Commentary

WRN Protein and Werner Syndrome

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Abstract

Werner syndrome is an autosomal recessive disorder associated with premature aging and cancer predisposition. Cells from Werner syndrome patients show increased genomic instability and are hypersensitive to DNA damage agents. Werner syndrome is caused by mutations of the *WRN* gene. WRN protein is a member of RecQ DNA helicase family. It not only contains a conserved 3'-5' helicase domain as other members of the RecQ family but also contains a unique 3'-5' exonuclease domain. WRN recognizes specific DNA structures as substrates which are intermediates of DNA metabolism. WRN interacts with many other proteins, which function in telomere maintenance, DNA replication, and DNA repair through different pathways.

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Werner Syndrome and its Cellular Phenotype

Werner syndrome is a human autosomal recessive disorder that displays symptoms of premature aging, including early graying and thinning of hair, wrinkling and ulceration of skin, atherosclerosis, osteoporosis, and cataracts. In addition, Werner syndrome patients exhibit an increased incidence of diabetes mellitus type 2, hypertension, and are highly disposed to the emergence of benign and malignant neoplasms.[1] Werner syndrome caused by mutation of the *WRN* gene, a member of the RecQ DNA helicase family.[2] There are five known human RecQ helicases.[3] Mutations in two other family members, BLM and RecQ4, are responsible for the two other premature aging syndromes, Bloom's[4] and Rothmund Thomson,[5] respectively. The *WRN* gene encodes a 1,432-amino acid protein[2] that contains both 3'→5' helicase and 3'→5' exonuclease activities.[6-9]

The cells from patients with Werner syndrome show premature replicative senescence compared with cells derived from normal individuals.[10] The Werner syndrome cellular phenotype suggests correlations among faulty DNA metabolism, genomic instability, and senescence. Werner syndrome cells show hypersensitivity to selected DNA-damaging agents including 4-nitroquinoline-1-oxide (4NQO),[11] topoisomerase inhibitors,[12] and certain DNA cross-linking agents.[13] Comparing with normal cells, Werner syndrome cells also exhibit increased genomic instability including higher levels of DNA deletions, translocations, and chromosomal breaks.[14,15] These studies suggest that WRN plays an important role in DNA repair, replication, and recombination pathways.

Werner Syndrome Mouse Model

Two knockout mouse models for Werner syndrome have been established by using different strategies, and displayed a distinct phenotype. Lebel and Leder targeted the exons that encode motifs III and IV of the helicase domain of mouse WRN. The targeted gene expresses a fully translated WRN protein that lacks 121 amino acids of the helicase domain. Therefore, it is expected that it lacks helicase activity but retains other putative functions of the WRN protein. The homozygous mice were born viable, and during the first year, the mice appeared normal. By 24 months, 62% of the homozygous mice had developed some kind of hyperplasia or tumor.[12] $WRN^{\Delta hel/\Delta hel}$ $p53^{-/-}$ double-knockout mice were shown to develop tumors more rapidly than the $p53^{-/-}$ parental line. Indeed, by 4–5 months, 50% of the double-knockout population had developed tumors.[16] The other knockout mouse, generated by Lombard and co-workers,[17] targeted the last exon of the helicase domain, which results in the expression of a truncated protein, a situation similar to that seen in most human Werner syndrome cases. Moreover, as is seen with many of the WS mutations in humans, these authors could not detect expression of the truncated protein. This mouse allele, therefore, can be considered as WRN^{-}.[17] The $WRN^{-/-}$ homozygous mutant mice were reported to be perfectly healthy. Also, $WRN^{-/-}$ embryonic fibroblasts from these animals showed no signs of sensitivity to 4NQO or camptothecin, and there was no significant decrease in the replicative lifespan of these fibroblasts. However, $p53^{-/-}$ $WRN^{-/-}$ double-knockout mice died earlier, and the lack of p53 accelerated the mortality of $WRN^{-/-}$ or $WRN^{-/+}$ mice. The lack of an obvious phenotype in this $WRN^{-/-}$ mice might be explained by the fact that telomerase is expressed constitutively in rodent cells, effectively masking any effect of a loss of WRN function.[17] The finding of the mice with WRN and Terc double knock out exhibiting the typical Werner syndrome phenotype indicates that WRN regulating telomere function.[18,19]

