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Helicases as Prospective Targets for Anti-Cancer Therapy

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Abstract

It has been proposed that selective inactivation of a DNA repair pathway may enhance anti-cancer therapies that eliminate cancerous cells through the cytotoxic effects of DNA damaging agents or radiation. Given the unique and critically important roles of DNA helicases in the DNA damage response, DNA repair, and maintenance of genomic stability, a number of strategies currently being explored or in use to combat cancer may be either mediated or enhanced through the modulation of helicase function. The focus of this review will be to examine the roles of helicases in DNA repair that might be suitably targeted by cancer therapeutic approaches. Treatment of cancers with anti-cancer drugs such as small molecule compounds that modulate helicase expression or function is a viable approach to selectively kill cancer cells through the inactivation of helicase-dependent DNA repair pathways, particularly those associated with DNA recombination, replication restart, and cell cycle checkpoint.

Keywords

helicase; chemotherapy; anti-cancer drug; DNA repair; genomic instability; cancer; RecQ; Fanconi anemia; Werner syndrome

Introduction

The emerging importance of helicases in various aspects of cellular nucleic acid metabolism and genome homeostasis has garnered significant support in the last couple of decades [1–6]. Genetic and biochemical studies have characterized the cellular and molecular roles of both prokaryotic and eukaryotic DNA helicases in replication, recombination, and DNA repair pathways. As shown in Table 1, a number of human DNA helicases have functions in specific DNA repair pathways, telomere maintenance or genomic stability. A new era in the study of helicases was ushered in with the discoveries of chromosomal instability disorders genetically linked to defects in DNA helicases. Despite the significant progress in understanding the biological importance and cellular roles of helicases, a real challenge exists for translating these basic research findings to clinical applications. The clearly evident connection between helicases and the DNA damage response and genomic stability suggests that this class of enzymes may be suitable targets for chemotherapy strategies. In this review, the rationale for targeting helicases implicated in DNA repair, or more generally the DNA damage response, will be provided along with a discussion of potential approaches in the area of small molecule inhibitors that either modulate helicase expression or function.

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Model DNA Repair Pathways Targeted for Anti-Cancer Therapy

Developing approaches to target DNA repair to improve cancer therapy has been an active area of research in recent years. It has been proposed that inhibition of DNA repair may provide an opportunity to selectively target cancer by enhancing the cytotoxicity of ionizing radiation (IR) or DNA damaging agents that are used as chemotherapy drugs. A number of excellent reviews have discussed the idea that specific DNA repair pathways may be modulated *in vivo* to either increase the sensitivity of cancer cells or improve the resistance of non-target cells upon treatment with chemotherapeutic agents [7–10]. Examples of small molecule compounds which inhibit the activities of proteins involved in the DNA damage response such as kinases or more directly in a DNA repair pathway (*e.g.*, base excision repair proteins) have been discussed in terms of potential avenues for anti-cancer treatment options.

From a DNA repair perspective, two excellent examples of DNA repair inhibitors being investigated for anti-cancer therapy are targeted toward the DNA damage response poly(ADP) ribose polymerase (PARP) or the Fanconi anemia (FA) proteins implicated in DNA interstrand cross-link repair, discussed in detail in an accompanying review (Litman et al.) in this series. Novel therapies for breast and ovarian cancer based on selective killing of BRCA-deficient cells by PARP inhibitors have been described [11;12].

Mammalian cells can only tolerate a very low threshold of interstrand DNA cross-links since this form of DNA damage effectively blocks DNA replication and transcription [13]. A number of compounds (nitrogen mustards, chloroethylnitrosureas, cyclophosphamides, mitomycin C, platinum molecules) that introduce DNA interstrand cross-links are clinically used to combat cancer (Fig. 1). However, their effectiveness for tumor reduction can be limited since certain types of cancerous tumors become resistant to the cross-linking agent. Improving the efficacy of cross-linker drug action will improve the clinical outcome of such therapy. A growing number of chromatin associated proteins with defined biochemical activities have been implicated in the process of DNA interstrand cross-link repair (Table 2). Although the mechanism of DNA cross-link repair remains to be completely understood, strong genetic evidence has established the prominent role of the FA/BRCA pathway in cellular resistance to DNA cross-linking agents including the bifunctional alkylating agent mitomycin C and the platinum analogues cisplatin and carboplatin that have been classically used to treat certain types of cancers (for review see [14]).

Based on their conserved ATPase/helciase domain sequence and catalytic functions, the FANCM and FANCI proteins are likely to be intimately involved in DNA damage recognition or processing steps of interstrand cross-link repair; therefore, these proteins may be useful targets for modulation by small molecules.

Anti-cancer agents such as topoisomerase inhibitors (*e.g.*, etoposide), bleomycin, or IR introduce toxic DSBs through direct or indirect mechanisms. Chemotherapy by such agents may be enhanced via direct inhibitors of DSB repair. Inhibitors of the ATM kinase which regulates HR and cell cycle checkpoints were found to sensitize normal cells to IR and chemotherapy drugs which introduce DSBs including etoposide, doxorubicin, and camptothecin (Fig. 2)(for review, see [9]). DNA helicases involved in nonhomologous end-joining or HR repair may be attractive targets for anti-cancer drugs.

The RecQ helicases, in particular, have been implicated in DSB repair by the HR pathway (for review, see [4]). Small molecule inhibitors of RecQ catalytic activities may be used to deter HR repair by a parallel pathway. In addition, the connection of DNA helicases to tumor suppressor and DNA damage response molecules p53, BRCA1, and BRCA2 may be exploited to advance current therapies to inhibit tumor progression [15]. The strong evidence for cross-talk between p53 and the human RecQ helicases WRN and BLM [4;16] sets the stage for

investigating how cancer therapies currently in place that affect p53 function [17] involve WRN or BLM. Chemotherapeutic treatments that saturate the HR repair machinery might be further enhanced by the simultaneous disruption of functional interactions between p53 and RecQ helicases. The cancer therapy strategy of altering p53 levels by inhibiting p53 protein-protein interactions with small molecules (e.g. nutlin-3) [18] may involve downstream partners of p53 such as the RecQ helicases. The apoptotic potential of DNA damage induced by chemotherapy drugs or radiation may be improved by the simultaneous treatment with small molecules that either stabilize the p53-WRN or p53-BLM interactions or mimic the p53 tumor suppressor-helicase interaction.

As an alternative to the small molecule approach, down-regulation of RecQ helicase expression by RNA interference may achieve similar outcomes (discussed later). Such approaches for targeting RecQ helicases involved in HR repair of DNA breaks could be tested in model systems for their cytotoxic effects in combination with DNA damaging agents or radiation that introduces DNA strand breaks either directly or indirectly. Once combinatorial treatments are characterized in model systems, protocols could be developed for preclinical trials.

