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WRN Rescues Replication Forks Compromised by a BRCA2 Deficiency: Predictions for How Inhibition of a Helicase that Suppresses Premature Aging Tilts the Balance to Fork Demise and Chromosomal Instability in Cancer

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Abstract

Hereditary breast and ovarian cancers are frequently attributed to germline mutations in the tumor suppressor genes *BRCA1* and *BRCA2*. BRCA1/2 act to repair double-strand breaks and suppress the demise of unstable replication forks. Our work elucidated a dynamic interplay between BRCA2 and the WRN DNA helicase/exonuclease defective in the premature aging disorder Werner syndrome. WRN and BRCA2 participate in complementary pathways to stabilize replication forks in cancer cells, allowing them to proliferate. Whether the functional overlap of WRN and BRCA2 is relevant to replication at gaps between newly synthesized DNA fragments, protection of telomeres, and/or metabolism of secondary DNA structures remains to be determined. Advances in understanding the mechanisms elicited during replication stress have prompted the community to reconsider avenues for cancer therapy. Insights from studies of PARP or topoisomerase inhibitors provide working models for the investigation of WRN's mechanism of action. We discuss these topics, focusing on the implications of the *WRN-BRCA2* genetic interaction under conditions of replication stress.

Graphical Abstract Text

Suppression of WRN helicase's action to remodel stalled DNA replication forks in BRCA2-deficient cancer cells causes genomic instability and cancer cell killing. Created with BioRender.com.

Keywords

WRN; BRCA2; synthetic lethality; replication stress; DNA repair; genomic instability; cancer; aging; genetic disease

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Introduction

The plethora of evidence for a causal relationship of DNA damage to cancer and aging has prompted the community of DNA repair experts, cancer biologists, gerontologists, and physician scientists alike to ask what are the key sources of endogenous and exogenously induced DNA damage that contribute to cancer, aging and age-related disease. Still an understudied area, replication stress is a strong driving force of mutagenesis and chromosomal instability that underlies cellular transformation, senescence, tissue malfunction, and clinical features of age-related disease, including cancer. An illuminating window for study in this area has been the characterization of mutated genes (e.g., DNA helicases [1, 2]) linked to diseases of premature aging that often display a propensity to cancer, as well as those mutations in tumor suppressors or proto-oncogenes associated with various cancers. Recent advances have helped to unravel the critical roles of WRN helicase and the BRCA2 tumor suppressor in fork protection and restart, the subject of this rubric: *BioEssays Problems and Paradigms*.

WRN and BRCA2 help cells cope with replication stress, a driving force in cancer and accelerated aging

Our most recent work [3] identified a genetic interplay between *WRN*, the gene in which bi-allelic mutations are linked to the premature aging disorder Werner syndrome (WS) [4], and the breast/ovarian cancer tumor suppressor *BRCA2* [5], both of which play an important role in the maintenance of genomic stability. While a significant body of work has provided experimental evidence that the WRN helicase-nuclease and BRCA2 are involved in DNA repair and replication, it is yet unclear how cells balance pathways involving WRN and BRCA2 to preserve chromosomal integrity. Our research elucidated mechanistically how WRN helicase helps to stabilize replication forks in cancer cells when BRCA2 is mutated or deficient (Figure 1). During replication stress, WRN helicase prevents irreversible fork collapse and promotes fork restart when forks lack the protection of BRCA2. This is essential for the suppression of deleterious double-strand breaks (DSBs) that directly contribute to increased chromosomal instability.

During replication stress, the forks are thought to become remodeled in a manner that allows cellular machinery to repair or bypass the DNA damage or region of replication stalling and restart DNA synthesis. BRCA2 helps to protect stalled replication forks or mediate repair of DSBs via homologous recombination (HR). Genetic complementation studies demonstrate that in BRCA2-deficient cells WRN helicase acts to rescue stalled forks by limiting hyper-degradation of the nascently synthesized DNA [3]. Supporting this model, treatment of *BRCA2*-mutant cells with a pharmacologically active WRN helicase inhibitor (WRNi), NSC617145, caused enhanced degradation of hydroxyurea (HU)-stalled forks; moreover, pharmacological inhibition of the MRE11 nuclease implicated in stalled fork degradation restored fork stability in the NSC617145-treated *BRCA2*-mutated cells. RNA interference (RNAi)-mediated depletion of cellular DNA translocases (SMARCAL1, ZRANB3, HLTF) that remodel stalled forks also restored fork stability in the WRNi-treated *BRCA2*-mutated cells, supporting a model that WRN acts to protect the stalled forks downstream of fork reversal [3].

In BRCA2-proficient cells, WRN helicase and exonuclease have distinct functions at stalled replication forks. WRN exonuclease protects stalled forks from uncontrolled nucleolytic degradation [6], whereas WRN helicase facilitates DNA2-mediated restart of stalled forks [7]. Mechanistically, WRN utilizes its ATP hydrolysis to unwind the regressed arm of a stalled fork which allows nucleolytic processing of reversed forks by DNA2 nuclease and subsequent replication restart [7]. Consistent with the WRN helicase/DNA2 nuclease model, WRN depletion results in the suppression of nascent strand degradation in BRCA2-proficient cells [3, 7].

Fork restart in *BRCA2*-mutated cells is severely impaired upon loss of WRN or WRN helicase inhibition [3]. The combined biochemical and cell biological results suggest that WRN helicase catalyzes fork restoration via its ATP-dependent branch-migration activity to limit MRE11 nuclease attack on the unprotected fork, thereby promoting fork restart [3]. Thus, unlike BRCA2-proficient cells, WRN helicase-mediated fork restart in a BRCA2-deficient setting is not attributed to its role in nucleolytic processing of reversed forks. In this manner, WRN helicase switches its role from stalled fork resection in BRCA2-proficient cells to fork protection and restoration in *BRCA2*-mutated cancer cells. Here, we will discuss some salient points pertaining to the crosstalk between WRN and BRCA2 as they relate to genome homeostasis in different scenarios and how they might be relevant for new ideas in cancer treatment modality.

Does WRN act to suppress replication gaps in BRCA2-deficient cells?

There is great interest in the molecular mechanisms of replication gap suppression or tolerance in the context of how cancer cells respond to therapeutic agents [8]. Replication stress and genomic instability can arise when forks are unrestrained and achieve an unregulated high speed [9]. BRCA2 restrains DNA synthesis and suppresses single-strand gaps when replication stress arises [10, 11]. Our studies define a role of WRN in recovery of stalled replication forks under condition of BRCA2 deficiency, but they do not exclude the possibility that WRN, like the chromatin remodeling factor CHD4 and its interacting proteins (EZH2, FEN1, ZFHX3) [11], may also help to suppress replication gaps, thereby contributing to chemoresistance in BRCA2-deficient cells. Indeed, the post-replicative gap filling/repair elicited in the wake of PRIMPOL repriming and DNA synthesis in human cells appears to be complex with multiple factors and mechanisms involved [12–14]. A role of WRN in gap suppression may be elucidated by DNA fiber experiments designed to detect single-stranded DNA regions by S1-nuclease or an antibody directed against a nucleoside analog incorporated into single-stranded DNA in BRCA2-deficient or BRCA2-proficient cells, depleted of WRN or exposed to WRNi. Determination if and how WRN is involved in gap suppression or gap filling will advance our understanding of the replication abnormalities apparent in WS patient cells. WRN may play a critical role in replication-centric mechanisms whereby cancer cells become resistant to chemotherapy treatments in defined genetic backgrounds (e.g., BRCA2).

WRN's role in telomere replication and potential interplay with BRCA2 at chromosome ends

One of the classic phenotypes of WS is compromised telomeric DNA replication. A significant role of WRN in mammalian telomere maintenance was first discovered with the observation that mice co-deficient in WRN and telomerase display premature aging features; moreover, the corresponding mouse embryonic fibroblasts (MEFs) display chromosomal instability characteristic of WS [15, 16]. Further studies with the mTERC$^{-/-}$ WRN$^{-/-}$ mouse model revealed elevated telomere-telomere recombination (exchange) of the sister chromatids (T-SCE) in the doubly deficient MEFs and dependence on the alternative lengthening of telomere (ALT) pathway for telomere maintenance [17]. These findings pointed toward an essential role of WRN to limit telomere-based pathogenesis in critically short telomeres using the genetic mouse models.

Closely following the discoveries with mice, WRN-deficient human cells were reported to display defective replicative synthesis of the telomeric lagging guanine (G)-rich strand [18]. It was subsequently shown that primary human fibroblasts deficient in WRN displayed elevated T-SCE but normal global SCE in telomerase-negative backgrounds [19]. It was suggested that the elevated T-SCE was a major contributing factor to replicative senescence that underlies the accelerated aging phenotypes of WS. The localization of WRN to human telomeres [20] and the demonstrated interactions of WRN with proteins involved in telomere maintenance [2, 21] suggest a unique role of WRN to facilitate replication of telomeric DNA. For example, WRN was found to interact with the structure-specific flap endonuclease 1 (FEN-1) [22–25], an enzyme that plays an essential role in processing of Okazaki fragments required for genomic stability. Furthermore, WRN's interaction with FEN-1 was indeed shown to be important for telomere maintenance [26]. WRN helicase was also shown to resolve telomeric D-loops (T-loops) [20] and G-quadruplex (G4) DNA [27, 28] *in vitro*, suggesting its biochemical role to smooth out unusually folded DNA structures at chromosome ends.

