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Identification of novel targets in prostate cancer progression

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CHAPTER 4

TARGETED RADIOSENSITIZATION IN PROSTATE CANCER

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ABSTRACT

Radiotherapy is one of the treatment options for locally or regionally advanced prostate cancer, but radioresistance of prostate cancer cells is a practical limitation of radiotherapy. The identification of molecular targets of radioresistance in prostate cancer is important to improve therapeutic intervention. The aim of this review is to give more biological insight into some well known processes involved in radioresistance of prostate cancer especially Apoptotic pathway; DNA damage response; and NF-kB signaling pathway. This review integrates salient, published, research findings with underlying molecular mechanisms, preclinical efficacy, and potential clinical applications of combining radiotherapy with these molecular targeted agents for the treatment of prostate cancer.

INTRODUCTION

The standard treatment regimen for clinically localized disease in prostate cancer is either radical prostatectomy or radiation therapy through external beam irradiation or local radioactive seed implants (brachytherapy) (1). A major reason for failure to eradicate local disease in prostate cancer and other solid tumors by radiotherapy (RT) is the radioresistance (2). Generally, there are two types of radioresistance in solid tumors: External mediated by interactions with microenvironment (Cell-cell and cell-matrix interactions (3) and local paracrine signaling), and internal (mediated by the general survival pathways like mutated p53) (4), amplification of DNA repair genes, overexpression of anti-apoptotic genes, increased levels of reactive oxygen species scavengers, activation of prosurvival/poor prognosis oncogenes such as Epidermal growth factor receptor (EGFR) (5,6) or c-MET(also known as hepatocyte growth factor receptor) (7,8). Molecular targeting of these survival mechanisms is now becoming a reality with new treatments designed to target processes that are thought to be tumor specific, or where there are quantitative differences in target expression between cancer and normal cells. The relative tumor-specificity of most molecular targeted agents may offer a theoretical advantage over chemotherapy, as overlapping toxicity with RT on normal tissue is potentially minimized. Furthermore, the intrinsic radiosensitivity of certain tumors may be modified by agents that target specific gene and protein expression. An illustrative example of this advantage is the targeting of EGFR expression to reduce proliferation of head and neck cancer cells without affecting the repopulation of normal mucosal epithelial cells required for healing during radiotherapy (9-11). This fundamental information has, in turn, suggested that targeting such radio response regulatory molecules can serve as a strategy for developing radiation sensitizers.

OVERCOMING RADIORESISTANCE IN PROSTATE CANCER BY TARGETING APOPTOSIS

Irradiation-induced tumor apoptosis can be enhanced by targeting of apoptotic machinery that involves a system of messengers.The challenge of apoptosis-targeting, as in all therapies, is to selectively target pathways operational in tumor cells over those operational in normal cells.