Regulation of Cell Cycle Checkpoints: A Potential Avenue for Helicase-Directed Cancer Therapy

BRCA1/2-mediated DSB repair and FA interstrand cross-link repair are two pathways that directly involve the action of helicases and may be potentially exploited in the development of a new class of small molecules directed against helicase function or expression. Understanding the precise roles of helicases and interacting proteins in DNA repair during specific stages of the cell cycle is a growing field of interest. For example, the sensitivity of BRCA1-deficient cells to DNA interstrand cross-linking agents is associated with defective HR repair and aberrant S-phase arrest [19]. Certain helicases function in the DNA damage response to regulate checkpoints which in turn modulate cell proliferation, growth, and division after DNA damage. Cell cycle regulation has an important role in tumorigenesis and chemoresistance; consequently, targeting the cell cycle has become a viable approach to cancer therapy (for review, see [20]). Delay or arrest of the cell cycle provides a protective mechanism for cancer cells to repair DNA damage. The ability of cancer cells to experience unregulated growth due to the absence of proper checkpoints has evoked interest from cancer biologists to search for novel targets in cancer therapy. Cyclin-dependent kinase inhibitors, currently used in preclinical studies, represent one important class of chemotherapeutic molecules that eliminate cell cycle checkpoints and render tumor cells more susceptible to apoptotic cell death. Proteins involved in cell cycle regulation, and more specifically during mitosis, such as G2-M kinases and kinesins are currently being evaluated as potential targets for anti-cancer therapy as well.

The direct involvement of DNA helicases or chromatin remodeling enzymes in cell cycle checkpoints suggest that these enzymes may be useful targets for anti-cancer therapy. Proteins of the minichromosome (MCM) helicase complex implicated in DNA replication initiation and elongation and the licensing checkpoint are up-regulated in human cells that are either undergoing malignant transformation or are in a state of malignancy [21;22]. MCM expression is down-regulated in differentiated somatic cells, further suggesting a role in proliferation. In one model for MCM checkpoint function, continuation of MCM helicase unwinding at the replication fork and stalling of the replicative polymerase leads to RPA-bound ssDNA which provides a critical signal for activation of the ATR checkpoint response [23].

A wealth of evidence exists for the direct involvement of lower eukaryote RecQ helicases in DNA damage checkpoints [24]. For humans, the prolonged S phase of WS and BS cells and the sensitivity of these mutant cells to either agents that induce DNA damage which impede

replication fork progression or slow replication by nucleotide depletion (e.g., hydroxyurea) suggest a role of the WRN and BLM helicases in the stabilization or restart of the replication fork [4]. The catalytic functions and protein interactions of WRN and BLM helicases suggest direct roles in the regulation of the initiation and/or progression of DNA replication, or replication restart. However, some evidence suggests a role of the stress-activated ATR kinase in checkpoint signaling during recovery from S phase arrest through its regulation of BLM function [25]. It has also been proposed that chromatin remodeling enzymes may facilitate replication restart and checkpoint activation [26]. Taken together, the involvement of molecular motor ATPases and helicases in transduction of cell cycle checkpoints suggests that anti-cancer approaches to target the roles of these enzymes in the cell cycle may be successful.

Cellular Consequences of Silencing the Expression of DNA Helicases Involved in Genome Stability Maintenance and the DNA Damage Response

The potency and specificity of RNAi therapeutics has led to its emergence as a novel class of pharmaceutical drugs [27]. Numerous clinical trials of RNAi therapeutic candidates have begun, leading to speculation that direct RNAi or systemic RNAi may be used to cure diseases including cancers of various organs. In the subsequent section, a rational basis for targeting helicase expression by RNA interference will be discussed as a promising chemotherapeutic approach.

Cellular Phenotypes of Helicase Mutant Cell Lines from Hereditary Genetic Disorders

There is increasing evidence that helicases genetically linked to human diseases of chromosomal instability are directly involved in specific DNA repair pathways or play a role in DNA damage signaling as a component of the DNA damage response. Cells from RecQ helicase disorders (Werner syndrome, Bloom syndrome, Rothmund-Thomson syndrome) have been reported to display sensitivities to a number of DNA damaging agents [4]. In a most recent report, cells from Rothmund-Thomson syndrome patients displayed irreversible growth arrest, decreased DNA synthesis, and reduced cells in S phase after oxidative stress [28]. Replication and recombination defects in human RecQ mutant cell lines may be a consequence of an aberrant response to replicational stress introduced by either endogenous or exogenous DNA damage. In addition to RecQ helicases, genetic defects in a number of other human DNA repair helicases are linked to human diseases. Xeroderma pigmentosum, Cockayne syndrome, and Trichothiodystrophy can arise from mutations in the XPB or XPD helicases implicated in the processes of nucleotide excision repair and transcription [29]. Cockayne syndrome with a deficiency in transcription-coupled repair can arise from a mutation in the *CSB* gene [30] which encodes a protein with DNA-dependent ATPase and chromatin remodeling activities [31]. Mutations in the genes encoding the FANCD1 helicase and FANCD2 ATP-dependent translocase are linked to FA and render cells hypersensitive to DNA cross-linking agents [32–35], suggesting a direct role of these proteins in DNA interstrand cross-link repair.

In addition to these well characterized DNA repair helicase disorders, other diseases associated with genomic instability are due to mutations in DNA helicase-like or ATP-dependent chromatin remodeling enzymes. For example, the gene mutated in ATRX syndrome, a disease characterized by X-linked mental retardation and α -thalassemia, encodes a chromatin remodeling enzyme that modulates gene expression [36–38]. A number of mammalian ATPase-dependent chromatin remodeling complexes (e.g., ATRX, SMARCA1, BRG1, BRM) genetically linked to human diseases are suggested to have roles in cell cycle progression, DNA replication, and/or transcription; however, the precise functions of chromatin remodeling enzymes remain to be fully characterized [36]. Given the emerging importance of DNA helicase-like proteins to stabilize the replication fork and other genomic DNA structures that emerge under stressed conditions, it seems likely that the list of helicases/

chromatin remodeling enzymes important for chromosomal stability is likely to expand in the near future.

DNA helicases directly involved in the recognition or processing of DNA damage or that mediate early checkpoint signaling are promising candidates for anti-cancer therapy through DNA repair inhibition. In recent years, significant progress has been made in the development of effective approaches to down-regulate human helicase expression. Measurable outcomes in terms of biological endpoints and cellular phenotypes have been achieved that are consistent with the known or proposed biological functions of respective helicases, particularly those engaged in the response to DNA damage or replicational stress. In the following sections, the results from studies using RNA interference-mediated knockdown of human helicase expression will be summarized. Overall, the findings provide a strong incentive for continued efforts in the targeting of DNA helicases by RNA interference technologies to sensitize cancer cells to DNA damaging treatments or radiation.

