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Werner Syndrome: Clinical Features, Pathogenesis and Potential Therapeutic Interventions

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Abstract

Werner syndrome (WS) is a prototypical segmental progeroid syndrome characterized by multiple features consistent with accelerated aging. It is caused by null mutations of the *WRN* gene, which encodes a member of the RECQ family of DNA helicases. A unique feature of the WRN helicase is the presence of an exonuclease domain in its N-terminal region. Biochemical and cell biological studies during the past decade have demonstrated involvements of the WRN protein in multiple DNA transactions, including DNA repair, recombination, replication and transcription. A role of the WRN protein in telomere maintenance could explain many of the WS phenotypes. Recent discoveries of new progeroid loci found in atypical Werner cases continue to support the concept of genomic instability as a major mechanism of biological aging. Based on these biological insights, efforts are underway to develop therapeutic interventions for WS and related progeroid syndromes.

1. Introduction

Werner syndrome (WS; OMIM# 277700) is a rare genetic disorder that displays clinical features suggestive of accelerated aging. WS was originally described by a German medical student, Otto Werner, in 1904 (Werner, 1985). Werner reported a family of four siblings, ages 31 to 40, who presented with “Cataracts in Connection with Scleroderma” as well as short stature and premature graying of hair. The term, “Werner’s syndrome” was first used in 1934 by Oppenheimer and Kugel who described a new case of WS (Oppenheimer and Kugel, 1934) and subsequently by Thannhauser in 1945, who provided a comprehensive review of “Werner’s syndrome (Progeria of the Adults)” (Thannhauser, 1945). The gene responsible for WS was discovered in 1996 through then-ground breaking positional cloning method (Yu et al., 1996). This review summarizes our current understandings of clinical

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phenotypes, normal functions of the *WRN* gene product and potential therapeutic approaches.

2. Classical Aspects of the Werner syndrome

WS is a rare autosomal recessive disorder characterized by an array of features consistent with accelerated aging (Fig 1)(Oshima et al., 2014; Takemoto et al., 2013). This is one of the few adult-onset syndromes of accelerated aging in which patients generally develop normally until they reach adolescence. The first sign, often recognized retrospectively, is a lack of a growth spurt and a relatively short stature as adults. Beginning in the early third decade of life patients begin to develop an aged appearance that includes skin atrophy, loss of subcutaneous fat and graying and loss of hair. Bilateral cataracts requiring surgery are seen in virtually all cases by the late 20s or early 30s (Huang et al., 2006; Takemoto et al., 2013). This is accompanied by a series of common age-related diseases that appear during middle age. These disorders include type 2 diabetes mellitus, hypogonadism, osteoporosis, atherosclerosis and malignancies. Several studies report that 30-40% of WS cases had children before gonadal atrophy leading to early loss of fertility in their 30s (Goto, 1997; Takemoto et al., 2013). Indolent deep ulcerations around Achilles tendons and, less frequently, at elbows, are almost pathognomonic to WS. These are associated with extensive subcutaneous calcifications and often lead to amputation of feet or lower extremities (Takemoto et al., 2013). Other features frequently seen in WS include a high pitched hoarse voice (recognizable over the phone), characteristic facial features (a “pinched” facial appearance), thin limbs, truncal obesity, and flat feet. The most common causes of death are cancer and myocardial infarction, at a median age of 54 (Huang et al., 2006). This is 7 years older than the median age of death reported in 1996 (Epstein et al., 1966), likely owing to improvements of medical care, as the median age for the extraction of cataracts (at age 31) were comparable in both eras.

There are clinical discordances in the presentation of age-related disorders between WS and normal aging. For example, systematic reviews of cancer in WS patients revealed a much higher incidence of sarcomas than expected for an age-matched control cohort (Goto et al., 1996; Lauper et al., 2013). The most common neoplasms in WS are thyroid follicular carcinomas, followed by malignant melanoma, meningioma, soft tissue sarcomas, primary bone tumors and leukemia/myelodysplasia. The elevated risk of these neoplasms ranges from 2 to 60-fold higher than population controls (Goto et al., 1996; Lauper et al., 2013). The arteriosclerosis of WS patients includes premature and severe forms of atherosclerosis, arteriolosclerosis and medial calcinosis. Hypertension, however, is not a common feature of WS. The cataracts seen in WS are almost always posterior sub-capsular, in contrast to those seen in normal aged people, which are typically nuclear cataracts. Osteoporosis in WS is more severe in distal limb bones than in the vertebral column, the opposite of what is seen in normal aged individuals. In addition, osteosclerosis of distal phalanges is highly characteristic of WS, though rarely observed during normal aging. There is no evidence for increased deposition of a variety of amyloids in WS, and dementias of the Alzheimer type are not a common feature of WS (Martin et al., 1999). Mental retardation, dysmorphology, skeletal anomalies and other developmental abnormalities are not features of WS; when present, they are likely due to co-existing disorders. The discordances above may be

attributed to a number of factors, such as differential expressions and regulations of the WRN protein in various cell types and tissues, rates of cell turnover, and variations in the replicative potentials of various types of stem cells. The presence or absence of the compensatory enzymes or signal transduction pathways among various tissues may also play important roles. It is clear, however, that further studies are needed to explain the characteristic distributions of phenotypes.

