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Review

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Enigmatic role of WRN-RECQL helicase in DNA repair and its implications in cancer

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Abstract

Werner (WRN) helicase belongs to the RECQL class of DNA helicases. Mutation in Werner (*WRN*) RECQL helicase leads to premature aging syndrome, Werner syndrome (WS), and predisposition to multiple cancers. WS patients exhibit heightened incidence of neoplasia, e.g., soft tissue sarcoma, osteosarcoma, malignant melanoma, meningioma, thyroid cancer, breast cancer, and leukemias. Extensive research on WRN helicase has revealed its important and diverse roles in DNA repair pathways, especially in double-strand break repair. Consequently, WRN deficiency is causally associated with genomic instability and cancer predispositions. In this review, we summarize recent studies unraveling the fundamental roles WRN helicase plays in DNA repair and genome stability and its implications in cancer therapy and resistance.

Keywords: RECQL, RECQ, WRN, genome instability, NHEJ, homologous recombination, aging, cancer

INTRODUCTION

In 1904, Otto Werner identified an autosomal recessive disorder characterized by premature aging. The disease, subsequently named Werner syndrome (WS), is caused by specific mutations in the *WRN* gene located on chromosome 8p12[1]. WRN, a large protein of 162 KDa, belongs to the five-member family of RECQ helicases in humans[1]. Structurally, WRN has four folded domains: exonuclease (bearing 3′→5′

exonuclease activity), ATPase, DNA binding RQC (RecQ C-terminal) domain, and a protein interacting HRDC (helicase-and-ribonuclease D C-terminal) domain [Figure 1]. WRN also has the distinction of being the only RECQL helicase to possess an exonuclease domain. WRN exonuclease acts in 3′-5′ direction, while helicase unwinds DNA duplex with 3′ or 5′ ssDNA overhang of minimum 10 nucleotides. The helicase and exonuclease domains act both independently and in a concerted manner to resolve a diverse array of DNA substrates, e.g., duplex DNA, D-loops, replication forks and bubble structures, G-quadruplexes, Holliday junctions, DNA flaps, *etc.*[2,3]. The RQC domain includes α2-α3 loop and β-wing motifs, which help in DNA binding and also facilitate interactions with many proteins. The WRN HRDC domain has weak DNA binding properties[4,5], while its hydrophobic pocket mediates interaction with multiple repair proteins at sites of DNA damage[6]. Several WRN-interacting proteins, such as MRN complex, KU heterodimer, RPA, and TRF2 (telomere protein), are known to boost its helicase activity. Interestingly, the exonuclease domain of WRN has structural homology with bacterial replication associated DNA-Q family exonucleases[7]. WRN has been shown to perform multifaceted roles in DNA repair-associated genomic stability (DNA editing, replication, processing of DNA ends, and switching repair process). The current review is focused on these recent reports on WRN and its role in various DNA damage response processes to limit genomic instability. Besides, targeting WRN deficient cancers as a strategy to enhance therapeutic outcomes is also discussed based on recent reports.