Several studies have implicated BRCA2 in maintenance of telomeres during replication. BRCA2 was found to associate with telomeres in the S/G2 phases of the cell cycle, suggesting that it loads onto telomeres during the replication process or onto replicated DNA ends at chromosomes [29]. Conditional loss of BRCA2 prevented Rad51 to load onto telomeres in MEFs, induced telomere shortening, and resulted in the accumulation of fragmented telomeric DNA. Interestingly, these defects were not observed in BRCA1-inactivated cells, suggesting a unique role of the tumor suppressor BRCA2 for telomere stability [29]. A similar important role of BRCA2 to suppress telomere instability was found independently, with the additional observations that the BRCA2-deficient MEFs accumulated common fragile sites most abundant in the G-rich lagging strand, as well as elevated T-SCE [30]. Moreover, both common fragile sites and T-SCE were even further increased in a significant manner in MEFs treated with replication inhibitors, suggesting a requirement for BRCA2 to maintain telomere stability during replication stress.

The common denominator of defective G-rich lagging strand synthesis in cells deficient in WRN or BRCA2 raises the possibility that the two proteins act to protect replication forks at telomeres in parallel pathways. One of the provocative DNA structures formed by the G-rich

telomeric strand is G4, which has been shown to interfere with smooth DNA synthesis by various DNA polymerases in vitro (for review, see [31]). A useful tool to investigate G4 DNA metabolism in cells has been the pharmacologically active G4 binding ligands [32]. G4 structures, as detected immunologically, accumulate in WRN-deficient cells exposed to G4 binding drugs, and show an enrichment at telomeres compared to genome-wide loci [33]. Pharmacological inhibition of WRN helicase activity in human cells sensitized them to the G4-binding drug telomestatin [34], supporting the biological importance of WRN to suppress accumulation of G4 DNA structures. Exposure of BRCA2-deficient cells to a G4 binding drug was also found to cause increased telomere fragility and replication defects [35]. It is plausible that WRN and BRCA2 play synergistic roles to protect telomeres during replication of their G4 structures that persist in the G-rich lagging strand. Upon replication fork stalling at telomeric G4 DNA structures that lead generationally to DNA damage in WRN-deficient cells, BRCA2 may be required for fork protection and/or HR to prevent fork collapse and fork-associated DSBs. This working hypothesis can be experimentally tested and may help to elucidate a role of WRN at telomeres in parallel to its involvement at microsatellite repeat elements (discussed below).

WRN as an alternative or ancillary target for PARP inhibitor induced synthetic lethality

When the Ashworth and Helleday labs demonstrated that *BRCA1/2*- mutated cancer cells are selectively killed by drug inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), a whole new field of cancer chemotherapy known as synthetic lethality (SL) began [36, 37]. The approval and use of PARP inhibitors for chemotherapy treatment of patients with *BRCA1/2*-mutated ovarian and breast cancers has established a paradigm for exploiting genomic DNA damage inflicted by chemotherapy drugs to fight cancer. PARP inhibitors act to cripple HR repair of DNA breaks or cause PARP to become trapped on DNA, forming a toxic complex requiring HR repair (for a perspective, see [38]). However, certain cancer cell types can be resistant to PARP inhibitors, either intrinsically or by adaptation, thereby compromising drug efficacy in the clinic [39]. Tumor resistance to PARP inhibitors has prompted research to identify other targets important for replication fork stability/DNA repair that may be suitable targets to achieve SL [40]. Our own experimental studies of WRN illustrate this principle [3]. As reported by others, we found that the *BRCA2*-mutated ovarian cancer cell line PEO1 displayed reduced sensitivity to the PARP inhibitor Olaparib, a chemotherapy drug that is currently used in the clinic. However, co-treatment of PEO1 cells with Olaparib and sub-lethal doses of the WRNi NSC617145 was SL. Similar observations were made for *BRCA2*$^{-/-}$ colorectal cancer cells [3], demonstrating that WRN helicase inhibition potentiates the cytotoxicity of a PARP inhibitor in cancer cells deficient for BRCA2.

While combination strategies for anti-cancer strategies have gained traction, it may be that targets other than or in addition to PARP are worthwhile to pursue for tumors of breast and ovary, as well as other tissues. For example, *BRCA2* mutations are known to exist in tumors of the pancreas and prostate [41]. Potential target genes are represented by those implicated in the replication stress response or DNA repair. A good example of the latter is *RAD52,* implicated in the healing of DSBs, which displays a SL interaction with mutations in

BRCA1, *BRCA2* or *MUS81* [42–44]. Of course, there are numerous DNA damage response factors that might be exploited to treat cancer.

Protein trapping by small molecule DNA repair inhibitors: a case for WRN following the path of the PARP inhibitor paradigm

Our recent work demonstrated that replication forks in BRCA2-deficient cancer cells were degraded to a significantly greater extent by treatment with the WRN helicase inhibitor compared to WRN depletion, suggesting that the WRN-drug complex exerts a more deleterious effect on cellular DNA replication than loss of WRN altogether [3]. Given the evidence that WRN directly binds the radiolabeled NSC617145 [3], we sought to address if toxic static WRN-DNA complexes were enriched in the NSC617145-treated cells. Indeed, chromatin fractionation experiments demonstrated a dose-dependent increase of WRN bound to chromatin in *BRCA2*-mutated cells [3]. In cells that were counterstained with the S-phase specific marker PCNA, WRN showed increased nucleoplasmic foci (as opposed to nucleolar where WRN primarily resides in unstressed cells [45]), indicative of WRN's preferential localization to replication forks in BRCA2-deficient cells. WRN's enrichment in the chromatin fraction was enhanced even further in cells co-treated with NSC617145 and HU, suggesting that WRN trapping preferentially occurs during replication stalling. Altogether, the results from DNA fiber and chromatin fraction experiments suggest that the pronounced fork instability in NSC617145-treated BRCA2-deficient cells is attributed to WRN helicase inhibition and formation of static WRN-DNA complexes that perturb the progression of the replication fork.

The formation of static WRN-DNA complexes induced by the small molecule WRN helicase inhibitor follows a paradigm set by other compounds that target DNA metabolic enzymes in cancer cells. For example, topoisomerase inhibitors [46] or PARP inhibitors [47] act as cytotoxic DNA damaging agents by trapping their target proteins on DNA, causing the formation of static protein-chromatin complexes refractory to repair. Recently, new insight was gained to explain the mechanism whereby the trapped PARP1-DNA complexes are processed in cells [48]. The interactomes of trapped PARP1 and non-trapped PARP-1 were determined. A ubiquitin-regulated p97 ATPase/segregase was identified to be a member of the trapped PARP1 interactome; furthermore, it was determined that p97 is implicated in the removal of trapped PARP1 from chromatin. PARP1 is first SUMOylated and then ubiquitylated, which promotes recruitment of the ATPase p97 that facilitates removal of trapped PARP1 from chromatin [48]. Small molecule inhibition of p97-catalyzed ATP hydrolysis causes prolonged residence of PARP1 on chromatin and elevates cytotoxicity in HR-defective cells treated with the PARP inhibitor, as well as patient-derived tumor organoids. It is plausible that the metabolism of trapped WRN-DNA complexes induced by cellular treatment with a WRN helicase inhibitor operates by a similar or related mechanism of action, prompting further study (Figure 2). It will be informative to determine what other proteins are trapped with WRN in the WRNi-treated cancer cells. Characterizing the chromatin trapped WRN interactome induced by exposure to the WRNi will provide insight to the cellular phenotypes, attributed to sequestration of key genome stability factors.

The parallel between WRN/DNA and PARP1/DNA complex trapping induced by small molecule inhibitor exposure suggests a potential generality of the sequestration mechanism and cytotoxicity that may cause more severe consequences for genomic DNA damage in cancer cells that have compromised DNA repair, as suggested [47]. Furthermore, like PARP inhibitors [47], the potential development of WRN helicase inhibitors for clinical translation will require evaluation of molecular tumor profiles and assessment of the relationship between WRN trapping and maximal tolerated dose in the clinic.