RNAi Therapeutics as a Mechanism to Target Helicases in Anti-Cancer Therapy

RNA interference is a potentially powerful approach for enhancing anti-cancer therapies since RNAi is a natural process that is not thought to perturb normal cell physiology. By efficiently silencing gene expression with extraordinary sequence specificity, RNAi-based therapeutic approaches may represent the new frontier of pharmaceutical drug treatments. For a discussion of the molecular mechanisms, design considerations, and delivery strategies of RNAi recent applications of the technology to treatment of human disease, see reference [27]. Depletion of DNA repair proteins may render cancer cells more sensitive to DNA damaging agents used in anti-cancer therapy, a hypothesis that was investigated by the DeWeese lab [39]. siRNA down-regulated expression of the DNA damage response kinases ATM, ATR, and DNA-PKCS resulted in an increased sensitivity of human prostate cancer cells to DNA damaging agents or radiation. Use of combination therapy to target a DNA repair system is a promising approach to anti-cancer therapy. In the following section, we will focus on DNA helicases as potential targets for RNAi in cancer treatment. siRNA-mediated inhibition of the expression of DNA repair helicases may be used as a mechanism to increase the vulnerability of rapidly proliferating cancer cells to therapeutically relevant DNA damaging agents. In a number of cases, effective protocols have been developed to render human cells deficient in the target helicase protein. Moreover, striking and unique phenotypes have been observed in cells that have reduced expression of DNA helicases that are important for the DNA damage response or maintenance of genomic stability.

FANCD1 (BACH1) and the Sequence-related ChIR1 Helicase

Several laboratories have developed successful strategies to knockdown FANCD1 helicase expression in human cell lines. siRNA reduction of FANCD1 expression was initially performed to evaluate the importance of the FANCD1-BRCA1 protein interaction [40]. γ -irradiated FANCD1-deficient cells, like BRCA1-deficient cells, failed to arrest in G2 and accumulate in M phase, suggesting a role in the G2-M checkpoint. Expression of a FANCD1 S990A mutant defective in the BRCA1 interaction in the siRNA depleted cells failed to correct the G2-M checkpoint defect, substantiating the importance of the phosphorylation-dependent interaction between the helicase and the BRCT domains of BRCA1 in the proper maintenance of the G2/M checkpoint.

siRNA knockdown of FANCD1 in HeLa cells resulted in significantly increased chromosomal breaks and exchange aberrations after treatment with the DNA cross-linking agent mitomycin C [41]. Similarly, siRNA knockdown of FANCD1 in MCF7 cells resulted in a much greater

sensitivity to mitomycin C compared to control cells [34]. Cells transfected with shRNA lentiviral vectors targeting FANCD2 displayed significantly greater genomic instability after mitomycin C treatment; however, FANCD2 monoubiquitination and Rad51 foci formation were found to be intact, suggesting that FANCD2 functions downstream of Rad51 in the DNA damage response [34].

RNA interference technology has also been employed to determine the biological consequences of reducing expression of the human CHL1-related (ChlR1) helicase which has strong homology to FANCD2 (24% identity and 42% homology) [42]. Tetracycline-inducible RNA interference of ChlR1 helicase expression resulted in a pro-metaphase delay and subsequent mitotic failure with chromosomes failing to segregate after a pro-metaphase arrest [43]. In addition, ChlR1 depletion led to abnormal sister chromatid cohesion, providing evidence that the ChlR1 helicase is required for normal mitotic progression.

WRN and BLM Helicases

Gene targeting via HR may serve as a mechanism for gene therapy treatment of various hereditary disorders and cancers. Since mutant cells from Bloom syndrome patients display elevated targeting efficiencies, So and colleagues [44] investigated the hypothesis that RNA interference of BLM expression may enhance gene targeting. Using a gene correction system based on a disrupted HPRT locus in a human HT1080 cell line, the authors observed that the gene targeting frequency of cells knocked down for BLM expression was increased three-fold, suggesting that the BLM helicase may be a viable target for improving gene targeting protocols in human cell lines.

A number of studies have utilized RNA interference technology to evaluate the consequences of WRN deficiency in specific biological endpoints, including the DNA damage response, telomere maintenance, and chromosomal stability. Depletion of WRN helicase expression in cancer cells by RNA interference exists as a possibility for anti-cancer strategies. Transduction of WRN+ hTERT+ immortalized fibroblasts with a retroviral vector expressing a short hairpin silencing RNA specific for the WRN gene demonstrated an almost complete abrogation of WRN protein from these cells [45]. As a consequence of WRN reduction, the cells proliferated poorly; furthermore, over expression of the c-MYC oncoprotein failed to rescue the poor growth of the WRN-depleted cells. Knockdown of WRN expression by RNA interference in cell lines derived from c-myc transformed Burkitt's lymphomas also resulted in slow cell proliferation and increased apoptosis [46]. The impaired proliferation of WRN-deficient cells is proposed to be a combinatorial effect of MYC deregulation and lack of WRN which together contribute to genomic instability. It was suggested the MYC-driven upregulation of WRN prevents cellular senescence and promotes tumorigenesis. Therefore, reduction of WRN helicase by RNA interference therapy may cause cancer cells to senesce or undergo apoptosis.

Cellular senescence can result from the accumulation of DNA damage induced by reactive oxygen species or other types of insult to chromosomal integrity. Several lines of biological evidence, including results from RNA interference studies [47–49], suggest that WRN protects cells from oxidative and other types of DNA damage. The collaborative role of WRN and BRCA1 in the cellular response to DNA interstrand cross-links may potentially be exploited to improve the efficacy of chemotherapeutic DNA cross-linking agents. Werner syndrome cells are sensitive to DNA interstrand cross-linkers [50]. Indeed, depletion of WRN by stable expression of WRN shRNA results in cells with elevated levels of psoralen-induced DNA cross-links as evidenced by results from comet assays [51]. Results from comet assays suggested that the processing of DNA interstrand cross-links required the helicase, but not exonuclease activity of WRN. Consistent with this, *in vitro* evidence also demonstrated that WRN helicase activity, but not exonuclease activity, is required for processing of psoralen interstrand cross-links [52]. Based on the mounting evidence for a role of WRN and other

helicase or helicase-like proteins (FANCM, FANCI) in the cellular response to DNA cross-linking agents, there is a strong incentive to determine the utility of these helicases as targets for the enhancing the effectiveness of chemotherapeutic DNA damaging treatments such as DNA interstrand cross-linkers.