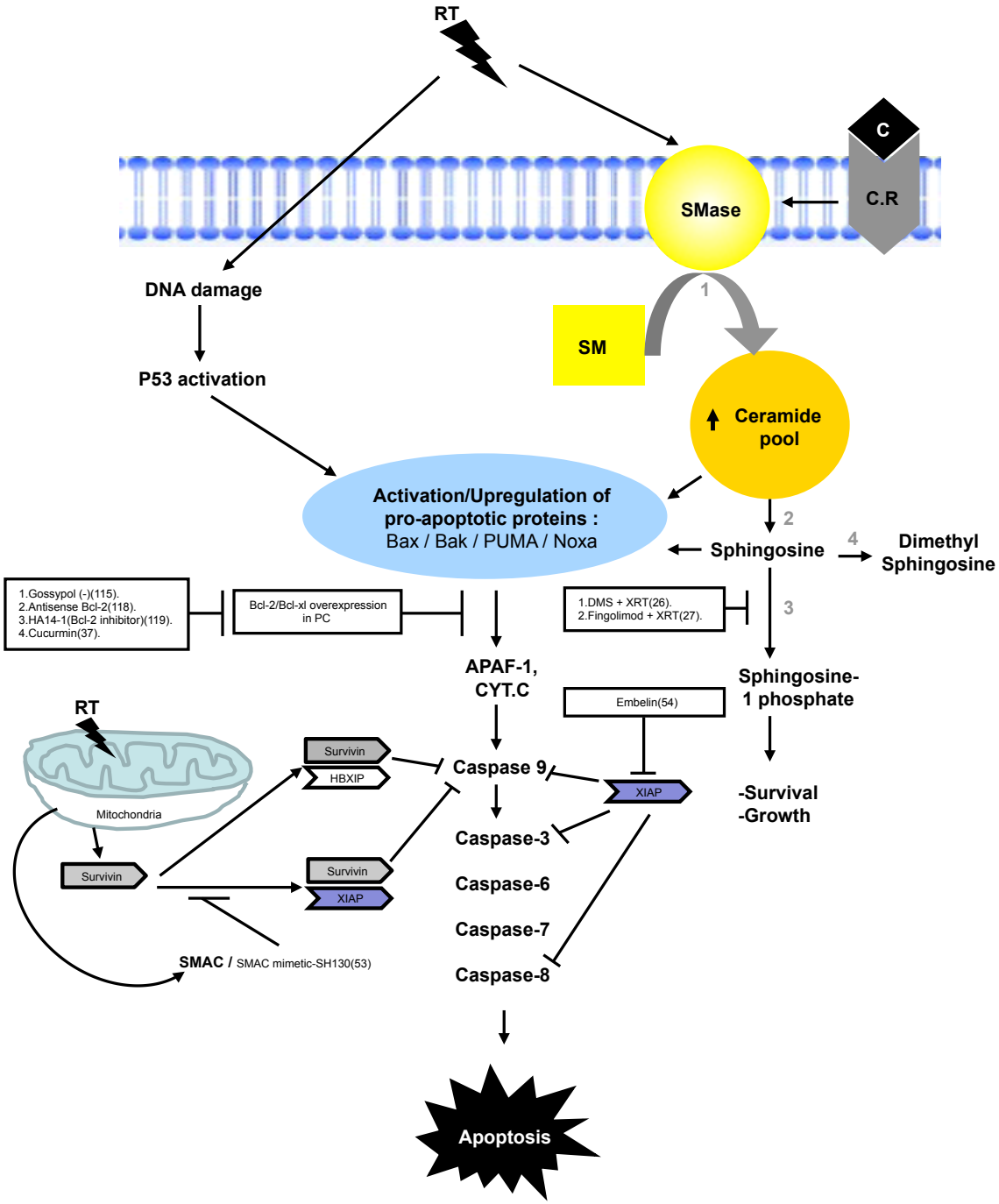

FIGURE 1.*Schematic model showing the activation of different pathways leading to apoptosis in the prostate cancer cell lines* (LNCaP/PC3). Pro-apoptotic signaling is activated via upregulation of p53, release of ceramide (e.g., by sphingomyelinase (SMase) due to XRT or by various cytokines like TNF- alpha. Ceramide is metabolized by a ceramidase to generate sphingosine. Sphingosine is phosphorylated by sphingosine kinase to form Sphingosine-1-Phosphate (S-1P). S-1P antagonizes ceramide-mediated apoptosis. All signals are integrated at the level of mitochondria by activation or upregulation of pro-apoptotic molecules belonging to the pro-apoptotic Bcl-2 family (Bax, Bak, Puma, Noxa). The relative level of pro and anti-apoptotic Bcl-2 molecules is the key decision point regarding cell death induction. In case of relative overweight of pro-apoptotic Bcl-2 members, cytochrome c is released from mitochondria and triggers execution of apoptosis by activation of caspase-9 and secondary caspases that cleave intracellular substrates, thereby inducing the apoptotic phenotype, including nuclear chromatin condensation and fragmentation. XIAP and survivin which belongs to the class of IAP (Inhibitor of apoptosis) proteins, inhibit the activation of the caspase cascade leading to the radioresistance.

Abbreviations: Smase: sphingomyelinase; SM: sphingomyelin; CS: ceramide synthase; C: Cytokine; CR: Cytokine Receptor; DMS: N,N-Dimethyl sphingosine 1. Smase 2. Ceramidase 3. sphingosine kinase; 4.Sphingosine N-methyl transferase

By Targeting Sphingomyelin-Ceramide Pathways

Radiation targets either the cell membrane or the nucleus to activate different apoptotic pathways (12-14). Sphingomyelin-Ceramide apoptotic pathway (Fig. 1) is initiated by hydrolysis of sphingomyelin through activated sphingomyelin-specific forms of phospholipase C, termed sphingomyelinases (SMases) that leads to generation of ceramide (12-14). Ceramide, in turn, can activate several pathways important for the induction of apoptosis (14). Also, Ceramide is further metabolized by ceramidase to generate sphingosine (Fig. 1), which in turn, can be phosphorylated by sphingosine kinase (Sphk) to form Sphingosine-1-phosphate (S-1P) (15,16). Conversely, S-1P has been implicated as a signaling molecule that antagonizes ceramide-mediated apoptosis (17). The modulation of ceramidase, sphingosine kinase, and S-1P phosphatase activities play a pivotal role in the regulation of apoptosis by regulating the intracellular ratio between ceramide, sphingosine, and S- 1P (17). Furthermore, Sphk1/S1P pathway has been linked to oncogenic transformation and cancer progression via increased rate of cell proliferation, and apoptotic resistance (18-20). Sphk1 is highly expressed in various human tumor tissues (21,22) and has been shown to be associated with poor prognosis in gastric cancer (23), glioma (24) and breast cancer (25). Radioresistance of Prostate cancer has been reported to be linked to sustained sphingokinase- 1(Sphk-1) activity (26). Recently, a new sphingosine analogue FTY720 (Finglimod) has been shown to induce radiosensitization and inhibition of tumor growth in in vitro and in vivo models (27).

Various in vitro studies (outlined in Table 1) have shown that the Sphingomyelin-Ceramide pathway is a very attractive target for radio-sensitization in prostate cancer. After critically analyzing all these in vitro studies it can be hypothesized that ceramide generation is a critical component of radiation-induced apoptosis in human prostate cancer cells and blockage of ceramide generation may provide a selective advantage in the development of radioresistance of prostate tumors. Because of the central role of Sphingomyelin-Ceramide pathway in radiation induced apoptosis, pharmacologic manipulation of the intracellular ceramide levels in conjunction with radiation could offer significant improvement to the clinical treatment of prostate cancer.