Clinically relevant helicase-inactivating mutations may share common pathways and consequences as that of DNA helicase inhibitors

Similar to the WRNi trapping model, helicase-inactivating missense mutations may lead to toxic helicase-dead: genomic DNA complexes that interfere with cellular nucleic acid transactions such as replication, transcription, and DNA repair (for a perspective, see [49]). There are a number of reported helicase-inactivating missense mutations genetically linked to diseases characterized by chromosomal instability [50]. In the case of FANCJ missense mutations, some were found to exert dominant negative effects in cell-based models [51, 52]; however, their biological mechanism of action remains to be characterized. Interestingly, patient-derived WRN mutations, with just a few exceptions, result in frameshift or early stop codons that yield truncated proteins which either destabilize the protein or negate its ability to localize to the nucleus because the nuclear localization sequence resides in the extreme carboxyl terminus [53]. For those genetic disorders in which helicase-inactivating mutations have been implicated, it will be valuable to determine the protein members of the putative chromatin trapped helicase interactome and relate this information to the mutant cellular phenotypes to determine if there are any causal relationships. In collaboration with Stewart, Harel, and others, we reported a new genome instability and progeroid disorder, RECON syndrome, linked to homozygous missense mutations located in the conserved zinc binding domain of RECQL1 that seriously compromise its catalytic activities but only modestly affect DNA binding or impair its ability to oligomerize [54]. In cells, the RECQL1 mutation interferes with the response to replication stress/DNA damage imposed by a topoisomerase poison. Future studies to characterize chromatin-enriched protein interactomes of helicase-disease states, like RECON syndrome, may yield insights to disease pathogenesis, diagnosis and potential therapies that compensate for the deficiencies of helicase-interacting proteins due to their stress-induced sequestration.

Fate of trapped WRN complexes: potential insights from DNA-protein cross-link studies

Encounters of replication forks with tight protein DNA complexes, such as those induced by certain PARP inhibitors, may be highly relevant to the trapped WRN-DNA complex induced by cellular exposure to a WRN helicase inhibitor. Experimental evidence using a model Xenopus reconstituted system demonstrated the repair of DNA-protein crosslinks (DPCs) by a replication-coupled proteolysis mechanism [55, 56], that subsequently implicated the SPARTAN (SPRTN) metalloprotease [57], which is conserved in humans [58]. Using a biochemical approach with purified recombinant human SPRTN and oligonucleotide-based model DPC substrates, the Stingele lab characterized a novel DNA structure-specific DPC cleavage mechanism by SPRTN protease [59]. Furthermore, results from the reconstituted

Xenopus egg extract system for replication-dependent DPC repair suggest that the replicative helicase collaborates with the Fe-S helicase RTEL1 to ensure SPRTN activation [57, 60], but the overall process(es) involved are quite complex.

Returning to the PARP story, SPRTN deficiency in human cells causes a hypersensitivity to those PARP inhibitors that act by forming trapped PARP1-DNA complexes [61]. The epistatic relationship of SPRTN with translesion (TLS) synthesis suggests a parallel pathway that cells use to bypass PARP1-DNA complexes during replication. Determination if SPRTN or a TLS pathway is directly involved in the cellular response to trapped WRN-DNA complexes will be informative and lead to insights into potential SL interactions.

Persistent covalent topoisomerase DNA protein crosslinks (TOP-DPC) resulting from cellular exposure to certain topoisomerase inhibitors used as chemotherapy drugs can result in DSBs and genomic instability [62]. TOP-DPC are removed by specific tyrosyl-DNA phosphodiesterases or structure-specific endonucleases, made accessible by members of the aforementioned proteasome and metalloprotease SPRTN family. Expanding the scope, a recent review from the Pommier lab provides a comprehensive discussion of the metabolism of TOP-DPC by proteasome, non-proteasome and non-proteolytic pathways [63]. Interestingly, but perhaps not surprisingly, like the PARP inhibitor story, SUMO modification followed by ubiquitylation targets TOP-DPCs for proteasome-mediated proteolytic degradation [64]. Indeed, recent evidence suggests that sumoylation of target proteins in the vicinity of the TOP-DPC fine-tunes the available DPC repair pathways to ensure faithful correction of the toxic and DNA damage-inducing lesion [65]. In another development, experimental evidence was presented that poly(ADP)-ribosylation (PARylation) of TOP-DPCs acts in a regulatory manner to deubiquitylate the complexes thereby suppressing their proteasomal degradation [66].

DPC repair need not always require replication interference to signal pathway activation. Recently, a mechanism whereby DPC repair of a DNMT-type methyltransferase covalently linked to genomic DNA in human cells was found to be mediated by a SUMO-RNF4 pathway independent of replication as a salvage mechanism when the interphase DNA damage checkpoint is not activated [67].

In contrast to the aforementioned studies, there is little known about the fate of trapped WRN-DNA complexes. We reported that exposure of HeLa cells to the WRNi NSC617145 not only induced WRN binding to chromatin, but also WRN degradation in a proteasome-mediated pathway, as evidenced by the suppression of WRN degradation with cellular exposure to the proteasome inhibitor MG132 [68]. Future studies should address if the pathways that metabolize trapped PARP or trapped topoisomerase DNA complexes become engaged to deal with trapped WRN-DNA complexes, and how they are regulated (Figure 2).

New library screens for WRN helicase inhibitors (e.g., [69]), advanced molecular docking approaches, and further detailed analysis of small molecule inhibitors of DNA helicases are warranted [70, 71]. Characterizing the mechanisms of action for helicase inhibitors and their basis for cytotoxicity in cancer cells should prove informative for translational efforts to develop new and improved cancer chemotherapy strategies. Characterization

of small molecule helicase inhibitors in preclinical models, such as the case for WRNi-induced shrinkage of BRCA2-deficient xenograft tumors in mice [3], will help to advance translational efforts.

WRN and genetic-based or chemically induced synthetic lethality

In addition to WRN being an alternative to PARP as a target for SL, we find the idea of combinatorial anti-cancer treatments that exploit WRN and other genes to be attractive. For example, dual inhibition of WRN and a genetic factor implicated in single-stranded or DSB repair or a unique pathway of fork protection may behave synergistically. In support of this idea, we found that HeLa cells were sensitized to a very low concentration of the PARP inhibitor KU0058948 (1 nM) by co-treatment with the WRNi NSC19630 [34]. The attractiveness of chemical induced SL should not overshadow the potential of a combined chemical and genetic SL in specific genetic mutant backgrounds. We determined that cancer cell lines from Fanconi Anemia (FA) patients displayed hypersensitivity to a very low concentration (9 nM) of the DNA cross-linking drug Mitomycin C (MMC) upon co-treatment with the WRNi NSC617145 [68]. Thus, the super-reliance on WRN helicase to deal with DNA damage that directly perturbs replication fork progression may serve as a lamppost for development of anti-cancer drug modalities dependent on the genetic background of the tumor.

One of the best characterized SL interactions of WRN is with the structure-specific endonuclease MUS81 [72]. Based on their experimental data, the Pichierri lab proposed a model that when replication forks collapse in WRN-deficient cancer cells, MUS81 becomes engaged and cleaves the collapsed replication forks, resulting in DSBs. These fork-associated DSBs undergo Rad51-mediated break-induced repair (BIR), allowing forks to restart but at the cost of genomic instability. These findings can be placed in a new context, given our recent work which showed that pharmacological inhibition of WRN helicase resulted in elevated MUS81-dependent DSBs, which in turn activate nonhomologous end-joining (NHEJ) and cause genomic instability in *BRCA2*-mutated cancer cells [3]. It will be of interest to assess the effect of WRN deficiency versus WRN helicase inhibitor treatment, in the genetic background of mutated *BRCA2* and MUS81 deficiency, to tease out their SL relationships and cellular reliance on BIR versus NHEJ to suppress DNA damage and retain viability. Interestingly, our earlier work demonstrated that pharmacological inhibition of WRN helicase activity caused an increased MMC sensitivity for cells deficient in both FANCD2 (a key protein in the FA pathway of interstrand cross-link (ICL) repair) and DNA protein kinase catalytic subunit (DNA-PKcs), a central player in NHEJ [73]. Thus, small molecule inhibition of WRN helicase activity exacerbated the cytotoxicity of MMC-induced DNA damage when both the FA ICL repair pathway and NHEJ were compromised. This led us to propose a model in which cells deficient in the DNA damage response or a specific DNA repair pathway exposed to a WRNi would be hypersensitive to replication stress, introduced by cellular exposure to different classes of agents (ICL-inducing compounds (MMC), nucleotide pool depleting drugs (HU) or base alkylating agents (e.g., Methylmethanesulfonate) (see Figure 3 in Ref. [73]). Our latest results which demonstrate a marked sensitization of BRCA2-deficient cells to WRN helicase inhibition in multiple contexts and biological endpoints support this model [3]. We can now better

appreciate how disruption of WRN helicase activity under conditions of replication stress leads to fork collapse and chromosomal instability.

Targeting WRN in cancers characterized by microsatellite instability

Mounting evidence from multiple laboratories using different experimental approaches [74–79] has solidified the essential role of WRN helicase in cancer cells characterized by microsatellite instability (MSI), a unique form of genomic instability involving repeated sequences of DNA from one to six base pairs in length. The tandem repeats are believed to arise during faulty DNA replication, leaving frame-shift mutations due to DNA polymerase slippage that fail to be recognized by the mismatch repair machinery. WRN might be a valuable target in mismatch repair-deficient tumors that are refractory to conventional treatments including radiation, chemotherapy and immunotherapy [80–85].