The downregulation of WRN expression by a siRNA pool resulted in reduced phosphorylation of p53 and histone H2AX in response to the treatment of cells with T-oligos homologous to the telomere 3' single-strand overhang [53]. Other lines of evidence indicate a direct role of WRN (and BLM) in telomere maintenance and the DNA damage response after telomere disruption. WRN and BLM mouse studies provide evidence that the complex phenotype of WRN or BLM deficiency relates to telomere shortening [54–56]. RecQ helicases are suggested to function in the Alternative Lengthening of Telomere (ALT) pathway [4;57–59]. The results from the T-oligo RNAi WRN knockdown studies [53] suggest that as an early component of the telomere-specific DNA damage response, WRN may signal ATM, p53 and other effector proteins to maintain genomic stability. Cells with long proliferative lifespans (cancer cells) require mechanisms to maintain telomeric integrity. The direct link of WRN and other helicases (BLM, DDX11, RTEL, PIF1) to telomere metabolism suggests a molecular target for anti-cancer therapy that exploits telomere maintenance as a critical process for cell proliferation [60] (see *Anti-Cancer DNA Binding Compounds* Section).

In a most recent study, the functional role of WRN helicase in human fibroblasts was explored by acutely depleting WRN using a retroviral expressed WRN-targeted shRNA [61]. WRN-depleted fibroblasts exposed to 2 μ M cisplatin displayed a three-fold reduction in cell survival and four-fold reduction in homology-dependent recombination. The proliferative defect of WRN-depleted cells was accompanied by senescence-associated β -galactosidase staining and an increase in cells with co-localizing γ H2AX and phosphoThr68-CBK2 DNA damage markers. The results from these WRN acute depletion studies indicate that the cell proliferation and DNA damage sensitivity phenotypes are a direct consequence of WRN loss and not reflective of the pre-existing genomic instability characteristic of WS cells. As suggested by Monnat and colleagues, the *in vivo* senescence of WS characterized by genetic instability, mutation accumulation, and increased cell loss may be exacerbated by DNA damage. Conceivably, acute WRN depletion in cancer cells that are also exposed to DNA damaging agents may lead to the accumulation of senescent and mutant cells and their ultimate elimination by cell death.

Human RTS and RECQ1 Helicases

siRNA knockdown of RECQL4 expression caused a significant reduction in RECQL4 nuclear foci formation [62]. RNA interference of RECQL1 expression in HeLa cells led to a significant increase in both spontaneous and MMC-induced sister chromatid exchange [63], consistent with our results that RECQL1 deficiency in mouse [64] or human cells (Sharma and Brosh, unpublished data) induces chromosomal instability and an impaired DNA damage response. The observation that RECQL1 efficiently catalyzes branch migration of Holliday Junction structures suggests a role of the enzyme in HR repair after DNA damage [63;65].

Genetic Alterations in DNA Repair Helicase Genes in Transformed Cells

The ability to specifically down-regulate expression of human DNA repair helicases suggests this approach may be potentially used to limit the chemo-resistance of cancer cells that use helicase-dependent DNA repair pathways to correct damage evoked by chemotherapeutic agents or IR. Certain cancer cell types or tumors may rely on the up-regulation of a given helicase for increased DNA replication, cell cycle progression, or transcription. In support of this notion, a number of studies suggest that helicase genes are indeed altered in terms of expression or function in transformed cell lines. Expression of the human helicases WRN,

BLM, RECQ4, and RECQ1 are sharply upregulated in human B cells transformed by Epstein-Barr virus and human fibroblasts or umbilical endothelial cells transformed by simian virus 40 [66], suggesting that RecQ helicases may be increased in transformed or actively proliferating cells. The BLM helicase is expressed in a variety of tumors, and a strong correlation exists between BLM expression and cell proliferation [67]. The proto-oncogene *c-myc* stimulates transcription of the *WRN* gene and WRN upregulation by *c-myc* is involved in promoting *c-myc* tumorigenesis by preventing cellular senescence [45;46]. Tumors that rely on the upregulation of a RecQ or related helicase for increased DNA replication or transcription may be more sensitive to modulation of helicase expression or function compared to normal cells.

The notion that WRN helicase may be a useful target for anti-cancer therapy is reinforced by the observation that epigenetic inactivation of the *WRN* gene by hypermethylation in colorectal tumors was a useful predictor for the clinical response to the topoisomerase inhibitor and camptothecin analogue irinotecan (Fig. 3) [68]. The results suggest that WRN epigenetic inactivation in cancer tissues may result in greater chromosomal instability and hypersensitivity to chemotherapy drugs.

Alterations in DNA repair genes may affect their respective functions and overall DNA repair proficiency, resulting in effects on cancer patients' response to agents such as the DNA cross-linker cisplatin. Genetic changes in helicase expression or function may affect the clinical outcome of chemotherapy. Interestingly, single nucleotide polymorphisms of RECQL1 were found to be associated with reduced survival of pancreatic cancer patients [69;70], raising the possibility that variant alleles of RECQ1 and other helicases may have an effect on the success of chemotherapy treatments. RECQ1 was found to be overexpressed in a screen of genes associated with cisplatin resistance in a human oral squamous cell carcinoma cell line [71]. In a screen of DNA repair genes, a polymorphism in the XPD helicase was found to provide a prediction of favorable clinical outcome among patients with advanced squamous cell carcinoma of the head and neck treated with cisplatin-based chemotherapy [72].

Targeting DNA Repair Proteins with Small Molecules to Enhance Anti-Cancer Therapy

As discussed earlier, small molecule approaches are currently being developed to target DNA repair systems in an effort to enhance chemotherapy or radiation treatments. With our improved understanding of the mechanistic basis for tumor resistance, strategies involving the use of small molecule compounds that potently and uniquely inhibit the function of a DNA repair protein in a specific DNA repair pathway are being either employed for cancer treatment or optimized in model systems. The combinatorial use of small molecule inhibitors of protein function and RNA interference of key proteins in the DNA damage response represent a viable approach. In the next sections, a rationale for small molecule approaches to target DNA repair helicases will be discussed.

Small Molecule Approach to Modulating Helicase Function

An important area of molecular cancer therapeutics is based on the ability to reversibly modulate protein functions in living cells and patients. The development of anti-cancer drugs is a growing discipline in the field of chemical and structural biology [73]. Small molecules offer a number of advantages for targeting specific macromolecules such as DNA repair proteins since the activity of an enzyme such as a helicase, for example, can be turned on or off temporally and may be targeted to certain tissues. Once a small molecule is identified that has a selective and potent ability to modulate its target, medicinal chemists can set to work to optimize the structure and properties of the small molecule to enhance its efficacy for therapeutic value. High-throughput screening of small molecules has become an

interdisciplinary field in the discovery of cancer-relevant therapeutics. The combined efforts of synthetic and analytical chemists, assay technicians and high-throughput engineers/robotics, structural and computational biologists, and computer software engineers encompass a production team for small molecule cancer therapeutics.