By Targeting Anti-apoptotic Bcl-2 Family of Proteins

Antiapoptotic Bcl-2 (B-cell lymphoma 2) protein is overexpressed in a variety of human cancers, including prostate cancer (28,29). Bcl-2 overexpression is frequently found in both primary and metastatic human prostate cancers (30,31). It is observed to be overexpressed in 30% to 60% of prostate cancer at diagnosis and in nearly 100% of hormone-refractory prostate cancers (31). Also, Bcl-xL (B-cell lymphoma-extra large) is found to be overexpressed in 80% to 100% of hormone-refractory prostate cancers, where it is associated with bad prognosis, shortened survival and advanced disease (32). Bcl-2/Bcl-xL overexpression decreases the pro-apoptotic response to such cellular insults as irradiation, chemotherapy, and androgen withdrawal, leading to resistance to treatment (33). Primary prostate tumors overexpressing Bcl-2 exhibit a high Gleason score and a high rate of cancer recurrence after radical prostatectomy (30,31). The most definitive evidence supporting a positive correlation between Bcl-2 and prostate cancer progression is that Bcl-2 overexpression leads to metastatic and chemo- or radioresistant phenotypes (34,35). Reversal of prostate cancer cell radioresistance in vitro has been achieved by downregulating Bcl-2 (36,37). Bcl-2 and Bcl-xL represent an attractive target for the development of new anti-prostate cancer agents that have either direct cytotoxic effects on prostate cancer cells or improve the efficacy of conventional radio- or chemotherapy by sensitizing

TARGET	APPROACH	CELL LINE	MECHANISM	REF.
SPHINGOSINE CERAMIDE PATHWAY	TNF- alpha + irradiation	LNCaP	↑Intracellular ceramide levels → Enhanced activation of the intrinsic mitochondrial apoptosis pathway. ↑Sphingosine and ↓SPP levels.	115
	Exogenous C2 ceramide+ irradiation	LNCaP	↑Intracellular ceramide levels → enhanced activation of the intrinsic mitochondrial apoptosis pathway	116
	Exogenous sphingosine + irradiation	LNCaP	↑Sphingosine levels → enhanced apoptosis of the radiation resistant prostate cancer cells.	26
	Sphingosine kinase inhibitor (N,N-DMS) + Irradiation.	LNCaP	↑Sphingosine levels → enhanced apoptosis of the radiation resistant prostate cancer cells.	26
	TPA +irrradiation	LNCaP	↑Ceramide synthase activity →ceramide levels (in- Vivo pathway) → enhanced apoptosis	115
TARGETING ANTIAPOPTOTIC BcL-2 FAMILY	(-)Gossypol+ irrradiation	- PC-3 - PC-3 xenograft	Blocks heterodimerization of Bcl-xL with Bax, Bad and Bim → enhanced response to radiation therapy.	115
	Antisense BcL-2+ irrradiation	-PC-3 -LNcaP	Reduction in Bcl-2 protein levels and a significant reduction in clonogenic survival.	117
	HA14 -1+ irrradiation	-PC-3 -LNcaP	HA14 –1 is an organic Bcl-2 inhibitor → enhanced response to radiation.	118
	Curcumin+ irrradiation	PC-3	Downregulation of endogenous and radiation Induced Bcl-2 protein expression → enhanced radiation induced apoptosis.	37
TARGETING IAP MEMBER OF PROTEINS	Smac mimetic SH-130	DU-145	Functional blocking of IAPs → enhanced radiation induced apoptosis.	53
	Embelin	PC-3	Inhibits XIAP → enhanced radiation induced apoptosis	54

TABLE 1. Shows preclinical studies, which demonstrate the potential of targeting Sphingomyelin-Ceramide pathway, Bcl-2 antiapoptotic and IAP family of proteins for prostate cancer radio-sensitization.

prostate cancer cells (Fig. 1). Various in vitro studies as shown in (Table 1) provide firm evidence, that targeting antiapoptotic Bcl-2 family of proteins represents an attractive target for prostate cancer radiosensitization.

Targeting the IAP (Inhibitor of Apoptosis) Member of Proteins

IAPs represent a class of apoptosis regulatory proteins consisting of eight family members: Neuronal apoptosis inhibitory protein (NAIP; also known as BIRC1), cellular IAP1 (c-IAP1; also known as BIRC2), cellular IAP2 (c-IAP2; also known as BIRC3), X chromosome-linked IAP (XIAP; also known as BIRC4), survivin (BIRC5), ubiquitin-conjugating BIR domain enzyme apollon (also known as BIRC6), melanoma IAP (ML-IAP; also known as BIRC7), and IAP-like protein 2 (ILP2; also known as BIRC8) (38,39). Among all human IAP proteins, XIAP and survivin have been reported to have the most prominent and strongest antiapoptotic function (40,41). IAPs function as potent endogenous apoptosis inhibitor (Fig. 1) due to their ability to bind and effectively inhibit two effector caspases (-3 and -7) and one initiator caspase-9 (42). A notable exception is-survivin which only inhibits active caspase 9 after binding to its cofactor hepatitis B-X-interacting protein (HBXIP) (43). Additionally, it has been shown that anti-apoptotic action of survivin could be mediated by its interaction with XIAP leading to increased stability of XIAP (44). IAPs suppress apoptosis against a variety of

