Effect of ubiquitin protease system on DNA damage response in prostate cancer (Review)

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Abstract. Genomic instability is an essential hallmark of cancer, and cellular DNA damage response (DDR) defects drive tumorigenesis by disrupting genomic stability. Several studies have identified abnormalities in DDR-associated genes, and a dysfunctional ubiquitin-proteasome system (UPS) is the most common molecular event in metastatic castration-resistant prostate cancer (PCa). For example, mutations in Speckle-type BTB/POZ protein-Ser119 result in DDR downstream target activation deficiency. Skp2 excessive upregulation inhibits homologous recombination repair and promotes cell growth and migration. Abnormally high expression of a deubiquitination enzyme, ubiquitin-specific protease 12, stabilizes E3 ligase MDM2, which further leads to p53 degradation, causing DDR interruption and genomic instability. In the present review, the basic pathways of DDR, UPS dysfunction, and its induced DDR alterations mediated by genomic instability, and especially the potential application of UPS and DDR alterations as biomarkers and therapeutic targets in PCa treatment, were described.

Contents

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1. Introduction

According to the International Agency for Research on Cancer GLOBOCAN Cancer Statistics for 2020, prostate cancer (PCa) is the second most prevalent malignancy in men worldwide (1). The detection of biomarkers for PCa may influence clinical decision-making, guide low-risk patients to avoid unnecessary biopsies and overtreatment, and design the best strategy for patients with high-risk diseases (2,3). The treatment for PCa includes androgen deprivation therapy (ADT), radiation therapy (RT), chemotherapy, newly emerging immunotherapies, and surgery. However, numerous patients cannot be cured after treatment and are prone to develop fatal metastatic Castration-Resistant PCa (mCRPC) (4). Currently, ~10 million men worldwide have the condition, of which ~700,000 are affected (5). It is well known that the occurrence and progression of PCa are androgen-dependent, and profiles of the PCa transcriptome and genome have identified chromosomal rearrangements and copy number increases or decreases, including androgen receptor (AR) amplification (6). Previous studies on PCa have mainly focused on AR mutations or related pathways, and ADT was once the most effective clinical treatment strategy. However, once patients enter a state of castration resistance, their cancer progression is difficult to control (7). Mutations in mCRPC have received increasing attention in recent years, with the highest mutation rate occurring in the DNA damage response (DDR)-associated BRCA2 gene (8,9). Studies have further found that mCRPC patients with mutations in the BRCA2 gene are effectively treated with the PARP inhibitor (PARPi) olaparib. In another study, PARPi was found to interact with the E3 ubiquitin-protein ligase TRIP12 (10), indicating an emerging crosstalk between the DDR and ubiquitination in PCa.

Numerous studies have shown that the ubiquitin-proteasome system (UPS) is essential for maintaining homeostasis *in vivo* by controlling a wide range of cellular functions. UPS dysfunction contributes to various human diseases, especially cancer and neurodegenerative disorders (11,12), Currently, the UPS is considered to be a very promising target for cancer therapy and is receiving increasing attention. Ubiquitin can be used not only as a signal molecule mediating proteasome degradation but also as a signal molecule for DNA repair, transcription factor activation and other biological processes (13).

Generally, ubiquitin binds covalently to substrates via various enzymes, modifying or degrading them to control various cellular processes. In addition to mediating proteasomal degradation of substrates, ubiquitin can also mediate non-degradative ubiquitination of substrates, which is often associated with the regulation of kinase activity. However, owing to the diversity and complexity of the UPS, the mechanisms of its physiological and pathophysiological actions are not fully understood. As the UPS is involved in a wide range of cellular functional activities, the role of the UPS in the DDR pathway was investigated in the present review, with a particular focus on PCa-related DDR.

2. DDR

It is estimated that a large number of cells in the human organism suffer tens of thousands of DNA lesions per day (14). Most lesions (75%) are DNA single-strand breaks (SSBs) that can be caused by oxidative damage during the process of metabolism or base hydrolysis. In addition, double-strand breaks (DSBs) form when two SSBs occur in close proximity or when the DNA replication apparatus encounters a single-strand break or other lesions, which are less frequent but challenging to repair and highly toxic (15). These lesions can prevent genome replication and transcription; if not repaired or repaired improperly, they can lead to mutations or broader genomic aberrations, threatening the viability of the cell or organism (16). Given the potentially destructive effects of genomic instability, cells have evolved a complex array of interlocking mechanisms to maintain their genomic integrity. The type and frequency of DNA lesions match the complexity of the mechanisms that counteract these threats to genomic integrity. These mechanisms are collectively referred to as DDR (17). In general, the DDR pathway consists of a similar set of closely coordinated processes, first detecting DNA damage, then recruiting a set of DNA repair factors at the site of the damage, and finally physically repairing the damage (14). DDR is a series of distinct but functionally intertwined pathways that depend on the type of DNA damage.

For example, base excision repair (BER) can rectify small base lesions caused by oxidation, deamination and alkylation, which do not significantly distort the DNA helix structure. When oxidative and alkylation damage occurs, the resulting base mutation is recognized by DNA glycosylase and excises the bases at the damaged site. A series of enzymes are used to complete the chain incision, cut the chain treatment to achieve DNA synthesis, fill gaps and connections, and complete BER (18). Nucleotide excision repair (NER) is used to repair damage to large segments of DNA in two ways: the global-genome NER (GG-NER) and the transcription-coupled NER (TC-NER), including lesions caused by solar radiation. GG-NER can correct DNA damage that occurs throughout the whole genome, whereas TC-NER specifically acts on DNA damage of the transcribed strand of transcriptionally active genes. DNA mismatch repair (MMR) is a highly conserved genomic pathway that can rectify DNA replication error, limit chromatin rearrangement, and mediate multiple types of DNA damage (19,20). The main mechanism of DSBs repair plays a crucial role in inhibiting genomic instability. There are two main mechanisms for DSBs repair in mammalian cells: Homologous recombination (HR) and non-homologous end joining (NHEJ) (21). The damaged site is identified by ATM/ATR, and BRCA1 is activated, which can recruit exonuclidenase MRE11 to excise a sequence near the damage of the two chains. The single chain was complementary to other homologous chains in the vicinity under the action of the recombinant enzyme RAD51, which is equivalent to DNA synthesis using the homologous chain as a template. After the synthesis of the original double strand can be partially complementary, this strand breaks off from the homologous chain, binds to another strand that was originally complementary to it, and uses it as a template for DNA synthesis by DNA polymerase. Finally, DNA ligase forms phosphate bonds to obtain a lossless DNA double strand, thus completing the HR (22). HR is an important process that is necessary to repair DNA DSBs, restart folding replication forks, and rearrange parental chromosome genetic information during meiosis (23).

