Testosterone in Androgen Receptor Signaling and DNA Repair: Enemy or Frenemy?

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Running Title: Testosterone in Androgen Receptor Signaling and DNA Repair
SUMMARY

Androgen suppression mediates transcriptional down-regulation of DNA repair genes. Stimulation with supraphysiological levels of dihydrotestosterone induces formation of lethal DNA breaks through recruitment of topoisomerase II enzymes to fragile DNA sites. Bipolar castration and stimulation that contributes to increasing DNA damage represents a novel strategy of sensitizing prostate cancer to cytotoxic therapies, including radiotherapy.
In this issue of *Clinical Cancer Research*, Hedayati and colleagues (1) report a novel, but possibly counter-intuitive, strategy of exploiting sequential androgen suppression and stimulation to increasingly sensitize prostate cancer cells to radiotherapy. They attributed this effect to the induction of transient DNA double-strand breaks (DSB) following a combination of androgen-deprivation and supraphysiological levels of dihydrotestosterone. This DNA damage response occurs through an exclusive interaction between the androgen receptor (AR) and topoisomerase II beta (TOP2B). This increased accumulation of DSB following irradiation was significant enough to inhibit tumor growth *in vivo*.

The combination of androgen suppression and radiotherapy is a time-honored treatment regime with proven efficacy of advancing cure rates in patients with high-risk and locally-advanced prostate cancer. Numerous randomized trials, testing a variety of castration approaches, have conclusively confirmed the synergism between androgen suppression and radiation, reporting better tumor control rates (pooled HR 1.67) and reduction of distant metastases (pooled HR 1.63) when compared to radiotherapy alone (2). Based on the documented effects on both local and systemic disease, it is widely perceived that combined modality therapy with suppression of the AR-axis simultaneously enhances the cytotoxic effects of radiotherapy on the primary prostate cancer and also targets occult metastases.

The mechanistic basis of this clinical observation is starting to mature as increasing evidence indicates that activation of the AR-axis exerts an influence on the cellular DNA repair machinery and overall DNA damage response (Figure 1). In response to genotoxic stress, androgen ligand binding to the AR triggers a cascade of signaling events that promotes the assembly of transcriptional elements leading to the over-expression of DNA repair genes involved in DNA damage sensing, DSB repair, base excision repair (BER), and mismatch repair (MMR) (3). The process of AR-dependent transcriptional regulation is further modulated by the receptor binding with repair proteins, such as Ku, DNA-dependent protein kinase (catalytic subunit)
(DNA-PKcs), and poly(ADP-ribose) polymerase 1 (PARP1); these proteins also function as AR co-activators (4).

Given the positive feedback circuit linking AR-axis stimulation and DNA repair, it would suggest in principle that targeting the AR-axis represents a very sound and attractive strategy for potentiating the DNA damaging effects of cytotoxic therapies in prostate cancer. Importantly, it was observed in primary prostate cancer specimens undergoing neoadjuvant androgen deprivation therapy (ADT), that inhibition of the AR-axis leads to a reduction of Ku protein expression in post-ADT prostate biopsies (5). In this first-in-human proof of mechanism study, Tarish et al. demonstrated longitudinally that castration primarily affected both Ku and DNA-PKcs expression in response to radiotherapy, leading to significant impairment of the non-homologous end-joining (NHEJ) pathway of DSBs (5). In parallel, Polkinghorn et al. and Goodwin et al. also observed a radiosensitization effect as a consequence of androgen blockade, and attributed this to the transcriptional down-regulation of DNA repair-related genes, with DNA-PKcs being a key target (6,7).

Stimulation of the AR-axis, particularly with supraphysiological levels of dihydrotestosterone, can also contribute to DSB formation, through an AR-driven recruitment of enzymes to common fragile sites in the genome that are prone to illegitimate rearrangements (Figure 1). As first observed by Lin et al., ligand-bound AR acts to foster chromosomal rearrangements. This work also demonstrated that AR binding promotes site-specific DSB formation through a novel enzymatic machinery comprising of activation-induced cytidine deaminase (AID) and LINE-1 repeat-encoded ORF2 endonuclease (LINE-1 ORF2) (8). Another mechanism proposed by Haffner et al. involves the co-recruitment of AR and TOP2B to sites of TMPRSS2-ERG genomic breakpoints, facilitating formation of transient DSB secondary to TOP2B catalytic cleavage (9). To add, NKX3-1 may be responsible for accelerating the repair of such breaks or increasing genetic instability with clonal selection of mutator phenotypes, and allelic deletion of this gene has been linked to clonogenic radioresistance and tumor recurrence post-radiotherapy (3). The induction of DSB with androgen stimulation offers an additional paradigm to AR
manipulation for therapeutic synergism when combined with cytotoxic cancer therapies, and may affect both primary tumor and metastatic phenotypes.

