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# Profiles of Radioresistance Mechanisms in Prostate Cancer

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## Abstract

Radiation therapy (RT) is commonly used for the treatment of localized prostate cancer (PCa). However, cancer cells often develop resistance to radiation through unknown mechanisms and pose an intractable challenge. Radiation resistance is highly unpredictable, rendering the treatment less effective in many patients and frequently causing metastasis and cancer recurrence. Understanding the molecular events that cause radioresistance in PCa will enable us to develop adjuvant treatments for enhancing the efficacy of RT. Radioresistant PCa depends on the elevated DNA repair system and the intracellular levels of reactive oxygen species (ROS) to proliferate, self-renew, and scavenge anti-cancer regimens, whereas the elevated heat shock protein 90 (HSP90) and the epithelial-mesenchymal transition (EMT) enable radioresistant PCa cells to metastasize after exposure to radiation. The up-regulation of the DNA repairing system, ROS, HSP90, and EMT effectors has been studied extensively, but not targeted by adjuvant therapy of radioresistant PCa. Here, we emphasize the effects of ionizing radiation and the mechanisms driving the emergence of radioresistant PCa. We also address the markers of radioresistance, the gene signatures for the predictive response to radiotherapy, and novel therapeutic platforms for targeting radioresistant PCa. This review provides significant insights into enhancing the current knowledge and the understanding toward optimization of these markers for the treatment of radioresistant PCa.

### Keywords

radiotherapy; prostate cancer; DNA repair; oxidative stress; EMT; HSP90

## I. INTRODUCTION

### A. Clinical Problem with Prostate Cancer Therapy

Prostate cancer (PCa) is the most commonly diagnosed cancer in males in Western countries. Risk factors for PCa development include age, race, and family history and

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possible risk factors include diet, lifestyle, androgens, and inflammation. One in six men will get PCa during his lifetime. The American Cancer Society estimated that there would be approximately 161,360 new cases of PCa diagnosed in the United States in 2017, resulting in an estimated 26,730 deaths.[1] Although serum markers for prostate malignancy may indicate the presence of cancer, they do not predict those patients in which localized cancer is predestined to form infiltrative or metastatic cancer.

Early-stage confined PCa can be treated by radical prostatectomy, radiotherapy (RT), or watchful waiting.[2] Surgery, RT, or a combination of both are the primary treatment of localized PCa. Androgen deprivation therapy (ADT) is the standard approach for patients with advanced metastatic PCa and poor prognosis.[3] Despite the initial success of ADT, castration-resistant prostate cancer (CRPC) will eventually evolve, resulting in tumor recurrence and patient morbidity. To date, survival of patients with CRPC has significantly improved as a result of the development of second-generation anti-androgen therapies such as abiraterone and enzalutamide.[4,5]

To minimize toxic effects from treatment, low-risk patients (Gleason scores ≤ 6, prostate-specific antigen [PSA] concentrations < 10 ng/mL, or T1-T2a) are often offered an active surveillance. In contrast, treatments of patients with intermediate-risk PCa (Gleason scores = 7, PSA concentrations of 10–20 ng/mL, or T2b—c) and high-risk PCa (Gleason scores ≥ 8, PSA concentrations ≥ 20 ng/mL, or T3/4) include radical prostatectomy or some type of RT.[6,7] To improve RT outcomes, adjuvant radiotherapy (co-administration of therapeutic agents with RT) and neoadjuvant therapy (administration of therapeutic agents before RT) are employed. RTOG 8610 was the first randomized phase III trial to evaluate neoadjuvant ADT that started 2 months before and then 2 months concurrently with external beam radiotherapy (EBRT) in men with locally advanced PCa.[8] The ADT plus EBRT arm had statistically significant improvements in 10-year overall survival (43% vs. 34%, $p = 0.12$) and disease-free survival (11% vs. 3%, $p < 0.001$) and statistically significant reduced 10-year PCa-specific mortality (23% vs. 36%, $p = 0.01$), distant metastasis (35% vs. 47%, $p = 0.006$), and biochemical failure (defined as a rise in the blood level of PSA after treatment with surgery or RT, 65% vs. 80%, $p < 0.0001$[9]) compared with the radiation alone arm. The 10-year results of the European Organization for Research and Treatment of Cancer support the addition of long-term ADT to EBRT in treating high-risk PCa.[10] Although these adjuvant therapies have shown significant clinical benefit, the side effects and late complications limited the full potential of RT.

The therapeutic response to RT is determined by the intrinsic radiosensitivity (differential response of cancers to low-dose RT), that is, levels of DNA damage-repairing enzymes, and/or acquired radiation resistance, that is, increased reactive oxygen species (ROS) or antioxidants, with factors driving radioresistance linked to oxidative stress. In this review, we consider the molecular and therapeutic effects of ionizing radiation (IR) on PCa and the underlying mechanisms that could result in an increased capacity of irradiated cancer cells to overcome the anti-proliferative effects of RT and/or repair radiation damage. We also discuss recent advancements in the identification of markers of radioresistance such as DNA repair enzymes, oxidative stress markers, and protein chaperones; the impact of the tumor microenvironment; and translational strategies that integrate cancer genomics, redox state,

and epithelial-mesenchymal transition (EMT) toward the development of therapeutic platforms for overcoming radioresistant PCa. The changes in these markers correspond to the resistant phenotype among different tumors.

## II. EFFECTS OF IR: THE GOOD AND THE BAD

The primary cellular target of IR is chromosomal DNA. Photon radiation damages intracellular molecules by direct ionization and through indirect ionizations mediated by water radiolysis products (Fig. 1).[11,12] The most common DNA damage is DNA breaks: single-strand breaks (SSBs), and double-strand breaks (DSBs).[13–15] Most DSB repair is accomplished by two mechanisms: homologous recombination (HR) and nonhomologous end joining (NHEJ). NHEJ is the predominant DSB repair mechanism during the $G_1$ phase; it repairs a direct, two-ended radiation-induced DSBs. HR is crucial for the repair of complex DNA DSBs.[16] *BRCA1* (a phosphoprotein that assists in 5' to 3' resection of DSBs and loading of RAD51) and *BRCA2* are large nuclear proteins that play an integral role in the HR pathway.[17] Mutations in BRCA genes have been associated with increasing the risk of PCa as well as outcomes and response to therapy.[18] Therefore, these DSBs, if not re-annealed at the site of the original break, may re-anneal with other breaks on the same chromosome or a different chromosome toform chromosomal aberrations.[19] γH2AX is often used as a biomarker for DBS.[20] Analysis of DSB repair kinetics revealed that cancer cells had increased levels of γH2AX formation 2 h after IR.[21]

As mentioned above, one of the indirect actions of radiation is a multicellular effect by water radiolysis that produces free radicals in a very short period of time (< 1 sec), such as hydrated electron-ionized water, the hydroperoxyl radical, the hydrogen radical, and the hydroxyl radical.[22,23] Hydroxyl radicals are highly reactive and can diffuse far enough to reach and damage biomolecules, including DNA.[24,25] At least 50% of radiation-mediated DNA damage is caused by indirect effects from free radicals derived from low linear energy IR.[26] To a large extent, these free radicals can initiate a chain of events that results in biological damage or further induce ROS. It has been demonstrated that ROS formation increased immediately after irradiation and continued for several hours.[27] Production of hydrogen peroxide and two important species, solvated electron and hydroxyl radical, occurs in ~$10^{-12}$ sec and these species remain in biological systems for times ranging from 0.1 to 1 ms.

It has been reported that IR induces secondary ROS generation via electron leakage from the mitochondrial electron transport chain complexes.[28,29] It was demonstrated that IR elicits state 4 respiration and mitochondrial hyperpolarization, which results in mitochondrial ROS production.[30] Therefore, IR-induced damage of mitochondrial integrity leads to mitochondrial dysfunction, ultimately inducing local and systemic oxidative stress and cell death. Conversely, McDermott et al. reported that mitochondrial ROS accumulation contributes to the survival of irradiated PCa cells.[31] Radiation-mediated mitochondrial ROS production was associated with reduced levels of DNA damage, CD44 expression, and apoptotic cells.

Other sources of secondarily generated ROS after IR include NADPH oxidase, Ras- related C3 botulinum toxin substrate 1 GTPase, and ER-stress-mediated ROS mechanisms.[32–34] Paradoxically, IR also promotes EMT to allow cancer cells to evade stress within the microenvironment.[35,36] IR-induced ROS generation can increase the activity of transcription regulators of EMT, such as Snail, hypoxia-inducible factor-1 (HIF-1), and transforming growth factor-beta (TGF-β).[37] This secondary ROS production leads to an array of biological effects, including aberrant apoptosis, genomic instability, and radiation-induced bystander effects, ultimately affecting cancer cell integrity and survival. Among the ROS, $H_2O_2$ is considered to be a molecule that activates cell signal or damages biomolecules due to its ability to diffuse across membranes.[24,25]

## III. BIOCHEMICAL MECHANISMS UNDERLYING RADIOADAPTIVE CHANGES

The development of resistance to radiation is one of the complications of PCa treatment. Several molecular entities associated with radioresistance have been identified in PCa[38–40]; nevertheless, the underlying mechanisms are still inconclusive. We proposed that radioresistance phenotypes are driven by diverse molecular mechanisms including altered DNA repair, adapted mitochondrial and cytoplasmic redox states, and favorable tumor environment (Fig. 1). It is worth noting that these mechanisms are not exclusive to radioresistant PCa development.