apoptotic stimuli, which include radiation, chemotherapy and immunotherapy in cancer cells (38). Specifically, radiation triggers release of mitochondrial proteins (Smac, cytochrome-c and survivin) into the cytoplasm (41,45). Consequently the released Smac binds to XIAP and other IAP proteins, thus abolishing their anti-apoptotic function (45). It has been shown that IAPs are highly expressed in many types of cancer including prostate cancer (39,42,46,47). Expression of cIAP1, cIAP2, XIAP, survivin, and NAIP has been examined in the NCI -60 human tumor cell line panel, which revealed widespread expression of cIAP1, XIAP, and survivin in tumor lines of diverse tissue origins (48). Genome wide analysis has confirmed the differential expression of survivin in tumors versus normal tissues (49). Survivin has been shown to be overexpressed in prostate cancer cell lines, aggressive prostate cancers with higher gleason grades, lymph node and distant metastasis (50-52). Because IAPs suppress apoptosis against a variety of apoptotic stimuli, including radiation, strategies targeting IAPs may prove to be highly effective in overcoming radiation resistance (Fig. 1). Against this background a number of different strategies have been developed to antagonize aberrant IAP protein function and/or expression in human prostate cancers as to overcome radioresistance (53,54). Some of these strategies are briefly outlined in the (Table 1).

TARGETING DNA DAMAGE RESPONSE PATHWAYS

Ionizing radiation (IR) leads to the formation of DNA single or double-strand breaks, altered or lost DNA bases and DNA-DNA or DNA protein cross-links (55). The DNA-damage response pathway begins with 'sensor' proteins that sense the DNA damage and/or chromatin alterations that occur after induction of DNA damage (56). These 'sensor' proteins convey the damage signal to transducers which in turn transmit it to numerous downstream effectors(56). The DNA-double strand breaks (dsbs) are first recognized by the (Telomere binding protein) TRF2 and Mre11– Rad50– Nbs1 (MRN) sensor complex. The MRN sensor complex is the most important sensor complex (Fig. 2) comprising the nuclease Mre11, the structural maintenance of chromosomes protein Rad50 and the protein Nbs1 (57,58). The transducers consist of a group of conserved nuclear protein kinases (59) and the 'phosphatidylinositol-3-OH kinase (PI (3) K)-related protein kinases' (PIKKs), which consists further of the DNA-dependent protein kinase (DNA-PK), ataxia-telangiectasia-mutated (ATM), the ATM and Rad3-related (ATR) protein and hSMG-1 (60,61). Current evidence suggest that the MRE11–RAD50–NBS1 complex (MRN complex) is the primary DSB sensor that recruits ATM to the DNA-dsbs (62). Another early step in the response to a DSB involves phosphorylation of the H2A histone family, member X, H2AX, which is redundantly carried out by ATM or DNA-dependent protein kinase (DNA-PK)(63). Phosphorylation of H2AFX produces discrete, microscopically detectable foci (64). The MRE-11 and H2AFX proteins further recruit DNA repair complexes and cell cycle checkpoint proteins (e.g. tumor protein 53 binding protein 1 (TP53BP1), mediator of DNA damage checkpoint 1 (MDC1), breast cancer 1, early onset (BRCA1), and check point kinase 2 (CHK2) (65). After initial sensing and activation of downstream pathways, parallel activation of human DNA-double strand break repair pathways homologous recombination (HR) and non-homologous recombination takes place, which can both interact or compete with each other during cell cycle transitions (66).

Cell cycle checkpoints or DNA repair pathways are commonly altered during the process of prostate cancer (66). Tumors having these genetic alterations are hypothetically sensitive to radiation upon further disruption of remaining checkpoint functions or remaining DNA repair pathways. The most important DNA-dsb damage