As a guide for the development of small molecules that might modulate the activities of DNA helicases involved in the DNA damage response to chemotherapies or radiation, a brief review of helicase structure-function relationships will follow that begin to provide a rationale for the design of small molecule helicase modulators. Although DNA repair helicases have not yet been reported to serve as useful targets for enhancing DNA damaging cancer therapies, it seems likely that this particular class of enzymes will join other DNA repair proteins, described in the accompanying papers of this journal issue, as candidates that can be targeted successfully by small molecule approaches.

Helicase Classification Based on Sequence Homology

A general classification of helicases based upon distantly related helicase proteins that share short, conserved amino acid sequence fingerprints, or helicase motifs was originally proposed [74]. The five groups are composed of three large superfamilies (SF-1, SF-2, and SF-3) and two smaller families (DnaB-like and Rho). SF1 and SF2 are the largest of the five, and encompass a number of DNA and RNA helicases from archaea, eubacteria, eukaryotes and viruses. Proteins of helicase SF-1 and SF-2 all contain a set of seven conserved motifs whose sequences, arrangements and predicted secondary structures are, in general, very similar. SF-3 includes small putative helicase domains of approximately 100 amino acid residues found in both DNA and RNA viruses. SF-3 has only three conserved motifs, including the two classical ATP-binding motifs. The DnaB-like family consists of helicases that are related in sequence to the *E. coli* replication fork helicase DnaB protein. These proteins have five motifs, unwind DNA in the 5' to 3' direction, and generally form hexameric ring structures. Lastly, the Rho family is exemplified by the transcription termination factor Rho. This family was recognized as a family with sequence similarity to the β subunit of proton-translocating ATPases.

The focus of this review is the potential of cancer drug discovery in terms of human helicase targets. The structural and functional aspects of the sequence-related SF1 and SF2 helicases will be discussed since members of these two families constitute the majority of helicases currently known to have important roles in genome stability maintenance. This background on helicase structure and mechanism will provide a rationalistic basis for the design of small molecules that modulate helicase function and can be potentially developed for molecular cancer therapeutics.

Mutational and Structural Analyses of the Conserved Helicase Motifs

A number of important studies provide the foundation for understanding the structural architecture of helicases and their molecular functions executed by conserved domains. The following sections will summarize the structural and functional evidence for the roles of the conserved helicase motifs that comprise the core ATPase domain motor used to fuel unwinding of nucleic acid structures.

Motifs I and II

Motifs I (Walker A) and II (Walker B) are conserved segments found in many NTP-binding proteins. Structural and mutational analyses implicate motifs I and II in nucleotide binding and/or hydrolysis. Motif I is composed of an α -helix containing two hydrophobic residues that interact with the adenine-ribose moiety of ATP and a conserved lysine at the end of the consensus sequence [75]. The lysine side chain occupies a pocket where the Mg^{+2} will bind.

Once the lysine binds the NTP-Mg²⁺ complex, the lysine side chain contacts the β phosphate of the bound NTP and may act to stabilize the transition state during catalysis. A mutation of this lysine to an uncharged amino acid abolishes ATP binding of RNA helicase eIF-4A [76] or ATP hydrolysis of the *E. coli* DNA repair enzyme UvrD [77]. A number of other helicase mutational analysis studies have confirmed the functional importance of motif I in nucleotide binding or hydrolysis (for review, see [78]). Mutations in motif I of the human RecQ helicase WRN [79] and other RecQ helicases (e.g., BLM [80]) seriously impairs their ATPase and DNA unwinding activities, suggesting a functionally conserved role of this motif.

Proteins containing motif II are called DEAD box-related proteins, a name derived from a highly conserved sequence [81]. Motif II contains a hydrophobic β -pleated sheet terminated by a negatively charged acidic residue (aspartic or glutamic acid) that flanks the triphosphate chain of the NTP-Mg²⁺ complex [75]. Mutational studies of this motif have been used to show that the ATPase activity is coupled to helicase activity [76;82]. Variations of the consensus sequence have been identified and arranged into different subgroups of SF2 such as DEAD, DEAH, and DEXH box proteins [78].

Motif III

Consensus sequences derived from an alignment of proteins that constitute helicases or putative helicases has revealed conserved motifs (Ia, III, IV, V, VI) in addition to the nucleotide binding pocket composed of motifs I and II. The consensus sequences derived from an alignment of proteins have been identified for SF1 and SF2 helicases. Among these conserved sequences, an understanding of motif III is emerging. Mutations in motif III of the HSV-I helicase UL5 were found to uncouple helicase and ATPase activity [83]. In these studies it was found that the protein retained its ATPase activity but lacked any significant helicase activity. Mutations in motif III of the RNA helicase eIF-4A resulted in a loss of its helicase activity while it retained its ATP binding and hydrolysis as well as RNA binding activities [76]. These results have suggested a link between motif III and the coupling of ATP hydrolysis to unwinding, an idea that is supported by mutational analysis of motif III in PcrA helicase [84]. Consistent with a role of motif III in energy coupling to DNA unwinding, the crystal structure analyses of SF1 helicases Rep [85] and PcrA [86] implicate a role of the motif in a network of interactions with ATP, ssDNA, and specific salt bridges or hydrogen bonds between subdomains of the helicase core structure.

To further define the functional significance of motif III, a highly conserved aspartic acid in motif III of *E. coli* UvrD was mutated and the enzyme extensively characterized both genetically and biochemically [87]. From a biochemical standpoint, UvrDD248N failed to form binary complexes with either DNA or ATP. However, UvrDD248N was capable of forming an active ternary complex when both ATP and ssDNA were present. The DNA-stimulated ATPase activity of UvrDD248N was reduced relative to that of wild-type UvrD with no significant change in the apparent K_m for ATP. The mutant protein also demonstrated a reduced DNA unwinding activity. The requirement for high concentrations of UvrDD248N to achieve unwinding of long duplex substrates likely reflects the reduced stability of various binary and ternary complexes that must exist in the catalytic cycle of a helicase. The data suggested that motif III acts as an interface between the ATP binding and DNA binding domains of a DNA helicase and is important in maintaining the proper alignment of the two domains.

E. coli RecQ contains a conserved aromatic-rich loop in its helicase domain between motifs II and III that maps to a similar tertiary position as found in the SF1 helicases [88]. The conserved aromatic-rich loop in motif III of SF1 helicases mediates both ATP and ssDNA binding [85; 86]. Mutational analyses of the RecQ aromatic-rich loop reveal that this region is critical for coupling ATPase and DNA binding/unwinding activities [88]. The crystal structure of *E.*

coli RecQ [89] has provided further insight to the functional importance of the conserved helicase motifs (see next section).