### B. Repairing of DNA Damage

DNA damage and DNA repair decide the fate of irradiated cells.[41,42] The DNA damage inducible checkpoints occur in all phases of the cell cycle; $G_1$, S, and G2 and are initiated by Ataxia telangiectasia mutated (ATM) and ATM and RAD3-related (ATR).[43] ATM, in cooperation with the trimeric protein complex composed of MRE11-RAD50-NBS1 (MRN), is the earliest responder to DNA DSBs.[44] Aberrations in ATM were reported in CRPC patients,[45] which affected the tumor suppressor functions of ATM; that is, the damage checkpoints, programmed cell death, and DNA repair pathways.[13,46] ATR is a DNA damage sensor and its absence in ATR results in deficient HR. Because RT triggers cell cycle checkpoints to block cells from entering mitosis, the apparent overexpression of ATM/ATR could simply reflect changes in the radioresistant activity of PCa. Interestingly, it has been reported that oxidative stress can also activate ATM by a mechanism independent of DNA DSBs.[47]

PARP-1, which is functionally involved in DNA damage repair and transcription factor regulation, is up-regulated in PCa and has been implicated in PCa radioresistance.[48,49] The elevated PARP-1 expression among the tumor cells undergoing EMT implicates that DNA repair mechanisms may potentially reverse the cytotoxic effects of RT-induced DNA breaks via phenotypic programming.[50,51] Moreover, the IR-mediated ROS is capable of inducing 8-hydroxy-2’-deoxyguanosine (80HdG) and reducing DNA repair[52] via cysteine modification of 8-oxogua-nine DNA glycosylase 1 (OGG1).[53] Accordingly, the interaction between OGG1 and PARP-1 has been described recently.[54] A further example of IR-induced PARP-1 is Apurinic-apyrimidinic endonuclease 1 (APE-1), which functions as DNA repair and

transcriptional regulatory proteins by facilitating binding of transcription factors to DNA.[55] IR-mediated ROS production after $Ca^{2+}$ mobilization via purinergic receptor-induced extracellular ATP stimulation is responsible for the localization of APE-1[56] and this interaction can stimulate PARP-1 activity and potentially cancer radioresistance.[57]

### C. Alteration of Cellular Dynamics

IR-induced mitochondrial dysfunction, especially decreased electron transport chain complex activities, produces a feed-forward loop that contributes to persistent oxidative stress.[58] Because the mitochondrion is an energy-generating organelle, mitochondrial dysfunction due to IR exposure would mediate alterations or adaptive responses of metabolic pathways involved in radioresistance development.[59–61] Up-regulation of OXPHOS and mitochondrial ROS are correlated with radiosensitivity in several cancer cell lines.[62,63] The increase in mitochondrial (mtDNA) content and mass after IR points to the up-regulation of mitochondrial biogenesis[64] with increased point mutations and deletions of mitochondria in patients treated with RT.[65] Petros et al. have demonstrated an association among four different mutations in cytochrome oxidase subunit I (m.6253T>C, m.6340C>T, m.6261G>A, and m.6663A>G), which has been implicated in PCa radioresistance, potentially via IR-induced increase of mitochondrial mass.[66,67] Studies from other investigators demonstrated an increase of mtDNA after IR.[28] Because mtDNA is prone to being attacked by IR-induced ROS, it is also potentially a source of the acquired radioresistant phenotype. Interestingly, cells depleted of mtDNA, $\rho^0$ (rho-zero) cells are radioresistant, possibly due to suppression of the G2 checkpoint.[68,69] Yamamori et al. demonstrated that IR increases mitochondrial membrane potential, mitochondrial respiration and content, and ATP production,[70] which eventually lead to up-regulation of mitochondrial OXPHOS. Consistent with other evidence,[70–72] Lu et al. revealed that cancer cells quickly adapt to radiation damage via mammalian target of rapamycin (mTOR)-mediated reprogramming of bioenergetics from predominantly aerobic glycolysis to mitochondrial OXPHOS via relocation of mTOR to the mitochondria and inhibiting hexokinase II.[62] Conversely, mitochondria are prone to radiation damage as a result of: (1) lack of histone protection and repair mechanisms[73,74] because mtDNA is 10 times more susceptible to DNA damage than nuclear DNA and (2) the proximity to the major source of these ROS. Therefore, a decrease in mtDNA content has been reported as a result of RT.[75] The dichotomous role(s) of mitochondria in radiation sensitivity has been investigated *in vitro* and *in vivo*.[76–79] Collectively, these data support the concept that IR targeting mitochondria plays a role, at least in part, in radiosensitivity.

A rapidly expanding body of evidence suggests that glycolysis might impede radiation treatment in radioresistant cancer cells.[80] In a study by Chang et al., the glycolysis pathway was found to be activated in radioresistant DU145 and PC3 cells and radioresistant PCa xenografts.[81] The investigators further found that ALDOA, a glycolytic enzyme that catalyzes the reversible reaction of fructose-1,6-bisphosphate to glyceraldehydes-3-phosphate and dihydroxyacetone phosphate,[82] is up-regulated using the label-free LC-MS method in radioresistant DU145 and PC3 cells. Shimura *et al.* found that AKT-mediated enhanced aerobic glycolysis causes acquired radioresistance by tumor cells.[83] Mechanistic evidence established that targeting H IF-lα and tumor glucose metabolism reduced the

antioxidant capacity of tumors, affected the microenvironment, and sensitized various solid tumors to IR.[84] Lactate dehydrogenase-5-isoenzyme, a marker of cancer anaerobic metabolism, is also significantly linked to highly proliferating prostate with biochemical failure and local relapse after RT.[85]

Metabolic alterations in radioresistant PCa may extend beyond an increase in glycolysis. A recent study indicated that PCa undergoes exacerbated endogenous fatty acid biosynthesis through inhibition of lipogenic enzymes such as the fatty acid synthase, ATP-citrate lysate, acetyl-coenzyme A, carboxylase 3, or stearoyl-CoA desaturase, resulting in decreased proliferation and increased apoptosis of radioresistant cancer cells.[86,87] Furthermore, using an integrative molecular epidemiology approach, Kelly et al. characterized metabolic signatures at the mRNA level in prostate tumors and identified pyrimidine metabolism as the most deregulated pathways in lethal cancers, including radioresistant phenotypes.[88] Taken together, these results indicate that glycolysis, lipid synthesis, and pyrimidine pathways are involved in radioresistance and targeting these pathways is likely to have broad therapeutic applications for cancer radioresistance.

### D. Oxidative Stress Overloaded

The association between PCa progression and oxidative stress has been well recognized. Altered mitochondrial bioenergetics likely underlies the development of PCa and the more aggressive phenotype is directly proportional to a degree of ROS generation. PCa cells have a higher level of ROS, including oxidative stress markers, compared with normal prostate cells and the level of oxidative stress is associated with PCa occurrence, recurrence, and progression (Fig. 2).[89–92] Studies have demonstrated that maintaining a balance of ROS levels in cancer is an important mechanism of radioresistance.[93] The level of ROS is also critical to the radiation response; therefore, IR tends to induce ROS levels and adaptive antioxidant defense systems, which may lead to radioresistance.[94–96] We have demonstrated that cancer cells are exposed to high oxidative stress at an early stage of tumorigenesis, in part due to the inhibition of various antioxidant enzyme activities such as manganese superoxide dismutase (MnSOD).[92,97,98] ROS/reactive nitrogen species (RNS) cause oxidative and nitrosative/nitrative stress in cells, functioning as secondary messengers in signal transduction cascades (Fig. 2). There is a close connection between radioresistance of PCa and oxidative stress. Oxidative stress activates DNA damage, which promotes an anti-survival role, and DNA damage repair, which promotes an anti-apoptotic role. It is well established that intracellular ROS levels are increased significantly after IR exposure and that elevated levels of ROS are persistent for several hours after initial IR.[99] In fact, oxidative stress in the G2/M phase lasts three times longer in irradiated cancer cells compared with non-irradiated cells.[100,101] Consistent with this, $H_2O_2$ levels are increased significantly in the radioresistant phenotype of PC3 and DU145 cells,[102] whereas increased lipid oxidation and malondialdehyde formation, as byproducts of IR-mediated ROS, are detected in both irradiated and bystander cells and are correlated with a significant decrease in MnSOD activity.[103] Although no IR-caused changes in cellular oxidative stress have been reported,[104,105] depending on tolerance level to oxidative stress, different cancer cells may exhibit a differential response to ROS elevation.

