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Human RECQ helicases: Roles in DNA metabolism, mutagenesis and cancer biology

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Abstract

Helicases use the energy of ATP hydrolysis to separate double-stranded nucleic acids to facilitate essential processes such as replication, recombination, transcription and repair. This article focuses on the human RECQ helicase gene and protein family. Loss of function of three different members has been shown to cause Bloom syndrome (BS), Werner syndrome (WS) and Rothmund–Thomson syndrome (RTS). This article outlines clinical and cellular features of these cancer predisposition syndromes, and discusses their pathogenesis in light of our understanding of RECQ helicase biochemical activities and *in vivo* functions. I also discuss the emerging role for RECQ helicases as predictors of disease risk and the response to therapy.

Keywords

RECQ helicases; Bloom syndrome; Werner syndrome; Rothmund–Thomson syndrome; DNA replication; DNA repair; Telomeres; Homologous recombination; Genetic instability; Cancer predisposition syndrome; Cancer chemotherapy; Premature aging syndrome

1. Prologue

Homer's *Odyssey* is a beguiling work, a bookend—literally—to Western literature together with the *Illiad*. The *Odyssey* is a literal and metaphorical travelogue, the forced decadal wanderings of Odysseus following the end of the Trojan war. Voyaging, wandering and sailing are recurrent themes in the *Odyssey*, and have served ever since as powerful metaphors for life's journey and all attempts to explore and to wrest meaning from the unknown. The muse in the following story is Nature; our goal is not Ithaca, home and rest, but an understanding of what experiments of Nature have revealed about our nature, the pathogenesis of disease and our fate.

2. Introduction

Helicase proteins are enzymes that use the energy of ATP hydrolysis to unwind double-stranded nucleic acids. DNA or RNA duplexes, together with DNA: RNA hybrid molecules all serve as physiologic substrates for these enzymes. Helicases can also perform a wider range of actions on nucleic acid or nucleoprotein templates to facilitate their metabolism. For example, helicases can act as DNA or RNA translocases, and as modulators of the

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structure and function of nucleoprotein filaments and complexes such as those involved in homologous recombination or replication fork restart [1,2]. The ubiquity of helicases reflects their important roles in virtually all aspects of nucleic acid metabolism.

This article focuses on the five members of the human RECQ helicase family, and their roles in DNA metabolism, human disease pathogenesis and the response to therapy. Mutations that lead to loss of function of three different human RECQ helicases cause different heritable cancer susceptibility syndromes. The observation that both Werner syndrome (WS) and Bloom syndrome (BS), two of the human RECQ helicase deficiency syndromes discussed below, were chromosomal instability and cancer predisposition syndromes provided early support for the idea that a mutator phenotype might provide particularly fertile soil for the emergence of cancer. We discuss and further elaborate on this idea below, and discuss how heritable or acquired loss of function of human RECQ helicase genes might promote genetic instability and cancer while, paradoxically, providing new opportunities to improve cancer therapy.

This review necessarily summarizes a large body of work from many different investigators. I have tried throughout to refer interested readers to recent reviews that cover some of the topics discussed here as well as other important areas that are not be discussed here. These reviews also provide fuller referencing of the primary literature on key points that could not be fully discussed and referenced here due to lack of space.

2.1. The RECQ helicase deficiency syndromes

The RECQ helicase deficiency syndromes were originally recognized and described on the basis of clinical findings and inheritance patterns. Bloom syndrome (BS), Werner syndrome (WS) and Rothmund–Thomson syndrome (RTS) are rare ($\leq 1/50,000$ live births), autosomal recessive Mendelian diseases that share an elevated risk of cancer together with additional, more variable features that include genetic instability and disease-specific developmental or acquired features. The clinical features of each syndrome are summarized below.

2.1.1. Bloom syndrome (BS)—Bloom syndrome (BS) was first identified in 1954 by David Bloom, who described three patients with congenital short stature and skin changes reminiscent of systemic lupus erythematosus [3,4]. Consistent features include marked intrauterine and post-natal growth retardation; congenital short stature; and a characteristic ‘butterfly’ rash across the bridge of the nose and cheeks that may extend to include the dorsum of the hands and forearms. This rash typically develops with sun exposure in the first years of life, then may become chronic with skin hyper- or hypopigmentation. Deficient cellular and humoral immunity is common, and may explain the elevated risk of otitis media and pneumonia. There is also an elevated risk of diabetes mellitus. BS patients have reduced fertility: males are typically infertile, whereas females are hypofertile but may give birth to normal offspring [5].

The most troubling aspect of BS is a high risk of cancer: BS patients are predisposed to a remarkably broad range of cancers, in contrast to almost all other genetically inherited cancer predispositions. There is an elevated risk of developing common adult epithelial tumors such as colon, breast and lung cancer; leukemias and lymphomas; sarcomas; and rare pediatric tumors such as Wilms' tumors [6]. Cancer is the most common cause of death in BS patients.

2.1.2. Werner syndrome (WS)—Werner syndrome (WS) alone among the human RECQ helicase deficiency syndromes has features strongly suggestive of premature aging. The key clinical findings, first reported by Otto Werner in 1904, include short stature; early graying and loss of hair; bilateral cataracts; and scleroderma-like skin changes [7–10]. The earliest

and most consistent change observed is graying and loss of hair. This typically begins in the second decade of life with the scalp and eyebrows, and is progressive. Cataracts in WS are often bilateral; appear beginning in the second or third decade of life; and differ from the common 'senile' cataracts by involving the lens posterior cortex and subcapsular regions as opposed to lens nucleus. Vision is otherwise normal, and can often be restored by cataract removal. The short stature of WS patients results from a failure to undergo an adolescent or pubertal growth spurt. There is no suggestion that this acquired growth deficit is caused by an underlying endocrinopathy or other disease state. Subcutaneous connective tissue atrophy and dermal fibrosis together give skin a 'tight, white and shiny' or contracted appearance that over time contributes to a progressive sharpening of facial features, foot and ankle deformation with ulceration, and soft tissue calcification. WS patients are at increased risk to develop premature atherosclerosis, myocardial infarction and stroke; osteoporosis; and diabetes mellitus. The CNS is typically spared, and WS patients are not at elevated risk of Alzheimer or other types of dementia apart from those associated with vascular disease. Fertility is reduced in males and females [7–9,11].

Unlike BS, WS confers an elevated risk of only selected types of cancer [12–14]. The most frequently observed neoplasms in WS patients are soft tissue sarcomas, follicular thyroid carcinoma, meningioma, acral lentiginous malignant melanoma, malignant or pre-neoplastic hematologic disease (chiefly leukemias) and osteosarcoma. This spectrum is broader than the misleading characterization of the cancer predisposition in WS being limited to sarcomas or soft-tissue tumors. Cancer and premature cardiovascular disease are the leading causes of death in WS patients [15].