Clinical criteria can be used to facilitate a diagnosis of WS. These are detailed at the International Registry of Werner Syndrome (Table 1) (www.wernersyndrome.org) (Oshima et al., 2014). Cardinal signs include bilateral cataracts (present in 99% of WS cases), premature graying and/or thinning of scalp hair (100%), characteristic dermatologic changes (96%) and short stature (95%) (Huang et al., 2006). Over 91% of affected individuals had all four cardinal signs (Huang et al., 2006). A related set of diagnostic criteria based on a national survey of 146 clinically diagnosed Japanese patients with WS lists progeroid changes of hair, cataracts, scleroderma-like changes of skin, intractable skin ulcers, soft-tissue calcifications, bird-like facies, and abnormal voice can serve as cardinal signs (Takemoto et al., 2013). Confirmation of a clinical diagnosis requires *WRN* gene testing.

3. *WRN* gene product and *WRN* mutations

Classical WS is caused by homozygous or compound heterozygous loss of function mutations in the *WRN* gene (Fig 2) (Yu et al., 1996). *WRN* is the only known gene in which mutations cause classical WS, and WS is the only known genetic disorder caused by null mutations of the *WRN* gene. The *WRN* locus is located on human chromosome 8p12, and consists of 34 coding exons spanning 140kb (Yu et al., 1996). The encoded WRN protein is a 1,432-amino acid, 160 kDa multifunctional nuclear protein with a 3'→5' exonuclease domain in its N-terminal region (Huang et al., 1998), an ATP-dependent 3'→5' helicase in its central region (Gray et al., 1997) and a nuclear localization signal in its C-terminal region (Matsumoto et al., 1997; Suzuki et al., 2001). There are two other consensus domains: the RecQ helicase conserved region (RQC) and the “helicase, RNaseD, C-terminal conserved region” (HRDC). Structural analysis and biochemical studies demonstrated that RQC, with its winged-helix (WH) domain, is critical for substrate-specific DNA binding to initiate unwinding (Kitano et al., 2010; Tadokoro et al., 2012; von Kobbe et al., 2003). The HRDC domain also plays a role in DNA binding, particularly for recruiting WRN protein to dsDNA breaks (DSBs) (Kitano et al., 2007; Lan et al., 2005; von Kobbe et al., 2003). The region between RQC and HRDC was also shown to possess single strand-DNA annealing activity and may influence oligomerization of the WRN protein (Muftuoglu et al., 2008).

More than 70 different disease mutations have been identified in classical WS patients from all over the world (Friedrich et al., 2010; Uhrhammer et al., 2006). A majority of the disease mutations are stop codons, small indels or splicing mutations which cause truncations with loss of the nuclear localization signal at the C-terminus of the WRN protein and/or promote nonsense-mediated mutant mRNA decay. Two amino acid substitution mutations within the exonuclease domain, p.Lys125Asn and Lys135Glu, render the WRN protein unstable and are functionally null mutations as well (Huang et al., 2006). Genomic rearrangements leading to loss of function with or without involvement of neighboring loci have been

identified by array comparative genome hybridization (array CGH)(Friedrich et al., 2010). Thus, virtually all *WRN* mutations in clinically ascertained patients are functional null alleles. There has been one report of possible genotype-phenotype correlation of the type of thyroid carcinoma with the location of *WRN* mutations in Japanese WS patients: follicular carcinomas were associated with C-terminal *WRN* mutations and papillary carcinoma with *WRN*-terminal mutations (Ishikawa et al., 1999). Further studies are needed to clarify the effect of residual mutant *WRN* proteins in WS patients.

Two *WRN* amino acid substitution mutations have been identified within the helicase domain, p.Gly574Arg and p.Arg637Trp, in compound heterozygotes in association with null mutations (Friedrich et al., 2010; Uhrhammer et al., 2006). Protein studies confirmed that the p.Gly574Arg change abolishes helicase activity, and that the p.Arg637Trp is predicted to inactivate helicase function (Tadokoro et al., 2013). The phenotypes of patients carrying p. Gly574Arg or p.Arg637Trp appear to be indistinguishable from those with *WRN* null mutations. (Tadokoro et al., 2013; Uhrhammer et al., 2006) These data support the idea that the helicase activity is a crucial function of the *WRN* protein. Consistent with this idea, a mouse model carrying a homozygous helicase domain deletion mutation (*Wrr^{Δhel/Δhel}*) showed various signs of genomic instability and metabolic abnormalities resembling those seen in WS patients, and had 10-15% shorter median lifespan compared to controls (Labbe et al., 2011; Lebel et al., 2003). In contrast, a mouse model with a homozygous null mutation (*Wrr^{-/-}*) appeared to live beyond two years of age without developing an obvious phenotype under normal dietary and husbandry conditions (Lombard et al., 2000; Moore et al., 2008). These findings raise the possibility that the presence of helicase-deficient *WRN* protein may render subtle but deleterious dominant negative effects that are not present in individuals with null mutations.