CELLULAR ROLES OF WRN-RECQL HELICASE

The role of WRN helicase in maintenance of DNA replication fork stability

Replication forks encounter multiple hindrances due to DNA damage, topological constraints, base modifications, nucleotide depletion, *etc.*, leading to replication stress stemming from fork slowing, stalling, or collapse. Classically, replication stress is elicited by exogenous chemotherapeutic agents, such as hydroxyurea, camptothecin, cisplatin, doxorubicin, *etc.*, during S-phase of the cell cycle. Extensive fork stalling during replication stress can cause loss of the replication machinery at the fork, leading to fork collapse. This in turn may jeopardize duplication and segregation of the entire genome with high propensity of genomic instability[8]. To cope with the dire consequences of replication stress, eukaryotic cells have evolved several elegant mechanisms to prevent and/or rescue stalled or collapsed replication forks. *In vitro* studies have shown that the RECQ helicases WRN and BLM, RAD54 translocase, and FANCM helicase trigger the generation of fork reversal, thereby preventing mutagenesis and neoplastic transformation[9]. Under unperturbed conditions, WRN primarily resides in nucleoli; however, under replication stress, it rapidly translocates to DNA damage sites. WRN has both NLS and NoLS sequences situated at the C-terminus, which enable sequestration of WRN in the nucleolus except during S-phase or upon DNA damage[10,11]. WRN localization is regulated through a balance between p300/CBP-mediated acetylation (which promotes redistribution of WRN to DNA damage sites) and SIRT1-mediated deacetylation (which favors re-localization of WRN to nucleoli on resolution of DNA damage)[12]. At collapsed replication forks, endonuclease activity of MRE11 introduces an internal nick followed by resection of double-strand break (DSB) ends and repair by HR pathway[13]. Besides, PARP1 is known to recruit MRE11 and promote fork reversal. Interestingly, RAD51 recognizes reversed forks and subsequently recruits and regulates the activity of MRE11 and other nucleases for the degradation and repair of reversed forks[14]. In the absence of RAD51, reverse forks are amenable to uncontrolled processing, leading to the disappearance of the regressed replication arm[14]. In another study, it has been shown that BRCA2 or BRCA1 protects reversed nascent replication forks from MRE11- and EXO1-mediated extensive degradation[15]. However, reverse forks with partially resected regressed arms are rescued by a MUS81- and POLD3-dependent mechanism[15]. WRN has been shown to prevent excessive degradation of stalled unprotected replication forks and facilitate efficient restart of forks in BRCA2-deficient cancer cells[16]. Intriguingly, in the BRCA1/2 proficient condition, DNA2/WRN promotes resection of degraded nascent strands[17]. Moreover, restart of collapsed replication forks is determined by RECQ1, by blocking PARP1 activity[18]. Hence, the status of BRCA1/2 determines the

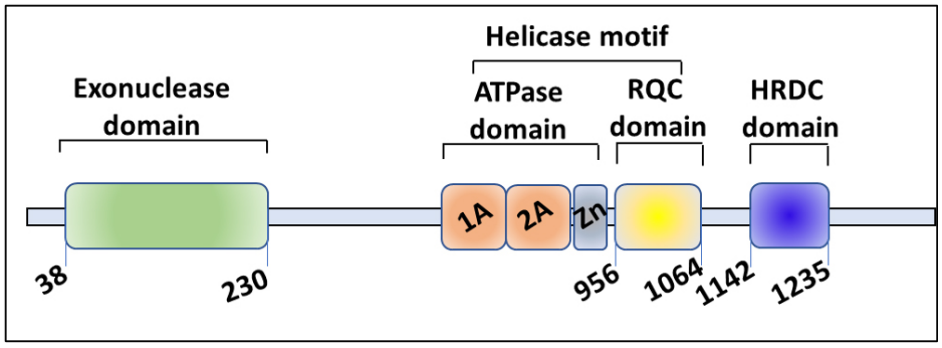

Figure 1. Functional domains of WRN RECQL helicase. RQC: RecQ C; HRDC: helicase-and-ribonuclease D/C.

involvement of the nucleases (MRE11, DNA2, EXO1, and WRN) for well-regulated degradation of nascent replication forks. In addition to the known roles of helicase and exonuclease functions of WRN in replication fork maintenance, the importance of individual catalytic (helicase/exonuclease) functions, as well as non-enzymatic functions of WRN, has also been demonstrated in replication stress. In response to a low dose of CPT, the exonuclease activity of WRN plays a crucial role in protecting the nascent replication forks from MRE11- and EXO1-mediated degradation[19]. In contrast, WRN helicase function is essential for stimulating regulated exonucleolytic degradation of nascent DNA strand and suppressing genome instability[19]. Besides, it has been demonstrated that WRN has a prominent non-enzymatic role in protecting nascent strands during replication stress[20]. At the sites of replication-associated DSBs, Nijmegen breakage syndrome protein 1 (NBS1) interacts with WRN and recruits MRE11. The physical presence of WRN at collapsed replication forks stabilizes the interaction of RAD51 with replication breaks, which blocks the access of MRE11 and hence suppresses MRE11 mediated fork degradation[20]. Hence, WRN is also critically involved in protecting replication forks to maintain genomic integrity in a non-enzymatic manner.