2.1.3. Rothmund–Thomson syndrome—Rothmund–Thomson syndrome (RTS) was first described by Rothmund in 1868 as a familial occurrence of unusual skin changes together with bilateral juvenile cataracts [16]. Subsequent cases were reported by Thomson in 1936, and in 1957 Taylor suggested that these reports were of patients with the same disease [16–18]. The characteristic skin changes of RTS typically appear within the first 3–6 months of life as a sun-sensitive rash with redness, swelling and blistering on the face. This rash spreads over the buttocks and extremities, while sparing the chest, back and abdomen. These skin lesions over time become variably pigmented with telangiectasias and areas of focal atrophy. Additional features include sparse or absent hair, eyelashes and eyebrows; congenital short stature in conjunction with frequent bone and tooth abnormalities; cataracts; and an elevated risk of cancer, most notably osteosarcoma [19].

The short stature of RTS patients is reminiscent, though not as severe, as that observed in BS: affected individuals are born small but proportionately developed and typically remain in the lower percentiles for height and weight throughout life. Bone and tooth abnormalities include dysplastic, malformed or absent bones, often involving the hand or thumbs; delayed bone formation or bone density loss; and malformed, missing or extra teeth. The cataracts originally noted by Rothmund have been found in only a minority of contemporary RTS patients [19,20]. Immunologic function appears to be intact, and fertility may be reduced although RTS females have given birth to normal offspring. Life expectancy in the absence of cancer appears to be normal [20].

Two additional, heritable human diseases have been associated with RTS, RAPADILINO syndrome and Baller–Gerold syndrome (BGS). RAPADILINO syndrome patients have joint dislocations and patellar hypoplasia or aplasia, but lack the characteristic poikiloderma seen in RTS patients. BGS patients have craniosynostosis with radial aplasia in addition to skin changes reminiscent of RTS [21,22]. Molecular analyses of clinically ascertained RTS patients have also identified RTS phenocopies, individuals whose clinical findings resemble RTS, though who lack *RECQL4* mutations (see below; [20,23]). The recurrent themes of

genetic heterogeneity, phenocopies, variable clinical expression of mutations in the same gene and ‘missing’ diseases are all discussed below: collectively these themes emphasize important work to be done to correlate clinical, pathologic and molecular findings in these diseases.

2.2. RECQ genes, deleterious mutations and SNP variants

Cloning of the genes causally linked to BS, WS and RTS immediately identified all three as members of a human *RECQ* helicase gene family [24]. *BLM* was cloned in 1995 by making clever use of the mitotic recombination phenotype of BS cells [25], and led to naming of the family after the helicase domain shared with the *E. coli* RecQ protein. Positional cloning of the *WRN* gene followed in 1996, guided by prior linkage analyses [26], and confirmed earlier speculation that the gene responsible for WS might be a helicase [27]. The cloning of *RECQL4* and *RECQL5* were based on sequence homology [28,29]. The remaining family member, *RECQL*, was identified independently by two groups in 1994 as encoding a potent ATPase and helicase activity in human cell extracts [30,31]. *RECQL* and *RECQL5* have not been linked to either heritable or acquired human disease states, although there is abundant evidence from biochemical, cellular and mouse modeling analyses that loss of function would likely lead to disease [32–34]. The five human RECQ helicase genes, their chromosomal locations, predicted protein products, conserved domains and encoded catalytic activities are shown in Fig. 1.

Cloning of the human *RECQ* helicase genes provided a powerful stimulus for further research and allowed the identification of pathogenic mutations and sequence variants in all of the human *RECQ* helicase genes. A summary of clinically ascertained pathogenic mutations in *BLM*, *WRN* and *RECQL4* is shown in Fig. 2 together with non-synonymous coding region SNP variants for each gene.

BLM mutations in BS patients invariably lead to a loss of helicase function [35], though do not in all cases eliminate expression of the mutant protein. This suggests that BLM helicase activity is the major determinant of BLM-associated phenotypes in human somatic cells, regardless of whether the mutant protein is lost or not. *WRN* mutations, in contrast, lead to loss of the WRN protein and its two associated catalytic activities [36]. Recently reported missense mutations affecting the WRN helicase domain (Gly574→Arg) or located 20 residues upstream of the C-terminal NLS (Met1350→Arg) were identified in heterozygous WS patients in conjunction with other clearly deleterious mutations [37]. Both of these missense mutations have a high likelihood of affecting protein stability, as did a previously reported pair of homozygous missense mutations in the WRN exonuclease domain (Lys125→Asn/Lys135→Glu; [15]). These results are consistent with the autosomal recessive inheritance pattern of WS, and with prior work demonstrating loss of mutant WRN protein expression from WS patient cells [36].

Mutation analyses have also allowed a clear distinction to be made between WS linked to pathogenic mutations in *WRN* and a clinically overlapping disease referred to as ‘atypical Werner syndrome’ that is caused by splice-disrupting mutations in the lamin A/C gene *LMNA* [38]. One interesting—and as yet unanswered—question is whether there is a ‘missing’ disease associated with *WRN* missense mutations that selectively inactivate the WRN exonuclease or helicase activities. In prior work we demonstrated a requirement for both the WRN exonuclease and helicase activities to suppress WS cellular phenotypes [39]. These experiments predicted that *WRN* missense mutational diseases should display autosomal recessive inheritance, be recombinant-deficient, and may be clinically expressed as either cancer susceptibility or DNA damage sensitivity syndromes. These experiments also failed to identify a dominant negative effect of expression of single- or double-mutant

WRN protein in control cells, a result that is again consistent with the autosomal recessive inheritance of WS [39].

A surprisingly large number of patients referred with potential WS lack either *WRN* or *LMNA* mutations. These patients represented 23% (41 of 176) individuals described in a recent update from the International Registry of Werner syndrome [37] (<http://www.wernersyndrome.org>). This group likely contains mistaken diagnoses; instances where WS was caused by *WRN* gene silencing, as opposed to mutation; individuals with mutations in proteins required for WRN function; or individuals with novel progeroid syndromes. Evidence for the existence of the latter group was first noted over a decade ago, in gene fusion experiments involving different WS patient-derived cell lines [40]. This patient cohort is thus a prime candidate for targeted exome sequencing to disentangle these possibilities.