The DDR pathway is complex and convoluted, and it is worth noting the DDR core components interact with cell cycle checkpoints and chromosome segregation mechanisms (17). These interactions allow DNA repair before mitosis and ensure the delivery of the correct complement of genetic material to daughter cells, which is essential for maintaining genomic stability (17). A large proportion of patients with PCa have DDR-associated gene alterations, and 19% of the 333 PCa patients' samples from The Cancer Genome Atlas had deleterious aberrations in the DDR-associated gene (24). The American Association for Cancer Research PCa Study Group identified 23% of DDR-associated gene alterations in 150 metastatic biopsies (25). Common aberrant DDR genes in PCa include *BRCA1/2, ATM, CDK12, FANCD2 and RAD51C*. Among these, *BRCA2* is the most commonly altered DDR-associated gene that results in aggression and poorer prognosis of PCa (26).

DDR in cancer. Maintaining genomic integrity and stability is crucial for intracellular DDR, and any disruption of this kinase-based signaling pathway can lead to the development of various diseases, especially cancer. A study has shown that one of the most common features of human tumors is genomic instability, which facilitates the development of driver mutations and expansion of tumor heterogeneity (27). Cytotoxic chemotherapy and radiation have long been the main treatments for tumors, and they cause severe DNA damage in proliferating cancer cells. However, tumor cells are often altered in the DDR-associated pathway, leading to genomic instability that can promote tumorigenesis and cancer cell growth such as driver mutations (28).

Although DDR defects in most cancers are unknown, a correlation between specific DDR dysfunction and tumor phenotype has been demonstrated in some cancers. For example, ~10% of breast cancer (BC) cases have been reported to be associated with germline defects in DDR-associated gene *BRCA1/2* and a small percentage of mutations in the genes encoding CHK2 and RAD51 (29). The expression of DNA-PKC was reduced in 57% of patients with early BC (30), and in >10% of aggressive BC samples, the *CDK12* gene was amplified or mutated (31). These findings have sparked extensive research and provided support for the use of DDR-targeted agents, such as PARPi, for treating BC.

DDR dysfunction has also been found in colorectal cancer (CRCA), and brain metastasis (BM) is a rare but fatal complication of CRCA. Patients with BM exhibit elevated mutational features of HR defects and MMR defects compared with primary CRCA (32). The importance of DDR in CRCA is supported by elevated levels of BM-specific mutations and microsatellite instability (MSI) in DDR-associated genes. MSI is observed in sporadic CRCA and familial hereditary non-polyposis CRCA, which is associated with loss-of-function mutations in MMR genes, such as MSH2 and MLH1 (33). In fact, MSI of CRCA is not only associated with DDR dysfunction but also with UPS-associated aberrations. A previous study has revealed that MSH2 acts as a critical DNA MMR protein and also functions as an E3 ligase that mediates MSH2 ubiquitination and degradation (34).

In conclusion, during tumorigenesis, DDR components are frequently dysfunctional, DNA damage cannot be efficiently repaired, and cells continue to have intact DNA damage during the cell cycle, which increases the chance of mutation occurrence. DDR disorders eventually lead to the occurrence and progression of cancer (17).

Although the treatment of PCa has progressed considerably in the past decades with the widespread use of ADT, AR antagonists, and androgen synthesis inhibitors, drug resistance often develops and progresses to mCRPC due to the amplification and overexpression of AR genes, AR mutations, and splice variants (28). In the case of mCRPC, AR function is reactivated and previous treatment options fail; new treatment strategies become the hope of patients, and DDR-related treatment strategies become particularly important. An increasing number of DDR-targeted drugs have rapidly spread to inhibitors of several members of the DDR pathway, including PARP, ATM, ATR, CHK1, CHK2, WEE1 and DNA-PK (35). Some of these are under clinical study, especially with PARPi olaparib and niraparib (36).

DDR-associated genes mutation in PCa. The incidence of germline mutations in the DDR-associated genes ranges from 11-33% in patients with metastatic PCa and was significantly higher than that in patients with localized PCa (8,9). DDR pathway impairment can be detected in a considerable proportion of cases, is more common in mCRPC, and is highly enriched in metastatic PCa (37). There is a wide range of DDR deficiencies in PCa, such as *TMPRSS2-ERG* gene fusion, Speckle-type BTB/POZ protein (SPOP) mutation, and loss of *PTEN* or *CHD1*, which are all related to DDR-related phenotypic damage. Functional defects in the DDR pathway may lead to sensitivity to genotoxic treatment programs, such as radiotherapy and chemotherapy. This can be further strengthened by molecular-targeting drugs to block the alternative DDR pathway (37). Alterations in the DNA damage repair pathway have recently been regarded as the main hallmark of PCa. Next-generation sequencing studies identified that ~10% of primary tumors and 25% of metastases from PCa have DDR defects, of which *BRCA2* mutation in BER pathway is considered to be the most common events (25).

In a landmark study, the most frequent aberrations in metastatic PCa patients were found to be *BRCA2* (5.3%), followed by *CHEK2* (1.9%), *ATM* (1.6%), *BRCA1* (0.9%) and *RAD51* (0.4%) (9). Another multi-institutional comprehensive clinical sequencing analysis found positive DDR-associated gene aberrations in 23% of 150 mCRPC biopsies. *BRCA2* was mutated in 13% of samples, followed by *ATM* (7.3%), *MSH2* (2%), *BRCA1, FANCA, MLH1* and *RAD51* (0.3%) (8). DDR-associated gene mutations usually increase during tumor progression (38). For example, CDK12, which plays an essential role in transcriptional regulation and genomic stability, is mutated in 1-2% of localized PCa and 4-7% of mCRPC (39). *CDK12* double allele inactivation mutations define a distinct subtype of advanced mCRPC. *CDK12* deletion is associated with genomic instability and localized tandem replication, leading to increased gene fusion and significant differential gene expression, especially in genes involved in cell cycle and DNA replication (39). Tandem duplication has also been described as an AR enhancer, possibly associated with disease progression in androgen pathway inhibitors (40).

