

# Werner syndrome as a hereditary risk factor for exocrine pancreatic cancer

## Potential role of WRN in pancreatic tumorigenesis and patient-tailored therapy

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**Key words:** Werner syndrome, WRN, RecQ helicase, exonuclease, pancreatic adenocarcinoma, tumor suppressor gene, adult-onset progeria, DNA repair syndrome

**Abbreviations:** WS, Werner syndrome; WRN, Werner syndrome RecQ helicase gene or protein; RecQ, *E. coli* recombination gene Q; TSG, tumor suppressor gene; HDAC, histone deacetylases; DNMT, DNA methyltransferases; LOH, loss-of-heterozygosity; PCR, polymerase chain reaction; ROS, reactive oxygen species; PanIN, pancreatic intra-epithelial neoplasia; CpG, cytosine in phosphodiester bond to guanine; siRNA, small interfering RNA; DSB, double-stranded DNA break

Advanced age is considered a risk factor for pancreatic cancer, but this relationship at the molecular and genetic level remains unclear. We present a clinical case series focusing on an association between pancreatic adenocarcinoma and Werner syndrome (WS) that is an autosomal recessive genetic disorder characterized by accelerated aging and cancer predisposition, and is caused by loss-of-function mutations in the WS RecQ helicase gene (*WRN*). Although pancreatic adenocarcinoma mostly occurs in a sporadic fashion, a minority of cases occurs in the context of susceptible individuals with hereditary syndromes. While WS has not been previously recognized as a risk factor for developing malignant tumors of the exocrine pancreas, the clinicopathologic features of three reported patients suggest a contributory role of WRN deficiency in pancreatic carcinogenesis. Molecular genetic analyses support the role of WRN as a tumor suppressor gene, although recent evidence reveals that WRN can alternatively promote oncogenicity depending on the molecular context. Based upon the clinico-pathologic features of these patients and the role of WRN in experimental models, we propose that its loss-of-function predisposes the development of pancreatic adenocarcinoma through epigenetic silencing or loss-of-heterozygosity of *WRN*. To test this hypothesis, we are investigating the mechanistic role of WRN in pancreatic cancer models including a pancreatic adenocarcinoma cell line generated from a human with WS. These studies are expected to provide new insight into the relationship between aging and pancreatic tumorigenesis, and facilitate development of novel strategies for patient-tailored interventions in this deadly malignancy.

### Introduction

Pancreatic cancer is a deadly malignancy with a rising incidence and a dismal prognosis.<sup>1</sup> Until preventive interventions and early detection of pancreatic cancer are feasible, the development of efficacious therapy is critical to improve the survival of afflicted patients. The vast majority of patients die of metastatic disease that is refractory to the conventionally used cytotoxic chemotherapeutic agents such as gemcitabine, 5-fluorouracil, and capecitabine. Rational design of molecular target-based and patient-tailored therapy can be facilitated by translational approaches that utilize the genetic alterations contributing to

pancreatic carcinogenesis.<sup>2,3</sup> Conceivably, genes that influence the aging process may contribute to development of pancreatic neoplasia and potentially serve as novel therapeutic targets, since the majority of patients with pancreatic cancer are diagnosed in the geriatric population.

Pancreatic carcinogenesis involves senescence of pancreatic epithelia associated with deregulation of developmental pathways, oncogenes and tumor suppressors.<sup>4</sup> Pancreatic intra-epithelial neoplasia (PanIN) initially exhibit genomic instability and senescence-associated telomere shortening.<sup>5,6</sup> Progression of PanIN to invasive adenocarcinoma is accompanied by aberrant activation of Hedgehog signaling, oncogenic *K-RAS* mutations

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**Table 1.** Clinical, pathologic and molecular features of WS patients with pancreatic tumors

Age/Gender	Risk factors	Clinical presentation	Histopathology	Mutation of <i>WRN</i>	References
43-y.o. male	none identified	Failure to thrive and obstructive jaundice	Moderately-differentiated ductal adenocarcinoma	009, 039	25
53-y.o. male	not reported	Obstructive jaundice	Carcinoma	Not reported	23
61-y.o. female	not reported	Obstructive jaundice	Papillary carcinoma	Not reported	24