As mentioned above, *RECQL4* mutations have been linked to at least three different clinical syndromes: RTS, RAPADILINO and Baller–Gerold syndromes (BGS). RTS itself is genetically heterogeneous, with only a portion of clinically ascertained patients have mutations in *RECQL4* [20,23]. BGS is similarly genetically heterogeneous: BGS patients have been identified that carry *FGFR2* or *TWIST*, as opposed to *RECQL4*, mutations [20]. Two other features of clinically ascertained *RECQL4* mutations are worth noting. First, there is an unusually high proportion of recurrent mutations that disrupt splicing. This is likely explained by the unusual genomic structure of the human *RECQL4* gene, where 13 out of 20 exons are short (<100 bp long) and prone to stochastic or mutation-induced mis-splicing [41]. A second mechanistically intriguing observation is mutational sparing of the N-terminal portion of RECQL4. This RECQL4 region shares homology with the yeast replication protein Sld2, which suggests that the RECQL4 N-terminus and/or the Sld2 homology domain may encode an essential function (reviewed in [42]).

All of the human *RECQ* genes have polymorphic variants of unknown significance in addition to the clearly pathogenic mutations summarized by molecular type and location in Fig. 2. One WRN polymorphic variant, R834C, has been shown thus far to affect WRN helicase activity [43], and has the potential to disrupt function if homozygous or found in combination with a second, clearly deleterious allele.

2.3. RECQ helicase biochemical activities and DNA metabolic function

The presence of a conserved RECQ helicase domain in each of the human RECQ helicase proteins provided both a common name for the protein family and predicted all of the family members would unwind duplex DNA in an ATP hydrolysis-dependent manner by translocating along one strand in the 3' to 5' direction (reviewed in [2,44]). This prediction was subsequently confirmed for all five of the human RECQ helicases using short oligonucleotide substrates *in vitro* (Fig. 3). *RECQL4* was initially thought to lack 3' to 5' helicase activity, though helicase activity was eventually documented when it was realized that RECQL4 possesses an unusually potent, competing strand annealing activity [45]. *In vivo* counterparts of the substrates used to define RECQ helicase catalytic activities are likely to be key intermediates in several important DNA metabolic processes (see below; Fig. 4).

BLM and WRN, but not RECQ1 or RECQL4, can unwind non-B-form DNAs such as G-quadruplex (G4) DNA in addition to B-form DNAs [2,24,47]. Likely genomic targets for RECQ activity to unwind G4 DNA include G-rich telomeres, ribosomal RNA genes and some G-rich simple repeat sequences that need to be unwound to facilitate replication, recombination, repair or transcription [48]. BLM and WRN alone can branch migrate (i.e. translocate) 3- and 4-stranded DNA junctions such as Holliday junctions (HJs) and

displacement loops (D-loops) along double-stranded DNA. HJs are strand exchange intermediates formed during homology-dependent recombination (HR), whereas D-loops form when a duplex DNA is invaded by another homologous, single-stranded 3' DNA end (Fig. 4).

RECQ1, WRN, and BLM can dissociate HJs into double-stranded DNA products, and WRN and BLM can in similar fashion dissociate D-loops [47]. RECQ5, in contrast, cannot efficiently dissociate D-loops [49]. BLM and WRN can also convert three-way junctions into a HJ by fork regression [2,47]. WRN can unwind flap structures (Fig. 3, top left that have 5' ends and may form during Okazaki fragment processing during lagging strand DNA replication. WRN can also unwind DNA:RNA duplexes, as well as substrates in which one strand has mixed RNA–DNA content similar to that observed in Okazaki fragments [50]. Okazaki fragments are short DNA:RNA hybrid fragments that have RNA on their 5' ends that are formed during lagging strand replication. All five RECQs unwind three-way DNA junctions resembling replication forks [2,24,47].

WRN alone among the human RECQ helicases contains a 3' to 5' exonuclease activity in addition to the canonical 3' to 5' helicase activity [51,52]. WRN exonuclease is active on many of the same substrates that can be unwound by WRN helicase activity, suggesting that the two activities might be coordinated either within a single polypeptide, or by interaction with other WRN molecules or proteins [53]. WRN exonuclease can degrade recessed 3' ends in double-stranded DNA, and can initiate DNA degradation from a nick or a gap in dsDNA. These types of activities might be particularly useful during DNA replication, where both nicked and gapped substrates are plentiful; in post-replication repair; and in other repair pathways (Fig. 4).

There are now several well-described examples of the activities of RECQ helicases modulating, or being modulated by, interactions with other DNA metabolic and DNA damage response signaling proteins. Some of these interactions are common to all five of the human RECQ helicases, e.g., interactions with RPA, whereas others are specific to individual RECQ proteins and thus provide mechanistic and functional clues. For example, BLM interacts with topoisomerase III α and two RMI (RECQ-Mediated Genome Instability) proteins to form a resolution complex. BLM is also part of the BRAFT supercomplex that includes Fanconi anemia-associated proteins [24,54]. BLM interacts with RAD51 [55], a crucial component of the homologous recombination (HR) machinery [56].

WRN-interacting proteins include XRRC4/Ligase IV [57] involved in DNA double strand break (DSB) repair; base excision repair (BER) proteins including DNA polymerase β [58]; and uniquely with topoisomerase I [59] and the HR proteins RAD54B and RAD52 [60,61]. WRN and BLM also been shown to interact with one another [47]. RECQ5, BLM and WRN all interact with the MRE11/RAD50/NBS1 (MRN) complex that detects and processes DSBs [47]. RECQL4 alone among the RECQ helicases forms a cytoplasmic complex with UBR1 and UBR2, and interacts with replication initiation components including MCM10, MCM2-7, GINS and CDC45 in the nucleus. These results reflect the important role for RECQL4 in replication initiation and, to a lesser extent, replication fork progressing [42,62,63].

Several general themes emerge from the above observations. First, RECQ helicases typically work in concert with other proteins to facilitate specific DNA transactions, and are rarely essential for any given process. These functional 'collaborations' may reflect direct physical interaction or functional interactions that are difficult to demonstrate using conventional protein–protein association assays. The best studied of the functional partnerships are between RECQ proteins and exo- and endonucleases, or different topoisomerases. Second,

RECQ function mediated by physical and/or functional interactions can promote or inhibit specific DNA metabolic processes. One good example is HR, that can be promoted by WRN and BLM, or alternatively antagonized by the actions of BLM and RECQL5 [64,65]. This and other observations suggest that RECQ protein activity may dynamically modulate DNA metabolic processes to favor specific outcomes under different circumstances. A third general observation is that RECQs are partially redundant for at least some specific functions. This conclusion is supported by recent work from my lab that analyzed the functional consequences of depleting WRN, BLM or both proteins from human cells on cell proliferation and cell survival after DNA damage [66]. Finally, it is important to remember that our suppositions about what RECQ helicase proteins do often reflect conclusions drawn initially—or solely—from *in vitro* biochemical data. A clear challenge now is to reinterpret these data in the context of cellular biochemistry and function.

2.4. RECQ helicase roles in cellular nucleic acid metabolism

The *in vivo* biochemical characterization of RECQ helicases proteins provided immediate clues to *in vivo* function. The following is a brief overview of functional roles for the human RECQ helicases in specific aspects of nucleic acid metabolism.