Ethnicity-specific *WRN* mutations have been reported in Japanese, Sardinian, Indian/Pakistani, Moroccan, Turkish and Dutch patients (Friedrich et al., 2010; Saha et al., 2013a). The prevalence of specific *WRN* mutations varies depending on the level of consanguinity. In the Japanese population, the heterozygote frequency is estimated to be 1/166 (Sato et al., 1999). Similarly, in the Sardinian population, a heterozygote frequency is estimated to be of the order of 1/120 (Masala et al., 2007). It is likely that other founder mutations and populations with high prevalence will be identified as the awareness of WS increases, and as genetic testing become more common worldwide.

4. Cellular functions of the *WRN* gene product

Extensive biochemical studies of the substrate preference of *WRN* protein have identified complex DNA structures such as G4 quadruplexes, Holiday junctions and bubble structures as preferred substrates (Brosh et al., 2006; Compton et al., 2008; Kamath-Loeb et al., 2012a; Kamath-Loeb et al., 1998; Shen et al., 1998). This suggests a role of *WRN* in resolving complex intermediate DNA structures generated either normally or accidentally during DNA metabolism. Two major characteristics of cells derived from WS patients are genomic instability and very limited replicative capacity (Oshima et al., 1995; Salk et al., 1985a; Salk et al., 1985b). At the chromosomal level, genomic instability of WS fibroblasts has been described as variegated translocation mosaicism, with characteristic multiple, variable,

predominantly stable chromosomal aberrations (Melcher et al., 2000). Biochemical and cell biological studies demonstrated that the roles of WRN protein in various DNA transactions, including DNA repair, replication, transcription and telomere maintenance, maintain genomic stability.

Double-strand breaks (DSBs) are potentially lethal DNA damages that can be generated by exogenous causes as well as by endogenous processes. DSBs are repaired through either homologous recombination (HR) or error-prone non-homologous end joining (NHEJ). It has been proposed that the choice between HR and NHEJ is determined in part by heterochromatin status and the chemical nature of the DSB, and that canonical NHEJ may be preferred for exogenous DSB repair during the G₀ and G₁ phases of the cell cycle (Kakarougkas and Jeggo, 2014; Keijzers et al., 2014). WRN physically interacts with a number of players in NHEJ such as Ku70/80, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and DNA ligase IV/XRCC4 (Kusumoto-Matsuo et al., 2014; Kusumoto et al., 2008). The potential physiologic significance of WRN in NHEJ repair is suggested by the demonstration of extensive deletions of reporter plasmids, particularly at 3' overhang ends, in cells lacking WRN protein (Oshima et al., 2002). Ku70/80 and Ligase IV/XRCC4 stimulate WRN exonuclease but not WRN helicase activity, again suggesting a specific requirement of WRN in NHEJ (Cooper et al., 2000; Li and Comai, 2001). HR repair of DSBs may predominate during late S and G₂ phases of the cell cycle, when a sister chromatid is available to serve as a repair template (Johnson and Jasin, 2000). Integrated recombination reporter substrates have been used to identify a role for the WRN helicase and exonuclease catalytic activities in promoting the resolution of recombinant DNA molecules during the postsynaptic phase of HR (Saintigny et al., 2002).

More recently, WRN was shown to participate in 5'→3' DNA end resection in human cells, a step during HR that creates a substrate for RAD51 binding and subsequent D-loop formation and strand exchange (Sturzenegger et al., 2014). WRN carries this function in cooperation with the DNA2 endonuclease, likely using its helicase activity to generate a substrate for DNA2. In this role, WRN is redundant with its close homolog, BLM, though it is conceivable that different cell lineages may preferentially utilize one or the other RECQ helicase. In fact, a recently identified heterozygous mutation in *RAD51*, p.T131P, which elicits a Fanconi anemia-like phenotype, revealed that WRN/DNA2 and not BLM/DNA2 is recruited to resect DNA at mitomycin C crosslinks (Wang et al., 2015). Together, these findings establish a role for WRN early in the HR pathway.

HR also plays a major role in the protection or resolution and repair of stalled or broken replication forks during S phase in eukaryotic cells (Kakarougkas and Jeggo, 2014; Petermann and Helleday, 2010). Stalled replication forks may exist in a dynamic, regulated equilibrium between a regressed form (a “chicken foot”) and its reversal. A RECQ helicase, RECQ1 (or RECQL1), reverses regressed forks in a manner that is blocked by PARP1 activation (Berti et al., 2013), and RAD51 may protect forks from regression (Zellweger et al., 2015), as well as limit resection of DNA at forks (see below)(Hashimoto et al., 2010). Both regressed and un-regressed forks may become substrates for nascent DNA end resection that may differ in its extent and in the identities of the proteins involved, but is, in

principle, similar to that occurring during HR (Neelsen and Lopes, 2015; Schlacher et al., 2011; Schlacher et al., 2012).