Note: Age refers to the age when pancreatic cancer was diagnosed. Clinical presentation preceding diagnosis of pancreatic tumor. Mutation of *WRN* refers to WS causative mutation number as listed in the Werner International Registry's mutational database (<http://www.pathology.washington.edu/research/werner/database>). y.o., years-old.

and inactivation of the tumor suppressors p16<sup>INK4A</sup>, SMAD4 and p53.<sup>7-9</sup> Important insights into the molecular mechanisms that govern tumorigenesis have also been gained through genetic analyses of pancreatic cancer in the context of hereditary syndromes.

About 5–10% of pancreatic adenocarcinoma develops in patients with genetic syndromes that cause a hereditary predisposition to a variety of neoplasia.<sup>10,11</sup> Werner syndrome (WS) is a disorder of accelerated aging with onset during adolescence that is inherited in an autosomal recessive fashion. It is caused by loss-of-function mutations of the *WRN* gene that encodes a DNA helicase of the RecQ family possessing an exonuclease domain.<sup>12-14</sup> This syndrome manifests by the development of the signs and symptoms of medical problems typically associated with aging during the second decade of life accompanied by a predisposition to a variety of cancers.<sup>15-20</sup> Cultured fibroblasts from WS patients exhibit genomic instability causing a variety of chromosomal abnormalities such as sister chromatid exchanges and variegated mosaicism.<sup>21,22</sup> To date, WS has not been considered as a predisposing hereditary syndrome for exocrine pancreatic cancer. However, emerging cases describing pancreatic adenocarcinoma in patients with WS suggest a potential role of *WRN* in the initiation and progression of pancreatic neoplasia.<sup>23-25</sup>

In this clinical case series, we will compare the clinical, pathological and molecular features of the three reported cases of pancreatic adenocarcinoma in patients with WS. This will be followed by a review of the biochemical function, molecular genetics, and role of *WRN* as a tumor suppressor gene (TSG), and a discussion of the potential role of *WRN* silencing in the pathogenesis of pancreatic adenocarcinoma. The predicted use of *WRN* as a diagnostic and prognostic biomarker as well as a preventive and therapeutic target in pancreatic adenocarcinoma will be addressed.

### Clinical Case Series

The clinical, pathological and molecular features of pancreatic cancer in three patients with WS are summarized in Table 1. All of these patients are of Japanese ethnicity consistent with the epidemiology of WS showing that greater than 75% of cases occur in this ethnic group.<sup>26</sup> The high prevalence of WS in the Japanese population is attributed to multiple founder mutations of the *WRN* gene in mountainous regions of Japan where geography has historically limited gene flow, thereby allowing amplification of mutant alleles. These patients developed the classical

signs of WS, including premature graying of hair, diffuse muscular and dermal atrophy, voice changes and classic “bird-like facies” (Fig. 1). Their medical history also includes cardiovascular disease, type 2 diabetes mellitus, bilateral cataracts and hypertension.

The clinical presentations of pancreatic adenocarcinoma in these patients are typical, with obstructive jaundice caused by a tumor in the pancreatic head (Table 1). However, the average age of these WS patients (52.3 ± 9) is younger than the average age at which sporadic pancreatic adenocarcinoma is typically diagnosed during the seventh decade of life.<sup>27,28</sup> A man with classic clinical findings of WS developed exocrine pancreatic cancer at age 53.<sup>23</sup> Another woman with genetically confirmed WS developed pancreatic papillary ductal carcinoma at age 61, and she was successfully treated by pancreaticoduodenectomy.<sup>24</sup> We have recently reported a case of metastatic pancreatic adenocarcinoma in a 43-year-old man who was a compound heterozygote with two different loss-of-function mutations of *WRN* (Fig. 2).<sup>25</sup> In our reported case, the pancreatic tumor was further characterized by molecular analyses. Of particular note in the 43-year-old patient's tumor was the absence of the oncogenic *K-RAS* mutations, especially as mutations that constitutively activate *K-RAS* signaling have been identified in more than 95% of sporadic pancreatic adenocarcinoma.<sup>4</sup> Taken together, the clinicopathologic findings and molecular features of pancreatic adenocarcinoma in these patients with WS raise the possibility of a causal relationship between development of pancreatic adenocarcinoma and their underlying WS.