The role of WRN in DSB repair in switching between c-NHEJ vs. alt-NHEJ and NHEJ vs. HR repair

In DSB repair, WRN plays a key role in intrinsic repair of DSBs by classical non-homologous end joining (c-NHEJ) and homologous recombination repair (HRR). Imperatively, WRN interacts with various crucial proteins, involved in pathway switch, to make temporal dynamic sub-complexes for making a choice of repair process[21]. In the c-NHEJ pathway, KU70/80 heterodimer and DNA-PK form a stable complex and initiate a cascade of events for DSB repair. Direct interaction of the KU70/80 heterodimer is known to stimulate the exonuclease activity of the WRN[22]. Two putative binding motifs, at N-terminus and C-terminus, of WRN interacts with KU proteins. N-terminal motif-mediated interaction with KU proteins is necessary for stimulation of its exonuclease activity[23]. At DSBs, DNA-bound KU70/80 interact and stimulate the kinase activity of DNA-PKc to mediate phosphorylation at serine-440 and serine-467 sites of WRN, leading to change in the exonuclease activity of WRN. This in turn helps in processing of DSB ends, amenable for XRCC4-DNA ligase IV-mediated ligation[24] [Figure 2]. C-NHEJ-mediated DSB repair is prevalent and dominant in all phases of cell cycle, while KU deficiency switches the DSB repair process from c-NHEJ to alternate NHEJ (alt-NHEJ)[25]. Several HRR proteins (MRN, CTIP, PARP, *etc.*) and other proteins (DNA ligase I and DNA ligase III) participate in alt-NHEJ for microhomology-dependent repair[26]. In alt-NHEJ repair, DSB ends are recognized by PARP and MRE11, while it is processed/resected by MRN complex and CTIP. DNA ligases I and III help in the sealing of the resected ends [Figure 2]. The role of WRN in the DSB repair process is quite complex and slowly evolving, as evident from recent findings. Shamanna *et al.*[27] demonstrated that catalytic activities of WRN are instrumental in stimulating c-NHEJ. Interestingly, non-enzymatic WRN inhibits MRE11-CTIP-mediated alt-NHEJ[27] [Figure 2]. WRN