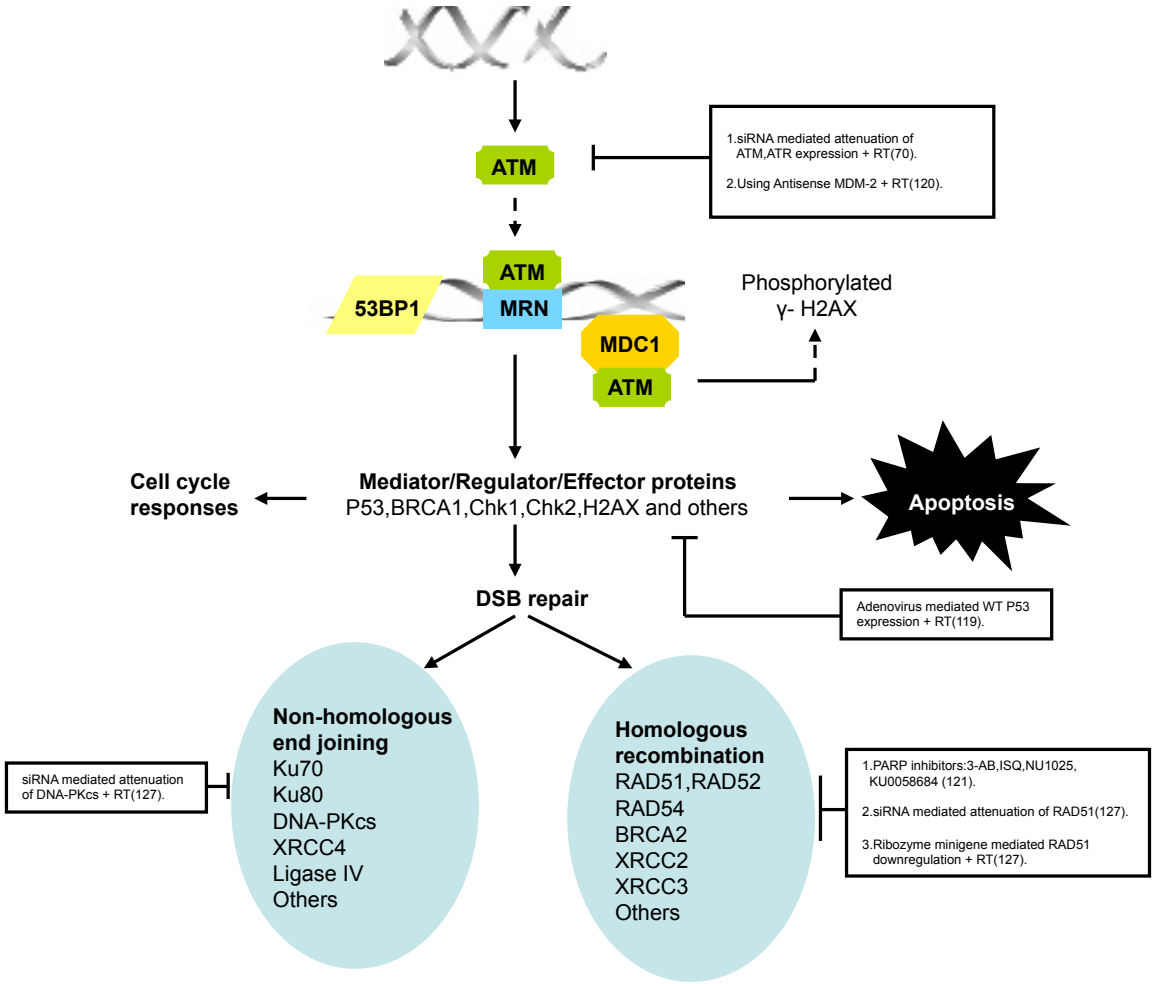

FIGURE 2. *Showing schematic outline of DNA damage response pathway and possible therapeutic strategies for radiosensitization in the prostate cancer.* For more information refer to the (Table 2).

TARGET	AGENT USED	MODEL USED	MECHANISM	REF.
ATM – P53 – MDM2 PATHWAY	Adenoviral mediated P53gene expression + XRT	-P53 defficient PC3 line -P53 wild type LNCAP cell line	Restoration/enhancement of normal p53 function ➔Enhancement of apoptosis.	119
	Antisense MDM2+AD+XRT	P53 wild type LNCAP cell line	- ↓MDM2 expression and ↑P53 and p21 expression. - ↑Apoptosis and ↓clonogenic survival.	120
	siRNA mediated attenuation of the ATM, ATR expression + XRT	- DU 145 - PC-3	↓Post-transfection levels of the ATM, ATR proteins ➔ decreased clonogenic survival	114
HR REPAIR PATHWAY	Genetically engineered PARP mutant, expressing dominant negative mutant of PARP in tumor cells+ XRT	Tumor xenograft	- PARP is required for the efficient repair of DNA singlestrand breaks (SSBs) during base excision repair and PARP inhibition leads to persistent singlestrand gaps in DNA➔these gaps are encountered by a replication fork, leading to arrest, and the single-strand gaps may degenerate into DSBs. - In the absence of BRCA1 or BRCA2, the replication fork cannot be restarted and collapse , causing persistent chromatid breaks Repair of these breaks by alternative error prone DSB repair mechanisms would cause large numbers of chromatid breaks and aberrations, leading to loss of viability.	121-126
	siRNA /antisense /Ribozyme minigene mediate attenuation of RAD51	LNCaP	Downregulation of RAD 51	127

TABLE 2. Showing various pre-clinical studies which have showed the potential of targeting DNA damage response pathway for prostate cancer radiosensitization.