WRN and DNA2 are thought to execute productive, limited resection at stalled forks that facilitates fork restart when conditions become permissive for resumption of DNA synthesis. These functions of WRN have been uncovered using DNA fiber technologies that quantify replication fork progression and can demonstrate, for example, that in hydroxyurea-arrested WRN-deficient human cells both fork resection during arrest and fork restart after hydroxyurea removal, are reduced (Ammazzalorso et al., 2010; Franchitto et al., 2008; Iannascoli et al., 2015; Sidorova et al., 2013; Sidorova et al., 2008; Thangavel et al., 2015). Both WRN helicase and exonuclease activities are implicated in preserving replication forks for restart. WRN may also have an additional role in facilitating DNA synthesis for the first moments after fork restart (Sidorova et al., 2013; Sidorova et al., 2008), which is thought to involve its functional interaction with specialized polymerases such as polymerase η (Kamath-Loeb et al., 2007). To add complexity to this already elaborate orchestra of activities surrounding stalled forks, it has recently been shown that WRN may have a non-enzymatic role in preventing unproductive nascent DNA resection at forks that developed double strand breaks due to an encounter with a camptothecin-induced topoisomerase I-DNA adduct (Su et al., 2014). Lastly, replication fork phenotypes of WRN deficiency are not limited to cases where replication progression is severely disrupted by genotoxic agents. An early study demonstrated increased stalling of nascent forks during an unperturbed S phase in primary fibroblasts deficient in WRN (Rodriguez-Lopez et al., 2002), and later work noted a subtle reduction in fork progression rate during a normal S phase in WRN-depleted transformed human fibroblasts (Sidorova et al., 2013). Chronic low-grade destabilization of forks that can drive the genomic instability phenotype of WRN deficiency, is also suggested by the increased common fragile site expression in WRN-deficient cells (Murfuni et al., 2012; Pirzio et al., 2008).

WRN may also participate in base excision repair through the interactions with APE endonuclease and DNA polymerase β (Sidorova and Monnat, 2015). There is a single publication showing the interaction of WRN and XPG, suggesting an involvement of WRN in nucleotide excision repair (Trego et al., 2011).

5. Telomere maintenance and Werner syndrome

Telomeres are DNA-protein structures that cap and protect the ends of chromosomes. Telomere complexes consist of TTAGGG repeats with 3' single strand overhangs which can strand-invade within the repeat region to form a T-loop. Telomeric DNA is then bound by several proteins to form the shelterin complex. These proteins include TRF2, TRF1, POT1, TPP1, Rap 1 and TIN2 (O'Sullivan and Karlseder, 2010; Palm and de Lange, 2008). In normal cells, the shelterin components TRF2, TRF1 and POT1 directly bind to WRN protein and modulate its enzymatic activities (Edwards et al., 2014; Opresko, 2008). Several lines of evidence argue that telomeres are physiological substrates of WRN protein, and that aberrant telomere maintenance in the absence of WRN may be an important disease mechanism leading to the progeroid phenotypes of WS patients. Tandem repeats of TTAGGG sequences can form G4 quadruplexes, which are preferred substrates of WRN helicase *in vitro* (Brosh

et al., 2006). In the absence of WRN protein, persistent G4 structures could lead to accelerated loss of telomere repeats if not resolved by other RECQ helicases in a timely fashion (Damerla et al., 2012). Replication of telomeres requires progressive resolution of four way junctions formed at the crosses of D- and T-loops, another preferred substrate of WRN (Opresko et al., 2009). Alteration of WRN function through expression of a dominant-negative WRN helicase mutant resulted in substantial loss of telomeric DNA that was associated with replication of the G-rich strand (Arnoult et al., 2009; Crabbe et al., 2004), consistent with the notion that cells lacking WRN helicase activity suffer delayed replication of the G4-forming telomere strand and subsequent shortening of telomeres from single sister chromatids. The increased vulnerability of telomeric G-rich DNA to WRN loss was explained by the consensus that these strands are replicated by the lagging mechanism only and thus spend more time in a single-stranded state, which facilitates G4 formation. Interestingly however, recent studies that combined telomere FISH and DNA fiber analysis of replication, showed that replication initiation events can in fact be detected within telomeric repeats, suggesting that a subset of G-rich strands are replicated by the leading strand mechanism (Drosopoulos et al., 2012; Sfeir et al., 2009). In mouse cells, where initiations within telomeric repeats are more prevalent than in human cells, it was possible to observe that the impact of WRN as well as BLM deficiency on fork progression was more pronounced in those DNA molecules where a G-rich strand was in fact copied by the leading strand mechanism (Drosopoulos et al., 2015). The same study confirmed that telomeric G4 signals, as visualized by a G4-specific antibody, were indeed elevated in WRN or BLM-deficient cells.

Whether or not WRN counteracts G4 formation in leading or lagging strand replication, it is clear that failure to replicate telomeric repeats properly will eventually lead to critically short telomeres, which are no longer end-protected, and can initiate a persistent DNA damage response and promote catastrophic chromosomal end fusions (Crabbe et al., 2007). Of note, in a *WRN* null mutant mouse model of WS, disease phenotypes were observed only in 3rd generation or later telomerase-deficient mice (*mTerc*^{-/-} *Wrrn*^{-/-}) having short telomeres (Chang et al., 2004). Interestingly, hTERT immortalization of WS fibroblasts abolishes both the proliferative defect and some of the genotoxic hypersensitivity displayed by WS cells (Hisama et al., 2000). Although the degree of damage accumulation at non-telomeric DNA was not assessed in these WS hTERT fibroblasts, this observation is consistent with the idea that telomeres are particularly vulnerable to a loss of WRN function and that telomere phenotypes may be important in WS disease pathogenesis.