2.4.1. Homologous recombination/other DNA repair pathways—Homologous recombination (HR) in somatic cells plays an important role in DNA double strand break repair, immunoglobulin class switching and the successful completion of DNA replication [56]. WS cells were first found to have a recombination resolution defect over a decade ago [67,68]. This defect is in the post-synaptic stage of recombination, after the generation of recombinant DNA molecules, and results in the loss of viable recombinant daughter cells containing conversion-type or non-crossover products. An HR resolution defect provides a plausible explanation for the chromosomal rearrangements and deletion mutator phenotype observed in WS cells: failed resolution products can still give rise to viable, albeit mutant, daughter cells following the breakage and end-joining of unresolved HR products.

BS cells were suspected to have a recombination defect on the basis of elevated sister chromatid exchanges, and evidence for a mutator phenotype driven by aberrant HR [4]. Consistent with these findings is the ability of BLM (and other RECQ helicases such as WRN) to unwind and/or branch migrate 3- and 4-way junctions such as D-loops or Holliday junctions (see above; Figs. 3 and 4). More recent data indicate that BLM participates both in early and late stages of HR to promote or antagonize recombination [69,70]. BLM can stimulate recombination by working with exonuclease 1/EXO1 to resect one strand of DNA duplex in the 5' to 3' direction to generate single-stranded DNA with a 3' end for formation of a RAD51 filament [71,72]. RAD51 filaments can invade homologous DNA to form a D-loop recombination intermediate (Fig. 4). BLM (but not WRN or QL) stimulates this strand exchange activity of active, ATP-bound RAD51 filaments, while disrupting inactive, ADP-bound RAD51 filaments [69,73]. Portions of this biochemistry, e.g., the interaction with EXO1, are also shared by WRN [74].

BLM has the unique property among the human RECQ helicases of being able to dissociate HJ intermediates while suppressing the generation of crossover products. This junction dissolution activity requires interaction with topoisomerase III α with RMI1 and RMI2 to form a functional 'dissolvasome' that branch-migrates and collapses HJs into a hemi-catenane, prior to removing the remaining linked single strands by making use of topoisomerase III α 's strand pass activity (Fig. 3). This dissolution reaction topologically resolves recombination products, and suppresses the generation of crossover products [75–77]. A failure to resolve double HJ intermediates to form non-crossover products could

explain the high levels of SCE's (sister chromatid exchanges), as well as the frequent appearance of UFBs (ultra-fine bridges) in BS cells (see below).

One surprise in light of the recent, rapid progress to understand the biochemical basis of recombination resolution has been the absence of new information on WRN: WRN was not found associated with any of the recently identified resolution proteins or protein complexes (e.g., GEN1, MUS81–EME1, and the SLX4 complex; [78,79]), and no new endonucleolytic activity has been found associated with or encoded by WRN that would allow direct resolution by strand cleavage. These results support initial suggestions that WRN resolves recombination products by strand unwinding or end degradation, as opposed to strand cleavage [67,68]. Consistent with this model, loss of either the WRN exonuclease or helicase activities confers an HR defect with preferential loss of the predominant conversion-type event in human cells, while still allowing the generation of cross-over or 'popout' type recombinants at reduced frequency [67,68].

RECQ helicases may be involved in DNA repair pathways in addition to HR. WRN has been implicated in nonhomologous end-joining (NHEJ) repair and in base excision repair (BER). These links are supported by WRN physical interaction with the MRN complex [80] and the KU70/80 protein [81], and with the APE1 endonuclease [82] and DNA polymerase β [58], respectively. Functional interactions have also been observed between WRN and NHEJ or BER factors [47]. Despite these observations, WRN-deficient cells are not appreciably radiosensitive, or highly sensitive to base damage as would be predicted if either appreciable DSB repair or BER-deficient. Moreover, WS patients do not display the immunodeficiency and variable radiation sensitivity observed in NHEJ-deficient patients, or the organ- or tissue-specific defects that can be predicted from the phenotypes of BER-deficient mice [83]. These observations suggest that roles for WRN in NHEJ or BER are likely to be subsidiary to the primary physiologic roles played by WRN in DNA replication, telomere maintenance and HR repair.

2.4.2. DNA replication—BLM and WRN interact with several proteins that play key roles in replication, e.g. FEN1 or flap endonuclease 1, DNA polymerase δ and proliferating cell nuclear antigen (PCNA). Both BS and WS cells show replication defects that may reflect in part the loss of these interactions [47,84]. Recent analyses have demonstrated that RECQ helicase deficiencies can affect unperturbed replication, and that RECQ function is critical during replication stress when fork progression is impeded by DNA damage or a shortage of dNTPs [66,84].

In contrast to BLM and WRN, RECQL4 and RECQL play important roles in replication initiation as opposed to fork progression, stabilization or restart. RECQL4, and to a lesser extent RECQL, bind directly to replication origins [62]. RECQL4 is found associated with the replicative MCM2-7 helicase proteins and other components of replication initiation complex via an interaction with MCM10 [63]. RECQL4 depletion reduces origin firing efficiency, and suppresses cell proliferation [62]. These observations point to an important role of RECQL4, and to a lesser extent RECQL, in origin unwinding and replication initiation. Cells deficient in BLM or RECQL show slight (10–20%) reductions in replication fork progression rates [62,85]. This may reflect transient fork pausing on the leading strand, as the fork encounters DNA secondary or tertiary DNA structures or bound proteins; or on the lagging strand if Okazaki fragment maturation is slowed.

The basal requirement for WRN and BLM, and perhaps RECQL during an unperturbed S-phase is likely to become more pronounced when replication forks encounter DNA template lesions, or are slowed or stalled by a shortage of dNTPs due to hydroxyurea (HU) treatment. Stalled replication forks need to be maintained in an active state if they are to resume

replication, or protected from breakage if they are to be passively replicated from adjacent active origins. Both WRN and BLM are required for the efficient resumption of replication in hydroxyurea-treated cells, where forks have been stalled by a dNTP shortage [86,87]. WRN and BLM may ensure replication restart by fork remodeling to protect nascent DNA strands, even if replicative DNA polymerase(s) have been lost from the fork. WRN is required for optimal fork progression after the resumption of HU-mediated replication arrest [87]. This may reflect a role for WRN in DNA polymerase proofreading, perhaps via its 3' to 5' exonuclease activity, as fork elongation after stalling may lead to elevated levels of nucleotide misincorporation.