PCa is a clinically heterogeneous disease that exhibits different responses to RT or chemotherapy, leading to different clinical outcomes. Several studies have investigated the prognostic role of BRCA2 (BRCA2 is often considered a central mediator of HR repair of DSBs) aberrations in patients with localized PCa and mCRPC receiving standard therapy (41). It is involved in initiating homology search, strand invasion, strand exchange, and limiting replication stress, and is a central regulator of genomic stability (42). In a retrospective study, *BRCA2* mutations were associated with higher Gleason scores, lymph node involvement, metastatic disease at diagnosis and T3/4 stage (26). In addition, *BRCA2* mutation is an independent prognostic factor associated with a poorer prognosis. In localized PCa, 5-year cancer-specific survival and metastasis-free survival were significantly shorter in *BRCA2* mutation carriers than in non-carriers (82 and 96%; 77 and 93%, respectively) (26). Disruption of *BRCA2* leads to defects in HR, resulting in a lack of sensitivity to DNA-damaging agents that induce DSBs and replication fork stall (43). In conclusion, among PCa-associated DDR defects, *BRCA2* mutations show relevant clinical significance by correlating with the poor clinical features of primary tumors and poor prognosis in patients with mCRPC.

Studies have shown that individuals with a reduced NER ability have an increased risk of PCa. In addition to the *BRCA2*, some of the established PCa-susceptibility genes include *RNASEL, ELAC2, MSR1, AR, CYP17* and *SRD5A2* (44). Germline mutations and polymorphisms of DDR genes [including *BRCA1,* 8-oxoguanine DNA glycosylase (*OGG1*), *XRCC1, CHEK2* and *ADPRT*] are associated with PCa risk (45,46). A previous study assessing NER polymorphisms and PCa risk revealed that the combined variant genotypes of *ERCC2/XPD D312N* in NER and *XRCC1 R399G* in BER significantly increased the risk of PCa tumorigenesis (47). NER and other repair pathways play essential roles in PCa risk (44).

A total of ~10-23% of PCa patients show high-level MSI associated with MMR gene mutations and corresponding altered MMR protein (48,49). Although the reduction or deletion of MSH2 protein expression may be associated with an increased risk of PCa tumorigenesis, it also appears to correspond to a hormone-sensitive phenotype. Compared with patients with moderate to strong MSH2 expression, the prognosis of patients with reduced or missing MSH2 expression is

relatively improved (50). Interestingly, in patients with PCa, elevated PMS2 expression, a component of the post-replicative DNA MMR, also appears to be negatively correlated with prognosis (48,50).

3. Effect of the UPS on the DDR pathway in PCa

The UPS is an essential component of DNA damage recognition and repair. The UPS plays an indispensable role in the recruitment and removal of proteins at DNA damage sites and in the regulation of downstream effectors. In addition, the UPS can participate in the arrangement and regulation of the assembly and disassembly of DDR-associated proteins at DNA damage sites to ensure the regulatory progress of DNA damage repair (51,52). DNA damage triggers corresponding cellular responses depending on the type of damage, ranging from cell cycle arrest to the activation of specific DNA repair mechanisms (53). Regulatory proteins such as E3 ligases (MDM2, Siah2, and Pirh2 in PCa) carry out the corresponding ubiquitination of p53, which determines the fate of cells, such as survival or apoptosis (54,55). SPOP mediates the non-degradative ubiquitination of HIPK2 and activates downstream targets of DDR (11,56). Ubiquitin-specific protease 14 (USP14) regulates recombinant ring finger protein 168 (RNF168) and is involved in recruiting the DDR effector protein TP53BP1 (57). HUWE1 induces non-degradative ubiquitination of KDM3A and enhances the transcription of DDR-associated genes, including *NBS1* and *RNF8* in HR repair, and *XRCC6* and *PRKDC* are involved in NHEJ repair (58). The deubiquitination function of USP14 reduces RNF168-induced γH2AFX ubiquitination signaling, which enhances cell sensitivity to ionizing radiation (IR). These UPS-associated proteins guarantee the timely repair of DNA damage, maintain the integrity of the genome, and prevent the development of a range of human diseases, including cancers and premature aging (59). Thus, the UPS-related DDR signaling pathway has been implicated in the occurrence and progression of PCa, and the specific mechanism is explained in each pathway (Fig. 1).

SPOP-HIPK2/53BP1 in PCa. SPOP is a well-known component of the E3 ligase complex and is frequently mutated in PCa. SPOP contains multiple domains, including an N-terminal MATH domain, internal BTB structure and C-terminal nuclear localization sequence, where the MATH structural domain is essential for substrate recruitment (60).

Multiple studies have confirmed that SPOP plays a tumor suppressor role in PCa by targeting a variety of proteins, but PCa-associated SPOP mutants often exhibit loss of function and negative dominant function, impairing tumor suppressor function and promoting the occurrence and progression of PCa. For example, SPOP induces ubiquitination and degradation of AR, repressing AR-mediated gene transcription and PCa cell growth. However, PCa-associated SPOP mutants abrogate this inhibition (61).

Previously, as a critical tumor suppressor in PCa, the relationship between SPOP and DDR has attracted attention. On the one hand, SPOP is associated with several proteins involved in transcription, mRNA splicing and export, including BRCA2, ATR, CHK1 and RAD51, promoting DDR and transcriptional expression of replication factors (62). By contrast, SPOP is phosphorylated by ATM kinase at Ser119 after DNA damage, which enhances SPOP binding to homologous interacting protein kinase 2 (HIPK2) and leads to the non-degradative ubiquitination of HIPK2 (11). Furthermore, SPOP-53BP1 interaction is enhanced in response to DNA damage by ATM-dependent phosphorylation of SPOP-Ser119 (56).

