signaling is the major contributor to radiosensitization. These data provide evidence for the clinical use of apalutamide in combination with EBRT for localized PCa patients.