2.4.3. DNA damage checkpoint signaling—DNA damage and replication stress are detected by signal transduction networks that arrest the cell cycle, initiate repair or recovery, or trigger cell death [88,89]. For example the ATM and ATR kinases, key components of the response to DNA damage and replication stress, phosphorylate BLM and WRN in a DNA damage/replication stress-inducible manner [90,91]. Other post-translational modifications likely also occur, e.g., ubiquitylation and SUMO addition (see, e.g., [92]). RECQ helicases may, in turn, modify checkpoint activity via protein interactions, or by generating or disrupting DNA substrates that modulate checkpoint signaling activity [93]. Impaired checkpoint signaling, or adaptation in the face of persistent, abnormal DNA metabolism may be an additional source of genomic instability in RECQ deficient cells.

2.4.4. Telomere metabolism—Telomeres, specialized structures that cap the ends of eukaryotic chromosomes, serve two important roles: they distinguish chromosome ends from DNA double strand breaks, and they facilitate the replication of chromosome ends. Telomeric DNA consists of tandem repeats of a short G-rich DNA sequence unit (TTAGGG in humans) that, together with the multiprotein shelterin complex, form a specialized telomeric D-loop (T-loop) structure [94,95]. Telomeres protect chromosome ends from being recognised and processed as double-strand breaks, but their very nature—a complex, highly structured G-rich nucleoprotein assembly—they impede normal replication and repair.

WRN and BLM may facilitate telomere replication by disrupting telomeric T-loops and G4 DNA [95,96]. The helicase activity of WRN is required for the efficient replication of the G-rich lagging strand of telomeric DNA [95,97,98]. During replication this G-rich strand may remain single-stranded, and thus prone to the formation of secondary structures. BLM appears to be partially redundant with WRN for telomere maintenance [47,96]. Telomere end replication of the lagging strand also requires telomerase that is able to add new, templated telomere repeats directly to chromosome ends. The interplay between telomere length and RECQ helicase activity has been best demonstrated in mice that are later generation (>G3) telomerase-deficient and lacking *Wrn* and/or *Blm* [99,100]. The corresponding story in human cells, however, appears more complicated (reviewed in [95]).

Although telomerase activity is absent from most human somatic cells, it can be readily detected in many cancer cells where it may serve as an important facilitator of neoplastic growth [101]. Telomerase-negative cancer cells take advantage of a second process, recombination-mediated 'alternative lengthening of telomeres' (ALT), that may also be modulated or facilitated by RECQ helicase proteins. In support of this idea, BLM and WRN have been found to colocalize with POT1, TRF1 and TRF2 in telomerase-deficient, ALT-positive immortalized cells, and BLM depletion from ALT cells leads to rapid telomere shortening [95,102].

2.5. Origins of cellular phenotypes

The RECQ helicase DNA metabolic functions outlined above provide mechanistic insight into the origins of common cellular phenotypes that have been defined for RECQ deficient cells. These include proliferative defects, DNA damage sensitivity and genetic or genomic instability.

2.5.1. Proliferative defects—These were first described for primary fibroblasts isolated from WS patients [103], and subsequently identified in BS cells as well. Slow growth in culture could reflect a prolonged cell cycle time, reduced growth fraction or increased cell death, or increased cellular senescence. The proliferative defect in WS cells reflects a combination of cell cycle abnormalities leading to longer cell cycle times, especially in response to DNA damage; a reduced growth fraction; and high levels of cellular senescence especially in long term cultures of primary cells [87,104]. Similar defects have been observed in BLM-deficient cells, and in comparative analyses the depletion of BLM has a stronger growth-suppressive effect than comparable levels of depletion of WRN. Of note, co-depletion of WRN and BLM in these experiments did not suppress proliferation beyond that observed in cells depleted of BLM alone [66]. Human cells depleted of RECQ1 and RECQL4 also display reduced proliferation in culture [32,62].

2.5.2. DNA damage sensitivity—Patient-derived BS and WS cells as well as normal cells depleted of BLM or WRN are sensitive to many DNA-damaging or replication-blocking agents (chemicals, radiation, reactive oxygen species). Some of these agents also selectively kill cells defective in RECQL, RECQL4 and RECQL5. These DNA damage sensitivity profiles provide additional mechanistic insight into functional roles of RECQ helicases in human cells. For example, the selective killing of WS cells by topoisomerase I inhibitors such as camptothecin or by DNA cross-linking agents such as mitomycin C or *cis*-Pt provides additional evidence that WRN is involved in replication and recombination pathways (see [66] for additional discussion).

2.5.3. Genomic instability—Genetic instability is a hallmark of both BS and WS, and this was first identified as chromosomal instability. Primary BS cells have elevated frequencies of DNA exchanges between different chromosomes, as well as breaks, gaps, and fusions in addition to sister chromatid exchanges (SCEs) [4]. Both spontaneous and DNA damage-induced SCEs are elevated in BS cells. In contrast, SCE frequencies are normal in WS cells, as predicted from our current understanding of functional roles of WRN in HR (see above). Regular and ultrafine (UFB) anaphase bridges are also increased in BS cells. Anaphase bridges form when segregating chromosomes remain partially linked as mitosis progresses, whereas UFBs are thin threads tethering otherwise segregated chromosomes [105,106]. WS cells accumulate clonal chromosome deletions and translocations, a characteristic cytogenetic phenotype that was originally termed ‘variegated translocation mosaicism’ [107,108]. These cytogenetic abnormalities in WS likely reflect the capture of stable karyotypic abnormalities that result from defective recombination, replication or telomere maintenance as described above.

The evidence for genomic instability in RTS and associated syndromes is still fragmentary, but there are interesting hints of a coherent story: *RECQL4* is located on chromosome 8q, and trisomy 8 or 8q isochromosomes have been identified in several RTS patients. Chromosome 8 abnormalities have been identified in osteosarcomas, as has upregulation of *RECQL4* mutations, and elevated levels of glycophorin-A variant red cells have been identified in the blood of one RTS patient [109,110]. It will be important in pursuing these clues to use cell lines and patient material that has been mutation-typed at the *RECQL4* locus to avoid the confusions of genetic heterogeneity and phenocopies described above.

2.6. Mechanistic origins of RECQ helicase deficiency syndromes

The cellular defects discussed above provide a way to link DNA metabolic defects resulting from RECQ deficiencies to pathogenesis of the RECQ helicase deficiency syndromes as illustrated in the general model outlined in Fig. 5.

The human RECQ helicases appear to be ubiquitously expressed during and after development in most or all cell lineages. Thus an inherited loss of function of a specific RECQ helicase will disrupt DNA metabolism during and after development. A key consequence of disrupted DNA metabolism is continued genetic instability and mutagenesis. Additional, less direct consequences include the potential for continued epigenetic 'drift' due to persistent cycles of DNA damage and repair due to 'low fidelity' DNA metabolism, and progressive loss of otherwise viable and useful cells due to replication defects, senescence or apoptosis. These cellular consequences of loss of function constitute 'intermediate phenotypes' that may limit the number or quality of cells needed to complete developmental tasks or to maintain tissue structure and function after development.