Persistent oxidative stress can regulate various transcription factors/activators, such as nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2), nuclear factor kappa-beta (NF-κB), and p53, thereby influencing the cell cycle and DNA repair expression and modulating cellular survival signaling pathways.[93] Among redox-sensitive transcription factors, Nrf2 is recognized as a key feature for protecting cells from apoptosis, xenobiotic metabolism enzymes, and drug efflux pumps leading to the radioresistant phenotype.[106] The up-regulation of Nrf2 target genes, such *as heme oxygenase-1*, *NAD(P)H dehydrogenase (NQO1),* and *peroxiredoxin (Prx),* have also been confirmed in relation to cancer radioresistance.[107] Overactivation of the Nrf2-mediated defense system leads to cancer cell protection from their inherently stressed microenvironment and anti-cancer treatments including PCa, lung, breast, gall bladder, ovarian, and colorectal cancers.[108] Accompanied Nrf2, NQO1, an ubiquitous enzyme involved in detoxifying pathways, can be activated by IR, thus contributing to the radioresistant phenotype.[108] Significantly enough, the expression of both N Q O l and Nrf2 is increased dramatically in PCa cells intrinsically resistant to R T[109] Prxs are thiol-specific antioxidant proteins that are classified largely on the basis of having either one (1-Cys) or two (2-Cys) conserved cysteine residues.[110] Prx1, a major member of the 2-Cys subfamily, is also a target gene of Nrf2.[111] Decreased Prx1 expression is associated with augmentation of radiosensitivity,[112,113] whereas activation of Prx enhances radioresistance of cancer cells.[114,115] Prx6 protein expression is increased significantly in radioresistant PC3 and D U −145 cells compared with parental cells.[81] Taken together, these results strongly support that IR-generated ROS can stimulate Nrf2-associated detoxification enzymes and antioxidants, which promote cell survival under conditions of oxidative stress.[108]

In addition to IR-induced Nrf2 activity, the elevation of NF-κB activity in certain cancers has been associated with resistance to treatment.[116] Mechanistically, ATM activation of NF-κB serves as an interexchange molecule for IR-activated NF-κB.[117] It was demonstrated that ATM is essential for activation of the entire NF-κB pathway, including IKK activation, IκB-α degradation, and induction of NF-κB DNA-binding activity, in both human cells and mouse tissues after DSB activation.[118] There is no direct evidence, however, to suggest that ATM-mediated NF-κB activation is required for the adaptive radioresistance phenotype. DNA-dependent protein kinase, a member of the phosphoinositide 3-kinase (PI3K)-like family of protein kinases, has also been implicated in the activation of NF-κB after exposure to IR.[119] Interestingly, inhibition of IR-induced NF-κB binding is cell-type specific. For example, in PCa cells, inhibition of NF-κB by a dom inant-negative IκB m utant after exposure to a radiation dose that is equivalent to medical diagnostic use enhances apoptosis in DU145 but not in PC3 PCa cells.[120,121] The induction of radiosensitivity is also mediated by several genes that are regulated by NF-κB, such as *XIAP, A20, FLIP,* and *Bcl-xL* (www.bu.edu/nf-kb/), to either regulate activation of survival pathways or to inhibit apoptotic signaling pathways.[122] Overall, the critical role of NF-κB in the emergence of radioresistance in tumors after RT and fractional IR is widely recognized.

Due to IR-mediated ROS production, depletion of intracellular antioxidants such as glutathione (GSH), thioredoxin, Prx, and superoxide dismutase (SOD), can actually enhance radiation sensitivity; in contrast, up-regulation of these redox-regulating enzymes can protect radiation damage.[123] Overexpression of antioxidant enzymes often occurs in cancer resistance to RT, so blocking these antioxidant enzymes enhances radiation sensitivity by

promoting radiation-induced apoptosis.[108,124] Radiation-resistant mice exhibit higher levels of SOD and catalase (CAT) activities compared with radiation-sensitive mice.[125] MnSOD is one of the most important antioxidant enzymes located exclusively in the mitochondria and modulates cellular redox status and influences the effects of radiotherapy.[126] MnSOD up-regulation has been implicated in adaptive response induced by fractionated doses of IR, leading to radioresistance.[38,127] We proposed that MnSOD is one of a key NF-κB effector genes in radioadaptive resistance. NF-κB and MnSOD expressions are up-regulated in PCa cells after exposure to low-dose IR.[128] Inactivation of NF-κB with an IKK-β inhibitor (IMD-0354) suppressed low-dose IR, induced expression of MnSOD, and diminished the adaptive radioresistance.[128] These results indicate that NF-κB-mediated MnSOD contributes to the scavenging of radiation-induced ROS and the signaling network leading to adaptive radioresistance. We demonstrated previously that selective inhibition of RelB (proto-oncogene, NF-κB subunit) after irradiation concomitantly decreased MnSOD and sensitized PCa cells to IR.[38,129–131]

In Fig. 3, we propose that radioresistant PCa cells maintain their redox state toward high levels of ROS and antioxidants to enable their escape and consistent proliferation post-radiation. Therefore, the ability of radioresistant PCa cells to survive and sustain a high proliferative activity is associated with persistent ROS production, up-regulation of redox-sensitive transcription factors, and higher antioxidant levels that scavenge ROS.

## E. EMT Landscape

EMT confers mesenchymal properties on epithelial cells. The ability for cancer cells to become established in regional and distant metastatic sites is dependent upon EMT. It is characterized by the loss of epithelial morphology and markers (i.e., E-cadherin, desmoplakin, Muc-1, and cytokeratin-18) and by the acquisition of mesenchymal markers (i.e., N-cadherin, vimentin, fibronectin, and vitronectin). IR is also known to enhance the metastatic potential of cancer cells by inducing EMT, which potentially mediates the emergence of the radioresistant phenotype.[132] Fractionated IR has been shown to induce EMT in prostate tumors by activation of the cAMP response element binding protein and cytoplasmic sequestration of the activating transcription factor 2.[133] Loss of E-cadherin is associated with attenuated radiation-induced DNA damages during hypoxia, thus contributing to radioresistance of tumor cells.[134] Recently, we identified a marked decrease in E-cadherin protein expression paralleled with an increase in N-cadherin and vimentin expressions in post-RT specimens compared with pre-RT of PCa.[135] The EMT phenotypic signature has been associated with biochemical recurrence and radioresistance.[136]

Mechanistically, IR-induced EMT is mediated by transcription factors (i.e., E12/E47, Snail/Slug, STAT3, Twistl/2, ZEB l/δEFl, and ZEB2/SIP1) that are activated by an array of signaling pathways (i.e., Hedgehog, Notch, TGF-β, Wnt, EGFR/PI3K/Akt, CXCL12/CXCR4, PAI-1, and MAPK) (Fig. 1). Among the transcriptional regulators of the EMT phenotypic landscape, Snail has been shown to play a crucial role in IR-induced EMT, with mitochondrial ROS stimulating activation of Snail and SMAD pathway through the MAPK/ERK cascade.[137,138] Sustained elevation of Snail promotes mesenchymal transition after irradiation of cancer cells, which is associated with the metastatic phenotype in

cancers. Furthermore, Snail shRNA prevented IR-induced mitochondrial repression and EMT switch, confirming that IR converts EMT via Snail activation.[139,140] Moreover, IR can indirectly induce Wnt/β-catenin signaling to promote Snail and EMT, which leads to the invasiveness of progeny from irradiated cancer cells.[141,142] Notch signaling is also implicated in IR-induced EMT; IR activates the interleukin-6 (IL-6)/JAK/signal STAT3 pathway to up-regulate Notch-1 and subsequently induces EMT.[143,144] IR-induced EMT is also mediated by EGF, TGF-β, vascular endothelial growth factor, ROS, and others.[22,145–147] For example, TGF-β-mediated EMT, in accordance with ATM, is capable of activating Homeobox B9 for enhancement of DNA damage and repair responses, leading to radioresistance.[148] M addition, TGF-β navigates disruption of the mitochondrial complex IV activity, thus prolonging the production of mitochondrial ROS which delay cell growth as a mechanism for cell death resistance.[149] Moreover, the balance between superoxide radical ($O_2^{\bullet -}$) and $H_2O_2$ can potentially determine pathways that drive the EMT process and its reversal to MET.[25] Work by Quiros-Gonzalez et al. showed that MnSOD affects EMT inter-conversion by modulating the rate of $H_2O_2$ production and the balance between $O_2^{\bullet -}$ and $H_2O_2$ in androgen-dependent prostate cancer LNCaP cells with stable expression of MnSOD.[150] Indeed, treatment with the N-acetylcysteine, a general ROS scavenger, prevents IR-induced EMT.[151] Activation of the redox-sensitive transcription factor NF-κB by ROS is also another route for ROS-mediated EMT,[152] supporting an inter-exchange role for ROS in IR-induced EMT.

## IV. PREDICTIVE MARKERS OF RESISTANCE TO RADIOTHERAPY

It is well recognized that substantial heterogeneity of the radiation response in normal tissues and PCa exists among individual patients.[153–155] Even within a single cancer, different regions can have a gradient of radiosensitivity depending on the microenvironment, distribution of cancer stem cells, and/or genetic alterations.[156] Several lines of evidence suggest difference in selected proteins of PCa patients after radiation exposure.[157,158] Identification of radiation response markers could be used for monitoring the progress of RT and prediction of RT therapeutic outcome.[159] The clinical and genomic databases can be used to develop and validate a gene expression. A signature to categorize patients as responders or non-responders to local therapy on initial diagnostic biopsies would be of significant clinical use.

### A. Gene Signature Predictive of PCa Radioresistance

Utilization of the genetically diverse NCI-60 cell lines, led to the first microarray analysis of gene expression in response to IR and correlation of the gene profiling with clonogenic survival. The following genes were identified: 22 genes associated with low survival after 2 Gy γ-rays, 14 genes associated with low survival after 8 Gy, and 25 genes with radiation responses dependent on wild-type p53.[160] Further, mapping of known DNA interactions with promoters of mitosis genes revealed a regulatory network centered on the transcription factors E2F4, E2F1, RBL2 (the p130 retinoblastoma-like protein), and TAF1. Among them, E2F4 and RBL2 act together as a repressor complex to prevent gene transcription.[161] Induction of E2F4 protein has been reported (8–24 h) after irradiation of PCa cell lines[162] and its activity is changes during normal cell cycle progression[163] and in response to IR.[164]

Recent studies in PCa patients who received either image-guided radiation therapy (IGRT) or radical prostatectomy as primary therapy revealed that RNA-based gene signatures could differentiate indolent and non-indolent and lethal PCa based on the alterations in the following genes: *MYC, NKX3–1, PTEN,* and *STAR*.[165–168] Interestingly enough, *TMPRSS2-ERG* fusion status does not predict prognosis after radical prostatectomy or IGRT[169,170] Zhao et al. utilized high-throughput gene expression and clinical data to develop a 24-gene postoperative radiotherapy outcomes score (PORTOS) for monitoring the likelihood of developing postoperative radiotherapy metastasis at 10 years.[171] PORTOS is further designed to enrich for intrinsic radiation response by including genes related to radiation or DNA damage response.[172] Accordingly, PORTOS (overexpression of the genes *DRAM1, GSEA, KRT14, PTPN22, ZMAT3, ARHGAP1, IL-1B, ANLN, RPS27A, MUM1, TOP2A, CDKN3, HCLS1, DTL, IL-7R, UBA7, NEK1, CDKN2AIP, APEX2, KIF23, SULF2, PLK2, EME1,* and *BIN2)* is the first validated gene signature developed to predict response to therapy in PCa, with a potentially high clinical value in selecting patients for radiotherapy.

