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Sensitization of prostate cancer to radiation therapy: Molecules and pathways to target

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ABSTRACT

Radiation therapy is used to treat cancer by radiation-induced DNA damage. Despite the best efforts to eliminate cancer, some cancer cells survive irradiation, resulting in cancer progression or recurrence. Alteration in DNA damage repair pathways is common in cancers, resulting in modulation of their response to radiation. This article focuses on the recent findings about molecules and pathways that potentially can be targeted to sensitize prostate cancer cells to ionizing radiation, thereby achieving an improved therapeutic outcome.

In western developed countries, prostate cancer is the most common cancer diagnosed in men [1]. Radiation therapy, which includes both external radiation such as photon and proton beams and internal radiation like brachytherapy, is a common treatment modality for prostate cancer. Based on 2010–2012 Prostate Cancer Treatment Patterns by Age in US, 23% of men less than 64 years of age, 36% of men between 65 and 74 and 33% over 75 years of age received radiation therapy as part of their prostate cancer treatment [2]. Radiation therapy is utilized mainly in patients where the cancer is localized or locally advanced with poor surgical indications [3]. The fundamental mechanism by which ionizing radiation achieves a therapeutic response is considered to be through initiating DNA damage in cancer cells, most notably double-strand breaks (DSBs), directly or indirectly through accumulation of reactive oxygen species [4].

Cancer cells are capable of utilizing DSB repair pathways to override the radiation-induced cytotoxicity [5,6], which is counter-productive to the therapy. As a result, radiation resistance can develop. Other pathways and molecules, which are not an integral part of the DSB repair pathways, can also affect the response of prostate cancer cells to radiation. Thus, while *in vitro* targeting certain molecules on relevant pathways has been demonstrated to sensitize prostate cancer cells to ionizing radiation, it is yet to be established if and how those molecules and pathways are associated with radioresistance. *In vivo*, there are other factors that need to be considered when interpreting a radiosensitizing effect, such as the immune system [7] and the tumor microenvironment, including angiogenesis [8,9]. Importantly, any increase in the efficacy of radiotherapy should not be at the expense of potential damage to surrounding normal tissues. Questions to ponder include whether pharmacological inhibiting or genetic silencing of DNA repair pathways *in vivo* will also disable the normal cells from recovering from the radiation damage. Also, we should ask whether cancer cells rely more on the repair pathway than normal cells so a differential response to suppressing DNA repair pathways can be expected. In this review, we summarize the pathways and representative molecules that have been successfully targeted to improve radiosensitivity in pre-clinical studies of prostate cancer.

DNA repair pathways: Ataxia telangiectasia mutated (ATM)

DSBs are detected by the Mre11, Rad50 and Nbs1 protein complex, which eventually activate ATM [10]. By rapidly phosphorylating downstream effectors, ATM initiates a cascade of DNA damage responses, resulting in activation of cell cycle checkpoints. The consequent halt in cell cycle progression enables the radiated

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cells to perform DNA repair by exploiting two major sets of machineries: the error-prone non-homologous end joining (NHEJ) and the more accurate homologous recombination (HR).

Fan et al. investigated if down-regulation of ATM would enhance radiosensitivity of prostate cancer cells [11]. To this end, PC-3 cells were infected with adenoviral vectors expressing antisenses to different regions of ATM gene. As a result, ATM was diminished at protein level within 2 days following the viral infection. Compared to control, the reduction in ATM protein abolished cell cycle checkpoint at S phase to a large degree after radiation, which was reflected by a minimized halt in DNA synthesis. This led to a radiosensitivity of the PC-3 cells as illustrated by reduced cell proliferation and colony formation.

Silibinin is a natural polyphenolic flavonoid and can be isolated from the seed extracts of the herb milk thistle [12]. Nambiar et al. reported that Silibinin can inhibit radiation-induced DNA repair involving ATM and downstream Chk1/2 [13]. In vitro, Silibinin sensitized PC-3 and DU145 cells to radiation, evidenced by diminished clonogenic formation, enhanced radiation-induced G2/M arrest, apoptosis and reactive oxygen species formation. Silibinin also suppressed radiation-induced nuclear translocation of DNA-PK, an important mediator of DSB repair, leading to a delayed resolution of phospho-H2AX (ser139) foci. *In vivo*, a combination of radiation and silibinin had greater inhibitory effect on DU145 cell xenograft growth compared to radiation alone, with a 10-fold increase in apoptotic response [13]. Thus, silibinin can enhance the radio-therapeutic response by suppressing radiation-induced DSB repair and pro-survival signaling (Fig. 1A).

DNA repair pathways: Poly (ADP-ribose) Polymerases (PARP)

PARP1 is the most highly expressed member among the PARP family and is implicated in DNA SSB repair that usually takes place during DNA synthesis [14]. Therefore, an inhibition of PARP1 would result in conversion of unrepaired SSBs to DSBs upon their collision with an ongoing replication fork, which triggers DNA repair by HR. Accordingly, the cancer cells with deficient HR (e.g., as a result of BRCA1 or BRCA2 mutation) are sensitive to PARP1 inhibition probably because the resultant DSBs are left unrepaired, leading to cytotoxicity [15].