-response genes associated with prostate cancer risk includes the ATM-p53 signalling axis, such as ATM, p53, and CHK2 (67). It has been shown that ATM expression is higher in the high gleason score prostate tumors, when compared to the normal tissues (68). Prostate cancer specimens have been shown to harbor p53 mutations that have been documented to be associated with androgen independence, metastasis, decreased disease free survival and radioresistance (59,69). Keeping this background in mind, ATM has been targeted in prostate cancer using specific antisense or siRNA approaches resulting in radiosensitization (70).Small molecular inhibitors or peptides have been generated to bind to mutant forms of p53 and reverting them to wild type conformation and leading to cell cycle arrest and apoptosis (69). Malignant prostate cancer cell lines express higher levels of RAD51, XRCC3, RAD52 and RAD54 genes involved in homologous recombination in comparison to normal prostate epithelial cells (71). Strategies that target DNA repair increase radiosensitization in vitro and in vivo after treating prostate, glioma and lung cancer cells with siRNA to RAD51 (72,73). Prostate cancer arising in BRCA2 mutation carriers display an aggressive tumor phenotype and present more poorly differentiated tumors when compared with non-carrier prostate cancer controls (74-76). It has been shown that cells defective in BRCA1 and BRCA-2 proteins exhibit reduced RAD51 activity and foci formation and show increased sensitivity to ionizing radiation (77,78). Furthermore, It has been observed that BRCA1 and BRCA2 deficiency sensitizes cells to the inhibition of Poly (ADP-

Ribose) Polymerase (PARP) enzymatic activity, which consequently leads to chromosomal instability, cell cycle arrest and apoptosis (79). PARP-1 (accounting for 80% of total PARP cellular activity) binds to both single and double strand DNA breaks and is involved in DNA single strand break (ssb) repair and break excision repair (80,81). It has been documented that inhibition of PARP increases the level of unrepaired DNA double strand breaks by a variety of mechanisms.Based on these principles, Ashworth and colleagues adopted an siRNA approach and observed an increased sensitivity to PARP inhibition in a variety of cells which were made deficient in proteins in HR or Fanconi's anemia pathway (like RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA or FANCC) (83). Targeting cancer cells harboring a specific DNA repair defect by inhibiting a second repair pathway is a representation of synergistic lethality (84), and this approach is rapidly being translated into effective treatments for hereditary BRCA1 or BRCA2 deficient cancers or with tumors harboring defects in HR repair pathways (85). Utilizing this principle, specific and potent inhibitors of PARP inhibitors (Fig. 2) have been developed that are very effective tumor radiosensitizers in in-vitro and in-vivo (84).

The various preclinical studies which have demonstrated the potential of targeting these pathways for prostate cancer radiosensitization are outlined in (Table 2). These studies support the concept that the predetermination of the repair capacity of tumor cells may help to select appropriate agents for use in combination with radiotherapy in prostate cancer.

BY TARGETING RADIATION INDUCED RADIOADAPTIVE NF- kB PATHWAY

Radio-adaptive response is assumed to be induced by activation of the specific prosurvival signaling network in irradiated mammalian cells leading to reduced cell sensitivity to a subsequent higher challenging dose when a smaller inducing radiation dose had been already applied (86). The earliest response of mammalian cells to ionizing irradiation consists of activation of transcription factors, like AP-1, p53 (also known as TP53), and NF-kB (87,88). Out of all these, NF-kB has served as a model system for inducible transcription in a broad range of physiological and medical effects. The mammalian NF-kB family of proteins consists of five members: RelA, RelB, c-Rel, p50 (NF-kB1) and p52 (NF-kB2) (89). All members of the NF-kB family posses a Rel-homology domain (RHD) containing a NLS (nuclear localization sequence), which is important for dimerization, DNA binding and its interaction with IkB proteins (most important inhibitors of NF-kB activation). In the majority of circumstances NF-kB is found in the cytoplasm where it is negatively regulated by its interaction with the IkB family of proteins. These IkB family of proteins possesses multiple ankyrin repeats which bind to the RHD and masks NLS of NF-kB (90). There are various stimuli that activate NF-kB, which results in the regulation of a myriad NF-kB target genes. The majority of proteins encoded by NF-kB target genes participate in the host immune response (91,92), cell adhesion and stress response (91), apoptosis regulators (93), growth factors (94), cell cycle regulators (95) and inflammatory cytokines (96).In cancer cells, it regulates the expression of many anti-apoptotic proteins (IAP1, IAP2, XIAP,cFLIP and BclxL). It also regulates the progression of the cell cycle by positively regulating the expresson of various cyclins (D1,D2, D3, and E) and c-myc (97). NF-kB is also known to stimulate invasion and angiogenesis by regulating the

expression of different matrix metalloproteinases (MMP-2, MMP-9) (98) and various angiogenic factors (IL-8 and VEGF) (94,96).

Moreover, the activation of NF-kB is considered to be the most important factor, involved in the inflammatory response generated by irradiation (99,100). It has been shown that increased basal NF- kB activity in certain cancers has been associated with tumor resistance to radiation and chemotherapy (101). NF-kB is activated after phosphorylation of IkB at two serine residues (Ser-32 and Ser-36) by IkB kinases, which is later polyubiquitinated, and then degraded by 26S proteasome (Fig. 3). The free NF-kB translocates to the nucleus and activates its target genetic programs (102) including manganese superoxide dismutase (MnSOD), an enzyme that catalyses the conversions of toxic superoxide radicals to hydrogen peroxide and molecular oxygen (103-105). The radioadaptive response mediated by the NF-kB members increases the expression of MnSOD leading to protection of tumor cells (106,107). NF-kB also modulates the apoptotic signals at various levels. The best example is found in the TNF receptor I signaling pathway (108). (Fig. 3) provides the schematic representation of NF-kB signaling network in radiation induced adaptive radioresistance in prostate cancer.