DNA repair disorders such as hereditary breast and ovarian cancer syndrome, ataxia telangiectasia and Lynch syndrome cause a hereditary predisposition to develop pancreatic cancer.<sup>10</sup> We propose that WS is a novel hereditary pancreatic cancer predisposition syndrome by causing a DNA repair defect and chromosomal instability in pancreatic epithelia (Table 2, Fig. 3). Although the possibility that pancreatic cancer and WS coincidentally occurred in these patients cannot be excluded, their early age of onset suggests that *WRN* mutations predisposed their cancer. Importantly, the absence of any identifiable risk factors in the 43-year-old whose pancreatic tumor harbored no oncogenic mutations in *K-RAS* argues for a role of *WRN* mutations in his cancer. Based on these observations, we hypothesize a causal relationship between loss-of-function of *WRN* and exocrine pancreatic cancer (Figs. 3 and 4). We are currently studying the functional significance of *WRN* in pancreatic neoplasia in order to test this hypothesis.

## Biochemical Functions and Molecular Genetics of WRN RecQ Helicase/ Exonuclease

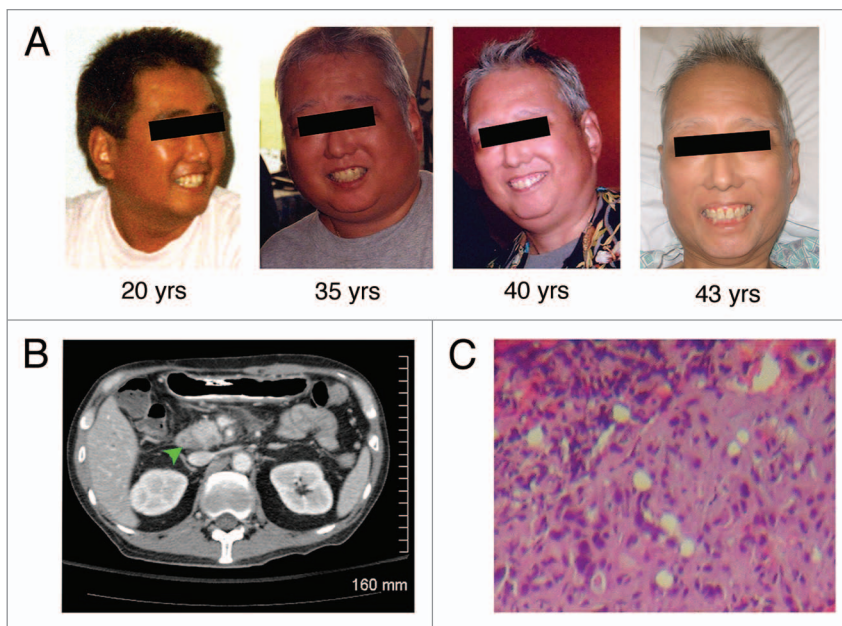
The *WRN* gene is located on chromosome 8p12, and it encodes a DNA helicase of the *E. coli* RecQ family. WRN is the only member of the RecQ helicase family that possesses an exonuclease domain.<sup>20</sup> In an ATP-hydrolysis-dependent fashion, WRN unwinds the DNA double helix in the 3' to 5' direction, thereby promoting DNA replication during the S-phase of the cell cycle.<sup>12-14</sup> During DNA synthesis, WRN binds to the large subunit of human replication protein A (RPA), resulting in the stimulation of its helicase activity.<sup>30</sup> WRN favors interaction with particular DNA substrates such as forked DNA, Holliday junctions, D-loops, triplexes, G4 tetraplexes and partial duplexes, suggesting highly specific roles in DNA replication and transcription.<sup>22</sup>