HIPK2 is a DNA damage-responsive kinase that activates downstream targets including p53 (63). HIPK2 phosphorylates p53 at Ser46, which activates apoptotic target genes such as *PUMA, BAX, NOXA* and *BID* in response to lethal DNA damage (64). When damage is mild, HIPK2 mediates p53 recruitment to the cyclin-dependent kinase inhibitor 1A (CDKN1A) promoter site, thus inducing cell cycle arrest followed by DNA repair (63). HIPK2 contributes to the DDR by regulating cell cycle arrest and apoptosis, thereby preventing mutations, genomic instability and carcinogenesis. In addition, it was found that SPOP-mediated ubiquitination of HIPK2 increases its phosphorylation activity on HP1γ, which further leads to the uncoupling of phosphorylated HP1γ from trimethylated H3K9me3 heterochromatin and the initiation of DNA damage repair (11). HHowever, PCa-associated *SPOP* mutants, such as $SPOP^{S119A/N}$ are defective in SPOP-HIPK2 interaction, which may lead to DDR abnormalities and genomic instability. 53BP1 is a DDR-associated protein that plays a role in the DSBs repair pathway selection. 53BP1 promotes NHEJ repair by facilitating long-range end joining of broken DNA and restricts HR by inhibiting DNA end resection. SPOP induces non-degradative polyubiquitination of 53BP1 and extracts 53BP1 from chromatin, which promotes DNA repair by more accurate HR over error-prone NHEJ. However, PCa-associated $SPOP^{S119N}$ promoted 53BP1 retention at DSBs sites and impaired DNA end excision. The lack of HR selection causes genomic instability in $SPOP^{S119N}$ cells (56).

These studies suggested that mutations in the PCa-associated SPOP-Ser119 locus impair the DDR, contributing to the genomic instability of PCa. PCa patients with SPOP-Ser119 mutations performed more sensitively to RT and chemotherapy, which may guide the clinical treatment of PCa patients with *SPOP* mutations.

USP12-MDM2-p53 regulates DDR in PCa. USP12 is similar to other USP family members, and contains a conserved catalytic cysteine/histidine structural domain (65). A previous study has found that USP12 can directly target AR, and induce its deubiquitination and stabilization, controlling the AR-AKT signaling network. The aberrant activity of AKT signaling is one of the most common features of mCRPC (66). MDM2 is a nuclear-localized E3 ligase consisting of a p53-binding domain, acid domain, zinc finger domain and ring finger domain. MDM2 targets p53 for proteasomal degradation to MDM2 to inhibit p53-mediated cell cycle checkpoint activation and DDR, thus promoting the tumorigenesis of PCa.

USP12 was previously identified as a deubiquitinase of histones H2A/H2B, Notch, PH domain leucine-rich repeat protein phosphatase 1 and AR (67). Previously, it was found that USP12 not only facilitates PCa progression by regulating AR but also regulates MDM2 deubiquitination, which in turn controls the protein level of p53 in PCa (65).

In addition to transmitting apoptotic signals, p53 can facilitate the clearance of DNA lesions by enhancing several