## B. Oxidative Stress Markers

**1. Oxidative Damage Products—**Oxidative damage products are a nonspecific systemic index of oxidative stress in the body. In cancer patients undergoing any cancer treatment, markers of oxidative stress in the form of lipid peroxidation and protein carbonyl content are often elevated.[173,174] Alteration of antioxidants and accumulation of oxidative damage products have been demonstrated during PCa development in human[91,92] and mouse *(Nkx3.1* or *Nkx3.1/PTEN* knockout) models.[175] Cells with *PTEN* loss exhibited decreased antioxidants and redox regulatory proteins and increased oxidative damage products.[176] Our data from PCa cell culture and human tissues clearly show an association between increased oxidative stress markers including 8OHdG, lipid peroxidation 4-hydroxylnonenal (4HNE), and 3-nitrotyrosine (3NT), with both high Gleason score and tumor stage.[91] Levels of 8OHdG were focally elevated in the nuclei of primary cancer compared with the benign epithelium, whereas metastatic PCa showed high levels of nuclear 8OHdG among cells. In contrast, metastatic PCa exhibited nuclear 4HNE protein adducts, whereas 3NT was detected in the cytoplasm. As a result, the persistent markers of increased oxidative stress may contribute to redox imbalance and promote PCa progression and recurrence after RT.

**2. Hypoxia—**Given the essential role of oxygen in the response of IR-induced ROS, hypoxia becomes a major problem with RT.[177,178] It is well established that tumor radio-resistance is conversely correlated with the distance from blood vessels; cancer cells near blood vessels are sensitive to IR compared with distant tumors.[179] IR resulted in acute hypoxia in the tumor due to transient changes in blood flow and this acute hypoxia increased radioresistance in the tumor.[180,181] Higher radiosensitivity of normoxic compared with hypoxic cells is most likely due tofixation of ROS under normoxic conditions.[182,183] Preclinical studies support that hypoxia leads to a radioresistant and metastatic phenotype of prostate tumors.[184] PCa patients with hypoxic tumors had recurrent disease after treatment with radical prostatectomy or IGRT.[185] A review by Deep and Panigrahi has summarized the crucial role of the major hypoxia signals, HIF, PI3K/Akt/mTOR, NOX, Wnt/β-catenin, and Hedgehog, in PCa growth and progression.[186] Among these signals, extracellular vesicles

called “exosomes” are the latest marker in mediating hypoxia-induced PCa progression, potentially via tumor microenvironment remodeling. Exosomes have been reported in hypoxia-induced angiogenesis, cancer sternness, activation of cancer-associated fibroblasts, and EMT.[187] The exosomes secreted under hypoxia enhance invasiveness and stemness of PCa cells by targeting adherent junction molecules and increased level of diverse signaling molecules (TGF-β2, TNFlα, IL-6, TSG101, Akt, ILK1, MMP, and β-catenin).[187] Therefore, exosomes serve as vehicles that transfer bioactive molecules between cells and mediate cell-cell communication during hypoxia-derived RT.

Recent radical prostatectomy mRNA cohorts (108 Memorial Sloan Kettering Cancer Center cohort patients and 110 Cambridge patients) by Lalonde et al. revealed that hypoxia RNA signatures are univariately prognostic.[188] However, when patients were separated into four groups on the basis of high versus low percentage of genome alterations and high versus low hypoxia values, it was found that hypoxia increased the prognostic accuracy of the percentage genome alteration for risk of biochemical relapse after radical prostatectomy.[185,189] Therefore, measurement of cancer hypoxia and genomic instability can improve the prognostic capability of a pretreatment biopsy.[189] Ample evidence supports the crucial role of these hypoxia RNA signatures. Evaluation of cancer hypoxia using [$^{18}$F] fluoromisonidazole-positron emission tomography prior to radiochemotherapy has become a powerful discriminator between groups with good versus poor prognosis after RT.[190] Because hypoxia diminishes the therapeutic efficacy of RT, the development of a platform to increase oxygen concentrations within the tumor, including fractioned RT, hypoxic cell sensitization, and pharmacological reduction of oxygen consumption, has been pursued.[191–193]

**3. IL-8—**IL-8, also known as CXCL8, is a chemoattractant chemokine that has oxidative stress as one of its secretion stimulators.[194] The effects of IL-8/IL-8 receptor signaling pathways in PCa progression and radiation sensitivity may be orchestrated by communication and/or interaction with chemokines and their receptors such as CXCR1–7.[195] Increased IL-8 expression is associated with a high Gleason score, tumor pathologic stage, and markers of advanced PCa including castration resistance and radioresistance.[196] Because the transition of PCa to radioresistant PCa is partially due to IL-8-signaling-induced HSP90 activation, identifying IL-8 expression and its down-regulated signaling pathways may be used as a marker for radioresistant PCa regardless of AR response.[197]

## C. “Marker Dictations” by the Tumor Microenvironment

**1. EMT Signature—**The expression of critical proteins of defining the phenotypic landscape of EMT, E-cadherin, N-cadherin, and vimentin are indicators of PCa cells with invasive characteristics. Based on knowledge about the EMT signaling pathway, several molecules could be up-regulated upon radioresistant phenotype development; these include TGF-β, Wnt, Hedgehog, Notch, EGFR/PI3K/Akt, MAPK, and p21-PAK1.[132] In turn, these signals contribute to the induction of Snail expression to promote EMT and invasion.[198] For example, vimentin, which is a symbol of the acquisition of mesenchymal characteristics, is found to be increased in radioresistant PCa cells (PC3-RR, DU145-RR, and LNCaP-RR) compared with their parental PCa counterpart (up to a 7-fold increase).[81] Slug, also known

to inhibit p53-mediated apoptosis, is up-regulated after IR of radioresistant cancers.[199] ZEB1 is known to confer radioresistance by binding to the USP7 deubiquitinase to stabilize CHK1, thereby activating the recombination-dependent DNA repair response.[200] Further, high expression of Wnt signaling activity is associated with increased radioresistance in colorectal cancer cells and intestinal stem cells,[201] whereas nuclear β-catenin correlates with poor clinical outcomes after RT.[202]

An EMT biomarker signature tailored to the emergence of radioresistance can be created and globally applied for the radioresistant response of various cancers, including PCa. It is well established that loss of E-cadherin is considered a hallmark of EMT. Therefore, loss of E-cadherin combines with other EMT signature markers that are correlated with a radioresistant phenotype would be potential candidates for radioresistant PCa markers. For instance, if E-cadherin is negative and Snail is positive, then the signals that contribute to induced Snail expression (i.e., Wnt, Hedgehog, Notch, EGFR/PI3K/Akt, and MAPK) maybe defined as surrogate markers of the radioresistant phenotype in cancer patients.

**2. TGF-β—**TGF-β is a ubiquitous growth factor that plays an important role in regulating injury response and in the progression of PCa. There are three TGF-β isoforms: TGF-βl, TGF-β2, andTGF- β3. Functionally, TGF-β is considered to be a molecular switch responsible for excess synthesis and deposition of collagen and other ECM proteins via the TGF-β receptors and the SMAD3 pathway. Once activated, SMADs translocate into the nucleus, where they regulate the transcription of genes involved in cell cycle arrest. During the early phases of tumor progression including tumor initiation, TGF-β plays role as a tumor suppressor.[203] Work from our group demonstrated that disruption of TGF-β signaling *in vivo* accelerated pathologic malignant changes in the prostate by altering the tumor growth kinetics and inducing EMT.[204] Interestingly, at the later stage, TGF-β promotes processes associated with tumor aggressiveness such as cell invasion, dissemination, EMT, and immune evasion.[205,206] IR induces the release and activation of TGF-β in cells and tissues.[207] A mechanistic study in a cell-free system demonstrated that oxidation of the TGF-β latent complex acts as a sensor of oxidative stress to mediate the release and activation of TGF-β and orchestrates cellular responses to IR.[208] In turn, TGF-β stimulates ROS production by activation of p22 (phox) subunit of NADPH oxidases/ATM/p53 axis, which initiates downstream signaling targets (e.g., SMADs and EGFR) and results in the expression of a subset of pro-fibrotic genes.[209] TGF-β also induces a stromal redox state imbalance as a result of elevated NADPH oxidase 4-dependent ROS production and inhibits the expression of the MnSOD and CAT.[210]

TGF-β exerts differential effects during the early versus late stages of PCa progression. As a predictive marker, TGF-β up-regulation has been associated with tumor grade, pathologic stage, and lymph node metastasis in PCa patients.[211,212] Although no difference in the serum levels of TGF-β has been detected between benign prostate hyperplasia and PCa, elevated levels of plasma and urinary TGF-β were found in patients with poor clinical prognosis.[213] Considering the role of TGF-β in radiation-induced PCa progression, TGF-β could serve as a marker of radioresistant PCa.