Subsequent to unwinding DNA substrates, WRN proofreads DNA with its exonuclease domain that similarly excises DNA base pairs in a 3' to 5' direction. This exonuclease function enables repair of DNA damage by facilitating resolution of hindered DNA replication forks during synthesis of complicated sequences, or by base pair excision and recombination when DNA polymerase encounters an obstruction.<sup>22,31</sup> Further supporting its dual roles in DNA replication and repair, the WRN protein is known to interact with DNA repair enzymes such as Pol $\beta$ , Ku, DNA-PK $\epsilon$ , PARP-1 and APE1.<sup>22</sup> In addition to directly proofreading DNA base pairs, WRN also plays roles in maintaining genomic integrity through a cell cycle "gatekeeper" function by inducing p53-dependent transcription of the cyclin-dependent kinase inhibitor *p21<sup>CDKN1A</sup>*.<sup>32</sup> By both directly regulating the fidelity of the DNA template through base pair excision and recombination, and by modulating cell cycle gatekeepers, WRN is considered to be a "genome caretaker" that maintains the structure and integrity of genomic and somatic DNA in proliferating cells.

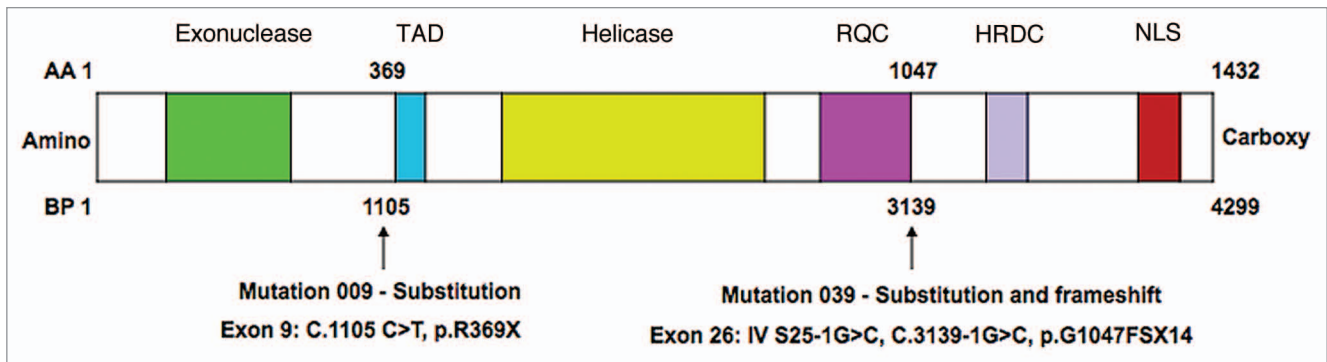
The structure of the WRN protein is large and complex, and ongoing efforts are underway to fully understand the functional significance of its structural domains. The *WRN* gene possesses 35 exons that encode a 1,432 amino acid protein with a molecular weight of 165 kDa. The carboxy (C)-terminus of the WRN protein contains its enzymatic functions, possessing both its helicase and exonuclease activity. Consistent with its enzymatic functions, a number of *WRN* mutations that truncate transcription of C-terminal exons are known to cause WS. The C-terminus has also been found to possess a transactivation domain located between its helicase and exonuclease regions, and its precise transcriptional targets are under investigation. While the C-terminus contains the enzymatic functions of WRN, its amino (N)-terminus contains its conserved RecQ helicase domain, nuclear localization signal, and p53 binding

component. Although the majority of causative WS mutations occur in the C-terminus, mutations that abolish the nuclear localization domain in the N-terminus have also been reported to cause WS.<sup>22</sup> While the biochemical functions of the WRN protein's structural domains have been characterized, further study is needed to establish whether their function is dependent upon specific cellular contexts. Of particular interest will be the precise transcriptional targets of WRN and whether it is a novel epigenetic modulator that allows transcriptional activation of TSGs by directly unwinding DNA substrates.