The roles of WRN protein at telomeres described above may in part explain the characteristic tumor spectrum of WS patients. The development of malignant neoplasms require a mechanism of telomere elongation, generally through re-activation of hTERT expression or through acquisition of the alternative lengthening of telomeres, referred to as ALT (an “alternative” mechanism). The ALT is mediated by homologous recombination of telomere sequences and is more frequently adopted by sarcomas (Bryan et al., 1997). Mouse embryonic fibroblasts (MEF) derived from the 5th generation (G5) of *mTerc*^{-/-} *Wrrn*^{-/-} mice showed elevated numbers of telomere sister chromatid exchanges (T-SCE). Tumors derived from the G5 *mTerc*^{-/-} *Wrrn*^{-/-} MEF showed the characteristics of ALT cells (Laud et al., 2005). Elevated SCEs, mainly localized to telomeres, were also observed in in

human WRN deficient cells (Gocha et al., 2014; Hagelstrom et al., 2010). For example, the widely used AG11395 human WS cell line is ALT-positive (Fasching et al., 2005). These findings support the hypothesis that WRN normally suppresses T-SCEs, and that the absence of WRN facilitates the activation of ALT as a telomere maintenance mechanism.

6. Translational approaches to WS

Currently there is no cure for WS. Clinical management has focused on treating manifestations, preventing secondary complications and screening for acquired diseases common to WS. Treatment of WS patients is similar to that of the general population, with the exception of neoplasia, where the use of DNA-damaging chemotherapeutic agents may be modified to reflect the sensitivity of WS cells to several classes of chemotherapeutic agents (Mao et al., 2010).

Several novel therapeutic approaches are also being explored that may more directly influence WS disease progression. One example involves mTOR inhibitors. The mTOR pathway is a key modulator of aging and age-related diseases across wide varieties of species. Important biological roles of the mTOR pathway include promoting cell proliferation in nutrient-rich environments (high mTOR activity), and the diversion of metabolic resources for stem cell maintenance in nutrient-poor environments (low mTOR activity)(Johnson et al., 2013). We reported that mTOR signaling and basal autophagy are upregulated in WS cells, and that long-term rapamycin treatment resulted in improved growth rate, reduced accumulation of DNA damage foci and improved nuclear morphology. Autophagy markers were also reduced to near-normal levels, possibly due to the clearance of damaged proteins (Saha et al., 2014). DNA damage accumulation in WS cells may compromise protein homeostasis, which in turn further contributes to impaired DNA repair. Support for this hypothesis comes from the independent observation that hydrogen sulfide can abrogate a protein aggregation phenotype and attenuate oxidative damage in WS cells via modulation of the mTOR pathway (Talaie et al., 2013).

Selective inhibitors of p38 mitogen-activated protein kinase (MAPK) have also been investigated as a potential pharmaceutical intervention for WS and other genomic instability syndromes (Davis et al., 2005; Tivey et al., 2013). The MAPK mediates stress-signaling, which activates two tumor suppressor pathways, p53/p21WAF1 and pRb/p16INK4A and induces premature senescence of primary fibroblasts (Yaswen and Campisi, 2007). A small molecule that targets p38 MAPK was shown to suppress accumulation of p21WAF1 and restore the replicative potential of WS fibroblasts (Davis et al., 2005; Tivey et al., 2013). Neither mTOR inhibitors nor p38 MAPK inhibitors have as yet been tested in WS patients.

There is a single case report of astaxanthin-treatment of a WS patient, in which administration of this keto-carotenoid markedly improved the patient's fatty liver possibly, via its anti-inflammatory and anti-oxidative capabilities (Takemoto et al., 2015).

The availability of human induced pluripotent cells (hiPSCs) has provided a new approach to disease modeling and treatment (Zhu and Huangfu, 2013). The hiPSCs are derived through the reprogramming of human somatic cells using ectopic expression of transcription

factors, including OCT3/4, SOX2, KLF4, MYC, NANOG and LIN28 (Shimamoto et al., 2014; Takahashi and Yamanaka, 2006). There have been two reports of hiPSC derived from WS patient fibroblasts (Cheung et al., 2014; Shimamoto et al., 2014), both of which showed reversion of senescence-related cellular phenotypes observed in fibroblasts. Comparison of the gene expression profiles of WS and control fibroblasts revealed numerous differences, most of which are believed to be involved in cellular aging. The gene expression profiles of WS hiPSCs were indistinguishable from those of control hiPSCs fibroblasts (Cheung et al., 2014; Shimamoto et al., 2014). While introduction of hTERT was shown to reverse some of the WS cellular phenotypes (Cheung et al., 2014; Hisama et al., 2000), the reversion of gene expression profiles of WS hiPSCs cannot be solely attributed to activation of hTERT (Shimamoto et al., 2014). Moreover WS hiPSCs were karyotypically stable (Shimamoto et al., 2014). The evidence of significant degrees of genomic stabilization in these WS hiPSCs is consistent with a hypothesis of early development gene actions that compensate, or at least partially compensate, for null mutations at the *WRN* locus. These precedents provide the basis for determining whether stem cell-based therapies may eventually be applied to WS patients to, e.g., promote the healing of the leg ulcers that often leads to amputation in many WS patients (Shimamoto et al., 2015). The first human iPSC-based therapy has already begun, in September 2014, for age-related macular degeneration (Kamao et al., 2014).