Particularly unstable TA-dinucleotide repeats in MSI cells act to stall replication forks and require WRN helicase-catalyzed resolution to prevent MUS81 nucleolytic cleavage and “massive chromosome shattering” [79]. The idea that WRN helps cells to deal with stalled replication forks at these difficult-to-replicate genomic foci is supported by earlier observations that WRN is recruited to stalled forks in a fashion that is dependent on the phosphorylation of the ATR checkpoint kinase [86]. Importantly, the model proposed by van Wietmarschen et al. implicates that longer uninterrupted TA repeats which occur in late-replicating regions and prone to form secondary DNA structures require WRN-catalyzed resolution to avoid MUS81-mediated DSBs and consequential genomic instability [79]. Van Wietmarschen et al. further suggest that once $(TA)_n$ repeats exceed a critical length threshold, the DNA sequence elements would assemble into cruciform-like structures that stall replicative DNA synthesis and activate the ATR checkpoint kinase, targeting WRN and other factors to enable completion of DNA replication. When WRN is deficient, the aforementioned massive chromosome shattering ensues.

Taking all the experimental evidence into account, it seems likely that WRN’s genetic interaction with BRCA2 to suppress replication fork instability at DNA sequence elements prone to form secondary structures is highly relevant. If the frontline fork protection measures mediated by BRCA2 and other fork-associated factors are compromised by mutation, then WRN becomes a paramount player to prevent uncontrolled nascent strand degradation and suppress chromosomal instability. This hypothesis, supported by our recent findings [3], requires further experimental investigation. Translational efforts may lead to a better assessment of the potential for personalized medicine to treat cancer in individuals with specific genetic mutant backgrounds.

Surprisingly, there is a deficit of experimental work that addresses the putative involvement of BRCA2 in its fork protection role to respond to replication stress caused by DNA sequences prone to form secondary structures. While genomic instability and cancer risk have been considered in the context of compromised DNA repair, including HR repair mediated by BRCA2 and other factors (see [87] for a recent review), the assessment of BRCA2’s fork protective role that might influence MSI is understudied. As we consider the genomic landscape of mutated cancer cells for predictive diagnosis, treatment and cure, it

will be valuable to undertake studies that assess the role of fork protection factors, and their genetic interactions, in cancer cells of distinct origins and lineages.

Returning to WRN, based on our studies of its role to safeguard unstable replication forks in *BRCA2*-mutated cancer cells, it is plausible that their genetic interaction at unstable genome sequence elements requires WRN to resolve persistent DNA structures that interfere with smooth fork progression. Furthermore, molecular characterization of pharmacologically induced toxic WRN-DNA complexes at sites of repeat sequence elements in mismatch repair defective cancer cells exposed to WRNi may prove to be informative.

WRN is considered a potential target for SL. Tumors with MSI are predictable targets for WRN [74–79]. However, beyond tumors characterized by MSI, we propose that targeted WRN inhibition by its genetic loss or pharmacological inhibition may be useful more broadly for SL strategies. In our work, we showed that small molecule inhibition of WRN helicase activity in a BRCA2-deficient background led to SL in multiple cell types/genetic backgrounds, including osteosarcoma, ovary, and colorectal adenocarcinoma [3]. For a more comprehensive discussion of the experimental evidence for SL interactions of WRN, see [81].

Conclusion

In this review, we have elaborated upon our recently published work to provide further mechanistic insight into stalled fork stabilization in cancer cells by WRN when the tumor suppressor BRCA2 is deficient. In addition, we have placed this field of study in the context of cancer biology and dynamic cellular DNA transactions at the replication fork to provide food-for-thought and working hypotheses that might be explored further. Replication stress is a driving force behind cancer and aging; consequently, understanding the complex interplay among factors that operate at the replication fork in rapidly dividing cells is both challenging and rewarding. Crosstalk between WRN and BRCA2 has significant implications for translational studies, which we hope will offer advances in cancer diagnosis and treatment. SL approaches, and more broadly personalized medicine, continue to benefit from basic research experimentation, which will inform future therapies in cancer and age-related disease. WRN, as well as other DNA helicases and DNA translocases involved in key aspects of replication fork protection, have emerged as a prioritized target to achieve genetic or chemically induced SL. This is highly relevant to treatment of tumors with MSI, but likely applies to other tumor-associated mutations. We predict that the field is poised for significant developments in targeting WRN and other helicases in translational efforts to enhance emerging therapies.

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References

1. Brosh RM Jr., DNA helicases involved in DNA repair and their roles in cancer. Nature reviews Cancer. 2013;13(8):542–58. Epub 2013/07/12. doi: 10.1038/nrc3560. PubMed PMID: 23842644; PubMed Central PMCID: PMCPMC4538698. [PubMed: 23842644]
2. Dhar S, Datta A, Brosh RM Jr., DNA helicases and their roles in cancer. DNA repair. 2020;96:102994. Epub 2020/11/03. doi: 10.1016/j.dnarep.2020.102994. PubMed PMID: 33137625.
3. Datta A, Biswas K, Sommers JA, Thompson H, Awate S, Nicolae CM, et al. WRN helicase safeguards deprotected replication forks in BRCA2-mutated cancer cells. Nature communications. 2021;12(1):6561. Epub 2021/11/14. doi: 10.1038/s41467-021-26811-w. PubMed PMID: 34772932; PubMed Central PMCID: PMCPMC8590011.
4. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, et al. Positional cloning of the Werner's syndrome gene. Science (New York, NY). 1996;272(5259):258–62. Epub 1996/04/12. PubMed PMID: 8602509.
5. Kass EM, Moynahan ME, Jasin M. When Genome Maintenance Goes Badly Awry. Molecular cell. 2016;62(5):777–87. Epub 2016/06/04. doi: 10.1016/j.molcel.2016.05.021. PubMed PMID: 27259208; PubMed Central PMCID: PMCPMC4966655. [PubMed: 27259208]
6. Iannascoli C, Palermo V, Murfuni I, Franchitto A, Pichierri P. The WRN exonuclease domain protects nascent strands from pathological MRE11/EXO1-dependent degradation. Nucleic acids research. 2015;43(20):9788–803. Epub 2015/08/16. doi: 10.1093/nar/gkv836. PubMed PMID: 26275776; PubMed Central PMCID: PMCPMC4787784. [PubMed: 26275776]
7. Thangavel S, Berti M, Levikova M, Pinto C, Gomathinayagam S, Vujanovic M, et al. DNA2 drives processing and restart of reversed replication forks in human cells. The Journal of cell biology. 2015;208(5):545–62. Epub 2015/03/04. doi: 10.1083/jcb.201406100. PubMed PMID: 25733713; PubMed Central PMCID: PMCPMC4347643. [PubMed: 25733713]
8. Cantor SB. Revisiting the BRCA-pathway through the lens of replication gap suppression: "Gaps determine therapy response in BRCA mutant cancer". DNA repair. 2021;107:103209. Epub 2021/08/23. doi: 10.1016/j.dnarep.2021.103209. PubMed PMID: 34419699.
9. Maya-Mendoza A, Moudry P, Merchut-Maya JM, Lee M, Strauss R, Bartek J. High speed of fork progression induces DNA replication stress and genomic instability. Nature. 2018;559(7713):279–84. Epub 2018/06/29. doi: 10.1038/s41586-018-0261-5. PubMed PMID: 29950726. [PubMed: 29950726]
10. Cong K, Peng M, Kousholt AN, Lee WTC, Lee S, Nayak S, et al. Replication gaps are a key determinant of PARP inhibitor synthetic lethality with BRCA deficiency. Molecular cell. 2021;81(15):3128–44.e7. Epub 2021/07/04. doi: 10.1016/j.molcel.2021.06.011. PubMed PMID: 34216544. [PubMed: 34216544]
11. Panzarino NJ, Krais JJ, Cong K, Peng M, Mosqueda M, Nayak SU, et al. Replication Gaps Underlie BRCA Deficiency and Therapy Response. Cancer research. 2021;81(5):1388–97. Epub 2020/11/14. doi: 10.1158/0008-5472.Can-20-1602. PubMed PMID: 33184108; PubMed Central PMCID: PMCPMC8026497. [PubMed: 33184108]
12. Kang Z, Fu P, Alcivar AL, Fu H, Redon C, Foo TK, et al. BRCA2 associates with MCM10 to suppress PRIMPOL-mediated repriming and single-stranded gap formation after DNA damage. Nature communications. 2021;12(1):5966. Epub 2021/10/15. doi: 10.1038/s41467-021-26227-6. PubMed PMID: 34645815; PubMed Central PMCID: PMCPMC8514439.
13. Taglialatela A, Leuzzi G, Sannino V, Cuella-Martin R, Huang JW, Wu-Baer F, et al. REV1-Polζ maintains the viability of homologous recombination-deficient cancer cells through mutagenic repair of PRIMPOL-dependent ssDNA gaps. Molecular cell. 2021;81(19):4008–25.e7. Epub 2021/09/12. doi: 10.1016/j.molcel.2021.08.016. PubMed PMID: 34508659; PubMed Central PMCID: PMCPMC8500949. [PubMed: 34508659]
14. Tirman S, Quinet A, Wood M, Meroni A, Cybulla E, Jackson J, et al. Temporally distinct post-replicative repair mechanisms fill PRIMPOL-dependent ssDNA gaps in human cells. Molecular cell. 2021;81(19):4026–40.e8. Epub 2021/10/09. doi: 10.1016/j.molcel.2021.09.013. PubMed PMID: 34624216; PubMed Central PMCID: PMCPMC8555837. [PubMed: 34624216]

15. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, et al. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. Nature genetics. 2004;36(8):877–82. Epub 2004/07/06. doi: 10.1038/ng1389. PubMed PMID: 15235603. [PubMed: 15235603]

16. Du X, Shen J, Kugan N, Furth EE, Lombard DB, Cheung C, et al. Telomere shortening exposes functions for the mouse Werner and Bloom syndrome genes. Molecular and cellular biology. 2004;24(19):8437–46. Epub 2004/09/16. doi: 10.1128/mcb.24.19.8437-8446.2004. PubMed PMID: 15367665; PubMed Central PMCID: PMCPMC516757. [PubMed: 15367665]

17. Laud PR, Multani AS, Bailey SM, Wu L, Ma J, Kingsley C, et al. Elevated telomere-telomere recombination in WRN-deficient, telomere dysfunctional cells promotes escape from senescence and engagement of the ALT pathway. Genes & development. 2005;19(21):2560–70. Epub 2005/11/03. doi: 10.1101/gad.1321305. PubMed PMID: 16264192; PubMed Central PMCID: PMCPMC1276730. [PubMed: 16264192]

18. Crabbe L, Verdun RE, Haggblom CI, Karlseder J. Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. Science (New York, NY). 2004;306(5703):1951–3. Epub 2004/12/14. doi: 10.1126/science.1103619. PubMed PMID: 15591207.

19. Hagelstrom RT, Blagoev KB, Niedernhofer LJ, Goodwin EH, Bailey SM. Hyper telomere recombination accelerates replicative senescence and may promote premature aging. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(36):15768–73. Epub 2010/08/28. doi: 10.1073/pnas.1006338107. PubMed PMID: 20798040; PubMed Central PMCID: PMCPMC2936608. [PubMed: 20798040]

20. Opresko PL, Otterlei M, Graakjaer J, Bruheim P, Dawut L, Kolvraa S, et al. The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2. Molecular cell. 2004;14(6):763–74. Epub 2004/06/18. doi: 10.1016/j.molcel.2004.05.023. PubMed PMID: 15200954. [PubMed: 15200954]

21. Estep KN, Brosh RM Jr., RecQ and Fe-S helicases have unique roles in DNA metabolism dictated by their unwinding directionality, substrate specificity, and protein interactions. Biochemical Society transactions. 2018;46(1):77–95. Epub 2017/12/24. doi: 10.1042/bst20170044. PubMed PMID: 29273621; PubMed Central PMCID: PMCPMC5863537. [PubMed: 29273621]

22. Brosh RM Jr., von Kobbe C, Sommers JA, Karmakar P, Opresko PL, Piotrowski J, et al. Werner syndrome protein interacts with human flap endonuclease 1 and stimulates its cleavage activity. The EMBO journal. 2001;20(20):5791–801. Epub 2001/10/13. doi: 10.1093/emboj/20.20.5791. PubMed PMID: 11598021; PubMed Central PMCID: PMCPMC125684. [PubMed: 11598021]

23. Sharma S, Otterlei M, Sommers JA, Driscoll HC, Dianov GL, Kao HI, et al. WRN helicase and FEN-1 form a complex upon replication arrest and together process branchmigrating DNA structures associated with the replication fork. Molecular biology of the cell. 2004;15(2):734–50. Epub 2003/12/06. doi: 10.1091/mbc.e03-08-0567. PubMed PMID: 14657243; PubMed Central PMCID: PMCPMC329389. [PubMed: 14657243]

24. Sharma S, Sommers JA, Brosh RM Jr., In vivo function of the conserved non-catalytic domain of Werner syndrome helicase in DNA replication. Human molecular genetics. 2004;13(19):2247–61. Epub 2004/07/30. doi: 10.1093/hmg/ddh234. PubMed PMID: 15282207. [PubMed: 15282207]

25. Sharma S, Sommers JA, Gary RK, Friedrich-Heineken E, Hübscher U, Brosh RM Jr., The interaction site of Flap Endonuclease-1 with WRN helicase suggests a coordination of WRN and PCNA. Nucleic acids research. 2005;33(21):6769–81. Epub 2005/12/06. doi: 10.1093/nar/gki1002. PubMed PMID: 16326861; PubMed Central PMCID: PMCPMC1301591. [PubMed: 16326861]

26. Saharia A, Guittat L, Crocker S, Lim A, Steffen M, Kulkarni S, et al. Flap endonuclease 1 contributes to telomere stability. Current biology : CB. 2008;18(7):496–500. Epub 2008/04/09. doi: 10.1016/j.cub.2008.02.071. PubMed PMID: 18394896; PubMed Central PMCID: PMCPMC2367431. [PubMed: 18394896]

27. Fry M, Loeb LA. Human werner syndrome DNA helicase unwinds tetrahelical structures of the fragile X syndrome repeat sequence d(CGG)n. The Journal of biological chemistry. 1999;274(18):12797–802. Epub 1999/04/23. doi: 10.1074/jbc.274.18.12797. PubMed PMID: 10212265. [PubMed: 10212265]

28. Mohaghegh P, Karow JK, Brosh RM Jr., Bohr VA, Hickson ID. The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases. Nucleic acids research.

2001;29(13):2843–9. Epub 2001/07/04. doi: 10.1093/nar/29.13.2843. PubMed PMID: 11433031; PubMed Central PMCID: PMCPMC55766. [PubMed: 11433031]

29. Badie S, Escandell JM, Bouwman P, Carlos AR, Thanasoula M, Gallardo MM, et al. BRCA2 acts as a RAD51 loader to facilitate telomere replication and capping. Nature structural & molecular biology. 2010;17(12):1461–9. Epub 2010/11/16. doi: 10.1038/nsmb.1943. PubMed PMID: 21076401; PubMed Central PMCID: PMCPMC2998174.
30. Min J, Choi ES, Hwang K, Kim J, Sampath S, Venkitaraman AR, et al. The breast cancer susceptibility gene BRCA2 is required for the maintenance of telomere homeostasis. The Journal of biological chemistry. 2012;287(7):5091–101. Epub 2011/12/22. doi: 10.1074/jbc.M111.278994. PubMed PMID: 22187435; PubMed Central PMCID: PMCPMC3281639. [PubMed: 22187435]
31. Sommers JA, Estep KN, Maul RW, Brosh RM Jr., Biochemical analysis of DNA synthesis blockage by G-quadruplex structure and bypass facilitated by a G4-resolving helicase. Methods (San Diego, Calif). 2021. Epub 2021/12/21. doi: 10.1016/j.ymeth.2021.12.005. PubMed PMID: 34929333.
32. Teng FY, Jiang ZZ, Guo M, Tan XZ, Chen F, Xi XG, et al. G-quadruplex DNA: a novel target for drug design. Cellular and molecular life sciences : CMLS. 2021;78(19–20):6557–83. Epub 2021/08/31. doi: 10.1007/s00018-021-03921-8. PubMed PMID: 34459951. [PubMed: 34459951]
33. Drosopoulos WC, Kosiyatrakul ST, Schildkraut CL. BLM helicase facilitates telomere replication during leading strand synthesis of telomeres. The Journal of cell biology. 2015;210(2):191–208. Epub 2015/07/22. doi: 10.1083/jcb.201410061. PubMed PMID: 26195664; PubMed Central PMCID: PMCPMC4508891. [PubMed: 26195664]
34. Aggarwal M, Sommers JA, Shoemaker RH, Brosh RM Jr., Inhibition of helicase activity by a small molecule impairs Werner syndrome helicase (WRN) function in the cellular response to DNA damage or replication stress. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(4):1525–30. Epub 2011/01/12. doi: 10.1073/pnas.1006423108. PubMed PMID: 21220316; PubMed Central PMCID: PMCPMC3029756. [PubMed: 21220316]
35. Zimmer J, Tacconi EMC, Folio C, Badie S, Porru M, Klare K, et al. Targeting BRCA1 and BRCA2 Deficiencies with G-Quadruplex-Interacting Compounds. Molecular cell. 2016;61(3):449–60. Epub 2016/01/11. doi: 10.1016/j.molcel.2015.12.004. PubMed PMID: 26748828; PubMed Central PMCID: PMCPMC4747901. [PubMed: 26748828]
36. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434(7035):913–7. Epub 2005/04/15. doi: 10.1038/nature03443. PubMed PMID: 15829966. [PubMed: 15829966]
37. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917–21. Epub 2005/04/15. doi: 10.1038/nature03445. PubMed PMID: 15829967. [PubMed: 15829967]
38. D'Andrea AD. Mechanisms of PARP inhibitor sensitivity and resistance. DNA repair. 2018;71:172–6. Epub 2018/09/05. doi: 10.1016/j.dnarep.2018.08.021. PubMed PMID: 30177437. [PubMed: 30177437]
39. Noordermeer SM, van Attikum H. PARP Inhibitor Resistance: A Tug-of-War in BRCA-Mutated Cells. Trends in cell biology. 2019;29(10):820–34. Epub 2019/08/20. doi: 10.1016/j.tcb.2019.07.008. PubMed PMID: 31421928. [PubMed: 31421928]
40. Ashworth A, Lord CJ. Synthetic lethal therapies for cancer: what's next after PARP inhibitors? Nat Rev Clin Oncol. 2018;15(9):564–76. Epub 2018/06/30. doi: 10.1038/s41571-018-0055-6. PubMed PMID: . [PubMed: 29955114]
41. Mersch J, Jackson MA, Park M, Nebgen D, Peterson SK, Singletary C, et al. Cancers associated with BRCA1 and BRCA2 mutations other than breast and ovarian. Cancer. 2015;121(2):269–75. Epub 2014/09/17. doi: 10.1002/cncr.29041. PubMed PMID: 25224030; PubMed Central PMCID: PMCPMC4293332. [PubMed: 25224030]
42. Cramer-Morales K, Nieborowska-Skorska M, Scheibner K, Padget M, Irvine DA, Sliwinski T, et al. Personalized synthetic lethality induced by targeting RAD52 in leukemias identified by gene mutation and expression profile. Blood. 2013;122(7):1293–304. Epub 2013/07/10. doi: 10.1182/blood-2013-05-501072. PubMed PMID: 23836560; PubMed Central PMCID: PMCPMC3744994. [PubMed: 23836560]