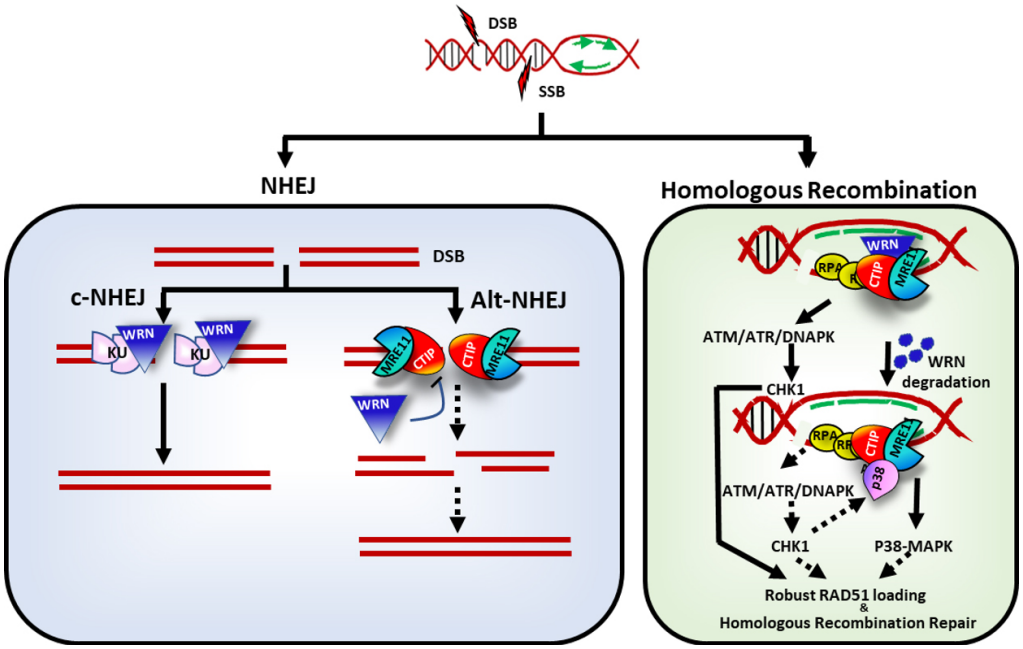

Figure 2. The role of WRN RECQL helicase in switching DSB repair pathway choice. WRN plays a crucial role in promoting c-NHEJ and suppressing alt-NHEJ. In HRR, WRN plays an indispensable role in late DSB resection and CHK1-mediated RAD51 loading in HRR. In the absence of WRN, regulation is switched to p38-MAPK-mediated RAD51 loading during HRR in cancer cells. The dashed arrow shows the compensatory pathway activated in the absence of WRN. DSB: Double strand break; SSB: single strand break; NHEJ: nonhomologous end joining; cNHEJ: canonical NHEJ; alt-NHEJ: alternative NHEJ; HRR: homologous recombination repair; CHK: checkpoint kinase 1; RAD5: DNA repair protein RAD51 homolog 1; MRE: meiotic recombination 11; CTIP: CtBP (carboxy-terminal binding protein) interacting protein; ATM: ataxia telangiectasia mutated; ATR: ataxia telangiectasia and Rad3 related.

favors NHEJ, especially in G1 phase, by inhibiting the recruitments of MRE11 and CTIP to the DSB sites. In contrast, cyclin-dependent kinase 1 (CDK1) phosphorylates at the serine-1133 site of WRN, which switches repair the process between HRR and NHEJ through regulation of MRE11 recruitment to DSB sites in S- and G2-phases[28]. During HRR, DSB end resection is initiated by MRN and CTIP initiate resection of DSB ends for shorter range, while DNA2, BLM, or EXO1 is required for extensive resection at a longer range[29]. It is also known that physical interaction of WRN and DNA2 influences their enzyme activities to stimulate DSB end processing and resection[30]. A recent study has shown that CDK2 also phosphorylates WRN at S426, which stabilizes its interaction with single-strand DNA binding protein RPA, leading to extensive resection at a long range at the DSB ends[31]. We observed that MRE11-CTIP, but not WRN, plays a key role in resection of DSBs, generated at early time points in response to ionizing radiation. In contrast, both CTIP and WRN are indispensable in resection of late and/or secondary DSBs generated in response to ionizing radiation[32] [Figure 2]. Therefore, the choice of proteins for resection may vary depending on the nature of DSB ends, generated under different conditions. Recently, we showed the role of WRN at different steps of HR. WRN also regulates CHK1-mediated RAD51 loading, while this was compromised by switching to CHK1-p38 MAPK regulated RAD51 loading in WRN-deficient cancer cells[32] [Figure 2]. Taken together, WRN plays a major and critical role in DSB repair and pathway choice[28].

The role of WRN in genomic maintenance and G4-DNA quadruplex regulation

Guanine-rich regions of the genomic DNA forms unusual G4-quadruplexes, which create obstacles for normal functioning of replication and transcription machinery[33]. Guanines are interlinked through Hoogsteen pairing in G4-DNA quadruplexes. Besides, non-B forms of DNA structures and fragile sites also pose hindrance to replication and transcription machinery. Several repair proteins are known to melt G4-

quadruplexes. WRN and Pol δ form a proofreading complex which mainly helps in dissolution of non-B DNA structures (e.g., DNA bubbles, four-way Holliday junctions, and D-loops) and processing of unmatched 3'-terminals[34]. At fragile sites (e.g., FRA16D), especially hairpins and microsatellite regions, DNA pol δ processive activity is promoted by the WNR helicase activity[35]. Thus, association of WRN and Pol δ plays a critical role in the maintenance of replication fidelity at the lagging strand. Moreover, WRN helicase facilitates hairpin repair at large (CTG)n repeats by promoting DNA pol δ-catalyzed DNA synthesis and preventing the genomic instability due to expansion or contraction of the microsatellite region[36].

