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PROSTATE CANCER

A variety of therapy: ARVs mediate DDR

> The effect of enzalutamide plus radiation on cell survival was enhanced by DNA-PKc inhibition

Androgen receptor splice variants (ARVs) that can be induced by androgen deprivation therapy (ADT) increase the clonogenic survival of prostate cancer cells after irradiation. Furthermore, irradiation triggers the binding of ARVs to DNA-dependent protein kinase c (DNA-PKc) and DNA-PKc inhibition enhances radiation-induced DNA damage. These observations provide insight into the mechanisms by which prostate cancer becomes resistant to combined ADT and radiotherapy and suggest that therapeutic targeting of DNA-PKc could be beneficial in this situation.

Combined ADT and radiotherapy is superior to radiotherapy alone; however, this combination therapy often fails. Thus, Yin and colleagues hypothesized that the DNA damage response (DDR) could be driven by ARVs induced by ADT.

Using R1-AD1 cells prostate cancer cell lines that express only the full-length androgen receptor (AR) or R1-D567 cells that express the constitutively active ARV ARv567es, the researchers found that enzalutamide (for androgen blockade) plus radiation delayed and decreased DNA repair in R1-AD1 cells, but not in R1-D567 cells. Moreover, enzalutamide treatment after radiation did not affect clonogenic survival of R1-DW567 or 22Rv1 cells (which express the full-length AR and AR-V7), but, in R1-AD1 cells and C4-2 cells (which only express full-length AR), survival was decreased. Knockdown of both the full-length AR and AR-V7 in 22Rv1 cells was required to enhance the effect of radiation on clonogenic survival.

Radiation induced expression of genes involved in homologous recombination and nonhomologous end joining (NHEJ); however analysis indicated that ARVs are unlikely to be the primary drivers of the transcriptional programme. Furthermore, radiation probably increases AR signalling at the pathway level, not the gene level, as slight upregulation of some pathway components occurred, but no considerable upregulation of a single component was observed.

Proximity ligand assays showed that ARv567es, AR-V7, and full-length AR quickly localized to sites of DNA damage after irradiation. Regardless of irradiation, DNA-PKc was the top-binder of ARv567es and radiation increased interaction of DNA-PKc with full-length AR and ARVs *in vitro*. Full-length AR and ARVs had different radiation-induced interactions with DNA-PKc, with the WHTLF domain critical for ARV interaction and the AR-LBD involved in full-length AR interaction. Treatment with enzalutamide abrogated the radiation-induced interaction of full-length AR with DNA-PKc, but not the ARV–DNA-PKc interaction; however, treatment with a DNA-PKc inhibitor did block this interaction. Furthermore, the DNA-PKc inhibitor increased DNA fragmentation and resulted in persistent DNA double-strand breaks and blocked NHEJ repair in irradiated R1-D567 cells. The effect of enzalutamide plus radiation on cell survival was enhanced by DNA-PKc inhibition, which was also associated with increased radiation-induced DNA damage and decreased cell survival whether cells expressed ARVs, full-length AR, or both.

In vivo, R1-D567 xenografts were implanted into male mice, which were castrated and then treated with a DNA-PKc inhibitor followed by radiotherapy or radiotherapy alone. Combination therapy plus DNA-PKc inhibition slowed xenograft growth to a greater extent than combination therapy alone, and DNA-PKc inhibition reduced the DDR in the tumours. Notably, R1-D567 subjected to short-course radiotherapy grew faster than R1-AD1 xenografts.

Overall, these data suggest that ARVs can interact with DNA-PKc and might contribute to the failure of combined ADT and radiotherapy. Moreover, radiation can enhance the interaction of AR and ARVs with DNA-PKc. Clinically, the addition of DNA-PKc blockade to combined ADT and radiotherapy could improve outcomes for men with localized prostate cancer.

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