Numerous studies have demonstrated the importance of the NF- kB pathway and its role as a cause of radioresistance in prostate cancer cell lines:

a). Josson and colleagues demonstrated that, constitutive nuclear level of RelB are significantly higher in PC-3 compared to LNCaP cells. They also showed that PC3 cells have a higher basal levels of MnSOD as compared to the LNCaP cells. These results suggest that comparatively higher levels of nuclear RelB and MnSOD protein may be responsible for the intrinsic radiation resistance of PC-3 cells (109). Selective inhibition of RelB decreased the levels of MnSOD leading to increase in the sensitivity of prostate cancer cells to radiation treatment (109). Xu and colleagues showed that interaction of 1-alpha, 25-dihydroxyvitamin D3 (1alpha, 25-(OH) 2D3) with the Vitamin D receptor (VDR) enhanced the radiosensitivity of prostate cancer cell lines at clinically relevant radiation doses. The radiosensitization effect of 1-alpha, 25-(OH)2D3 is partly mediated by selectively suppressing IR-mediated RelB activation, leading to decreased expression of manganese superoxide dismutase (MnSOD), suggesting that suppression of MnSOD is a mechanism by which 1-alpha, 25-(OH) 2D3 exerts its radiosensitization effect. Therefore, 1-alpha, 25-(OH) 2D3 is a high potential effective pharmacologic agent for selectively sensitizing prostate carcinoma cells to irradiation via suppression of antioxidant responses in mitochondria (110). Yulan Sun and colleagues showed that inhibition of NF-kB pathway is also a common mechanism for the radiosensitization effect of parthenolide in prostate cancer cells LNCaP, DU145, and PC3 (111). Radiation-induced NF-kappaB DNA-binding activity is inhibited by parthenolide leading to the decreased transcription of the sod2 gene, the gene coding for an important antiapoptotic and antioxidant enzyme (manganese superoxide dismutase) in the three prostate cancer cell lines (111). Using immunohistochemical studies, Lessard and colleagues demonstrated that all the members of the NF-kB family were expressed in normal prostate tissues, prostatic intraepithelial neoplasia and prostate cancer. However, only the nuclear localization of RelB correlated with the prostate cancer patient's Gleason scores (112), suggesting that the level of RelB is associated with prostate cancer progression. These studies provide us convincing evidence that RelB plays an important role in redox regulation of the cell and protects