The role of WRN in telomeric maintenance and senescence

In WS patients, WRN-deficient cells have slower growth coupled with higher rates of telomere attrition, thus conferring a fatal growth disadvantage to these cells. This telomeric dysfunction contributes to aging as well as cancer[1,2]. Telomeric integrity is faithfully guarded by large telomeric DNA structures, e.g., T-loops, D-loops, and G-quadruplexes, which prevent exonuclease-mediated telomere degradation[37]. Moreover, TRF1 associates with TTAGGG repeats of telomeres, forming the shelterin complex with TRF2, TIN2, TPP1, POT1, and RAP1[38]. The shelterin complex protects telomeres from various exonuclease-mediated insults, damages, and DSB repair-mediated chromosome fusions[39]. WRN interacts with several proteins of the shelterin complex; for instance, WRN is known to interact with POT1, leading to its enhanced helicase activity for D-loop structures[40]. Interestingly, WRN recruitment to D-loops is facilitated by TRF2, leading to stimulation of its DNA unwinding activity. TRF2 can be displaced from telomeric regions by WRN, independent of its ATPase and helicase activities[41,42]. In the case of oxidative damage at telomeric regions, WRN is recruited at the N-terminal of TRF1 (which is PARylated) and repairs the oxidative insults at telomeric regions. Hence, WRN plays a critical role in limiting telomeric loss-induced cellular senescence[38]. Recently, WRN has been shown to be degraded by MDM2- or MIB1-mediated ubiquitin proteasome pathway in response to etoposide and camptothecin, respectively, leading to cellular senescence through p53-dependent or -independent pathways. Hence, it is evident that degradation of WRN by the MDM2/MIB1-mediated ubiquitin proteasome pathway eventually promotes senescence by telomeric loss[43,44]. Interestingly, pathological features and premature aging phenotype were not observed in WRN knockout mice[1]. This may be attributable to abundant and long mouse telomeres, which may suppress the manifestation of telomere instability.

The role of WRN in transcription and RNA metabolism

It has been well established that WRN is essential for the activation of ATR/CHK1 and ATM signaling pathways under replication stress[45,46]. Recently, a study demonstrated that WRN helicase limits genomic instability arising from transcription-associated R-loops[47]. The authors demonstrated that ATR/CHK1 pathway regulates the Ddx19 RNA helicase, which is responsible for the removal of R-loops derived from replication-transcription conflicts. However, the ATR/CHK1 pathway is impaired in WRN-deficient cells[45]; this leads to disruption of Ddx19 nuclear localization, resulting in defective R-loop clearance[47]. Under mild replication stress, R-loop-associated genomic instability is suppressed by DSB-induced ATM activation in WRN-deficient cells[47]. Oncogene-induced replication stress is known to trigger transcription-replication conflicts, which in turn are linked to genomic instability. Interestingly, RNA polymerase I/II-mediated transcription is influenced by WRN, as evidenced by reduced transcription in WRN deficient cells[48,49]. Together, the role of WRN in transcription, replication stress, and resolution of R-loop is important to suppress genomic instability mediated cancer.

The role of WRN in cellular metabolism, energy homeostasis, and autophagy

It is interesting to note that WS patients suffer from severe metabolic dysfunctions, and several recent findings have unraveled the causal link of WRN with metabolism. Fang *et al.*[50] showed low NAD^+ levels and defective mitophagy in WS models (WS patient samples, *Caenorhabditis elegans,* and *Drosophila*

melanogaster). WRN is associated with the transcription of nicotinamide nucleotide adenylyl transferase 1, which is a key enzyme for biosynthesis of NAD^+[50]. Interestingly, supplementation of NAD^+ delayed or suppressed WS phenotypes, e.g., aging and stem cell dysfunction, and extended the lifespan of WS models in *C. elegans* and *D. melanogaster*. Interestingly, supplementation of vitamin C extended the lifespan and rescued other age-related dysfunctions (inflammation and adipose and liver tissue abnormalities) in *WRN* mutant mice[51]. Mechanistically, it has been shown that vitamin C suppresses expression of genes which are overexpressed in WS fibroblasts and cancers[51]. In support of the role of WRN in autophagy, the acidic domain of WRN was shown to positively regulate the transcription of key autophagic proteins such as BECLIN-1, ATG5, and LC3II, which promote autophagy and prevent aging[52]. PARP inhibition and DNA damage are known to activate autophagy mediated *de novo* resistance in BRCA proficient cancers[53,54]. Since WRN plays a pivotal role in interacting with PARP and fork protection in BRCA-deficient cells[16], it is imperative to understand the precise role of WRN in crosstalk for regulating autophagy and its therapeutic implications in BRCA1/2-proficient and -deficient cancers.