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Biochemical Characteristics of the WRN Protein

The lack of phenotypes of WRN knockout mice underscores the need to study human WRN functions at biochemical and cellular level. WRN is a bipartite and bifunctional enzyme. In addition to the ATP-dependent 3'→5' helicase and DNA-dependent ATPase activities, it also possesses a functional 3'→5' exonuclease domain, which is similar to the exonuclease domain of *E. coli* DNA polymerase I.[20] The two enzymatic functions are separable from each other. The mutation WRND82A and E84A, disables exonuclease activity, but retains its helicase activity.[7] Similarly, the ATPase and helicase activities are diminished when the Lys577 residue in the ATPase domain is mutated, but the exonuclease activity is not affected by this substitution.[7,21] The helicase and exonuclease activities are physically separable, recombinant N-terminal fragments WRN1–368,[22] and the minimal exonuclease domain WRN70–240[23] display exonuclease activity without helicase activity. Similarly, N terminal deletion derivatives, which lack the exonuclease domain, retain helicase activity. Although the two enzymatic activities are not affect each other, full function and regulation of catalytic activities require the presence of other regions of the protein, which could modify the activity of the minimal enzymatic domains.[24]

The helicase activity of WRN shows very poor processivity as it preferentially unwound bubble substrates, forked structures and G-quadruplex DNA.[25] WRN was also capable of branch-migrating Holliday junctions over distances as great as 2.7 kb.[26] The 3'→5' exonuclease activity also displays low processivity. Exonucleolytic cleavage results in the production of 5'-deoxymonophosphates.[8] It prefers 5' overhangs, bubbles, loops and Holliday junctions structures, but has little or no activity on double-stranded duplexes with blunt ends, partial duplexes with 3' overhangs, or single-strand DNA.[24]

Functions of WRN Protein and its Subcellular Localization

The multi-functional nature of WRN, as well as the range of different symptoms seen in Werner syndrome patients, suggests that WRN may be a versatile enzyme with an involvement in diverse cellular processes. The finding that WRN interacts physically and functionally with other proteins required for DNA metabolism supports this notion. WRN was found to interact directly with numerous proteins, including proliferating cell nuclear antigen and Topoisomerase I,[27] Replication Protein A (RPA),[28] p53,[29,30] the Ku complex,[31,32] and DNA Pol δ.[33] In this regard, WRN may be a functional component of various cellular processes, including DNA replication, transcription, recombination, and repair.

WRN protein shows dynamic translocalization within the nucleus under different conditions. The WRN protein localizes to the nucleoli in a variety of cell types,[34] and this localization is modulated by DNA damage during cell cycle. Upon serum starvation or treatment with hydroxyurea (HU), aphidicolin, 4NQO, etoposide or camptothecin, WRN migrates from nucleoli to discrete nuclear foci.[26,30,35-37] The fact that DNA damage also induces the formation of RPA and RAD51 foci, and these co-localize with WRN almost fully (RPA), or partially (RAD51),[37] emphasizes the potential role of the WRN protein in DNA replication and DNA repair.

Conclusion Mark

The increasing number of WRN interacting proteins involving DNA replication, recombination and repair provide the strong evidence that WRN functions in multiple DNA metabolic processes. WRN participates in several DNA repair pathways including double strand break repair for both homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways and base excision repair (BER) pathway. While it may not be essential in any individual process, WRN appears to have significant functional roles in these pathways. WRN resolves DNA intermediates that arise normally as a result of DNA repair and replication processes. However, the precise role of WRN protein in these processes still remains unclear. How the mutations of WRN protein contribute to the plethora of premature aging symptoms in Werner Syndrome is yet to be ascertained. Further study to address these important questions should lead to new insights regarding aging process.

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