43. Feng Z, Scott SP, Bussen W, Sharma GG, Guo G, Pandita TK, et al. Rad52 inactivation is synthetically lethal with BRCA2 deficiency. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(2):686–91. Epub 2010/12/15. doi: 10.1073/pnas.1010959107. PubMed PMID: 21148102; PubMed Central PMCID: PMCPMC3021033. [PubMed: 21148102]
44. Murfuni I, Basile G, Subramanyam S, Malacaria E, Bignami M, Spies M, et al. Survival of the replication checkpoint deficient cells requires MUS81-RAD52 function. PLoS genetics. 2013;9(10):e1003910. Epub 2013/11/10. doi: 10.1371/journal.pgen.1003910. PubMed PMID: 24204313; PubMed Central PMCID: PMCPMC3814295.
45. Marciniak RA, Lombard DB, Johnson FB, Guarente L. Nucleolar localization of the Werner syndrome protein in human cells. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(12):6887–92. Epub 1998/06/17. doi: 10.1073/pnas.95.12.6887. PubMed PMID: 9618508; PubMed Central PMCID: PMCPMC22674. [PubMed: 9618508]
46. Thomas A, Pommier Y. Targeting Topoisomerase I in the Era of Precision Medicine. Clinical cancer research : an official journal of the American Association for Cancer Research. 2019;25(22):6581–9. Epub 2019/06/23. doi: 10.1158/1078-0432.Ccr-19-1089. PubMed PMID: 31227499; PubMed Central PMCID: PMCPMC6858945. [PubMed: 31227499]
47. Pommier Y, O'Connor MJ, de Bono J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. Sci Transl Med. 2016;8(362):362ps17. Epub 2016/11/01. doi: 10.1126/scitranslmed.aaf9246. PubMed PMID: 27797957.
48. Krastev DB, Li S, Sun Y, Wicks AJ, Hoslett G, Weekes D, et al. The ubiquitin-dependent ATPase p97 removes cytotoxic trapped PARP1 from chromatin. Nature cell biology. 2022;24(1):62–73. Epub 2022/01/12. doi: 10.1038/s41556-021-00807-6. PubMed PMID: 35013556; PubMed Central PMCID: PMCPMC8760077. [PubMed: 35013556]
49. Wu Y, Brosh RM Jr., Helicase-inactivating mutations as a basis for dominant negative phenotypes. Cell cycle (Georgetown, Tex). 2010;9(20):4080–90. Epub 2010/10/29. doi: 10.4161/cc.9.20.13667. PubMed PMID: 20980836; PubMed Central PMCID: PMCPMC3055193.
50. Suhasini AN, Brosh RM Jr., Disease-causing missense mutations in human DNA helicase disorders. Mutation research. 2013;752(2):138–52. Epub 2013/01/02. doi: 10.1016/j.mrrev.2012.12.004. PubMed PMID: ; PubMed Central PMCID: PMCPMC3640642. [PubMed: 23276657]
51. Wu Y, Sommers JA, Loiland JA, Kitao H, Kuper J, Kisker C, et al. The Q motif of Fanconi anemia group J protein (FANCJ) DNA helicase regulates its dimerization, DNA binding, and DNA repair function. The Journal of biological chemistry. 2012;287(26):21699–716. Epub 2012/05/15. doi: 10.1074/jbc.M112.351338. PubMed PMID: 22582397; PubMed Central PMCID: PMCPMC3381133. [PubMed: 22582397]
52. Wu Y, Sommers JA, Suhasini AN, Leonard T, Deakyne JS, Mazin AV, et al. Fanconi anemia group J mutation abolishes its DNA repair function by uncoupling DNA translocation from helicase activity or disruption of protein-DNA complexes. Blood. 2010;116(19):3780–91. Epub 2010/07/20. doi: 10.1182/blood-2009-11-256016. PubMed PMID: 20639400; PubMed Central PMCID: PMCPMC2981534. [PubMed: 20639400]
53. Yokote K, Chanprasert S, Lee L, Eirich K, Takemoto M, Watanabe A, et al. WRN Mutation Update: Mutation Spectrum, Patient Registries, and Translational Prospects. Human mutation. 2017;38(1):7–15. Epub 2016/09/27. doi: 10.1002/humu.23128. PubMed PMID: 27667302; PubMed Central PMCID: PMCPMC5237432. [PubMed: 27667302]
54. Abu-Libdeh B, Jhujh SS, Dhar S, Sommers JA, Datta A, Longo GM, et al. RECON syndrome is a genome instability disorder caused by mutations in the DNA helicase RECQL1. The Journal of clinical investigation. 2022. Epub 2022/01/14. doi: 10.1172/jci147301. PubMed PMID: 35025765.
55. Duxin JP, Dewar JM, Yardimci H, Walter JC. Repair of a DNA-protein crosslink by replication-coupled proteolysis. Cell. 2014;159(2):346–57. Epub 2014/10/11. doi: 10.1016/j.cell.2014.09.024. PubMed PMID: 25303529; PubMed Central PMCID: PMCPMC4229047. [PubMed: 25303529]
56. Stingele J, Schwarz MS, Bloemeke N, Wolf PG, Jentsch S. A DNA-dependent protease involved in DNA-protein crosslink repair. Cell. 2014;158(2):327–38. Epub 2014/07/08. doi: 10.1016/j.cell.2014.04.053. PubMed PMID: 24998930. [PubMed: 24998930]