Two examples illustrate how this model could explain developmental features of BS and RTS, or the development of progeroid features in WS patients. BS and RTS patients are typically born small though are proportionately developed. This finding is particularly striking in BS, where affected individuals are often born, and typically remain, at or below the 5th percentile for height and weight throughout life [111]. Cell loss due to DNA replication defects in BS and RTS could lead to proportionate dwarfing by providing too few cells to complete otherwise normal developmental processes. This quantitative deficit, if further compounded by ongoing genetic instability and cellular dysfunction, provides a partial explanation for the elevated risk of developmental abnormalities in both BS and RTS patients.

Persistently abnormal DNA metabolism may have similar long term consequences. These include the seeding of all cell lineages with mutant cells during development; persistent DNA damage signaling; epigenetic 'churning' and drift due to higher levels DNA turnover and associated DNA- or chromatin protein-associated epigenetic marks; and the suppression of global regulatory pathways such as the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) signaling pathway that regulates metabolism and longevity in many organisms [112,113]. The combination of genetic instability, epigenetic drift and cell loss in continuously or conditionally replicating cell lineages would provide a fertile environment for the emergence of pre-neoplastic cells with proliferative advantage. Some cell lineages may be particularly susceptible to this combination of events, e.g., the osteoblast lineage that gives rise to osteosarcoma in BS, WS and RTS and RAPADILINO patients. A systematic comparison of genetic and epigenetic alterations in osteosarcomas arising in WS, BLM and RTS/RAPADILINO patients would be of considerable interest, as it might identify mutations in each syndrome that reflected underlying genetic instability in addition to common mutations that promoted osteosarcoma.

The conceptual model in Fig. 5 also provides an explanation for the striking, prematurely aged appearance of many WS patients. The proliferative defects, genetic instability and DNA damage sensitivity of WRN-deficient cells would lead to the progressive accumulation of high levels of senescent cells in many cell lineages, though this alone would not be of sufficient magnitude during development to produce a high risk of developmental defects. Loss of WRN function substantially increases the probability of generating senescent cells, as cellular senescence is one important outcome of DNA damage sensitivity and disrupted DNA metabolism [104,114]. The progressive accumulation of senescent cells has been documented in aging primates [115], and one testable prediction is that senescent cells are present—and likely substantially elevated—in many tissues in WS patients. Cell loss and

elevated levels of cellular senescence could compromise tissue or organ structure and function with time, and may further compromise function by negative trophic effects on surrounding normal cells [116]. These senescent cell-associated ‘trans’ effects represent an important area for further exploration in both WS and normal aging, as they may represent an avenue for therapeutic intervention or disease prevention.

The dominant role of cellular senescence in WS might have one modest silver lining: senescence is an effective, non-specific mechanism to suppress the emergence of tumors, even in the face of persistent genetic instability [117]. It should be possible to gain additional insight into these different aspects of disease pathogenesis by examining ‘intermediate’ phenotypes in patients with RECQ helicase deficiency syndromes, and by making judicious use of mouse models of several of the human RECQ deficiency syndromes.

2.7. RECQ helicase roles in sporadic cancer

Heritable loss of RECQ function is associated with an elevated risk of cancer in BS and WS patients, and the subset of RTS and RAPADILINO patients who carry *RECQL4* mutations. Less clear is whether heterozygous carriers of known pathogenic *RECQ* mutations are at elevated risk of cancer, or of enhanced toxicity following cancer therapy with DNA damaging agents. *WRN* heterozygotes carrying known pathogenic mutations have *in vivo* genetic instability [118], and cell lines from these individuals show intermediate sensitivity to killing by DNA damaging chemotherapeutic agents that selectively kill *WRN*-deficient cells [119]. Persistent genetic instability associated with inherited or somatic mutations and haploinsufficiency for *WRN*—and perhaps for other human *RECQ* genes—might be sufficient to initiate a vicious cycle with frequent loss of remaining intact *RECQ* alleles and DNA metabolic and cellular defects. Resulting fully RECQ-deficient cells would drive the pathogenetic sequence outlined in Fig. 5, and the associated organismal endpoints of tumorigenesis and tissue dysfunction and hypofunction. These arguments suggest that heterozygote phenotypes associated with partial loss of RECQ function may be of considerable practical significance. Moreover, they may be common as current estimates of the frequency of individuals carrying single known deleterious *WRN* mutations, of 1 in 250 individuals in the U.S. [120], predicts that there may be $\sim 1.5 \times 10^6$ individuals at risk of this type of deleterious positive feedback loop that could lead to an elevated risk of cancer, therapy-related toxicity or other diseases.

Few somatic mutations in *RECQ* genes have been reported in human tumors, or identified by large scale cancer genome sequencing. Loss or silencing of expression of *RECQ* genes, in contrast, may be frequent in common adult epithelial cancers such as breast and colorectal cancer. Promoter region or gene methylation have been suggested as one mechanistic explanation for the loss of RECQ expression [121,122]. However, the relationship between methylation and loss of expression does not appear to be consistent enough, in our and others' hands, to use methylation alone as an expression marker. Thus new reagents or assays will be needed to reliably determine whether loss of RECQ expression is common in sporadic human cancer.

RECQ expression loss in tumors is practically important to identify, as it would provide a potentially useful therapeutic biomarker of immediate utility in conjunction with the large body of data on the drug sensitivity of RECQ-deficient human cells [66]. The idea of targeting RECQ helicase proteins directly to treat cancer has also been suggested, as the helicase and exonuclease catalytic activities of the human RECQ helicase proteins provide ready targets for the identification of new drugs or small molecule inhibitors [123]. One advantage of this approach is that the consequences of RECQ helicase inhibition are readily predictable (see above). Direct targeting of tumors with RECQ helicase inhibitors might

provide a clear tumor-specific therapeutic advantage if combined with conventional chemotherapy, or with pre-existing genetic instability or higher levels of replication stress in tumors [124,125]. The targeting of survival pathways specific to one—or common to several—RECQ helicases could provide a second approach to improve the therapy of patients with tumor-specific RECQ defects. These pathways could be identified by the use of RNAi or drug/small molecule screens to identify pathways or proteins that are synthetically lethal with RECQ deficiencies [126–129].

2.8. Epilogue: RECQ futures

Loss of function of three different members of the human RECQ helicase family, BLM, WRN and RECQL4, lead to distinct diseases with developmental and acquired features including a markedly elevated risk of specific cancers. These ‘experiments of nature’ have, after a decade of work, begun to provide useful insight into the role of the human RECQ helicases in cellular DNA metabolism, the origins and consequences of DNA metabolic defects following loss of function, and how acquired loss of RECQ function may provide new opportunities to improve cancer therapy.