TARGETING WRN FOR DEVELOPMENT OF CANCER THERAPEUTICS

WRN as a synthetic lethal target in microsatellite unstable cancers

With the advancement in high throughput sequencing techniques, functional genomics have been thoroughly explored by screening a large number of cancer cell lines. Four large recent independent studies, based on silencing or CRISPR technology, showed that knocking out *WRN* gene or depletion of WRN protein led to selective or synthetic lethality in cancer lines with higher microsatellite instability, due to defective DNA mismatch repair (MMR)[55-58]. Further, Project DRIVE and Project Achilles were conducted by Novartis[59] and Broad Institute[60], respectively, to analyze two large-scale cancer cell line encyclopedias. These studies analyzed large numbers of human cancer cell lines of different tissue origin and identified various synthetic lethal interactions between a large, curated set of genes. Besides, Project Achilles and Project DRIVE validated several novel druggable targets and narrowed them down to the two best targets: PRMT5 and WRN. Behan *et al.*[55] reported that the growth of colon xenograft tumors derived from MSI^+ HCT116 cells is drastically reduced upon depletion of WRN through CRISPR-Cas9. Mechanistically, WRN deficiency led to enhanced DSBs, cell cycle arrest, genomic instability, and apoptosis in MSI^+ cells[56-58]. It is quite interesting to note that the synthetic lethal interaction of MMR deficiency is associated specifically with WRN but not with other RECQL helicases. MSI-high cells harbored few endogenous DSBs but had extensive DSBs at specific genomic loci following shRNA- or siRNA-mediated WRN depletion; again, this effect was not seen in MSS (microsatellite stable) cells. Because of defective DNA mismatch repair, some cancer cells exhibit microsatellite instability, a predisposition to frequent mutations resulting in expansion or contraction of DNA elements called microsatellites, which are short, repetitive sequences scattered throughout the genome[61]. Mechanistically, a protein complex containing the structure-specific endonuclease subcomplex MUS81-EME1 and the scaffold protein SLX4 is the source of DNA DSBs observed around $(TA)_n$ dinucleotide repeats[61]. Detection of stalled replication forks around $(TA)_n$-repeat loci induced by the generation of non-B form DNA secondary structures in these genomic regions triggers phosphorylation-based activation of the checkpoint kinase ATR, which subsequently recruits WRN to resolve stalled forks *via* its helicase activity. Notably, $(TA)_n$-repeat regions undergo extensive repeat expansion in MSI-high cells, leading to accumulation of non-B form DNA structures throughout the genome, thus explaining the indispensability of WRN in these cells[61]. This observation was further translated to preclinical and clinical models, by testing 60 heterogeneous dMMR colorectal cancer preclinical models, which confirmed WRN as a promising synthetic lethal target in dMMR/MSI-H colorectal cancer tumors as a monotherapy or in combination with targeted agents, chemotherapy, or immunotherapy[62]. Collectively, these results reveal the molecular mechanism underlying WRN dependency in MSI-high cancer cells and suggest that WRN may be an effective therapeutic target for personalized/precision cancer treatment.