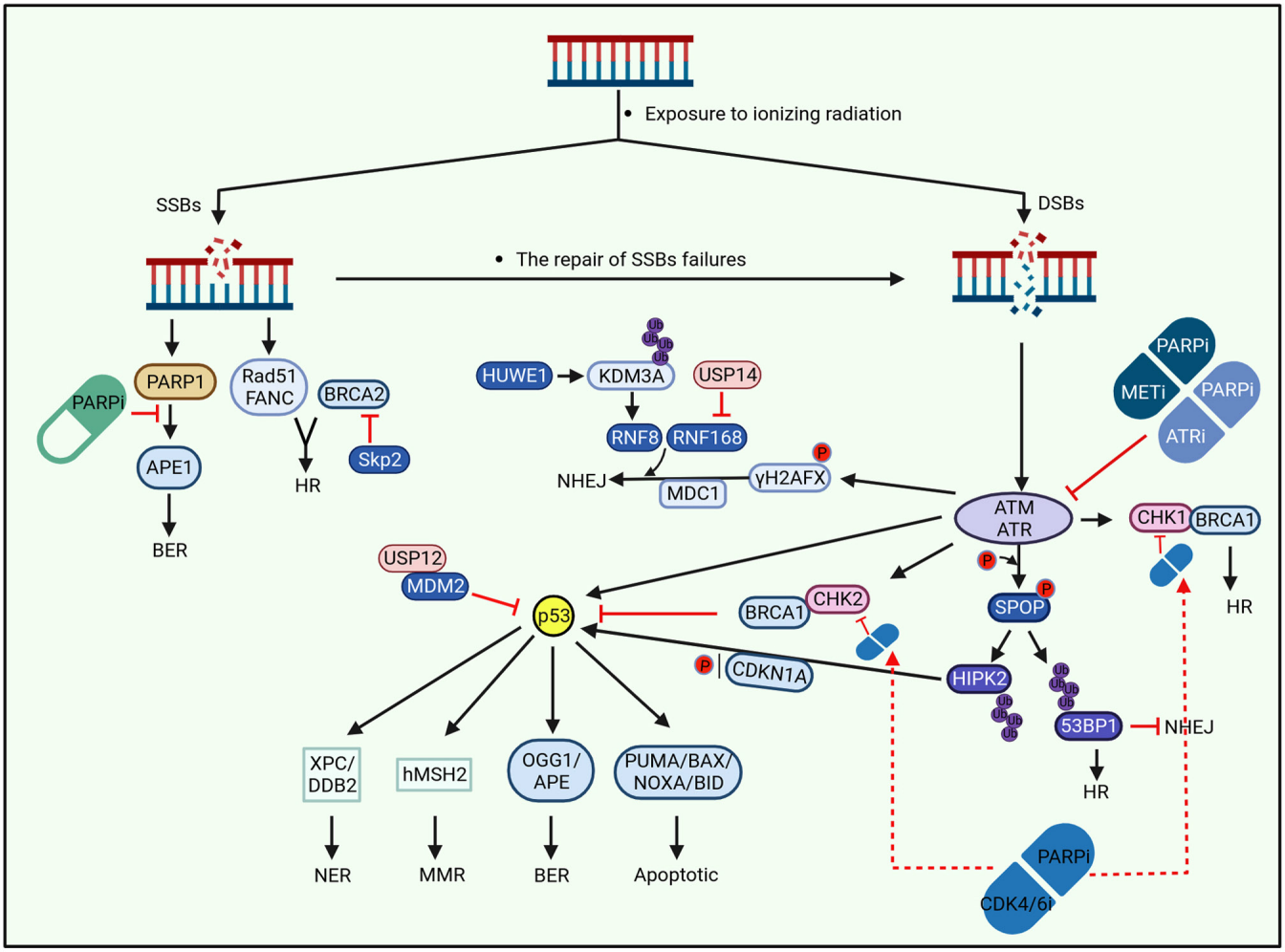

Figure 1. DDR is caused by exposure of DNA double strands to ionizing radiation. Due to the different damage degrees of ionizing radiation exposure, DNA exhibits DNA SSBs when the damage was weak. When the damage is severe, DSBs appear. SSBs are repaired by HR and BER, and if the repair fails, it can be further developed into more severe DSBs. DSBs can be repaired in various ways, including HR, NHEJ, BER, NER and MMR. The specific repair process is complex and varied, and ubiquitin protease system can participate in DDR by influencing the ubiquitination of related proteins. Drugs can also affect the progression of DDR, with PARPi, ATRi, METi and CDK4/6i drugs affecting multiple processes of DDR as demonstrated in the figure. DDR, DNA damage response; SSBs, single strand breaks; DSBs, double strand breaks; HR, homologous recombination; NHEJ, non-homologous end joining; BER, base-excision repair; NER, nucleotide excision repair; MMR, mismatch repair.

DNA repair pathways (53). p53 is involved in NER through transcriptional upregulation of xeroderma pigmentosum complementation group C and damage-specific DNA binding protein 2, both critical damage recognition factors required to initiate global genomic NER (68). However, it has also been implicated in the transcriptional control of the MMR component human muts homolog 2 during DNA damage (69). In addition to transcriptional regulation, p53 interacts directly with the critical BER enzymes, OGG1 and apurinic/apyrimidinic endonuclease, to enhance their activity, thereby increasing the efficiency of DNA lesion excision to regulate BER (53,70).

However, a high USP12 protein level in mCRPC correlates with a poor prognosis in PCa (53). USP12 stabilizes MDM2 and positively correlates with MDM2, leading to p53 degradation (53). Reduced abundance of p53 impairs its function, leading to DDR disruption and genomic instability. Thus, MDM2 inhibition may represent an attractive and feasible strategy for treating PCa. MDM2 inhibitors can increase the sensitivity of PCa to IR and ADT, thereby improving treatment outcomes.

Skp2-BRCA2 regulates DDR in PCa. Skp2, a member of the F-box protein family, is a substrate recognition component of the SCF^{Skp2} E3 ligase complex and plays a role in phosphorylation-dependent ubiquitination. It recognizes phosphorylated cyclin-dependent kinase inhibitor 1 B (also known as p27 or KIP1), mainly in the S-phase, and is overexpressed in several cancers including BC, gastric cancer and PCa (71,72).

Overexpression of Skp2 and decreased p27 abundance are often associated with aggressive PCa (73,74). Skp2, RB transcriptional corepressor 1 and p53 can trigger the degradation of p27, and overexpression of Skp2 mediates the ubiquitination and degradation of p27 and blocks cell cycle arrest and apoptosis in PCa (73). Skp2 inhibitors would lead to the accumulation of p27, activate the p27-E2F1-p73 axis, and induce apoptosis to inhibit PCa (74,75). In addition, Skp2 can activate AR directly or indirectly via TRAF6-EZH2/H3K27me3 to contribute to PCa progression (76,77). Skp2 downregulates TRAF6-mediated lysine demethylase 5B (KDM5B) ubiquitination, inhibits H3K4me3 stabilization, and promotes mCRPC migration (78).

Previously, with more attention paid to DDR-associated studies in PCa, the effect of Skp2 on BRCA2 has been gradually discovered (79). In PCa, abnormal upregulation of Skp2 leads to hydrolytic degradation, reduced abundance and impairment of BRCA2 protein-related functions. On the one hand, impairment of BRCA2 forms a complex with Rad51/FANC, which participates in HR repair of SSBs and coordinates the function of HR repair of DSBs (80). However, the functions of BRCA2 in stabilizing DNA replication forks, centrosome replication and transcriptional regulation are impaired. In addition, BRCA2 deficiency is closely associated with migratory behavior and tumor growth during PCa development, while Skp2 overexpression impairs DDR and promotes PCa progression (79,80).

These studies suggested that Skp2 is at least a key regulator determining BRCA2 abundance in PCa cell lines and that novel inhibitors targeting Skp2 activity or specifically counteracting Skp2 BRCA2 interaction, providing a new idea of therapeutic treatment for PCa patients (79,80).

USP14-RNF168 regulates DDR in PCa. USP14 is a deubiquitinating enzyme that interacts with the proteasome to regulate substrate ubiquitination (81). It contains an N-terminal ubiquitin-like (UBL) domain and C-terminal USP domain (81). The UBL domain regulates proteasome activity, whereas the USP domain displays deubiquitinase activity. USP14 protects the substrate from degradation by removing ubiquitin chains through the cooperative function of these two domains (81).

Previous studies have confirmed that USP14 overexpression accelerates PCa cell proliferation by deubiquitinating and inhibiting AR degradation in androgen-responsive PCa cells (82). Moreover, it can enhance the stability of the cancer-associated AR mutant protein AR-V7 by deubiquitination modification, promoting the progression of PCa (83,84). USP14-mediated deubiquitination of activating transcription factor 2 upregulates its abundance, which functions as an oncogenic transcription factor in PCa, thereby leading to the proliferation of PCa cells (85).

Apart from affecting AR stability to influence the occurrence and progression of PCa, it has been reported that UPS14 also affects RNF168-associated ubiquitination signaling, playing a role in NHEJ repair of DDR in PCa (57).

