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PROSTATE CANCER

Understanding why

> DNAPKcs expression is induced in the presence of androgen and IR

For patients with high-risk prostate cancer, treatment with both androgen deprivation therapy (ADT) and ionizing radiation (IR) improves progression-free and overall survival and has long been the standard of care. Two papers published in *Cancer Discovery* unveil the molecular mechanisms that underpin this clinical response.

Previous findings have suggested a link between the androgen receptor, and DNA damage and repair. For example, two proteins, DNA protein kinase catalytic subunit (DNAPKcs; encoded by *PRKDC*) and Ku70 (encoded by *XRCC6*) that are involved in DNA repair pathways, might also be coactivators of the androgen receptor.

William Polkinghorn and colleagues and Jonathan Goodwin and colleagues found that castration-sensitive and castration-resistant prostate cancer (CRPC) samples and cell lines have reduced viability in response to treatment with both ADT and IR compared with either treatment alone.

Using gene set enrichment analysis (GSEA) Polkinghorn and colleagues initially found that activation of the androgen receptor alone increased levels of DNA damage in prostate cancer cell lines and that, in CRPC xenografts treated with ARN-509, a second-generation ADT, the expression of genes involved in DNA repair was reduced compared with untreated controls. Further work using human primary prostate cancer samples identified 144 DNA repair genes the expression of which was significantly associated with known androgen receptor target genes. Of these DNA repair genes, 32 contained a classic androgen receptor binding site.

Using quantitative PCR to identify substantial changes in specific genes that are part of the DNA repair transcriptome after treatment of CRPC cells with IR in the presence or absence of androgen, Goodwin and colleagues found three genes that were induced in the presence of androgen and DNA damage: *PRKDC*, and two RAD51 homologues *XRCC2* and *XRCC3*. Previously published chromatin immunoprecipitation and sequencing (ChIP–seq) data, as well as new ChIP–seq studies, verified that the androgen receptor was recruited to these genes after the treatment of hormone-stimulated cells with IR. Further experiments showed that DNAPKcs expression is induced in the presence of androgen and IR, and that DNAPKcs is phosphorylated and therefore active. These authors also noted that CRPC cells treated with ADT or another second-generation ADT drug, MDV3100, and cells treated with ADT and IR, had reduced levels of phosphorylated DNAPKcs, which were restored on treatment of the cells with dihydrotestosterone (DHT). Ku70, identified by Goodwin and colleagues as a potential androgen receptor target gene, can bind DNAPKcs and is involved in its activation. Small interfering RNA (siRNA)-mediated depletion of Ku70 decreased the levels of phosphorylated DNAPKcs in response to IR, indicating that activated androgen receptor might contribute to DNA repair by inducing *PRKDC* transcription and Ku70-mediated activation of DNAPKcs. Inhibition of DNAPKcs activity using a chemical inhibitor resulted in increased levels of nuclear foci containing γH2AX and 53BP1 (markers of DNA double-strand breaks) in the presence of IR. The number of foci was reduced in cells pretreated with DHT, and repair rates increased within two hours post-irradiation compared with cells not pretreated with DHT.

Both groups examined the effect of androgen signalling on homologous recombination-mediated DNA repair and non-homologous end joining (NHEJ) DNA repair. Goodwin and colleagues found that pretreatment of CRPC cells with DHT prior to DNAPKcs inhibition (to limit NHEJ repair) and exposure to IR increased repair rates by homologous recombination. Activation of the androgen receptor also increased NHEJ repair rates. These authors also found that DNAPKcs is involved in controlling the transactivation potential of the androgen receptor, and based on all their findings they have proposed a model in which activation of the androgen receptor directly induces transcription of *PRKDC* and indirectly induces the activation of DNAPKcs, resulting in positive feedback on the expression of the androgen receptor, increased efficiency of DNA double-strand break repair and increased cell survival. Polkinghorn and colleagues found that inhibition of androgen receptor signalling reduced NHEJ after exposure to IR, but that it had no effect on homologous recombination when NHEJ repair pathways were intact.

Both groups conclude that the links they have uncovered between DNA repair and the androgen receptor go some way to explaining the synergy between ADT and radiation therapy.

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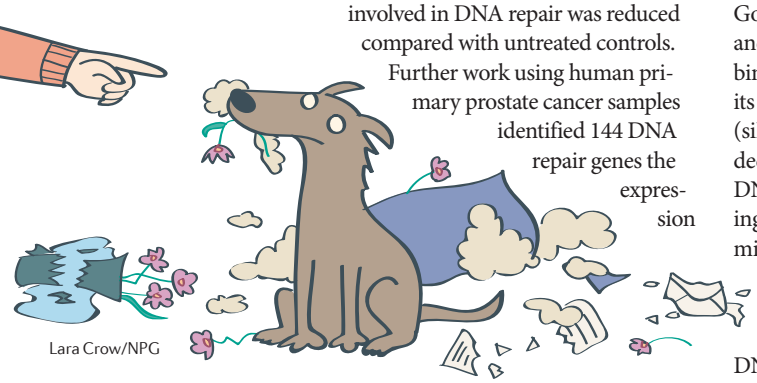

Lara Crow/NPG

ORIGINAL RESEARCH PAPERS Goodwin, J. F. *et al.* A hormone–DNA repair circuit governs the response to genotoxic insult. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-13-0108 (2013) | Polkinghorn, W. R. *et al.* Androgen receptor signaling regulates DNA repair in prostate cancers. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-13-0172 (2013)