WS human embryonic stem cells (hESCs) have also been generated by *WRN* knockout in hESCs. The analysis of *WRN*^{-/-} hESCs and derived MSCs (mesenchymal stem cells) compared to *WRN*^{+/+} cells again showed cellular phenotypes associated with accelerated aging (Zhang et al., 2015). Epigenomic and chromatin analyses of *WRN*^{-/-} MSCs revealed reduced levels of H3K9me3 as well as reduced expression of heterochromatin protein 1 α (HP1 α), suppressor of variegation 3-9 homolog 1 (SUV39H1), and lamina-associated polypeptide 2 β (LAP2 β). Interestingly, knockdown of SUV39H1 or HP1 α in wild-type MSCs led to accelerated cellular senescence without induction of DNA damage makers, raising a possibility that chromatin disorganization, not DNA damage, may be responsible for the pathogenesis of WS (Zhang et al., 2015). In fact, loss of heterochromatin and the resulting transcriptional “leakage” have now been implicated as a factor in cellular aging, providing support for this novel understanding of WS etiology (O’Sullivan and Karlseder, 2012).

7. International Registry of Werner Syndrome and atypical Werner syndrome

The International Registry of Werner Syndrome (Department of Pathology, University of Washington, Seattle, WA) (www.wernersyndrome.org) was established in 1988 to recruit WS patients from all over the world for the positional cloning of the *WRN* gene (Yu et al., 1996). Following the cloning of the *WRN* gene, our Registry has expanded its scope to search for causative mutations and mechanisms responsible for a broader range of progeroid syndromes. One prominent example is our work to better define patients with “atypical Werner syndrome (AWS)”. These patients were suspected to have WS on clinical grounds, but were found on subsequent analysis to lack *WRN* mutations. As of 2015, our

International Registry has enrolled 71 AWS cases, in addition to 149 WS patients with documented *WRN* mutations.

Our analyses of AWS patients have revealed several potential candidate genes. Among the first candidate genes examined in AWS was the *LMNA* gene, which encodes nuclear intermediate filaments, lamin A/C, and is known to be mutated in the Hutchinson-Gilford Progeria syndrome (HGPS) and other laminopathies (Eriksson et al., 2003). *LMNA* mutations identified in AWS are either dominant heterozygous amino acid substitutions, or are splicing mutations which result in the generation of amounts of progerin that are much smaller than those responsible for HGPS (Chen et al., 2003; Hisama et al., 2011). *LMNA* mutant cells show evidence of genomic instability such as accumulations of double strand breaks, redistribution of ATM/ATR, and accelerated telomere shortening (Benson et al., 2010; Liu et al., 2006; Saha et al., 2013b). Another gene mutated in a small subset of AWS is *POLD1*, which encodes the catalytic subunit of the replication DNA polymerase δ , a known *WRN*-interacting protein complex (Kamath-Loeb et al., 2012b; Lessel et al., 2015; Szekely et al., 2000). The primary role of *POLD1* is in lagging strand synthesis, and in translesion synthesis (TLS) of lagging strands as well as leading strand synthesis (Johnson et al., 2015). The *POLD1* mutations found in AWS are dominant heterozygous mutations, p.Ser605del and p.Arg507Cys, which perturb polymerase activity, unlike cancer-disposing germline mutations that abolish its exonuclease activity (Lessel et al., 2015; Palles et al., 2013). The p.Ser605del was previously described in patients with a multisystem disorder known as the MDPL syndrome (mandibular hypoplasia, deafness, progeroid features, lipodystrophy) (Weedon et al., 2013). Despite the obvious connection with genomic instability, malignancies leading to death are not reported among *LMNA* mutant and *POLD1* mutant AWS in our Registry.

Next generation sequencing of members of two AWS pedigrees have also revealed homozygous and compound heterozygous mutations in *SPRTN* (SprT-like N-terminal domain), a gene previously associated with Ruijs-Aalfs syndrome (RAS) (Lessel et al., 2014b; Ruijs et al., 2003). RAS is characterized by developmental retardation, skeletal abnormalities and a progeroid appearance. Of note, *SPRTN* is an adaptor protein that binds to a TLS polymerase η (encoded by *POLH*) that functions in post-replication repair of DNA leading strands (Lessel et al., 2014b). Both of the *SPRTN* mutant individuals we studied developed hepatomas. We have also identified homozygous *SAMHDI* (SAM domain- and HD domain-containing protein 1) mutation in a patient with progeroid feature and progressive ataxia (Lessel et al., 2014a). *SAMHDI* encodes a dNTP pool regulator with 3'→5' exonuclease activity, has been implicated in DNA damage response (Beloglazova et al., 2013), and when mutant has been linked to a subset of Aicardi-Goutieres syndrome (AGS). AGS is a genetically heterogeneous neurological disorder known for pronounced phenotypic variability. Our *SAMHDI* mutant AWS patient carried a heterozygous *WRN* mutation which might have synergistic effects with *SAMHDI* mutation (Lessel et al., 2014a). Searches for novel genetic alterations in additional AWS cases are currently in progress. The Registry now welcomes inquiries from clinicians who believe they have identified patients with either classical WS, or those with atypical features. We particularly welcome cases for which members of nuclear pedigrees are available for genetic analysis.