In the presence of a genotoxic agent or radiation causing DSB of DNA, PARP1 inhibition would sustain the damage as a result of suppressing a backup DNA repair mechanism in HR deficient cancer cells with BRCA1 or BRCA2 mutation, leading to synthetic lethality [16]. Notably, the approach to inhibit PARP1 should be more effective to the cancer cells where PARP1 is hyperactivated in the presence of HR defect [17].

It has been reported that the deleterious mutations in BRCA1 and BRCA2 (diagnosis of prostate cancer at $\leq$65–69 years) are respectively 0.45% [18] and 1.20% [19]. Although certain benefits of PARP inhibition were observed in patients with BRCA2 mutation [20], an efficacy of PARP inhibition has been also documented in patients without BRCA mutations. Therefore, a strong need is required for defining other factors that contribute to PARP inhibition sensitivity in prostate cancer.

Nonhomologous end joining (NHEJ) encompasses classical NHEJ and alternative NHEJ [21]. PARP1 has been found to promote alternative NHEJ [22]. Consistently, sensitization to radiation was reached with PARP inhibition in mouse embryo fibroblasts where ligase IV, a component of classical NHEJ, was absent [23]. In a panel of cancer cells including prostatic LNCaP and PC-3 cells in which DSB-repair is switched to alternative NHEJ, radiosensitization was achieved by suppression of DNA damage repair with a PARP inhibition. In contrast, the radiosensitization was not evident in DU145 cells where the switch was not obvious [24].

To evaluate genetic determinants that may govern radiosensitization with PARP inhibition, clonogenic survival test was conducted in a panel of prostate cancer cells following radiation and PARP inhibition with rucaparib. The combination index revealed the most prominent synergy occurred in the cancer cells expressing ETS gene fusion proteins (VCaP) or absence of PTEN (LNCaP), both correlated with persistent DNA breaks as determined by phospho-H2AX, p53BP1, and Rad51 foci as well as senescence indicated by β-galactosidase activation. Clinically, a sizeable portion of prostate cancers harbor a gene fusion consisting of the 5′ region of androgen-regulated TMPRSS2 and a 3′ end ETS family transcription factor, most commonly ERG [25,26]. TMPRSS2-ERG blocks NHEJ DNA repair by inhibiting DNA-PKcs. However, due to rescue mechanism related to PARP1, TMPRSS2-ERG alone did not result in radiosensitization. In combination with PARP inhibition, radiation then enhances DNA damage in TMPRSS2-ERG-expressing cells due to the inhibition of PARP-related rescue mechanism. As rucaparib-induced radiosensitization was more effective in the presence of TMPRSS2-ERG or absence of PTEN, the combinatory therapy could be applicable to the patients bearing these genetic alterations (Fig. 1A).

It has been reported that hypoxia is related to an earlier relapse of prostate after radiotherapy [27]. To test whether a PARP inhibitor AZD-2281 functioned as a radiosensitizing agent under hypoxic condition, Gani et al. treated 22Rv1 cells by radiation in the presence or absence of the inhibitor [28]. Radiosensitization was achieved with AZD-2281 under acute hypoxia or chronic hypoxia as well as normoxia *in vitro*. Consistently the combination of fractionated radiotherapy with the PARP inhibition brought about a delayed growth of tumor *in vivo* with a reduced survival fraction measured by an ex vivo clonogenic survival assay.

Taken together, the studies aforementioned illustrate that radiosensitization of prostate cancer cells can be materialized by administration of PARP inhibitors and it is worthwhile to determine the clinical efficacy of the combination.

Androgen receptor (AR) and DNA repair

Radiation therapy is one of the mainstays in the treatment of prostate cancer. However, the treatment usually activates AR activity and renders subsequent disease relapse. Spratt et al. showed that AR expression, its nuclear translocation and transcriptional activity were increased following radiotherapy in a durable manner under both *in vitro* and *in vivo* conditions [29]. Notably, the amplitude of increase in AR gene expression following radiation compared to baseline was correlated with a lower degree of DNA damage measured by immunofluorescence of phospho-H2AX and comet assay, a higher clonogenic survival fraction and a shorter time to tumor progression. Clinically, the patients with higher AR activity, reflected by serum levels of AR-regulated hK2 protein following radiation, had an increased likelihood to experience biochemical relapse of the disease [29]. These observations indicate that radiation-induced activation of AR may lead to an enhanced transcription of genes that are involved in DNA repair, suggesting a better clinical outcome could be achieved by a combination of androgen-deprivation therapy (ADT) and radiation compared to radiation alone [30].

To gain an insight into the relationship between AR functional status and expression of genes implicated in DNA repair, Polkinghorn et al. first illustrated that antiandrogen ARN-509 down-regulated transcription of DNA repair genes in a xenograft model of castration-resistant prostate cancer. Likewise, they also found that there was a correlation of canonical AR output and enriched DNA repair genes in clinical samples of prostate tumor. Subsequently, they delineated 32 genes that were direct targets of AR

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