**3. PARP- 1—**PARP-1 is a nuclear protein with multiple cellular functions including DNA damage repair.[14] Herein, we redirected our focus to PARP-1 as an IR-mediated EMT marker. A recent study demonstrated that inactivation of PARP-1 by gene-targeted deletion in an *in vivo* model of PCa leads to EMT induction toward high-grade PCa.[214,215] PARP-1 immunostaining increased dramatically in PCa cells associated with the EMT phenotype (mesenchymal) in response to RT.[48] Statistical evaluation of PARP-1 expression data among the mesenchymal cells in PCa after RT revealed that loss of PARP-1 is associated with biochemical recurrence and poor response to RT.[216] Work from our group revealed that loss of PARP-1 is strongly associated with prostate tumorigenesis *in vivo* via TGF-β-mduced EMT[48] and poor therapeutic response to RT.[135] Recent clinical studies utilizing PARP-1 inhibitors in the treatment of CRPC support targeting PARP-1 as a means to sensitize PCa to AR-directed therapeutics.[217] PARP-1 inhibitors can be of clinical value in combination with chemotherapy or RT to sensitize cancer cells to genotoxic insult.[218] Evidence on tumor radiosensitization by PARP inhibition provides indirect support for a role of PARP-1 in promoting radioresistance in PCa.[50]

### D. Chaperone Proteins: Heat Shock Protein 90

Among the chaperone proteins, HSP90 is of particular interest due to the ~100-fold elevation in human cancers.[219] HSP90 is an ATP-dependent chaperone that plays a central role in refolding and maintains the stability of diverse proteins (~725 client proteins), many of which are essential for cancer growth and confer therapeutic resistance (e.g., Akt, Src kinase, mutant p53, cyclin-dependent kinase 4, and H IF-lα[220,221]). HSP90 is often overexpressed in cancer due to mutation, amplification, deletion, methylation, and post-translational modifications (PTMs).[222] According to the Cancer Genome Atlas, the copy number of an HSP90AA1 gene is not altered significantly, but its protein expression is increased significantly in PCa.[223] Using a proteomics approach, it was shown that HSP90-mediated PI3K/Akt/mTOR signaling pathway is an important signaling pathway regulating radioresistant PCa cells.[224] The HSP90-mediated PI3K/Akt/mTOR signaling pathway was identified as a main pathway associated with PCa cell radioresistance.[81] In addition, Quanz et al. reported that HSP90α is both a chaperone and substrate of DNA-dependent protein kinase (DNA-PK). After TRAIL-induced apoptosis, DNA-PK is the sole kinase that phosphorylates HSP90α (at the threonine-5 and −7 positions).[225]

## V. NOVEL TARGETING TO OVERCOME PCA RADIORESISTANCE

Identification of a patient's susceptibility for developing adverse effects from RT is an important prerequisite for the personalized treatment era. Previously, p53/MDM2, Bcl-2/Bax, PTEN/Akt, and COX-2 were investigated extensively as radiation sensitization targets of PCa (as reviewed by Palacios et al.[226]). From 1997 to 2008, 11 biomarkers for radiation resistance were studied by the Radiation Therapy Oncology Group.[227] mRNA microarray studies revealed more genes altered after IR, ineluding IL-2 and sequence-specific DNA-binding proteins.[160] Targeting a single signaling pathway that drives radioresistance is not sufficient to improve RT efficacy, but a multi-targeted approach by simultaneously targeting DNA repair and chaperone protein would improve therapeutic outcome for radioresistant PCa.

### A. PARP-1 in Charge of DNA Repair

The lethality of unrepaired DNA DSBs, commanded the development of agents to target selectively proteins involved in the DNA damage response. Gavande et al. developed a radiation sensitivity molecular signature based on gene expression profiling datasets and identified 10 genes as DSB repair genes.[228] Several clinical trials combining PARPI's with radiation have been established.[13,229] Agents that inhibit DNA repair indirectly are being combined with PARP inhibitors (PARPi's), such as inhibitors of EGFR, PI3K, HSP90, and agents that inhibit the $G_2$ checkpoint, CHK1, and WEE1.[230–232] Agent pairs such as HSP90-PARP are more effective when combined with RT,[233] indicating that radiation-induced DNA damage can potentiate therapeutic response. The comprehensive genetic characterization of PCa identified a significant number of germline and somatic mutations in DNA repair genes.[234] Given the frequency of these DNA mutations in metastatic CRPC, the paradigm of PARPi synthetic is a potentially important addition to the management of patients with radioresistance disease.[235] Current PARPi's undergoing clinical evaluation are veliparib, olaparib, niraparib, talazoparib, rucaparib, CEP-9722, and SC10914.[236] A clinical benefit of PARPi's in PCa was detected in a phase I trial of olaparib in 60 patients with solid tumors who were germline *BRCA* mutation carriers.[237] Selected mutations could inhibit PARPi effectiveness; for example, the somatic loss of functional mutations in the tumor suppressor PTEN results in impaired PARPi sensitivity in PCa cells.[238–241] Moreover, PARPi's can impair AR recruitment to target promoters.[242] In contrast, a phase II trial (NCI 9012), reported in the *Journal of Clinical Oncology*, found no benefit of adding veliparib to abiraterone (Zytiga) plus prednisone in CRPC patients with ETS fusion-positive tumors. Therefore, ETS status was not predictive of response to veliparib. Interestingly, exploratory analysis led to the novel and unexpected finding that a DNA-damage repair defect (not PARP-1) was associated with improved outcomes with abiraterone (Zytiga) plus prednisone treatment, possibly through induction of a synthetic lethality in the context of HR defects.[243]

### B. Cellular Redox State: MnSOD, the Player

MnSOD is one of the important antioxidant enzymes that is located exclusively in the mitochondria[244] and its expression and activity modulate cellular redox status, influencing the effects of radiotherapy. *In vivo*, the administration of MnSOD-liposome (MnSOD-PL) reduced GPx activity significantly in conjunction with radiation, but did not affect radiation-induced reduction of GSH in oral cavity orthotopic tumor tissue. Intraoral administration of the MnSOD-PL therapy decreased the number of ulcerations significantly 5 days after irradiation compared with control mice.[245] Overexpression of MnSOD failed to prevent bladders from the early off-target effects of IR, but enhanced recovery at 4 weeks after IR.[246] *In vivo*, intraperitoneal administration of recombinant MnSOD (rMnSOD) after irradiation with subsequent daily injection of rMnSOD led to a significant decrease of IR-induced off-target injury and increased survival up to 30 days after IR compared with controls, which died 7–8 days after IR.[247] Radioprotective thiols can modulate TNF-α-induced MnSOD gene expression with NF-κB activation.[248] MnSOD affects the release of cytochrome C from mitochondria due to alterations in ROS levels in mitochondria.[249] Our recent studies, which are supported by the work of other investigators, demonstrated that selective inhibition of RelB-induced MnSOD after irradiation can sensitize PCa cells to radiation treatment.[130,250–252]

Given the significance of MnSOD, manganese porphyrin compounds have been developed with variable $O_2^{\bullet -}$-scavenging properties, pharmacokinetics on tissues, and subcellular localization.[253] The impact of SOD mimetics on increasing radiosensitivity in cancers stems from evidence that Mn(III) 5.10.15.20-tetrakis(N-ethylpyridinium −2-yl)por- phyrin) (MnP-2-PyP5+) inhibits the radioprotective effects of cancer-cell-conditioned medium on endothelial cells *in vitro* and enhanced radiation-induced damage to tumor vasculature and delayed tumor growth *in vivo*.[254] These results suggesting that MnP-2-PyP5+ may disrupt communication between cancer and endothelial cells, leading to the breakdown of tumor vasculature and disruption of the microenvironment after IR. Furthermore, Mn(III) 5.10.15.20-tetrakis(N-hexylpyridinium-2-yl)por phyrin) (MnTnHex-2-PyP5+), a more potent SOD mimetic due to its greater lipophilicity and higher biodistribution,[255] exerted a radiosensitizing effect on HeLa cells, murine mammary carcinoma 4T1 cells, and melanoma B16 cells *in vitro* and *in vivo* via attenuated DNA damage repair and triggered a shift from pro-survival pathways to apoptosis.[127,256] In addition to their radiation enhancer effects, SOD mimetics exert radioprotective effects in normal cells, the most powerful SOD mimetic, Mn(III) meso-tetrakis(N-n-butoxyethyl-pyridinium −2yl) porphyrin, MnTnBuOE-2-PyP5+) (MnP), has been demonstrated to protect bone marrow suppression from irradiation via inhibiting oxidative damage.[257] Recent studies by Oberley-Deegan et al. demonstrated that MnP protects against prostate radiation-induced injury to male reproductive system via up-regulation of Nrf2.[258] Therefore, SOD mimetics boost ROS generation in cancer cells selectively toward oxidative stress overload while stimulating adaptive responses in normal cells and thus emerge as attractive candidates for adjuvant therapy with RT in PCa patients (Fig. 4).