8. Conclusions

A combination of clinical and biological analyses of WS as a disease phenotype and the consequences of *WRN* mutations have led us to propose a simple model for the pathogenesis of WS (Fig 3)(Monnat, 2006). In this model, lack of WRN protein causes genomic instability, with the accumulation of somatic mutations, accelerated telomere attrition and cell dysfunction and cell loss in many cell lineages. These cellular consequences result in either atrophic or hyperplastic changes in many tissues or cell lineages, leading to progressive progeroid features accompanied by the emergence of tumors. Mutations of the *WRN* gene and various AWS genes appear to lead to overlapping yet distinct disease phenotypes, particularly as regards patterns of neoplasia. One can anticipate that further discoveries of novel segmental progeroid syndromes will also share features of genomic instability as a common pathogenetic mechanism underlying the accelerated appearances of a range of geriatric disorders, including a distinct disease- or syndrome-specific spectrum of neoplasia.

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Fig. 1. Werner syndrome patient with homozygous null WRN mutations. Although apparently normal at age 8, cataracts were removed at age 36 and severe ankle ulcerations were recorded at age 56. (Registry# SANAN1010) (Hisama et al., 2006)

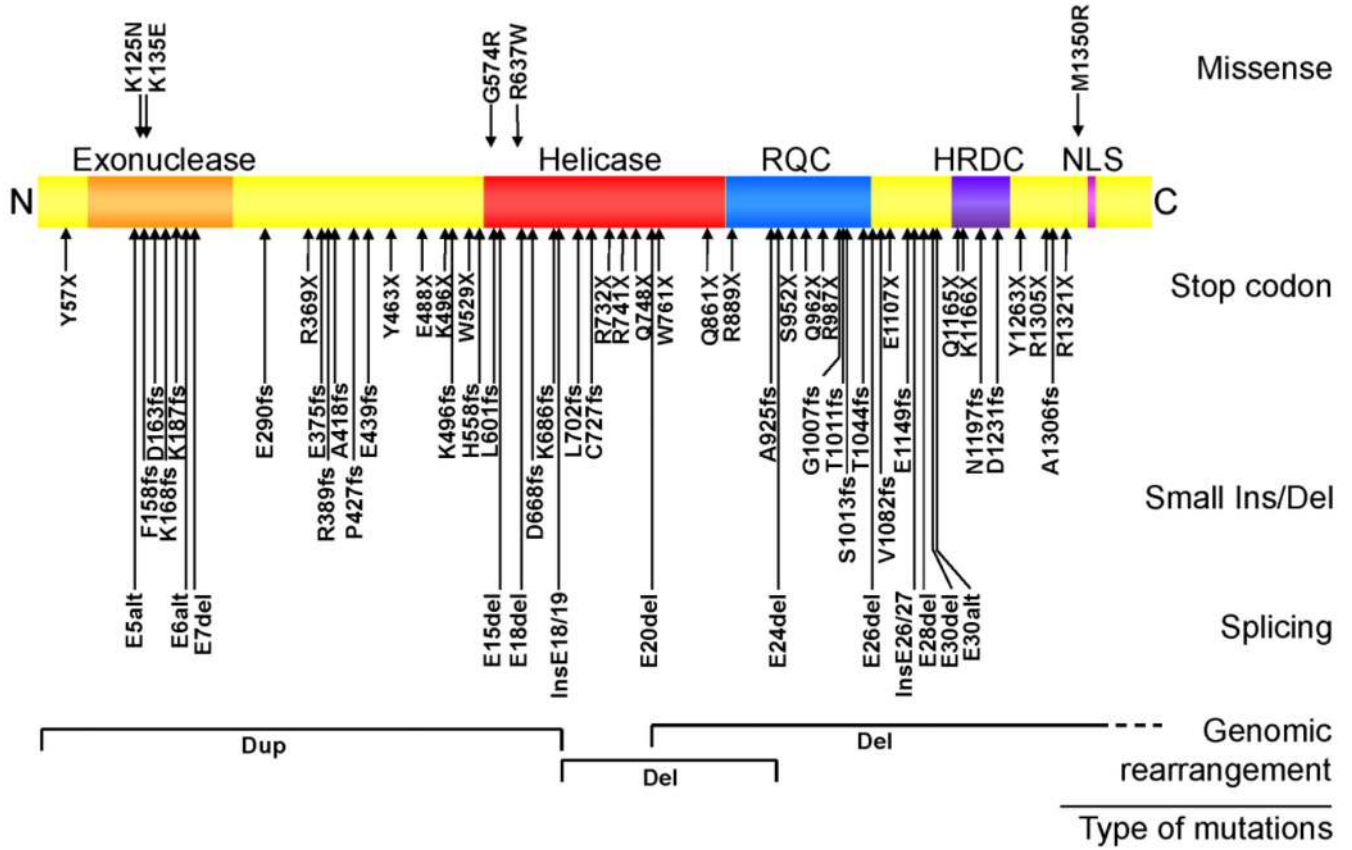


Fig. 2. *WRN* disease mutations in classical WS patients. The rectangular box shows the *WRN* protein. Known functional domains are: exonuclease region (Exo), helicase region, RecQ C-terminus consensus region (RQC), helicase and RnaseD consensus region (HRDC) and the nuclear localization signal (NLS). Disease mutations are grouped based on the types of mutations. Splicing mutations that result in identical exon skipping are combined and indicated by the number of unique mutations as in (2). Splicing mutations that create new splice sites are indicated as “nss”. Modified from (Friedrich et al., 2010).