Motifs Ia and IV

Motifs Ia and IV have been shown by crystal structure studies of SF-1 and SF-2 helicases to have an involvement in binding ssDNA [85;86;90]. Specific mutations in motif Ia in UL9 of HSV-1 have been characterized both genetically and biochemically [91]. The mutations were made in specific amino acids thought to be involved in ssDNA binding (R112A and R113A/F115A). These mutants exhibited moderate to severe defects in both ssDNA binding, ssDNA-stimulated ATPase activity, and helicase activity; however, they were able to dimerize and bind the HSV-1 origin of replication as well as wild-type UL9. The results from these mutants demonstrate a requirement for motif Ia in ssDNA binding [91].

In addition to nucleic acid binding, motif IV also has a role in nucleotide binding. Contacts between specific motif IV residues and nucleotide were demonstrated by the nucleotide-helicase co-crystal structures of Rep [85] and PcrA [86]. Mutation of an invariant arginine in motif IV resulted in a dramatic reduction in the K_m for ATP binding by UvrD, but normal DNA binding [92]. Collectively, these studies suggest that a complex network of interactions with nucleotide and nucleic acid can be mediated by the residues within a conserved motif.

Motif V

Motif V of Rep helicase makes direct contacts with the bound oligonucleotide [85]. Mutation of a highly conserved glycine in motif V of UL5 significantly impaired the affinity of the enzyme for ssDNA and decreased the rate of ATP hydrolysis [93]. These results suggest that motif V also plays a role in DNA binding. Replacement of a conserved amino acid in motif V of the yeast pre-mRNA splicing factor Prp22 inactivated RNA helicase activity [94], consistent with the importance of this motif in the unwinding reaction.

Motif VI

Mutational studies of this motif in the mammalian RNA helicase eIF-4A have revealed a role of motif VI in nucleic acid binding [76]. Mutations in *E. coli* UvrD DNA helicase in motif VI have shown that there is a variety of effects including reduced ssDNA binding, ATP hydrolysis, and ligand-induced conformational changes [95]. A change in the histidine residue to arginine in motif VI of HSV1 UL5 reduced the RNA binding activity as well as reduced the ATP hydrolysis [96]. These results suggest that motif VI has an involvement in nucleic acid binding and a role in ATPase activity. The structure of PcrA complexed with AMPPNP implicates a guanidinium group of a conserved arginine in motif VI for salt bridge formation with the γ phosphate of the nucleotide [86].

Summary of Helicase Motif Arrangement

A simple take-home message from the mutational and structural studies of the various SF1 and SF2 helicases is that these enzymes all bind and hydrolyze an NTP molecule and harness the chemical energy for nucleic acid transactions, ultimately hydrogen bond disruption and strand separation, by a collective functional unit of the seven conserved helicase motifs. The solved crystal structures of SF1 and SF2 helicases, some of which are highlighted in the next section, indicate a close three-dimensional proximity of all the conserved motifs. Thus, inter-functional activities (nucleotide binding, nucleic acid binding) among the linearly but not spatially distant helicase motifs are responsible for nucleotide hydrolysis and DNA unwinding. Efficient coupling of these two processes by residues in the conserved motifs is likely required for efficient strand translocation and helicase activity.

New Insights to Helicase Mechanism and Potential Cancer Drug Design from Recently Solved High-Resolution Crystal Structures of DNA Helicases

Although there has been a significant amount of information pertaining to the genetic and biochemical functions of helicases in DNA metabolism, only a limited understanding of the mechanism of DNA unwinding catalyzed by these molecular motor enzymes existed until recently. Tremendous advances in the understanding of molecular functions of helicases have been made with the solution of a number of helicase structures. The steps forward in comprehending the structural biology of helicases raise the possibility that helicases may serve as targets for new approaches to molecular cancer therapeutics since this particular class of enzymes is so intimately linked to genomic integrity. In the following sections, a summary of several recently solved helicase crystal structures highlights potential avenues for rational cancer drug discovery based on small molecule modulation of the activity of helicase protein domains.

Superfamily 2 RecQ Helicases: Modular Domains that May Be Targeted

The Keck lab determined the crystal structure of a catalytically active *E. coli* RecQ domain fragment lacking the C-terminus [89]. The solved structure revealed a trilobed modular molecule that has a 'Y' shape with an apparent cleft on the surface. The amino terminal and carboxyl terminal regions constitute each half of the cleft which is lined with the conserved helicase motifs. The RecQ helicase crystallized with ATP γ S indicated that the ATP analogue is packed into a binding site that includes residues from motifs 0, I, and II. The compact and modular arrangement of the ATP binding pocket suggests a potential target for small molecules designed to interfere with ATP binding and/or hydrolysis, a prerequisite for DNA unwinding. This topic is discussed in the next section.

The crystal structure of the *E. coli* RecQ helicase domain also revealed a Zn²⁺ metal binding site in the conserved RQC region found in a number of other RecQ helicases [89]. The RQC motif also contains the structural helix-turn-helix fold which is termed a winged helix domain. A recent solution structure of the human WRN RQC region was also shown to contain a Zn²⁺ binding domain and winged helix-like motif [97]. A combined number of studies have provided strong evidence that the winged helix domain of WRN serves as a multi-functional domain that interacts with structure-specific DNA and a variety of DNA-processing enzymes [4]. The coordinated action of RecQ helicases with structure-specific nucleases on key DNA replication, repair and recombination intermediates, mediated by a protein interaction with the RQC region, is likely to be a critical function for the maintenance of genomic stability. The design of small molecules which specifically bind to the RQC winged helix domain of WRN and related RecQ helicases (e.g., BLM) may prove to be a useful approach to generate biologically active compounds that impair the biochemical mechanism involved in the DNA damage response of these helicases to replicational stress induced by chemotherapeutic DNA damaging agents.

Other RecQ protein domains whose structures have been determined are the HRDC domain proposed to target RecQ helicases to different types of DNA structures [98] and the WRN exonuclease domain which has an overall structural similarity to the DnaQ family of 3' to 5' exonucleases [99].

Superfamily 1 UvrD Helicase: Implications for Small Molecule Nucleotide Binding Inhibitors

Co-crystal structures of the DNA repair helicase UvrD complexes with DNA and ATP hydrolysis intermediates revealed a DNA unwinding mechanism in which the binding of a single ATP molecule enables UvrD to unwind one base pair at a time by a two-part power stroke with a combined wrench-and-inchworm mechanism [100]. The authors suggest that the

similar ATPase domain motor of other SF1 and SF2 helicases represents a unifying unwinding mechanism in which protein domain rotation with various single- or doubled-stranded states of bound nucleic acid is used for cellular processes.