### C. Redox-Sensitive Transcription Factor: RelB

NF-κB transactivation is induced in many types of cancers by therapeutics, including radiation. The non-canonical dimer p52/RelB plays a more important role in protecting PCa cells against radiation compared with p50/RelA.[130] Studies from our group indicated that selective inhibition of RelB enhances the radiosensitivity of PCa cells.[129,252] Mechanistic evidence indicates that selective blockade of RelB nuclear import by a cell-permeable SN52 peptide, a variant of the SN50 peptide blocking nuclear import of NF-κB family members, is highly effective in sensitizing PCa to radiotherapy.[251,258] The RelB/sirtuin (SIRT3)/MnSOD axis has been identified as a critical contributor to ascorbic-acid-induced radiosensitization of PCa cells and radioprotection of normal prostate cells. RelB suppression decreases expression of SIRT3 and MnSOD, which in turn increases oxidative and metabolic stresses in PCa cells; in contrast, ascorbic acid enhances RelB expression in normal prostate, improving antioxidant and metabolic defenses against radiation. Therefore, targeting RelB in PCa is a viable approach to enhance RT efficacy selectively.[61]

### D. TGF-β in Control of the EMT Landscape

Radioresistance augments prostate tumor progression by promoting EMT, metastatic spread, and evasion of immune surveillance, all processes that are regulated by TGF-β. Considering that IR induces the release and activation of TGF-β and the association of poor prognosis in PCa and blood levels of TGF-β,[259] the use of TGF-β inhibitors to ameliorate radiosensitization in PCa is expected. A small-molecule inhibitor of the TGFβ type I

receptor kinase (LY364947) has been shown to increase radiosensitivity *in vitro* and *in vivo*.[260] LY364947 promotes clonogenic cell death and decreases radiation-induced phosphorylation of γH2AX and p53. Several studies indicated that inhibition of TGF-β signaling with a selective inhibitor of a TGF-β type I receptor, ALK5, enhances radiosensitivity in glioblastoma by disrupting EMT and self-renewal capabilities, blocking DNA damage response, and increasing apoptosis.[261] Administration of a 2G7-neutralizing pan-TGF-β IgG2 antibody blocked IR-induced circulating levels of TGF-β 1, circulating tumor cells, and lung metastases in transgenic model of metastatic breast cancer.[262] Reduction in metastasis is attributed to auto crine TGF-β production in the tumor microenvironment.[263] Galunisertib (a TGF-β receptor inhibitor; www.clinicaltrials.gov identifiers NCT01246986 and NCT01373164) is undergoing a phase II trial for pancreatic ductal adenocarcinoma and hepatocellular carcinoma.[264] A putatively hypofunctional TGF-β 1 haplotype is associated with lower TGF-β plasma levels and increased sensitivity to radiation-induced chromosomal aberrations and apoptosis in lymphoid cells.[265,266] Increased circulating TGF-β levels during RT have been correlated with poor prognoses in patients with non-small-cell lung cancer.[267] RT-induced TGF-β is implicated in tissue damage such as fibrosis[268] and the use of TGF-β inhibitors has been shown to ameliorate IR toxicity to normal tissues.[269] Blocking of TGF-β signaling before irradiation attenuates DNA damage, increases cell death, delays tumor growth, and prolongs survival in several human cancers.[60,260] Taken together, this evidence suggests that targeted disruptions of TGF-β signaling can radiosensitize prostate tumor cells while inhibiting metastasis.

### E. Protein Homeostasis: All Eyes on HSP90

HSP90 has emerged as a promising target in cancer treatment, including RT, for more than a decade. To date, there are at least 20 HSP 90 inhibitors in oncology trials (www.clinicaltrials.gov). Numerous studies have indicated that HSP90 inhibitors can sensitize tumor cell lines and tumors growing *in vivo* to clinically relevant doses of X-ray or photon exposure.[270] The HSP90 inhibitors NVP-AUY922 and 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin have been shown to induce $G_2M$ arrest by down-regulating Cdk1 and Cdk4 and alter cell cycle distribution by affecting cell cycle checkpoints.[271,272] Currently, ganetespib, which is safe and well tolerated, has the potential to become the first-in-kind HSP90 inhibitor to be approved as a radiosensitizer in PCa[273] because it was shown to be effective in clinical trials of rectal cancer (www.clinicaltrials.gov identifier NCT01554969) and esophageal cancer (www.clinicaltrials.gov identifier NCT02389751).

If HSP90 is cleaved, cross-linked, or changed in conformation due to site-specific PTM, it could prevent the binding of ATP, client proteins, or co-chaperones and activate ubiquitination, sumoylation, or selected AAA-type proteinase, leading to radioresistant cancer cell death.[222,274] Therefore, utilizing ROS to modify the HSP90 protein level and function via ROS-mediated PTM is a logical tactic to additionally inhibit HSP90. Clinically, ErbB3 expression is correlated with resistance to HSP90-inhibitor-induced tumor cell radiosensitization.[275] The distinct pattern of HSP90 subcellular compartmentalization in the cytoplasm, nucleus, endoplasmic reticulum, and mitochondria can regulate different sets of client proteins dynamically.[276] In PCa, mitochondrial HSP90 interacts with proteins

essential for survival and metabolism.[277] Gamitrinib, a mitochondria-targeted, small-molecule HSP90 inhibitor, is active against all tumor types tested and efficiently killed metastatic, CRPC, and multidrug-resistant PCa cells characterized by overexpression of the ATP-binding transporter P-glycoprotein.[278] Targeted inhibition of mitochondrial HSP90 suppresses localized and metastatic PCa in the TRAMP mouse model of PCa progression.[279] Shown in Fig. 5 is the approach of simultaneously targeting cytoplasmic and mitochondrial HSP90 during RT. Inhibition of HSP90 can sensitize cancer cells to fractionated IR and block selection of residual tumor-radioresistant cells.

## VI. CONCLUSION

PCa is one of the most lethal cancers in men in the United States today. Understanding the underlying mechanisms of therapeutic resistance in PCa patients, including radioresistance, will aid in strategies to overcome treatment failure, minimize toxicity, and increase patient survival. The challenges surrounding increased radiation intensity to improve total control of cancer cell growth in prostate tumors relate to significant risks of major side effects associated with radiotherapy. An attractive radiation enhancer would provide exploitation of the intrinsic differences in the radioresponse of cancer epithelial cells and normal cells. DNA repair systems, oxidative stress, HSP90, and EMT are critical for the clinical radiation response of PCa. The multilayered complexity and the functional interactions of these networks support a multi-targeted platform to improve the therapeutic outcomes for radioresistant PCa. Concurrent targeting of the expression and the topological localizations of effectors such as HSP90 in mitochondrial and cytoplasmic compartments (Fig. 5) may provide concomitant protection of normal tissues while sensitizing PCa to IR. ROS, at least in part, regulate DNA repair systems, HSP90, and EMT, thus pushing oxidative stress beyond the cancer cell antioxidant capacity to alter multiple ROS-associated proteins. This represents a promising platform for enhancing RT efficacy by killing cancer cells while protecting normal cells (Fig. 4).

In summary, several combination strategies are currently in clinical trials with great anticipation for overcoming radioresistant PCa and improving patient survival and quality of life. As technology advances, the framework of molecular signatures could be embedded commercially in the clinical platform, integrating it as a predictive factor for the outcome of post-RT to facilitate biomarker-driven treatment approach toward personalized therapies.

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## ABBREVIATIONS:

| | |
|---|---|
| **3NT** | 3-nitrotyrosine |
| **4HNE** | 4-hydroxylnonenal |
| **ADT** | androgen-deprivation therapy |

| | |
|---|---|
| **APR-1** | apurinicapyrimidinic endonuclease 1 |
| **AR** | androgen receptor |
| **ATM** | ataxia telangiectasia mutated |
| **ATR** | ATM and RAD3-related |
| **BER** | base excision repair |
| **BRCA1** | phosphoprotein that assists in 5’ to 3’ resection of DSBs, loading of RAD51 |
| **BRCA2** | phosphoprotein that assists with RAD51 loading |
| **CAT** | catalase |
| **CRPC** | castration-resistant prostate cancer |
| **DSB** | double-strand breaks |
| **$e^-aq$** | hydrated electron |
| **EBRT** | external beam radiotherapy |
| **EMT** | epithelial mesenchymal transition |
| **GSH** | glutathione |
| **$H^\bullet$** | hydrogen radical |
| **$H_2O^+$** | ionized water |
| **$H_2O_2$** | hydrogen peroxide |
| **HIF-lα** | hypoxia inducible factor 1 alpha |
| **$HO_2$** | hydroperoxide radical |
| **HR** | homologous recombination |
| **HSP90** | heat shock protein 90 |
| **IGRT** | image-guided radiation therapy |
| **IL** | interleukin |
| **IMRT** | intensity-modulated radiation therapy |
| **IR** | ionizing radiation |
| **LNCaP** | androgen-dependent prostate cancer cell |
| **MDC1** | mediator of DNA damage checkpoint protein 1 |
| **MnP** | Mn(III) meso-tetrakis(N-n-butoxyethyl-pyridinium-2yl)porphyrin |

| | |
|---|---|
| **MnP-2-PyP5+** | (Mn(III) 5,10,15,20-tetrakis(N-ethylpyridinium-2-yl)porphyrin |
| **MnSOD** | manganese superoxide dismutase |
| **MnSOD-LP** | MnSOD-liposome |
| **MnTnHex-2-PyP5+** | Mn(III) 5,10,15,20-tetrakis(N-hexylpyridinium-2-yl)porphyrin |
| **MRN** | MRE11-RAD50-NBS1 |
| **mtDNA** | mitochondrial DNA |
| **mTOR** | mammalian target of rapamycin |
| **NEIL2** | nei-like DNA glycosylase |
| **NF-κB** | nuclear factor kappa-beta |
| **NHEJ** | nonhomologous end joining |
| **NQO1** | NAD(P)H dehydrogenase |
| **Nrf2** | nuclear factor (erythroidderived 2)-related factor 2; O2•−, superoxide radical |
| **O H•** | hydroxyl radical |
| **OXPHOS** | oxidative phosphorylation |
| **PARP** | polyadenosine diphosphate (ADP) ribose polymerase |
| **PARPi** | PARP1/2 inhibitor |
| **PCa** | prostate cancer |
| **PI3K** | phosphoinositide 3-kinase |
| **Prx** | peroxiredoxin |
| **PrxSO3** | oxidation form of Prx |
| **PTEN** | signaling, phosphatase and tensin homolog |
| **PTM** | posttranslational modification |
| **rMnSOD** | recombinant MnSOD |
| **RNS** | reactive nitrogen species |
| **ROS** | reactive oxygen species |
| **RT** | radiotherapy |
| **SBRT** | stereotactic body radiotherapy |

| | |
|---|---|
| **SIRT3** | sirtuin |
| **SOD** | superoxide dismutase |
| **SSB** | single-strand break |
| **TGF-β** | transforming growth factor-beta |
| **$\rho^0$** | rho-zero cells |