Sharma *et al* (57) examined the role of autophagy in regulating the DDR in response to IR using PCa cell lines as a model system and observed that RNF168 protein levels were reduced, and DSBs were not repaired when PCa autophagy-deficient cells were damaged by continuous IR. IR is a canonical DNA-damaging method, and the DDR network recognizes induced DSBs, which subsequently recruit ATM kinase, thus triggering downstream phosphorylation of histone H2AFX at Ser139. Phosphorylated H2AFX (γH2AFX) recruits E3 ligases RNF8 and RNF168 via MDC1, which initiates γH2AFX ubiquitination required for recruiting DDR factors, such as TP53BP1, to DSBs sites to coordinate NHEJ repair (57). The researchers further found that USP14 directly interacted with RNF168 and affected its associated ubiquitination process, and RNF168-mediated ubiquitination decreased in the presence of USP14 (57). In summary, USP14 negatively regulates DDR signaling, inhibits NHEJ repair, and enhances cellular sensitivity to IR by suppressing RNF168-induced γH2AFX ubiquitination.

Notably, a previous study has suggested that nuclear-localized p62 is a major factor regulating RNF168 in tumor cells, such as HeLa cells from cervical cancer and HCT116 cells from CRCA (86). However, in more advanced PCa, p62 is mainly located in the cytoplasm and is barely detectable in the nucleus (57). Additionally, USP14 was confirmed to be a novel autophagic substrate that accumulated in PCa autophagy-deficient cells, and p62 interacted with USP14 to regulate its autophagic degradation (57). Therefore, regulation of RNF168 by USP14 may be an effective mechanism to stabilize NHEJ to avoid genomic deranging in PCa cells that lack nuclear p62 (57).

This finding has significant implications in guiding PCa treatment. Firstly, the detection of UPS14 can be used to predict radiosensitivity (57). Second, autophagy signaling is usually enhanced in advanced PCa, and the application of autophagy inhibitors or p62 inhibitors to regulate the abundance of UPS14 to inhibit DDR signaling may enhance IR sensitivity.

HUWE1-KDM3A regulates DDR in PCa. HUWE1 is an evolutionarily conserved E3 ligase belonging to the HECT family. HUWE1 contains a HECT domain, UBA domain, Bcl-2 homology region 3 domain and UBM1 domain (87). The C-terminal HECT domain is the primary domain that acts as an E3 ligase that mediates ubiquitination and subsequent proteasomal degradation of substrates (87). HUWE1 is a crucial regulator of DDR transcription, autophagy and apoptosis (88,89).

HUWE1 may play different roles in different cancers as it is upregulated as an oncogene in non-small cell lung cancer (NSCLC) and downregulated as a tumor suppressor in colon adenocarcinoma (COAD) (90,91). In NSCLC, HUWE1 directly binds to and degrades the tumor suppressor p53, and an increase in HUWE1 expression is significantly associated with a worse prognosis in patients with NSCLC (91). The inactivation of endogenous HUWE1 is essential for p53 stability, and the HUWE1-p53 axis may be a potential target for NSCLC therapy (91). HUWE1 is a critical COAD suppressor that destabilizes MYC-MIZ1 and prevents DNA damage accumulation and tumor initiation (90). Notably, there are studies indicating a relationship between HUWE1 and DDR in PCa. KDM3A, a histone demethylase, has been reported to be overexpressed and play a tumor-promoting role in PCa. It was found that HUWE1 induced the non-degradative ubiquitination of KDM3A and enhanced its transcription of DDR-associated genes, including *NBS1* and *RNF8* involved in DSBs HR repair, and *XRCC6* and *PRKDC* involved in NHEJ repair. However, PCa cells expressing the KDM3A^{K918R} mutant, which cannot be ubiquitinated by HUWE1, exhibit DSBs repair defects and sensitivity to genotoxic stress (92).

The aforementioned study suggested that KDM3A modification by HUWE1 is an important event affecting DDR-associated gene expression and DSBs repair in PCa. This interference with non-degradative ubiquitination of KDM3A by HUWE1 may be a means of regulating DSBs repair, which may improve DSBs repair and the response to RT in advanced PCa.

4. Treatment for DDR defects in PCa

Currently, DDR-associated treatments for patients with PCa are mainly focused on PARPi, ATR inhibitors (ATRi) and

platinum-based chemotherapy (7). Among the currently approved PCa regimens, PARPi and platinum-based chemotherapy are effective in other cancer types associated with *BRCA1/2* alterations, and several PARPi have been clinically studied in patients with mCRPC. Other DDR inhibitor targets, such as *ATM, ATR, CHK1, CHK2* and *WEE1*, have been extensively studied (35,41). PARPi combined with RT is commonly used to treat CRCA and glioblastoma (93,94). Currently, the combination of PARPi is often used in clinical practice for the treatment of mCRPC. The currently ongoing clinical practice is summarized in Table I. In the present review, the selection of these agents for improved treatment was discussed.

PARPi is the first class of drugs to enter the clinic targeting DDR, and is a successful example of the concept of selective targeting of cancer cells introduced by precision medicine (36). PARPi causes the conversion of SSBs gaps to DSBs by blocking BER, which can result in BRCA1/2 aberrations and HR-deficient cell death (95). Notably, PARPi was first approved for treating BC and OC with BRCA aberrations through the synthetic killing effect of DDR-associated gene mutations and has been further applied to PCa treatment (36). ADP ribosylation is involved in various cellular processes including cell growth and differentiation, transcriptional regulation and apoptosis. In addition, ADP ribosylation plays a crucial role in DNA repair by promoting DSBs repair via HR (96). PARPi takes advantage of genomic instability induced by oxidative and replicative stress and defects in DDR pathways to destabilize replication forks by entrapment of PARP DNA and to induce cell death by mitotic disasters induced by replication stress (36).

Olaparib, a representative drug, was the first PARPi drug to enter a PCa clinical trial. One clinical study found that the application of olaparib was associated with prolonged progression-free survival (PFS), improved response measures and patient-reported endpoints compared with patients with mCRPC who received ADT, such as enzalutamide or abiraterone, while still experiencing disease progression (97). The aforementioned study suggested that the clinical benefit of olaparib is promising and that its combination with PARPi is a hot topic in current research. For example, the combination of olaparib and the CDK4/6 inhibitor (CDK4/6i) drugs palbociclib or abemaciclib for mCRPC and neuroendocrine PCa has been demonstrated to synergistically inhibit the p-RB-E2F1 signaling axis at the transcriptional and post-translational levels, leading to the disruption of cell cycle progression and inhibition of E2F1 gene targets (CHK1 and CHK2), including genes involved in DDR signaling damage repair CDK1 (98). The combination of PARPi and CDK4/6i not only inhibits the growth of PCa cells but also promotes apoptosis, giving greater play to the ability of PARPi to induce cell death.