The observation that ATP binding alone to UvrD helicase results in strand separation by one base pair reinforces the principal that suitable competitive inhibitors of nucleotide binding will directly inhibit helicase-catalyzed unwinding in an efficient manner. Because of their essential roles in the DNA damage response, helicases may be a suitable target of chemotherapeutic molecules that interfere with nucleotide binding.

By analogy, structural biology approaches have revealed distinct nucleotide binding clefts and modes for protein kinases and the heat shock protein 90 (HSP90) which are considered druggable targets in molecular cancer therapeutics. As detailed nicely in a recent review [73], structure-based design has proven to be useful in identifying and optimizing HSP90 inhibitors. Recently, a class of small molecule chemotherapeutic geldanamycin-like inhibitors of the molecular chaperone HSP90 was identified which interfere with ATP binding to the N-terminal ATP binding site of HSP90 [101]. Clinical trials with 17-AAG, a derivative of the natural product geldanamycin (Fig. 4), demonstrated targeted inhibition in tumor tissue and biological activity for treatment of individuals with melanoma [102]. The prospect for success of new HSP90 agents currently in Phase I and Phase II clinical trials suggest that a similar approach might be used to prevent the critical steps of nucleotide binding and/or hydrolysis by DNA repair helicase enzymes. Continued work in this area will elucidate if small molecules may be used to modulate nucleoside triphosphate binding by a DNA repair helicase and provide a mechanistic opportunity for cancer therapy. An in-depth understanding of the structural biology of target DNA repair helicases should help to reduce the probability of encountering cross-reactive 'frequent hitters' during the process of lead generation, as discussed in general terms for the development of cancer therapeutic targets by Collins and Workman [73]. Selective inhibition of a target helicase by engineered designer drugs that block nucleotide binding may become an important area of anticancer drug discovery.

Superfamily 2 XPB Helicase: DNA Damage Recognition Domains

XPB is an essential helicase subunit of the TFIIH particle implicated in transcription initiation and nucleotide excision repair. Recently solved crystal structures of an *Archaeoglobus* XPB homolog (AfXPB) have provide new insights to the recognition of DNA damage by XPB helicase during nucleotide excision repair [103]. The Tainer lab discovered that the coordinated actions of conserved XPB domains within a functional core that includes a DNA damage recognition domain provide a DNA unwinding mechanism with implicated roles in the verification of DNA damage during nucleotide excision repair. It was suggested that the region of XPB protein harboring the damage recognition domain is able to recognize a broad spectrum of DNA lesions and likely facilitates the initiation of DNA unwinding by the XPB/TFIIH complex during NER. The AfXPB crystal structures reveal that reorientation of the two helicase domains would be required in order for the functional helicase motifs to form the active cleft. Identifying small molecules which interfere with the DNA damage recognition domain or reorientation of the helicase domains may prove to be a viable approach to modulating the role of XPB in its DNA repair capacity. Sensitizing cancer cells to helix-distorting DNA damage induced by currently used anti-cancer drugs may improve cancer therapy. Conceivably, helicases that are involved in DNA damage recognition or the initiation of DNA processing may be useful targets.

Helicase-directed Peptide-based Chemotherapy Approaches

Cellular sensitivity to DNA cross-linking agents such as cisplatin can be enhanced by defects in proteins that facilitate HR repair. One mechanism to affect protein function can be to

modulate critical protein interactions of the DNA repair machinery. This can potentially be achieved by peptides that block interactions between HR repair proteins. This approach was successfully used by the Bishop lab recently in which phage display was implemented to identify peptides with sequence similarity to RAD51C that bind XRCC3 [104]. A synthetic peptide representing a hot spot for RAD51C interactions fused to a membrane transduction sequence was found to inhibit RAD51 subnuclear assembly, and sensitize hamster cells to cisplatin.

The human single-stranded DNA binding protein RPA implicated in the processes of DNA replication, recombination and repair, and transcription interacts with several human RecQ helicases that have important roles in maintaining genomic stability (for review see [4]). It is likely that the robust enhancement of DNA unwinding by the human RecQ helicases in the presence of RPA is critical to the functions of these proteins in the response to DNA damage or replicational stress. Using a combination of approaches that included yeast two-hybrid, WRN affinity pull-down, and ELISA assays with purified WRN recombinant fragments, an N-terminal highly acidic perfect 27 amino acid direct repeat sequence in the WRN protein harbors the high affinity protein interaction site with RPA that is necessary for the physical and functional interaction with the single-stranded DNA binding protein [105] (Fig. 5). Given the evidence that WRN plays an important role in the DNA damage response to a variety of agents including DNA cross-linking agents, disruption of the WRN-RPA interaction by a WRN peptide approach may be a useful approach to sensitizing cancer cells to DNA damaging agents used in chemotherapy. The notion that disruption of WRN protein interactions may interfere with the role of WRN in the DNA damage response was suggested by the observation that overexpression of the PARP-1 binding domain of WRN strongly inhibits the poly(ADP-ribosyl)ation activity in H₂O₂-treated control cell lines [106].

The interaction of RecQ helicases with structure-specific nucleases (FEN-1, EXO-1) may be an avenue to exploit for cancer therapy since these interactions are likely to be important for the replicational stress response induced by DNA damaging agents [4]. The fine mapping of the WRN: FEN-1 interaction site to the RQC region of WRN protein [107] and the extreme C-terminal 20 amino acids of FEN-1 [108] raises the possibility that the WRN: FEN-1 interaction may be modulated by peptide or small molecule therapy through the interference of the protein interaction. Presumably, such an approach may be used to modulate the physical and functional interactions of the helicase with the nuclease, and present a strategy for modulating an important RecQ interaction, one that was previously shown to have functional importance in the DNA damage response *in vivo* [109].

Anti-Cancer DNA Binding Compounds That Inhibit Helicase Function

A number of small molecule DNA-interacting compounds currently used as chemotherapy drugs potentially inhibit the cellular processes of DNA replication, recombination, repair or transcription. Understanding their molecular targets and precise mechanisms of action *in vivo* should help researchers to design improved compounds through their cooperative efforts in chemical synthesis and biological evaluation. Since helicases have essential roles in virtually all aspect of cellular DNA metabolism, a number of studies, reviewed in reference [60], have characterized the effects of small molecule DNA-interacting compounds used in chemotherapy on the functions of DNA helicases. Depending on the chemical structure of these compounds and the nature of their interactions with DNA structures, chemotherapy molecules exert markedly different effects on the catalytic activities of the DNA helicases that have been studied.