The biochemical roles of WRN as a helicase/exonuclease are supported by the clinical phenotype of patients with WS. In these individuals, proliferating tissues undergo premature senescence and neoplastic transformation, whereas non-proliferating tissues are spared from age-related pathologies. These clinical observations are supported by analysis of fibroblasts in cell culture from WS patients that exhibit decreased proliferative capacity, hindered progression through the S-phase of the cell cycle,<sup>33</sup> and enhanced sensitivity to the clastogenic effects of DNA damaging agents such as ionizing radiation and DNA cross-linking agents. By contrast, the non-proliferating tissues are spared from senescent changes as suggested by the failure to develop senile dementia or cerebral atrophy in WS. For example, histologic analysis of the neural tissue from our recently reported patient with WS and pancreatic adenocarcinoma at autopsy lacked any signs of neuronal atrophy or neurodegenerative changes.<sup>25</sup> These biochemical roles of WRN as a genome caretaker along with the tumor predisposition seen in WS support the rigorous



**Figure 1.** The 43 year-old patient with WS developed accelerated aging with onset during the second decade of life and subsequently pancreatic adenocarcinoma. (A) on physical examination, the patient appeared older than his stated age and developed gray hair and "bird-like" facies due to atrophy of subcutaneous fat. (B) computed-tomographic image of the abdomen. The arrowhead points to the hypodense lesion/tumor in the head of pancreas. (C) histologic examination of the primary tumor in the pancreas revealed moderately-differentiated ductal adenocarcinoma.



**Figure 2.** A schematic diagram of WRN with the loss-of-function mutations identified in the 43-year-old patient who developed pancreatic adenocarcinoma. The *WRN* gene encodes a helicase of the RecQ family containing an exonuclease domain, and also possesses a transactivation domain (TAD), RecQ conserved domain (RQC), helicase and RNase D C-terminus domain (HRDC) and nuclear localization sequence (NLS). This patient possessed mutations 009 and 039 as defined by the Werner International Registry. Mutation 009 caused a stop codon at exon 9 through a cytosine-to-thymine transition (C1105T), leading to the substitution of arginine by a stop codon (R369stop), and resulting in the truncation of the helicase and exonuclease domains. Mutation 039 caused a guanine-to-cytosine transversion (G3139-1C) at the junction of intron 25 and exon 26, leading to the substitution of phenylalanine for glycine (G1047F), and resulting in a frameshift and insertion of a stop codon 14 amino acids distal to the nucleotide change that abolished the NLS domain. AA, amino acid; BP, DNA base pair.

**Table 2.** Genetic syndromes with mutations in repair of DNA damage as predisposing risk factors of progeria and/or cancer

Disorders	Mutated protein	Progeria	Cancer	References
Werner syndrome	WRN RecQ helicase/ exonuclease	Premature aging	Soft tissue sarcoma; gastric, colorectal, thyroid and pancreatic carcinoma	22–25, 37, 46
Fanconi anemia	BRCA2	NA	Breast, leukemia, genitourinary, head and neck	47
Hereditary non-polyposis colon cancer	MSH and MLH	NA	Colon, gastric, genitourinary, ovarian, skin, hepatobiliary, pancreatic	48
Xeroderma Pigmentosum	*XPA, XPB, XPC, XPD, XPE, XPF and XPG	Premature aging	Skin, central nervous system	50, 51
Xeroderma Pigmentosum- DeSantis Cacchione's syndrome	XP-DSC	Premature aging	Internal organs	52
Cockayne's syndrome	CSA and CSB	Premature aging	NA	53
Bloom's syndrome	BLM	Premature cancer at early age	Breast, gastric, cervical, leukemia, skin, colorectal, hepatocellular	54
Rothmund-Thomson's syndrome	RECQ4	Premature aging	Osteosarcoma, skin	55
Hutchinson-Gilford Progeria	Lamin A	Premature aging	NA	56
Trichothiodystrophy	XPD, XPB, TTDA	Premature aging	NA	57