Additionally, the proto-oncogene mesenchymal-epithelial transition (MET) is highly expressed in human mCRPC tissues, and MET is critical for tumor cell growth, proliferation, migration and invasion. A trial combining PARPi olaparib with the MET inhibitor (METi) crizotinib found that olaparib and crizotinib jointly downregulated the ATM/ATR signaling pathway. Drugs enhance the antitumor effects of olaparib-induced DU145 and PC3 in PCa cells by inhibiting the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway to induce apoptosis, increase mCRPC sensitivity to PARPi, and provide a new combination treatment option for Mcrpc (99).

In addition to PARP, the targeting of other DDR-related proteins is an attractive therapeutic strategy. ATR, a DDR kinase, plays a key role in preventing excessive genomic instability in tumors. ATR is responsible for sensing replication stress and sending it to the S and G2/M checkpoints to promote repair. When the DNA damage load is high enough, loss or inhibition of ATR can lead to genomic instability or cell death (100). A recent study found a new type of ATRi induction that differs from PARPi, ATRi, through abrogation of the ATR-CHK1-CDK1 regulated G2-M cell cycle checkpoint, which leads to cell death and activation of cGAS-STING signaling (101). Moreover, in contrast to PARPi, ATRi-induced abrogation of ATR-CHK1 signaling and activation of CDK1 results in the activation of the CDK1-SPOP axis, which leads to destabilization and degradation of PD-L1 in PCa cells. This difference in mechanisms provides new opportunities for combination therapy with ATRi and PARPi (101).

The main ATRi currently entering clinical oncology studies are Berzosertib, Ceralasertib, RP-3500, ART-0380, ATRN-119, M-4344, M-1774, M-6620 and Elimusertib. The ATRi drugs currently entering clinical studies in PCa have been summarized in Table II.

ATM is the most commonly mutated DDR-associated gene for PCa, except for *BRCA1/2*. Previous research data suggested that patients with *ATM* mutations may be less likely to benefit from PARPi treatment than patients with *BRCA1/2* alterations, and PCa patients with harmful *ATM* mutations are more likely to benefit from ATRi treatment. This may be a manifestation of the different mechanisms of action of ATRi and PARPi.

As aforementioned, platinum-based chemotherapy is a popular research topic for mCRPC treatment. A study evaluated the response of mCRPC patients with multiple DDR-associated gene mutations, including *BRCA1/2, ATM, PALB2, FANCA* and *CDK12* to platinum-based chemotherapy and found that a subgroup of patients with DDR-associated gene alterations may benefit from platinum-based chemotherapy (102). DDR aberration carriers exhibited improved response to platinum-based chemotherapy, indicating that DDR status deserves further validation as a potential biomarker for patient selection (102,103).

5. Discussion

The role of DDR in cancer has received increasing attention in recent years, and numerous clinical trials of DDR-related drugs are underway; however, they remain very limited in terms of clinical application. The application of DDR-related drugs is limited by the presence of specific genetic aberrations. For example, PCa cells with *BRCA1/2* mutations are more sensitive to PARPi, PCa cells with *ATM* aberrations are more sensitive to ATRi drugs, and cells without the corresponding aberrations are less sensitive. DDR-related drugs often have limited effects owing to their high drug specificity. Therefore, effectively blocking the various escape routes of cancer cells is key to deciphering the limitations of these drugs.

There is no doubt that targeted DDR is an important clinical strategy for the PCa patients treatment, but previous research is also a solid basis for improving the efficiency of PCa treatment.

Table I. Ongoing clinical trials assessing the role of PARPi in metastatic castration-resistant prostate cancer.

NCT number	Phase	PARPi	Interventions	Primary endpoint
NCT05171816	III	Olaparib	Drug: Olaparib, Abiraterone acetate	rPFS
NCT03732820	III		Drug: Olaparib, Abiraterone acetate	rPFS
NCT05457257	IV		Drug: Olaparib, Enzalutamide, Abiraterone acetate, Prednisone	rPFS
NCT03874884	I		Drug: Olaparib. Combination Product: 177Lu-PSMA	DLT, MTD, RP2D
NCT02987543	III		Drug: Olaparib, Enzalutamide, Abiraterone acetate	rPFS
NCT04556617	I/II		Drug: Olaparib, PLX2853, Abiraterone acetate, Prednisone	DLT
NCT01972217	II		Drug: Olaparib, Placebo, Abiraterone, Prednisone	AEs, DLT, rPFS
NCT03012321	II		Drug: Olaparib, Abiraterone acetate, Prednisone	PFS
NCT03834519	III		Drug: Olaparib, Abiraterone acetate, Prednisone, Enzalutamide. Biological: Pembrolizumab	OS, rPFS
NCT05005728	II		Combination Product: XmAb20717 + Olaparib. Combination Product: XmAb20717 + Carboplatin + Cabazitaxel. Biological: XmAb20717 monotherapy	AEs
NCT02861573	I/II		Drug: Olaparib, Docetaxel, Prednisone, Enzalutamide, Ebiraterone acetate, Lenvatinib, Carboplatin, Etoposide. Biological: Pembrolizumab, Pembrolizumab/Vibostolimab coformulation	PSA, AEs, ORR
NCT05262608	II		Drug: Olaparib	ORR
NCT03317392	I/II		Drug: Olaparib. Other: Laboratory Biomarker Analysis, Quality-of-Life Assessment Radiation: Radium Ra 223 Dichloride	rPFS
NCT02893917	II		Drug: Olaparib, Cediranib	rPFS
NCT03787680	II		Drug: Olaparib, AZD6738	ORR
NCT03568656	I/II		Drug: Olaparib, CCS1477, Abiraterone acetate, Enzalutamide, Darolutamide, Atezolizumab	AEs
NCT04038502	II		Drug: Olaparib, Carboplatin	PFS
NCT05252390	I/II		Drug: Olaparib, NUV-868, Enzalutamide	RP2D
NCT03903835	III	Niraparib	Drug: Niraparib plus Abiraterone acetate plus Prednisone, Enzalutamide Oral Capsule, Abiraterone Oral Tablet, Carboplatin, Docetaxel Injectable Solution, Radium Chloride Ra-223	PFS
NCT02854436	II		Drug: Niraparib	ORR
NCT03431350	I/II		Drug: Niraparib, Cetrelimab, Abiraterone acetate, Prednisone	ORR, AEs, RR
NCT02924766	I		Drug: Niraparib, Apalutamide, Abiraterone acetate, Prednisone	RP2D
NCT03748641	III		Drug: Niraparib, Abiraterone acetate, Prednisone, Placebo, New Formulation of Niraparib and Abiraterone acetate	rPFS
NCT04179396	I	Rucaparib	Drug: Rucaparib, Enzalutamide, Abiraterone	AEs, SAEs
NCT04253262	I/II		Drug: Rucaparib, Copanlisib	MTD
NCT02952534	II		Drug: Rucaparib	ORR
NCT02975934	III		Drug: Rucaparib, Abiraterone acetate or Enzalutamide or Docetaxel	rPFS
NCT03442556	II		Drug: Rucaparib, Rucaparib Camsylate, Carboplatin, Docetaxel. Other: Laboratory Biomarker Analysis	rPFS
NCT03338790	II		Drug: Rucaparib, Docetaxel, Enzalutamide, Prednisone Biological: Nivolumab	ORR, RR
NCT04455750	III		Drug: Rucaparib camsylate, Enzalutamide, Placebo, Leuprolide acetate,Goserelin acetate, Degarelix. Other: Quality-of-Life Assessment. Other: Questionnaire Administration	rPFS, OS
NCT04676334	III		Drug: Rucaparib	AEs, SAEs
NCT05425862	I	Talazoparib	Drug: Talazoparib, Pidnarulex	MTD
NCT04846478	I		Drug: Talazoparib, Tazemetostat	DLT, AEs
NCT04703920	I		Drug: Talazoparib, Belinostat	DLT
NCT03148795	II		Drug: Talazoparib	ORR
NCT04052204	I/II		Drug: Talazoparib, Avelumab, Bempegaldesleukin, Enzalutamide	DLT
NCT03395197	III		Drug: Talazoparib with enzalutamide, Placebo with enzalutamide	MTD, rPFS
NCT04824937	II		Drug: Talazoparib, Telaglenastat	ORR