RecQ helicases may serve to resolve the G-rich 3' overhang tetraplex structure or a D-loop structure that arise through a recombination-based pathway of telomere metabolism [4] (Fig.

6). In addition, a Pif-1-like helicase may inhibit telomerase-mediated lengthening of the 3' overhang [1]. In addition to compounds that bind various structural motifs (minor groove, major groove, intercalated bases) of B-form duplex DNA, small molecules have also been identified and characterized that specifically bind G-quadruplex DNA structures (Fig. 6) that are proposed to form in G-rich sequences of the human genome such as promoter elements or telomeres [110]. An example of a G-quadruplex interactive compound that represents a potential anti-cancer drug is Telomestatin tetra-(N-methyl-4-pyridyl) porphine (TMPyP4) (Fig. 6). Certain DNA helicases, particularly those of the RecQ family, efficiently resolve G-rich tetraplex structures; moreover, small molecules under evaluation as chemotherapy drugs, have been shown to inhibit the resolution of these structures by DNA helicases (for review, see [60;111]). Tetraplex-binding compounds which modulate promoter activation or telomere maintenance *in vivo* and represent potential chemotherapy drugs are likely to exert their biological effects through the inhibition of cellular DNA helicases which act to resolve the higher order DNA structures. In the future stages of anti-cancer drug discovery, it will be imperative for chemical biologists to tailor the specificity of these compounds such that their molecular targets (e.g., RecQ helicases) are specifically affected in tumor tissues and general cytotoxicity is avoided.

Potential Caveats for Targeting Helicases as a Chemotherapeutic Option

As discussed in other reviews on targeting DNA repair proteins for cancer therapy, a potential drawback is that DNA repair inhibitors might adversely affect repair mechanisms in normal tissues. Increased susceptibility of tumor cells to a helicase inhibitor may be evident for those tissues which are actively replicating and transcribing their DNA since helicases play fundamentally important roles in these processes. For example, RecQ helicases may be up-regulated to maintain genomic stability in cells that are transformed or actively proliferating [66;67]. Innovative approaches to selectively introducing DNA damaging chemotherapy drugs or radiation to tumor tissues without adversely affecting normal cells is an active area of investigation.

Therapy-related carcinogenesis can arise from the genomic instability induced by DNA damage in the face of compromised DNA repair [112]. Understanding the cellular roles of DNA helicases can help to elucidate therapy options that minimize secondary cancers which pose a significant risk to cancer survivors. The potentially functional redundancy of certain DNA helicases (e.g., RecQ helicases) may make it difficult to target a single helicase in a defined step of a DNA repair pathway. This may be particularly true for the current DNA-interacting compounds that are used in chemotherapy treatments. Improving drug design and our understanding of the molecular functions of target DNA helicases or other DNA repair proteins will hopefully lead to successful non-surgical treatments of cancer.

Summary

This is an exciting era for cancer therapy in which DNA repair targets may become more mainstreamed as a chemotherapeutic option. Research to develop anti-cancer therapies that incorporate small molecule modulation of helicase function or down-regulation of helicase expression, in combination with DNA damaging treatments is likely to be an active and fruitful area of investigation. Cancer survival can be improved with the joint efforts of chemical biologists and clinicians. Novel targets such as helicases may provide innovative mechanisms to compromise the DNA damage response and improve the efficacy of cancer treatments.

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Chloroethylnitrosurea Crosslink

Mitomycin C Crosslink

Fig. 1. Structures of DNA interstrand cross-links introduced by chemotherapeutic agents
Panel A, chloroethylnitrosurea. *Panel B*, mitomycin C. *Panel C*, 8-methoxypsoralen. *Panel D*, platinum.

Doxorubicin **Camptothecin** **Etoposide****Fig. 2.** Chemical structures of the chemotherapy drugs camptothecin, etoposide, and doxorubicin

Irinotecan

Fig. 3. Chemical structure of the chemotherapy agent and topoisomerase I inhibitor Irinotecan

Fig. 4. Chemical structure of the HSP90 inhibitor Geldanamycin

Fig. 5. The single-stranded DNA binding protein RPA increases WRN helicase processivity in a specific manner by a direct protein interaction

Panel A, schematic of DNA unwinding reaction catalyzed by WRN helicase with RPA coating ssDNA that is generated by duplex unwinding. *Panel B*, DNA and protein interactions of RPA and WRN helicase. Note that the physical interaction between RPA70 and the WRN acidic repeats is required for RPA stimulation of WRN helicase activity on long duplex DNA substrates.

Fig. 6. Telomeric DNA structures that RecQ helicases are proposed to act upon
Panel A, Intra-telomeric (t-loop) and inter-telomeric structures that are created through strand invasion of the 3' telomeric overhang into the duplex region of the telomere. *Panel B*, secondary planar structure of G-quadruplex DNA, stabilized by Hoogsteen hydrogen bonds, that may form in the G-rich 3' ssDNA overhangs of telomeres. *Panel C*, G-quadruplex interactive compound and potential anti-cancer drug Telomestatin.

Table 1
Human DNA Helicases with (Proposed) Roles in DNA Repair, Genomic Stability, or Telomere Maintenance

Helicase/ATP-dependent Translocase	(Proposed) Function
BACH1/BRIP1/FANCI	DNA Interstrand Cross-link Repair Homologous Recombinational Repair
BLM	Homologous Recombinational Repair Telomere Maintenance
CHL1	Chromosome Segregation Sister Chromatid Cohesion
CSB	Transcription-Coupled Nucleotide Excision Repair
DDX11 (putative)	Telomere Maintenance
FANCM	DNA Interstrand Cross-link Repair
HEL308	DNA Interstrand Cross-link Repair
PIF1	Telomere Maintenance
RECOL (RECQ1)	Double Strand Break Repair
RECQ4	Recombinational Repair
RECQ5	?
Senataxin (putative)	?
TWINKLE	Mitochondrial DNA Maintenance
XPB	Nucleotide Excision Repair
XPD	Nucleotide Excision Repair
WRN	Homologous Recombinational Repair Base Excision Repair Nonhomologous End-Joining Telomere Maintenance

See text for details

Table 2
Eukaryotic Proteins with Proposed Functions in DNA Interstrand Cross-link Repair

Protein	Biochemical Activity
FANCI	5' to 3' Helicase
FANCM	ATPase/Translocase
BLM	3' to 5' Helicase
WRN	3' to 5' Helicase
DNA polymerase ζ	Translesion Synthesis DNA Polymerase
MRN-RAD50-NBS1	Nuclease
MUS81-Eme1	Endonuclease
XPF-ERCC1	Endonuclease
RAD51-BRCA2/FANCD1-DSS1	Recombinase
BRCA1	DNA Binding
FANCD2	DNA Binding

See text for details