57. Larsen NB, Gao AO, Sparks JL, Gallina I, Wu RA, Mann M, et al. Replication-Coupled DNA-Protein Crosslink Repair by SPRTN and the Proteasome in Xenopus Egg Extracts. Molecular cell. 2019;73(3):574–88.e7. Epub 2019/01/01. doi: 10.1016/j.molcel.2018.11.024. PubMed PMID: 30595436; PubMed Central PMCID: PMCPMC6375733. [PubMed: 30595436]
58. Reinking HK, Hofmann K, Stingele J. Function and evolution of the DNA-protein crosslink proteases Wss1 and SPRTN. DNA repair. 2020;88:102822. Epub 2020/02/15. doi: 10.1016/j.dnarep.2020.102822. PubMed PMID: 32058279.
59. Reinking HK, Kang HS, Götz MJ, Li HY, Kieser A, Zhao S, et al. DNA Structure-Specific Cleavage of DNA-Protein Crosslinks by the SPRTN Protease. Molecular cell. 2020;80(1):102–13.e6. Epub 2020/08/28. doi: 10.1016/j.molcel.2020.08.003. PubMed PMID: 32853547; PubMed Central PMCID: PMCPMC7534798. [PubMed: 32853547]
60. Sparks JL, Chistol G, Gao AO, Raschle M, Larsen NB, Mann M, et al. The CMG Helicase Bypasses DNA-Protein Cross-Links to Facilitate Their Repair. Cell. 2019;176(1–2):167–81.e21. Epub 2019/01/01. doi: 10.1016/j.cell.2018.10.053. PubMed PMID: 30595447; PubMed Central PMCID: PMCPMC6475077. [PubMed: 30595447]
61. Saha LK, Murai Y, Saha S, Jo U, Tsuda M, Takeda S, et al. Replication-dependent cytotoxicity and Spartan-mediated repair of trapped PARP1-DNA complexes. Nucleic acids research. 2021;49(18):10493–506. Epub 2021/09/23. doi: 10.1093/nar/gkab777. PubMed PMID: 34551432; PubMed Central PMCID: PMCPMC8501961. [PubMed: 34551432]
62. Sun Y, Saha S, Wang W, Saha LK, Huang SN, Pommier Y. Excision repair of topoisomerase DNA-protein crosslinks (TOP-DPC). DNA repair. 2020;89:102837. Epub 2020/03/23. doi: 10.1016/j.dnarep.2020.102837. PubMed PMID: 32200233; PubMed Central PMCID: PMCPMC7188568.
63. Sun Y, Saha LK, Saha S, Jo U, Pommier Y. Debulking of topoisomerase DNA-protein crosslinks (TOP-DPC) by the proteasome, non-proteasomal and non-proteolytic pathways. DNA repair. 2020;94:102926. Epub 2020/07/17. doi: 10.1016/j.dnarep.2020.102926. PubMed PMID: 32674013.
64. Sun Y, Miller Jenkins LM, Su YP, Nitiss KC, Nitiss JL, Pommier Y. A conserved SUMO pathway repairs topoisomerase DNA-protein cross-links by engaging ubiquitin-mediated proteasomal degradation. Sci Adv. 2020;6(46). Epub 2020/11/15. doi: 10.1126/sciadv.aba6290. PubMed PMID: 33188014; PubMed Central PMCID: PMCPMC7673754.
65. Serbyn N, Bagdiul I, Noireterre A, Michel AH, Suhandynata RT, Zhou H, et al. SUMO orchestrates multiple alternative DNA-protein crosslink repair pathways. Cell reports. 2021;37(8):110034. Epub 2021/11/25. doi: 10.1016/j.celrep.2021.110034. PubMed PMID: 34818558.
66. Sun Y, Chen J, Huang SN, Su YP, Wang W, Agama K, et al. PARylation prevents the proteasomal degradation of topoisomerase I DNA-protein crosslinks and induces their deubiquitylation. Nature communications. 2021;12(1):5010. Epub 2021/08/20. doi: 10.1038/s41467-021-25252-9. PubMed PMID: 34408146; PubMed Central PMCID: PMCPMC8373905.
67. Liu JCY, Kühbacher U, Larsen NB, Borgermann N, Garvanska DH, Hendriks IA, et al. Mechanism and function of DNA replication-independent DNA-protein crosslink repair via the SUMO-RNF4 pathway. The EMBO journal. 2021;40(18):e107413. Epub 2021/08/05. doi: 10.15252/embj.2020107413. PubMed PMID: 34346517; PubMed Central PMCID: PMCPMC8441304.
68. Aggarwal M, Banerjee T, Sommers JA, Iannascoli C, Pichierri P, Shoemaker RH, et al. Werner syndrome helicase has a critical role in DNA damage responses in the absence of a functional fanconi anemia pathway. Cancer research. 2013;73(17):5497–507. Epub 2013/07/23. doi: 10.1158/0008-5472.can-12-2975. PubMed PMID: 23867477; PubMed Central PMCID: PMCPMC3766423. [PubMed: 23867477]
69. Sommers JA, Kulikowicz T, Croteau DL, Dexheimer T, Dorjsuren D, Jadhav A, et al. A high-throughput screen to identify novel small molecule inhibitors of the Werner Syndrome Helicase-Nuclease (WRN). PloS one. 2019;14(1):e0210525. Epub 2019/01/10. doi: 10.1371/journal.pone.0210525. PubMed PMID: 30625228; PubMed Central PMCID: PMCPMC6326523.
70. Banerjee T, Aggarwal M, Sommers JA, Brosh RM Jr., Biochemical and cell biological assays to identify and characterize DNA helicase inhibitors. Methods (San Diego, Calif). 2016;108:130–41. Epub 2016/04/12. doi: 10.1016/j.ymeth.2016.04.007. PubMed PMID: 27064001; PubMed Central PMCID: PMCPMC5035559.

71. Datta A, Brosh RM Jr., New Insights Into DNA Helicases as Druggable Targets for Cancer Therapy. Frontiers in molecular biosciences. 2018;5:59. Epub 2018/07/13. doi: 10.3389/fmolb.2018.00059. PubMed PMID: 29998112; PubMed Central PMCID: PMCPMC6028597. [PubMed: 29998112]
72. Franchitto A, Pirzio LM, Prosperi E, Sapora O, Bignami M, Pichierri P. Replication fork stalling in WRN-deficient cells is overcome by prompt activation of a MUS81-dependent pathway. The Journal of cell biology. 2008;183(2):241–52. Epub 2008/10/15. doi: 10.1083/jcb.200803173. PubMed PMID: 18852298; PubMed Central PMCID: PMCPMC2568021. [PubMed: 18852298]
73. Aggarwal M, Banerjee T, Sommers JA, Brosh RM Jr., Targeting an Achilles' heel of cancer with a WRN helicase inhibitor. Cell cycle (Georgetown, Tex). 2013;12(20):3329–35. Epub 2013/09/17. doi: 10.4161/cc.26320. PubMed PMID: 24036544; PubMed Central PMCID: PMCPMC3885643.
74. Behan FM, Iorio F, Picco G, Goncalves E, Beaver CM, Migliardi G, et al. Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature. 2019;568(7753):511–6. Epub 2019/04/12. doi: 10.1038/s41586-019-1103-9. PubMed PMID: 30971826. [PubMed: 30971826]
75. Chan EM, Shibue T, McFarland JM, Gaeta B, Ghandi M, Dumont N, et al. WRN helicase is a synthetic lethal target in microsatellite unstable cancers. Nature. 2019;568(7753):551–6. Epub 2019/04/12. doi: 10.1038/s41586-019-1102-x. PubMed PMID: 30971823; PubMed Central PMCID: PMCPMC6580861. [PubMed: 30971823]
76. Kategaya L, Perumal SK, Hager JH, Belmont LD. Werner Syndrome Helicase Is Required for the Survival of Cancer Cells with Microsatellite Instability. iScience. 2019;13:488–97. Epub 2019/03/23. doi: 10.1016/j.isci.2019.02.006. PubMed PMID: 30898619; PubMed Central PMCID: PMCPMC6441948. [PubMed: 30898619]
77. Lieb S, Blaha-Ostermann S, Kamper E, Rippka J, Schwarz C, Ehrenhofer-Wolfer K, et al. Werner syndrome helicase is a selective vulnerability of microsatellite instability-high tumor cells. eLife. 2019;8. Epub 2019/03/27. doi: 10.7554/eLife.43333. PubMed PMID: 30910006; PubMed Central PMCID: PMCPMC6435321.
78. Picco G, Cattaneo CM, van Vliet EJ, Crisafulli G, Rospo G, Consonni S, et al. Werner Helicase Is a Synthetic-Lethal Vulnerability in Mismatch Repair-Deficient Colorectal Cancer Refractory to Targeted Therapies, Chemotherapy, and Immunotherapy. Cancer Discov. 2021;11(8):1923–37. Epub 2021/04/11. doi: 10.1158/2159-8290.Cd-20-1508. PubMed PMID: 33837064. [PubMed: 33837064]
79. van Wietmarschen N, Sridharan S, Nathan WJ, Tubbs A, Chan EM, Callen E, et al. Repeat expansions confer WRN dependence in microsatellite-unstable cancers. Nature. 2020;586(7828):292–8. Epub 2020/10/02. doi: 10.1038/s41586-020-2769-8. PubMed PMID: 32999459. [PubMed: 32999459]
80. CRISPR Screens Single Out WRN. Cancer Discov. 2019;9(7):Of6. Epub 2019/05/12. doi: 10.1158/2159-8290.Cd-nb2019-055. PubMed PMID: 31076483.
81. Datta A, Dhar S, Awate S, Brosh RM Jr., Synthetic Lethal Interactions of RECQ Helicases. Trends Cancer. 2021;7(2):146–61. Epub 2020/10/13. doi: 10.1016/j.trecan.2020.09.001. PubMed PMID: ; PubMed Central PMCID: PMCPMC7855770. [PubMed: 33041245]
82. Feng FY, Gilbert LA. Lethal clues to cancer-cell vulnerability. Nature. 2019;568(7753):463–4. Epub 2019/04/20. doi: 10.1038/d41586-019-01086-w. PubMed PMID: 31000834. [PubMed: 31000834]
83. Hahn WC. A CRISPR Way to Identify Cancer Targets. The New England journal of medicine. 2019;380(25):2475–7. Epub 2019/06/20. doi: 10.1056/NEJMcibr1905048. PubMed PMID: 31216404. [PubMed: 31216404]
84. van Wietmarschen N, Nathan WJ, Nussenzweig A. The WRN helicase: resolving a new target in microsatellite unstable cancers. Current opinion in genetics & development. 2021;71:34–8. Epub 2021/07/21. doi: 10.1016/j.gde.2021.06.014. PubMed PMID: 34284257. [PubMed: 34284257]
85. Yamamoto H, Watanabe Y, Maehata T, Imai K, Itoh F. Microsatellite instability in cancer: a novel landscape for diagnostic and therapeutic approach. Arch Toxicol. 2020;94(10):3349–57. Epub 2020/07/08. doi: 10.1007/s00204-020-02833-z. PubMed PMID: 32632538. [PubMed: 32632538]
86. Ammazzalorso F, Pirzio LM, Bignami M, Franchitto A, Pichierri P. ATR and ATM differently regulate WRN to prevent DSBs at stalled replication forks and promote replication fork recovery.