Table I. Continued.

NCT number	Phase	PARPi	Interventions	Primary endpoint
NCT04019327	I/II		Drug: Talazoparib, Temozolomide	AEs, ORR
NCT03330405	I/II		Drug: Talazoparib, Avelumab	DLT, OR
NCT01576172	II	Veliparib	Drug: Veliparib, Abiraterone Acetate, Prednisone. Other: Laboratory Biomarker Analysis	RR
NCT01085422	I		Drug: Veliparib, Temozolomide	PSA test

MTD, maximum tolerable dose; PFS, progression-free survival; rPFS, radiographic PFS; pCR, pathological complete response; OS, overall survival; AEs, adverse events; SAEs, serious AEs; ORR, overall response rate; RR, composite response rate; DLT, dose-limiting toxicities; RP2D, recommended phase II dose; PK, pharmacokinetic; DRPro, patients with mCRPC who are DNA repair proficient; DRDef, patients with mCRPC who are DNA repair deficient; PSA test, protein-specific antigen test.

Table II. Ongoing clinical trials assessing the role of ATR inhibitors in prostate cancer.

NCT number	Phase	ATR iinhibitors	Interventions	Primary endpoint
NCT03787680	II	Ceralasertib	Drug: Ceralasertib, Olaparib	CR, PR
NCT03682289	II		Drug: Ceralasertib. Drug: Olaparib. Drug: Durvalumab	ORR
NCT04564027	II		Drug: Ceralasertib	ORR
NCT03517969	II	Berzosertib	Drug: Berzosertib, Carboplatin, Docetaxel. Other: Laboratory Biomarker Analysis	PAS test, PFS, rPFS
NCT04267939	I	Elimusertib	Drug: Elimusertib, Niraparib	TEAEs, TESAEs, MTD, DLT

CR, complete response; PR, partial response; ORR, overall response rate; PSA test, protein-specific antigen test; PFS, progression-free survival; rPFS, radiographic PFS; TEAEs, treatment emergent adverse events; TESAEs, treatment emergent serious adverse events; MTD, maximum tolerable dose; DLT, dose-limiting toxicities.

Therefore, a combination of drugs with different mechanisms of action is key to PCa treatment. As shown in Table I, some clinical trials have already tested the combined application of PARPi and ADT, which may be a promising approach. Several trials have suggested an increase in radiographic PFS of 5.6 months in the abiraterone combined with olaparib group compared with the ADT drug abiraterone alone group in some patients with mCRPC, but there was also a more severe incidence of adverse events (AEs) (104). Therefore, phase III clinical trials (NCT03732820) are ongoing to assess the feasibility of abiraterone in combination with olaparib as a first-line agent for mCRPC. Future combination applications could not be limited to ADT. ATRi, epidermal growth factor receptor, vascular endothelial growth factor (VEGF) and immunotherapy are also important research directions for PCa treatment, but their combination applications are much less frequent in PCa than in BC and OC. For example, combining PARPi and ATRi overcomes PARPi and platinum resistance in an OC model and significantly improves patient survival (105). Combination immunotherapy of PARPi with immune checkpoint inhibitors for mCRPC has been poorly studied; although previous studies suggested improved overall survival, the high incidence of AEs cannot be ignored, and further exploration of effective combination immunotherapy strategies with few adverse effects is warranted (106). VEGF pathway inhibition enhanced the efficacy of PARPi in OC and reduced growth and survival in OC models, irrespective of HR repair mutation status (107).

Overall, existing research provides support for new drug combination therapies with molecular mechanisms that offer more opportunities for the treatment of patients with advanced PCa. Of course, research needs to overcome the current limitations and build on existing studies to more thoughtfully apply drug combinations to further contribute to the treatment and prognosis of PCa.

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Authors' contributions

YL drafted the manuscript. XJ and YL made substantial contributions to interpretation, drafting the study, and revising it critically for important intellectual content. XJ and YL were the major contributors to the manuscript. Both authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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