One important ‘take-home’ from the past decade's work on RECQ helicase deficiency syndromes is that genetic instability requires context-dependent information to predict phenotypic effects. The RECQ helicase deficiency syndromes illustrate how different DNA metabolic defects may lead to a mutator phenotype, and how genetic instability may promote developmental defects, the early emergence of tumors, or progeroid features in different contexts. Tumors represent a practically important focus for further developing context-dependent analyses of mutator phenotypes: many of the needed data are in hand or are being rapidly acquired, and cancer therapy represents an important practical opportunity to determine how mutator phenotypes can be identified, rapidly characterized and turned to therapeutic advantage to aid individual patients. The progress summarized here provides hope that our voyage, though far from over, has prospect for a satisfying conclusion.

Web links

Bloom syndrome/BLM

Entrez gene record with reference sequence (RefSeq) gene and protein links:
<http://www.ncbi.nlm.nih.gov/gene/641>.

On-line Mendelian inheritance in man (OMIM) record:
<http://www.ncbi.nlm.nih.gov/omim/210900>.

GeneClinics gene review:
<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=bloom>

Werner syndrome/WRN

Entrez gene record with reference sequence (RefSeq) gene and protein links:
<http://www.ncbi.nlm.nih.gov/gene/7486>.

On-line Mendelian Inheritance in Man (OMIM) record:
<http://www.ncbi.nlm.nih.gov/omim/277700>.

GeneClinics gene review:
<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=werner>.

Werner syndrome locus-specific mutation database:
<http://www.pathology.washington.edu/research/werner/database/>

International registry of Werner syndrome:
<http://www.pathology.washington.edu/research/werner/index.html>.
<http://www.pathology.washington.edu/research/werner/registry/registry.html>.

Rothmund–Thomson syndrome/RTS

Entrez Gene record with reference sequence (RefSeq) gene and protein links:
<http://www.ncbi.nlm.nih.gov/gene/9401>.

On-line Mendelian inheritance in man (OMIM) record:
<http://www.ncbi.nlm.nih.gov/omim/268400>.

GeneClinics gene review:
<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=rts>.

RAPADILINO syndrome

On-line Mendelian inheritance in man (OMIM) record:
<http://www.ncbi.nlm.nih.gov/omim/266280>.

Baller–Gerold syndrome

On-line Mendelian inheritance in man (OMIM) record:
<http://www.ncbi.nlm.nih.gov/omim/218600>.

GeneClinics gene review:
<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=bgs>.

RECQL

Entrez gene record with reference sequence (RefSeq) gene and protein links:
<http://www.ncbi.nlm.nih.gov/gene/5965>.

RECQL5

Entrez Gene record with reference sequence (RefSeq) gene and protein links:
<http://www.ncbi.nlm.nih.gov/gene/9400>.

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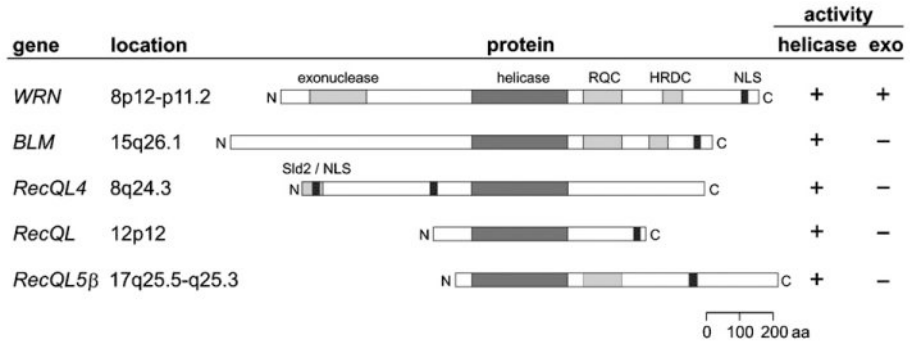


Fig. 1. Human RECQ helicase gene and protein family. The five human RECQ helicase proteins are shown as boxes (center). Gene symbols and gene chromosomal locations are given to the left, and encoded catalytic activities to the right, of each protein diagram. All five proteins share a central, conserved RECQ helicase domain that encodes a 3'-5' helicase activity. Three family members contain RECQ Consensus (RQC) domains, and two a Helicase and RNase D C-terminal (HRDC) domain. Nuclear localization signals (NLS) are depicted as short filled boxes. The 3'-5' exonuclease domain is unique to WRN, whereas the Sld2 homology domain is found only in RECQL4.