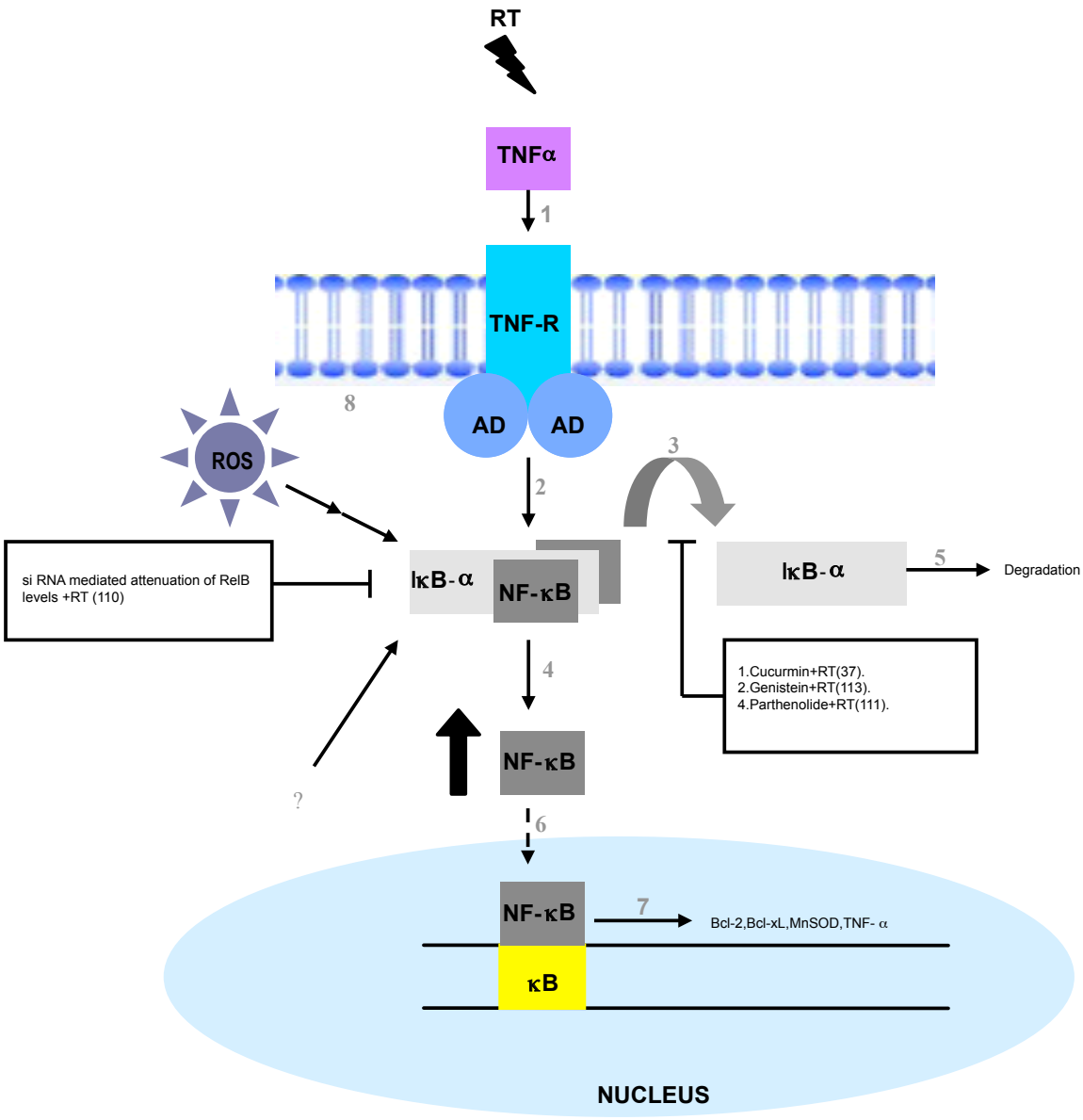

FIGURE 3. *Schematic presentation of the NF-kB signaling network in radiation-induced adaptive radioresistance in prostate cancer.* 1. Radiation induces TNF-alpha ligand expression. Binding of TNF-alpha to its receptor, leading to recruitment of adaptor proteins (AD). 2/3. Activation of NF-kB by phosphorylation of IkB by IkB kinases; 5. Polyubiquitination, and degradation of IkB by 26S proteasome. 4.increased free NF-kB levels in the cytoplasm. 6. Translocation of NF-kB to the nucleus. 7. Activation of NFkB target genetic programs. 8. ROS also stimulates the formation of free NF-kB.

Abbreviations: RT = Radiotherapy; ROS=Reactive Oxygen Species

aggressive prostate cancer cells against radiation-induced cell death. Thus, inhibition of RelB could be a novel mechanism to radiosensitize prostate cancer.

b). Damodaran and colleagues demonstrated using p53 deficient PC-3 cell line, that radiation also causes an induction of TNF- alpha protein expression, NF-kB activity and Bcl-2 upregulation (37). They also showed that radiation-induced NF-kB activity depends on radiation induced expression of TNF-alpha. Curcumin in combination with radiation caused inhibition of TNF- alpha - mediated NF-kB activity resulting in downregulation of bcl-2 protein leading to the enhanced radiation-induced clonogenic inhibition and radiation-induced apoptosis in p53 deficient PC-3 cells (113). Curcumin inhibits NF-kB activation by inhibiting phosphorylation of IkB-alpha, which is required to export NF-kB from cytosol to nucleus as to activate its target genes (113). Together, these mechanisms strongly suggest that the natural compound Curcumin is a potent radiosensitizer, and that it acts by overcoming the effects of radiation-induced prosurvival gene expression in prostate cancer.

c). Julian and colleagues showed that Genistein (4', 5,7 trihydroxyisoflavone) combined with radiation causes greater inhibition in PC-3 colony formation compared to genistein or radiation alone due to the strong inhibition of the NF-kB activity (113). Their findings support the novel strategy of combining genistein with radiation for the treatment of prostate cancer. All these studies demonstrate the role of NF-kB as a stress factor in prostate cancer cells. NF-kB is a crucial element of the cell's protective response to radiation and represents therefore an attractive target in new therapeutic approaches to fight prostate cancer. Inhibition of NF-kB is expected to increase the therapeutic efficiency of radiation.

CONCLUSIONS AND REMAINING QUESTIONS

The identification of molecular targets of radioresistance in prostate cancer cells is very important to improve therapeutic intervention in prostate cancer. Anyhow, an ideal molecular targeting agent should improve the therapeutic efficacy of radiotherapy by targeting specific pathway(s), in practice, but this may be difficult to achieve with predictability because of the complex molecular cross-talk between signaling pathways (113). The most challenging part for a clinical investigator is to interpret this large amount of preclinical data and, then to select the most promising molecular targeting agent suitable for human clinical trials. Moreover, there are other challenges that are faced during the designing of the early clinical trials for molecular targeted agents. It is unknown whether expression of the molecular target can adequately predict clinical response to molecular agents (55). There are also several issues in studying radiosensitizers at the molecular level using prostate cancer cell lines, including their heterogeneity, different growth properties, hormone responsiveness, originated from metastatic tissue,etc. Nevertheless, these cellular models have provided considerable understanding of the biology of prostate cancer and are important for the initial investigation process. Moreover, our current knowledge of radiation-induced pathways is incomplete, and it does not provide direct proof for improving the efficacy of radiation therapy. In this context, the advent of RNA interference technology (114) can provide us a better insight into the radiation induced biomolecular pathways by virtue of its high selectivity for molecular targets. Functional genomic studies utilizing siRNA high throughput libraries can produce unbiased information regarding the molecules involved in the prostate cancer radioresistance. This ge-

nome wide approach will reveal new molecules involved in prostate cancer radioresistance, and it will lead to the development of new molecular targeted radiosensitizing strategies in prostate cancer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest

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