This concept was most recently clinically tested by Schweizer et al. where men with low to moderate metastatic burden castrate-resistant prostate cancer (CRPC) were exposed to spikes of supraphysiological levels of dihydrottestosterone in the background of continuous castration therapy (10). In this study of 16 men, some of whom had progressed on second generation anti-androgen therapies, clinical responses (both biochemical and radiological) were recorded in 50% of the treated cohort. Consistent with Haffner et al. (9), the authors linked the clinical efficacy to incremental accumulation of DSB, as a result of stabilization of AR-induced transient DSB following etoposide (a TOP2 inhibitor). AR ‘over-stabilization’ contributing to loss of DNA relicensing, and subsequent mitotic death was also proposed as another mechanistic cause for tumor growth inhibition in vivo. When taken together, both experimental and clinical evidence support the therapeutic synergism between bipolar androgen stimulation and castration in prostate cancer through modulation of DSB induction and repair.

To validate these findings, trials will need to be conducted to address issues pertaining to patient selection, and scheduling of androgen deprivation and stimulation. In clinical practice, many men will receive at least two months of LHRH agonist prior to commencing radiotherapy. One combinatorial approach could be to initiate androgen stimulation 1-2 days before radiotherapy, and repeating every 2-4 weeks during treatment, given that stimulation lasts for approximately two weeks following an injection of supraphysiological dihydrottestosterone and track DSBs using in situ DSB biomarkers such as γH2AX or 53BP-1. Note that the transient nature of DSB induced by androgen stimulation is also an important mechanistic consideration, since majority of these DSB are repaired within 24 hours (9). However, the repression of NHEJ by continuous castration should also impede the repair of these site-specific DSB, resulting in prolonged stabilization of these lethal lesions. These are testable hypotheses with clinical trial specimens.
Of note, the field also needs molecular biomarkers that can identify specific patients with AR-dependent prostate cancers that are aggressive and at risk for recurrence following radiotherapy alone so that they can be offered combined ADT and radiotherapy. Our group recently reported that *NBN* copy number gain and high percent genome aberration (PGA) are highly predictive for biochemical relapse following radiotherapy, and such patients may be suitable for intensification with added androgen modulation (11,12). The safety profile of this more intensive treatment, if incorporating androgen stimulation as well, requires detailed evaluation in prospective clinical studies. Lastly, the proposed mechanism(s) of radiosensitization with ADT relies on a functional AR-axis; whether these novel approaches will also be as efficacious in hormone-insensitive tumor clones in later-stage disease (e.g., metastatic castrate-resistant or neuroendocrine prostate cancers) requires close study.

In conclusion, the synergism between androgen suppression and radiotherapy that has been observed for the past 15 years can now be partially explained by modulation of repair of radiotherapy-induced DSB. New mechanistic insights into the complex interplay between androgen manipulation and DNA repair are now giving rise to novel treatment strategies with radiotherapy or other agents to sensitize aggressive prostate cancers and improve cure.

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References


**Figure legend**

Figure 1. Interplay between androgen receptor (AR)-axis manipulation and DNA repair in prostate cancer. AR is over-expressed in response to radiotherapy (RT), and mediates radioresistance by transcriptional up-regulation of DNA repair genes. Ku70, DNA-PK, and PARP1 partake in a positive-feedback circuit by acting as transcriptional co-activators. AR-axis suppression counters this process, and radiosensitizes by inhibiting DNA double-strand break (DSB) repair. Paradoxically, AR-axis stimulation with supraphysiological levels of dihydrotestosterone (DHT) triggers the assembly of enzymes at genomic fragile sites, contributing to DSB formation. Such androgen manipulation can be a potential radiosensitization strategy through increased generation of DSB. (Pink-circled) Copy number alterations of NBN (11) is a potential molecular biomarker of radioresistant prostate cancer; Ku70 and DNA-PK are key proteins involved in the synergism between AR-axis suppression and RT (5,7); PARP1 represents a ‘druggable’ target.

Abbreviations: luteinizing hormone-releasing hormone (LHRH); single-strand break (SSB), hormone response element (HRE), prostate-specific antigen (PSA), homologous recombination (HR), base excision repair (BER), mismatch repair (MMR).
Figure 1:

Interplay between androgen manipulation and DNA repair in prostate cancer

**Sensitization through suppression of AR-axis**
- Androgen suppression
  - Orchiectomy or LHRH agonists (e.g., goserelin) and antagonists (e.g., degarelix) or ARN509, abiraterone
- Antiandrogen therapy
  - Bicalutamide, enzalutamide

DHT

Membrane

Nucleus

DNA damage (RT, chemotherapy)

**Sensitization through stimulation of AR-axis**

AR AR

Supraphysiological DHT

AR AR

AR AR

HRE PSA

Coactivators
- Ku70
- DNA PKcs
- PARP1

AR-regulated transcriptional machinery of DNA repair genes

AR-induced enzymatic machinery contributes to DSB formation

DSB at genome fragile sites

TOP2B

LINE1 ORF2

AID

SSB/bases/adducts

PARP1 BER

NHEJ XRCC5 XRCC4 Ku70 DNA PKcs

HR RAD54B RAD51C

BER PARP1 Lig3

MMR MSH2 MSH6

DNA repair genes

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