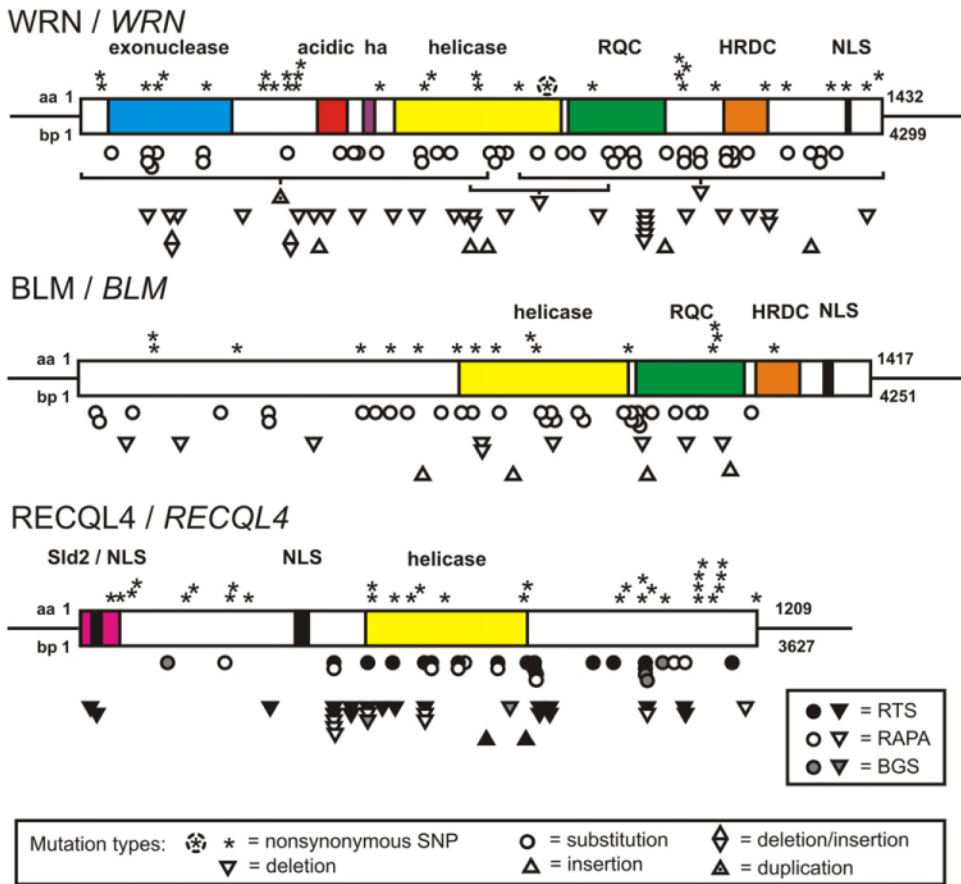


Fig. 2. Disease-causing mutations in human *RECQ* helicase genes. WRN, BLM and RECQL4 open reading frames are depicted as boxes with domains indicated as in Fig. 1. Two additional acidic domains are shown for WRN: the acidic repeat domain (acidic) and a short hyperacidic (ha) stretch consisting of aspartic and glutamic acid residues preceding the helicase domain. Residue and bp coordinates are shown to the left of the beginning of each protein. Coding region non-synonymous SNP polymorphisms are shown above, and clinically ascertained mutations below, each RECQ protein. Mutations, not consequences, are shown; and only single examples of specific mutations. The WRN R834C SNP polymorphism is circled (see text), and large deletions and a duplication are indicated by a horizontal line linked to the appropriate type symbol. *RECQL4* mutations identified in Rothmund–Thomson, RAPADILINO or Baller–Gerold syndrome patients are further indicated by the symbol fill, with the key to the lower right.

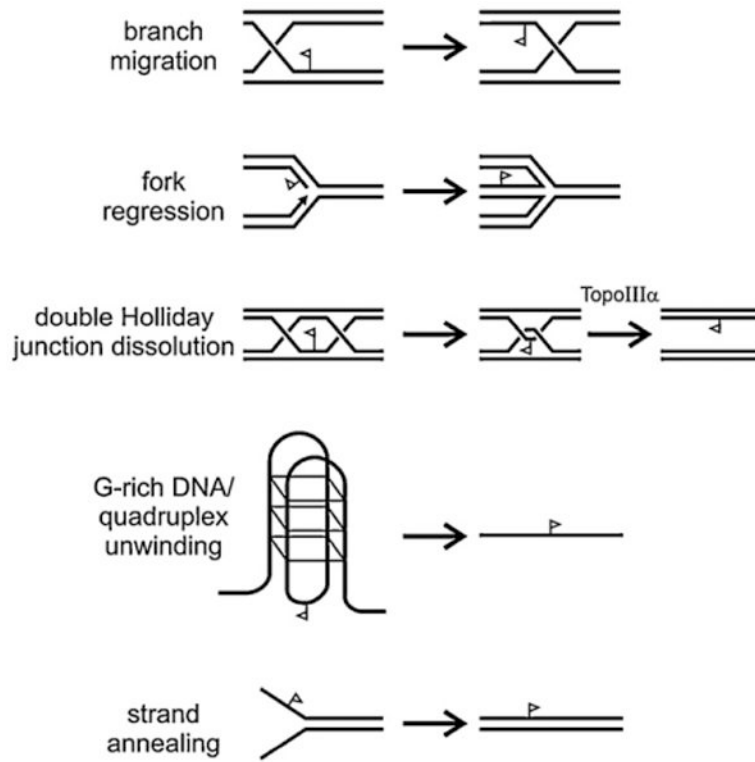


Fig. 3. DNA metabolic activities of human RECQ helicases. Activities of human RECQ helicases on model DNA substrates is depicted, and can be thought of as different combinations of unwinding, translocation/displacement, strand annealing and, in the case of WRN exonucleolytic degradation activities. Different human RECQ helicases encode different combinations of the activities shown (see text for detail). The flag symbol on DNA strands provides a reference point to aid visualization of the consequences of RECQ-mediated biochemical activities.

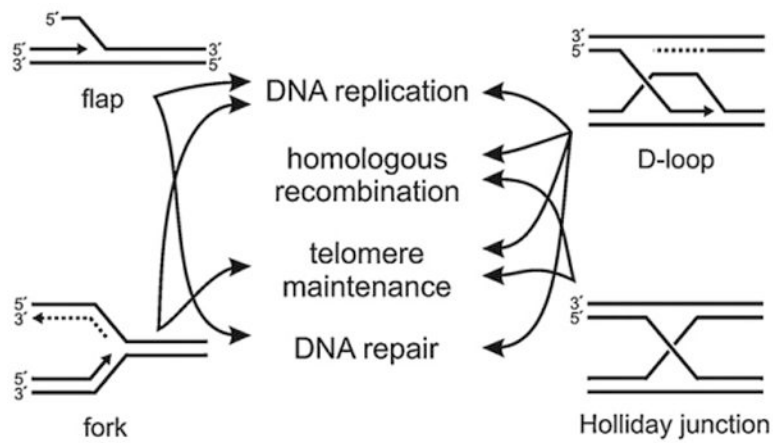


Fig. 4.

Inferred RECQ helicase substrates and their roles in DNA metabolism. The common model substrates depicted in Fig. 3 and used to define RECQ helicase biochemical activities have direct counterparts in cellular DNA metabolism. RECQ helicases are able to unwind and release DNA flaps (upper left); promote replication fork progression, regression or remodeling (lower left); release an invading 3' DNA tail in a D-loop (upper right); and branch migrate and, in the case of BLM in conjunction with topoisomerase III α , resolve separate DNA duplexes joined in a Holiday junction (lower right).

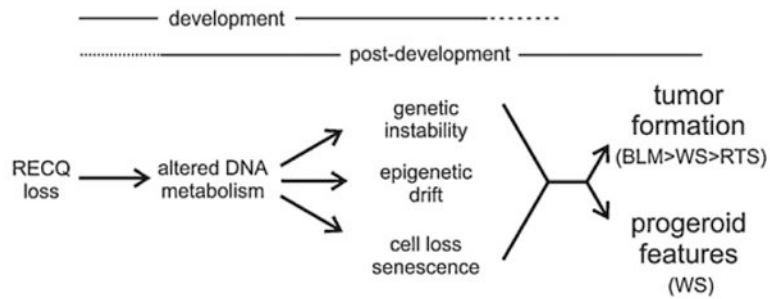


Fig. 5. Pathogenesis of human RECQ helicase deficiency syndromes. A model is depicted that summarizes cellular and organismal consequences of loss of RECQ helicase function during and after development. Heritable loss of RECQ function leads to altered DNA metabolism in most or all cell lineages during and after development. Altered or aberrant DNA metabolism, in turn, leads to genetic instability, epigenetic ‘drift’ and cell loss or senescence that over time may compromise tissue structure and function while promoting the emergence of cells with a proliferative advantage to form specific neoplasms (upper right). Tumor generation is strongest in BS. Loss of WRN function also strongly promotes cellular senescence that contributes to global progeroid changes and may provide a non-specific tumor suppressive mechanism that limits tumor formation to a few susceptible cell lineages such as osteoblasts.