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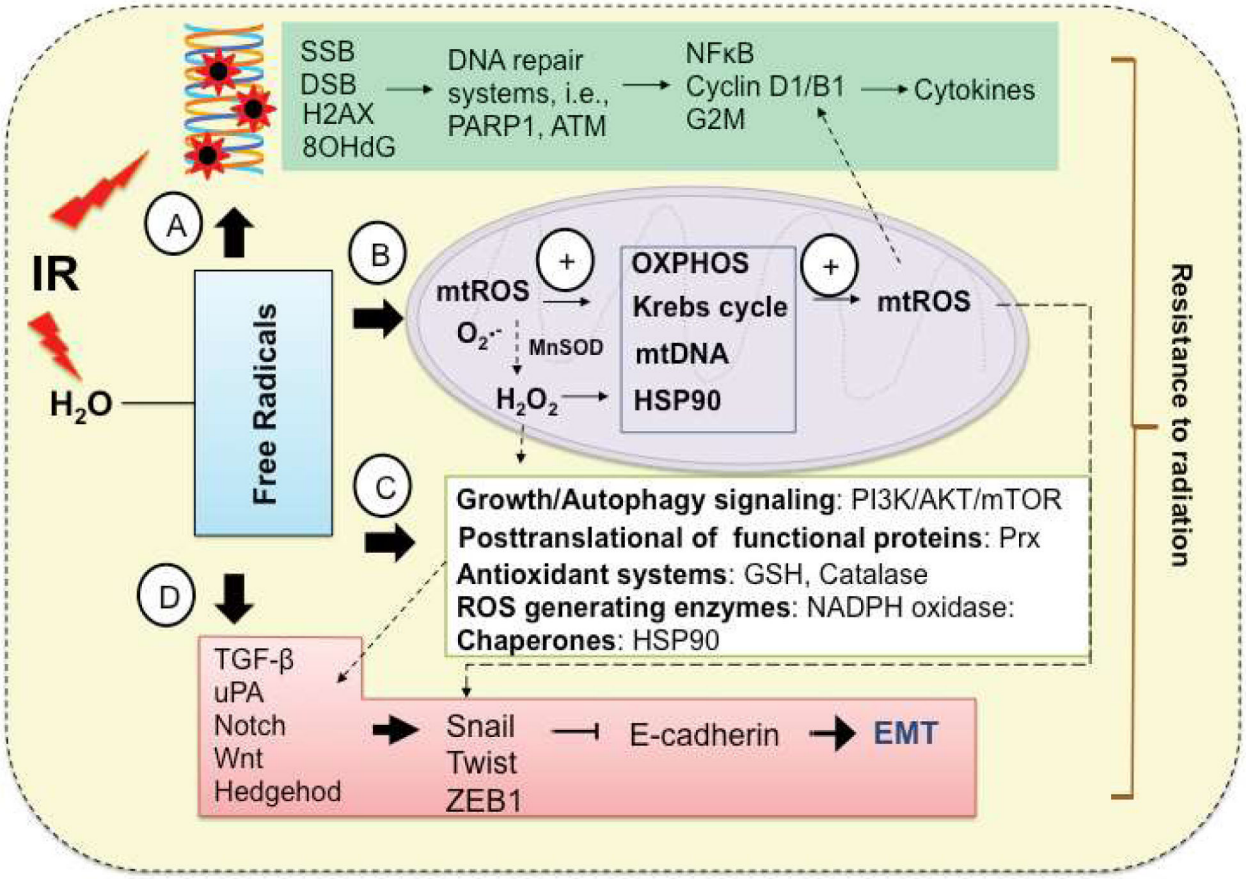


**FIG. 1:**

Effect of radiotherapy on DNA repair, mitochondrial function, cellular ROS, and EMT. IR initially causes ionization and excitation of water, leading directly and indirectly to the formation of free radicals. (A) Signaling of IR-induced DNA damage and repair. DNA can either be damaged directly by IR or indirectly through radiation-induced free radicals, which causes radical base formation, SSBs, DSBs, and DNA damage. (B) Signaling pathway of IR-induced modification of mitochondrial function. IR induces mitochondrial leakage of electrons, which interact with 0 2 to generate ROS. ROS can travel into the nucleus to cause DNA damage and $G_2$M arrest and activate EMT signaling or to the cytoplasm to activate growth signaling and other cellular responses. Up-regulation of the mitochondrial content and mitochondrial OXPHOS subsequently leads to amplification of mitochondrial ROS and ATP production. (C) Signaling pathway of IR-induced cellular responses. Other cellular responses, including activation of ROS-generating enzymes, antioxidant proteins, growth/autophagy signaling, and chaperone proteins, are activated to contest IR-mediated cell death. (D) Signaling IR-induced EMT. ROS are implicated in IR-induced EMT via activation of signaling pathways (i.e., TGF-β, Wnt, Hedgehog, Notch, and EGFR/PI3K/Akt), which in turn activate transcription factors (i.e., Snail, Twist, and ZEB1) and induce a loss of epithelial morphology.

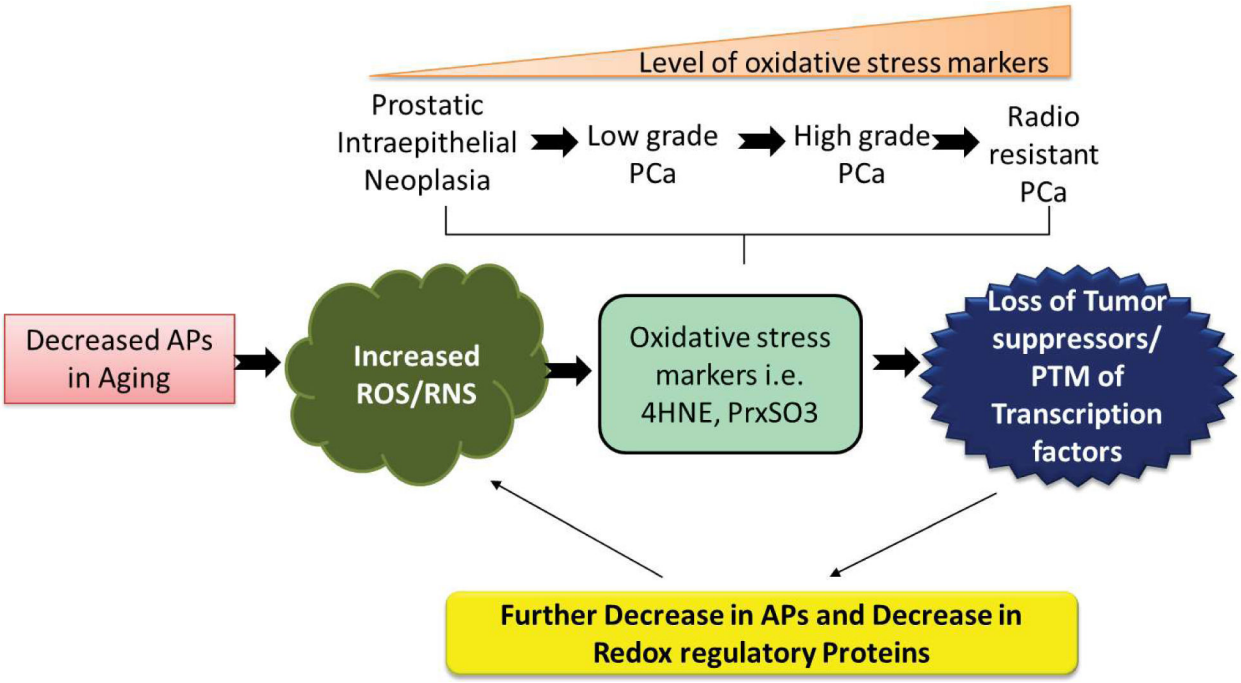


**FIG. 2:**

Proposed regulation of PCa radioresistance by the redox state. During the aging process, expression of antioxidant proteins (APs) decreases. The high influx of ROS/RNS and $H_2O_2$ and inactive APs secondary to aging lead to increasing oxidizing condition, oxidative stress markers (4HNE, PrxSO3), and PTMs of cysteine (Cys) residues. This PTM has a profound effect on tumor suppressors and transcription factors, which leads to mutations of redox-regulated proteins and a further decline of APs. The persistent influx of ROS/RNS drives the progression of prostatic intraepithelial neoplasia to low-grade PCa and subsequently to high-grade PCa, as indicated by higher oxidative stress markers correlated with PCa stage. A better measurement of baseline levels of the redox state would enable the development of more sensitive biomarkers and more effective redox-related treatment of PCa emerging as radioresistant disease.