The EMBO journal. 2010;29(18):3156–69. Epub 2010/08/31. doi: 10.1038/emboj.2010.205. PubMed PMID: 20802463; PubMed Central PMCID: PMCPMC2944071. [PubMed: 20802463]

87. Yoshioka KI, Kusumoto-Matsuo R, Matsuno Y, Ishiai M. Genomic Instability and Cancer Risk Associated with Erroneous DNA Repair. International journal of molecular sciences. 2021;22(22). Epub 2021/11/28. doi: 10.3390/ijms222212254. PubMed PMID: 34830134; PubMed Central PMCID: PMCPMC8625880.

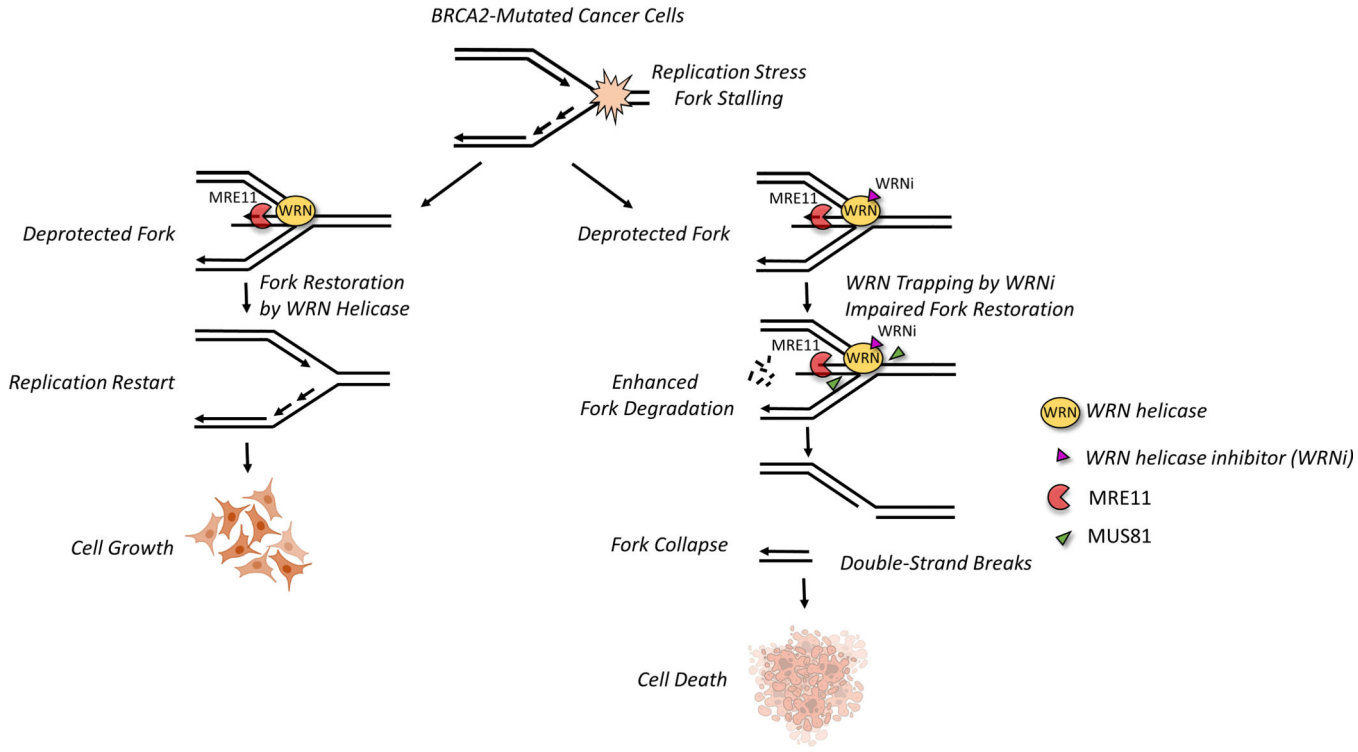

Figure 1. Targeted WRN helicase inhibition results in defective replication restart and fork degradation in BRCA2-deficient cancer cells.

Left side, WRN helicase helps BRCA2-deficient cancer cells to protect stalled forks and restart replication by converting the regressed fork to an intact fork, thereby limiting MRE11-mediated fork degradation and allowing replication restart. *Right side*, In contrast to WRN deficiency, pharmacological inhibition of WRN helicase causes rapid sequestration of inactive WRN on replicating chromatin leading to increased fork stalling and engagement by MRE11 and MUS81 nucleases leading to elevated fork degradation. This results in accumulation of DSBs at collapsed forks in *BRCA2*-mutated cancer cells and lethality. Thus, suppression of WRN helicase's action to remodel stalled DNA replication forks causes genomic instability and cell killing in a BRCA2-deficient background. Created with BioRender.com.

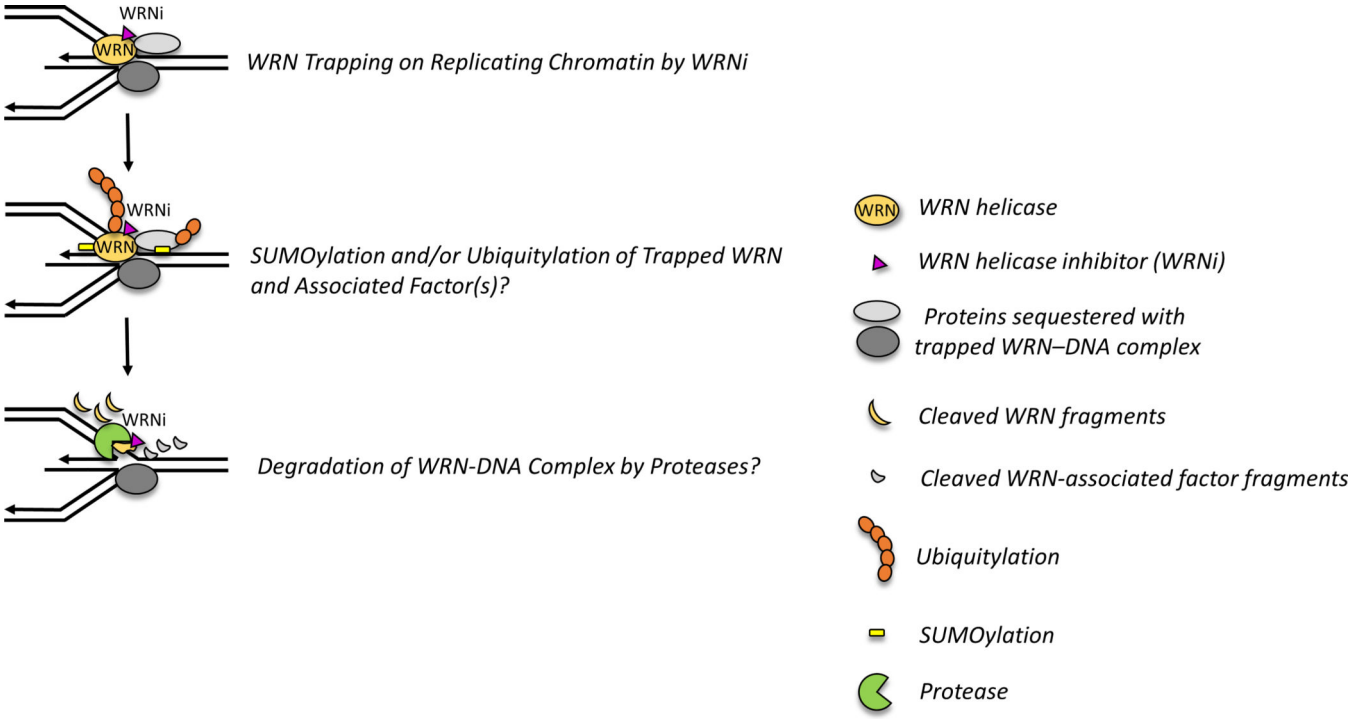

Figure 2. Potential fate of trapped WRN-DNA complexes.

Following the example of how trapped PARP complexes are metabolized, we propose a working model to study the fate of trapped WRN-chromatin complexes induced by cellular exposure to a WRNi. Other proteins may become sequestered with the trapped WRN-DNA complex on chromatin. Post-translational modifications (e.g., SUMOylation, ubiquitylation) of WRN, and perhaps associated factors, may regulate their proteolytic degradation by pathways which remain to be characterized.