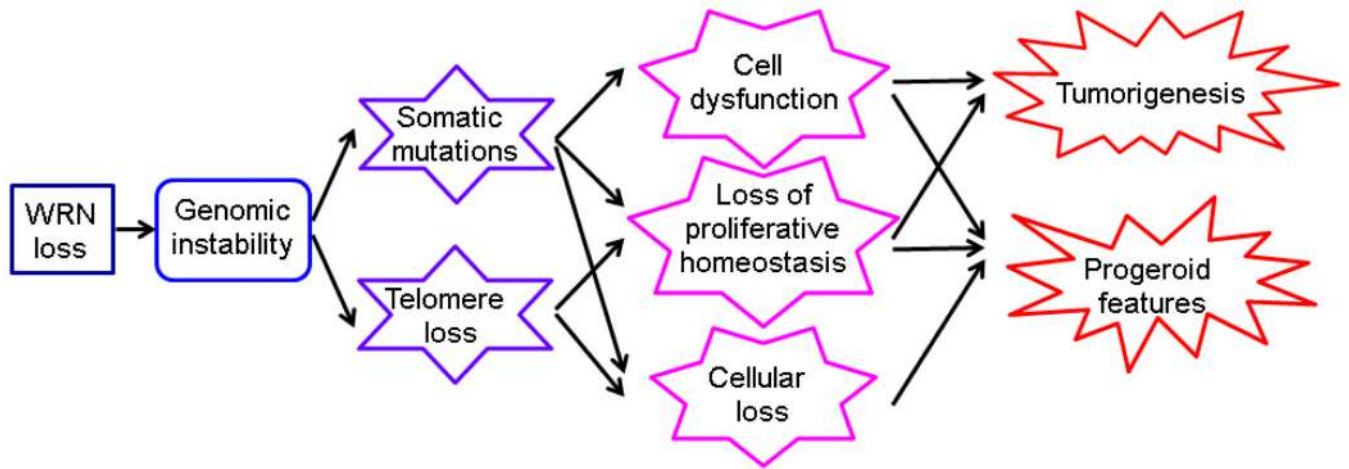


Fig. 3.
Model for the pathogenesis of WS. Modified from (Monnat, 2006). See text.

Diagnostic criteria of Werner syndrome

Table 1

International Registry of Werner Syndrome (Oshima et al., 2014)		Japanese Registry (Takemoto et al., 2013)	
I. Cardinal signs and symptoms (onset over 10 years old)			
1	Cataracts (bilateral)	1	Progeroid changes of hair (Gray hair, baldness, etc)
2	Characteristic dermatological pathology (tight skin, atrophic skin, pigmentary alterations, ulceration, hyperkeratosis, regional subcutaneous atrophy) and characteristic facies ('bird' facies)	2	Cataract (Bilateral)
3	Short stature	3	Changes of skin, Intractable skin ulcers (Atrophic skin, tight skin, clavus, callus)
4	Parental consanguinity (3d cousin or greater) or affected sibling.	4	Soft-tissue calcification (Achilles tendon, etc)
5	Premature greying and/or thinning of scalp hair.	5	Bird-like face
		6	Abnormal voice (High pitched, squeaky, hoarse voice)
II. Further signs and symptoms			
1	Diabetes mellitus.	1	Abnormal glucose and/or lipid metabolism (High pitched, squeaky, hoarse voice)
2	Hypogonadism (secondary sexual underdevelopment, diminished fertility, testicular or ovarian atrophy)	2	Deformation and abnormality of the bone (Osteoporosis, etc)
3	Osteoporosis.	3	Malignant tumors (Non-epithelial tumors, thyroid cancer, etc)
4	Osteosclerosis of distal phalanges	4	Parental consanguinity
5	Soft tissue calcification	5	Premature atherosclerosis (Angina pectoris, myocardial infarction)
6	Evidence of premature atherosclerosis (e.g. history of myocardial infarction)	6	Hypogonadism
7	Mesenchymal neoplasms, rare neoplasms or multiple neoplasms.	7	Short stature and low bodyweight
8	Voice changes (high pitched, squeaky or hoarse voice)		
9	Flat feet		
III. Genetic testing			
Definite: All the cardinal signs and two others.			
Probable: The first three cardinal signs and any two others			
Possible: Either cataracts or dermatological alterations and any four others.			
Exclusion: Onset of signs and symptoms before adolescence (except stature)			
Addendum: Mental retardation is seldom found in WS and cognitive function is often appropriate for the age			
Confirmed: All cardinal signs are present or a gene mutation in addition to at least three cardinal signs.			
Suspected: Two or more cardinal signs or 1-2 cardinal signs in addition to other signs.			