Other synthetic lethality interactions of WRN

WRN deficient cells were observed to be hypersensitive to replication fork inhibitors such as hydroxyurea in the absence of MUS81. During replication fork stalling in WRN deficient cells, MUS81 cleaves stalled forks and generates DSBs, which initiate RAD51-mediated recombination, resulting in restarting of the replication fork and preventing cell death. Hence, WRN shows synthetic lethality with MUS81[63]. Further, the recruitment of RAD51 to stalled replication forks is crucial for protecting nascent DNA strands from degradation. Therefore, WRN and RAD51, acting independently on replication forks, display a synthetic lethal interaction. Recently, we showed that HR is a target for cancer treatment with IR[32]. We showed an important role of the CHK1-p38-MAPK axis in loading of RAD51 during HRR, which is significantly downregulated in WRN-silenced cells. Moreover, targeting CHK1 or p38-MAPK through pharmacological inhibitors rendered WRN-deficient cells hypersensitive to IR. IR treatment along with a CHK1 inhibitor (undergoing various clinical trials) showed strong synergy in reducing the growth of WRN-deficient melanoma tumors *in vivo*. This suggests that the therapeutic response of radiation treatment of WRN-deficient cancer patients may be enhanced under specific clinical settings[32].

WRN inhibitors

High-throughput screening of chemical compound databases and various synthetic lethal vulnerability tests against a diverse variety of tumors have led to the discovery of two novel WRN inhibitors, NSC19630 and NSC617145. NSC19630 inhibits the helicase activity of WRN. This leads to enhanced replication stress-associated DNA damage[64,65]. In addition, WRN deficiency acts synergistically with topoisomerase I inhibition, thus leading to increased cell death in combination with topotecan at sublethal doses[66]. A recent study has suggested that NSC19630 induces apoptosis and acute cytotoxicity in normal human epithelial and primary mesenchymal cells in a caspase-9-dependent and p53-independent manner[67]. NSC617145 inhibits WRN helicase to induce DSBs and chromosome abnormalities in cells harboring defects in the Fanconi anemia (FA) pathway in response to low doses of mitomycin C[68]. It is implicated that inhibition of WRN by NSC617145 leads to abrogation of HR and elicitation of NHEJ in FA defective cells. WRN plays an important role in the repair of interstrand cross link in FA-defective cells[69]. Therefore, FA-defective tumors may be sensitized by simultaneous targeting of WRN along with cross-linking agents.

CONCLUSION AND FUTURE PERSPECTIVES

The current review summarizes some of the interesting involvements of WRN in DNA repair (replication forks, DSB, TOP1-DNA cross-links, G4-DNA quadruplexes, and telomeres), transcription, and autophagy. Besides, this review also covers the role of WRN in genomic instability, cancer resistance, and the possibility of pharmacological targeting of WRN for better therapeutic outcomes. Growing evidence from recent literature not only suggests important molecular roles of WRN helicase in several DNA repair processes and maintenance of genomic stability, but it also highlights several hitherto putative roles of WRN helicase in DNA repair. Considering the following seminal roles of WRN helicase in various cellular functions, future research may yield interesting answers for some of the open-ended questions: (1) WRN plays a critical role in DSB repair pathway choice in NHEJ, alt-NHEJ, and HRR. Interestingly, WRN-depleted cells are dependent on a critical but compromised CHK1-mediated HRR pathway for repairing ionizing radiation (IR)-induced DSBs for their survival. It would be worth exploring the role of WRN in DSB repair pathway choice at single-ended DSBs, which are generated during the recovery of IR treatment and/or replication stress. (2) Growing evidence suggests that, in addition to its enzymatic functions (exonuclease and helicase) in DNA repair, non-enzymatic functions of WRN helicase are also involved in key DNA repair and signaling processes. It would be interesting to dissect the enzymatic and non-enzymatic functions of WRN in various DNA repair processes, e.g., DSB resection and pathway choice, resolution of TOP1-DNA complexes and G4 quadruplexes, maintenance of microsatellite stability, and DDR signaling (e.g., ATM and ATR activations). (3) WRN is known to regulate autophagy-mediated resistance in cancer, but it would be

interesting to address how nuclear WRN regulates a very important process in cytoplasm and how it cross-talks and regulates autophagy. Extensive work is required to glean an in-depth understanding of the molecular mechanisms of the involvement of WRN in various DNA repair and cellular processes. This will help in developing personalized therapy for patients with deregulated WRN expression in cancer.

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Authors' contributions

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All authors declared that there are no conflicts of interest.

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Consent for publication

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