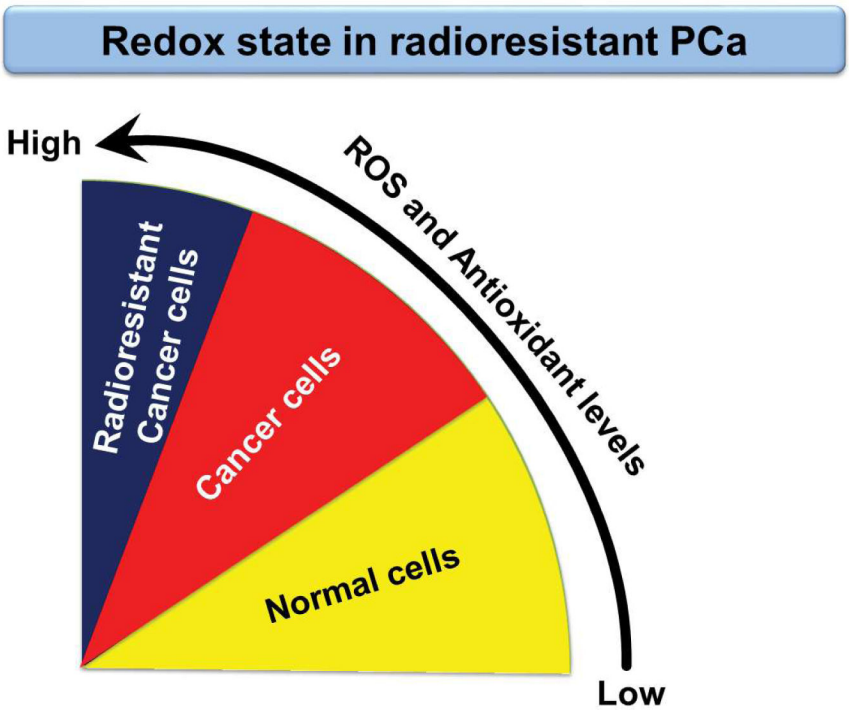


**FIG. 3:**

Mechanistic models of redox state in radioresistant PCa. Cancer cells have higher ROS and antioxidant proteins compared with normal cells; an even higher level of ROS and antioxidants are detected in radioresistant PCa. ROS at low levels is physiologically functional, whereas at high levels, it is toxic to cells. ROS production is also activated by radiation exposure, leading to more persistent oxidative stress in cancer cells. We propose that radioresistant PCa cells adapt their redox state into higher oxidative stress via up-regulation of endogenous ROS-generating enzymes and antioxidant proteins.

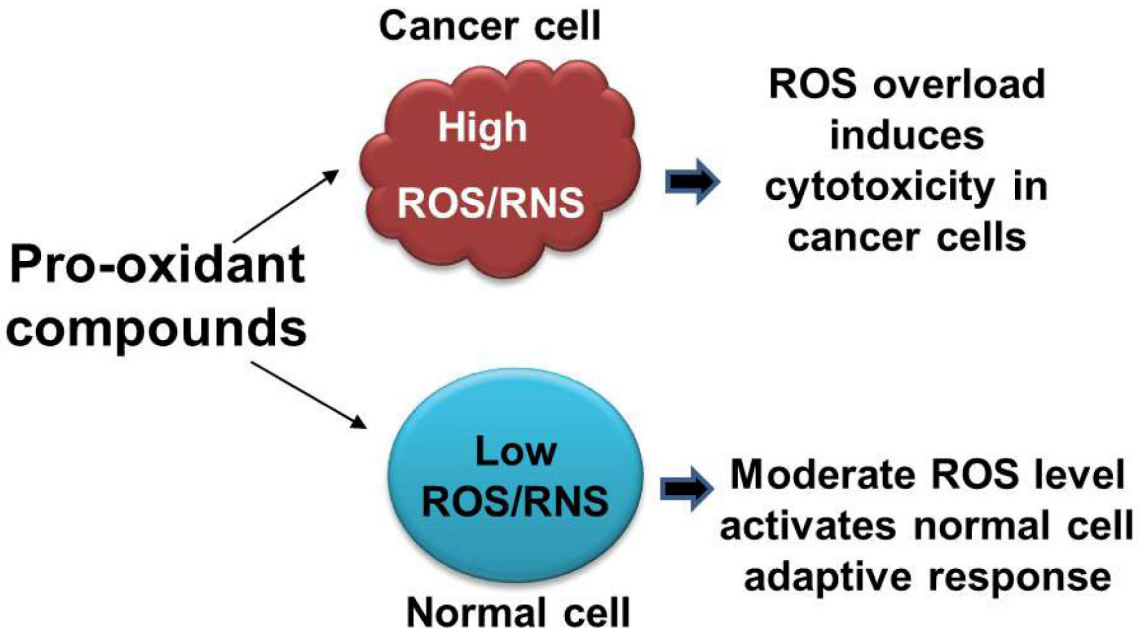


**FIG. 4:**

Schema of cell death induction by pro-oxidants. Generally, levels of ROS/RNS are significantly higher in cancer cells than in normal cells. Pro-oxidant treatments with, for example, SOD mimetics prompt an influx of ROS/RNS into cells and shift the cellular redox state of cancer cells into oxidative stress and that of normal cells into a mild pro-oxidant state. Moderate levels of ROS in normal cells induce adaptive responses that activate Nrf2-mediated antioxidant proteins, which protect normal cells from ROS overload. Cancer cells harboring endogenously high levels of ROS, and with further increased ROS, could undergo apoptosis consequential to protein degradation and DNA damage via intrinsic mitochondrial-mediated apoptosis signaling.

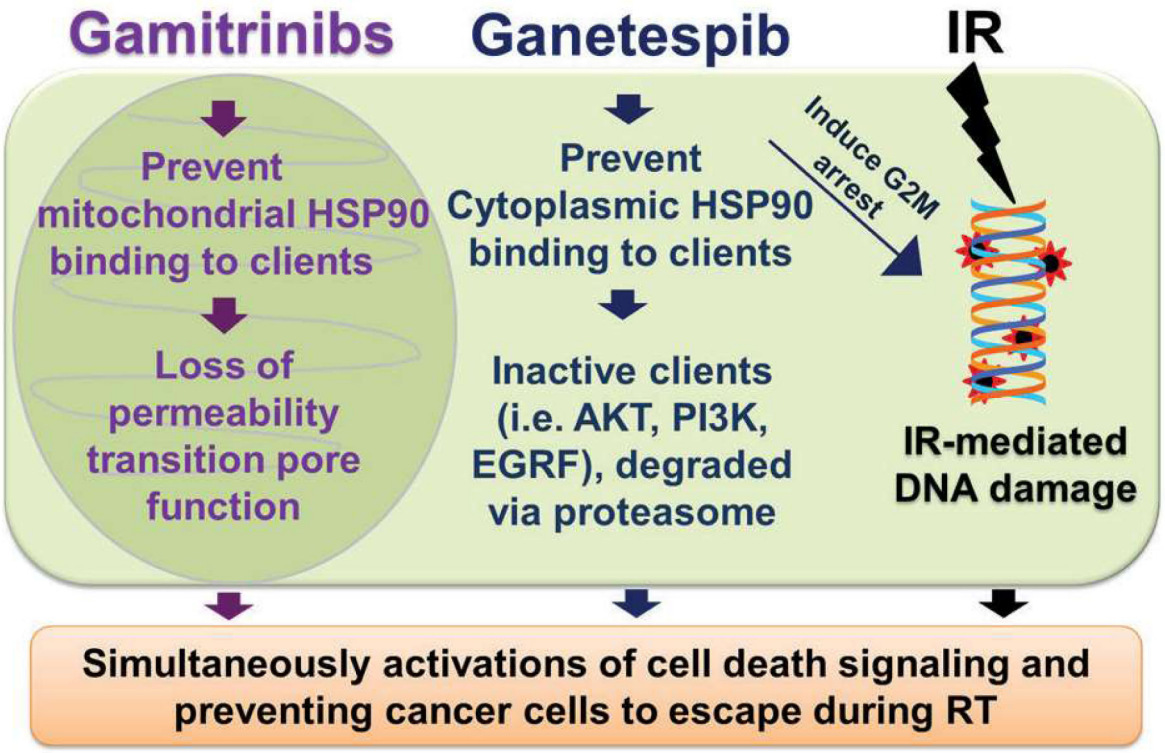


**FIG. 5:**

Multi-targeting HSP90 therapeutic approach. Mechanistically, gamitrinib prevents mitochondrial HSP90-binding activity, thus inhibiting mitochondrial function by inducing acute mitochondrial dysfunction in PCa cells, with a loss of organelle membrane potential and release of cytochrome C and caspase activity independently of proapoptotic Bcl-2 associated proteins, Bax/Bak. In contrast, ganetespib prevents cytoplasmic HSP90-binding activity and thus inhibits multiple processes, including: (1) growth signaling; cyclin proteins, and MET/EGFR; (2) angiogenesis: HIF-lα, vascular endothelial growth factor (VEGF), and Src; and (3) survival signaling navigated by AKT and IGF-1R. Systemic administrations of both gamitrinib and ganetespib during RT would target effects of radioresistance and prevent the adaptive feedback response or compensation mechanism due to targeting a single HSP90.