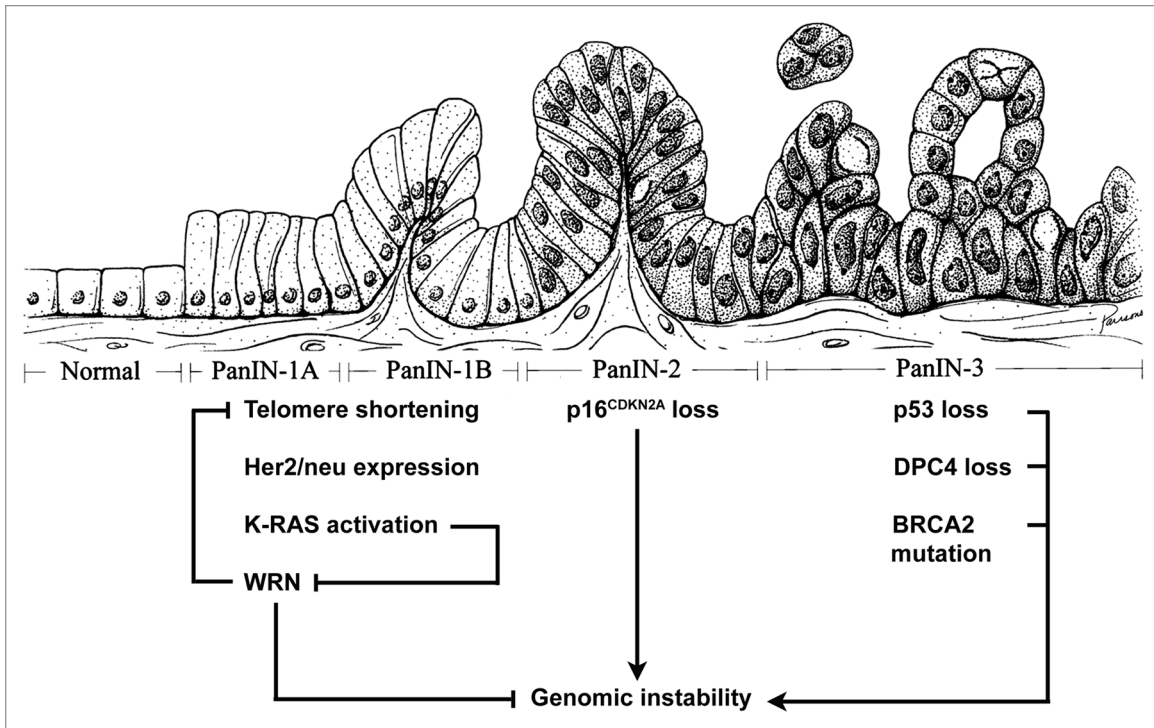
\*XP is mostly caused by mutations in XPA. NA, not associated. Note: In some of the above syndromes caused by defects in DNA damage repair, progeria is not associated with cancer predisposition. Tentatively, those afflicted individuals die at young age, such that the multi-step development of cancer that requires lengthy period of time is precluded. On the other hand, those syndromes predisposing cancer are not associated with progeria, suggesting complex genetic involvement in premature aging and cancer.

study of its role in epithelial malignancies such as pancreatic adenocarcinoma.

### Role of WRN in Tumorigenesis

Several lines of evidence suggest that WRN possesses a TSG function in the initiation, progression and maintenance of a variety of neoplasia. With respect to tumor initiation, the genomic instability in WS is consistent with the observation that patients develop a variety of malignancies including colorectal, thyroid,

pancreatic and skin cancers, and they have a very high incidence of mesenchymal malignancies such as soft tissue sarcomas.<sup>17</sup> Another role that WRN silencing may play in the initiation of neoplasia is by regulating telomere length, especially as shortened telomeres are characteristic of both pre-cancerous lesions and in WS.<sup>34,35</sup> The physical interaction of WRN with telomeres allows the maintenance of telomere length,<sup>36</sup> thereby preventing loss of telomere caps that normally protect chromosomes from genetic instability at their 3' ends. Furthermore, forced exogenous expression of telomerase abolishes the WS phenotype in



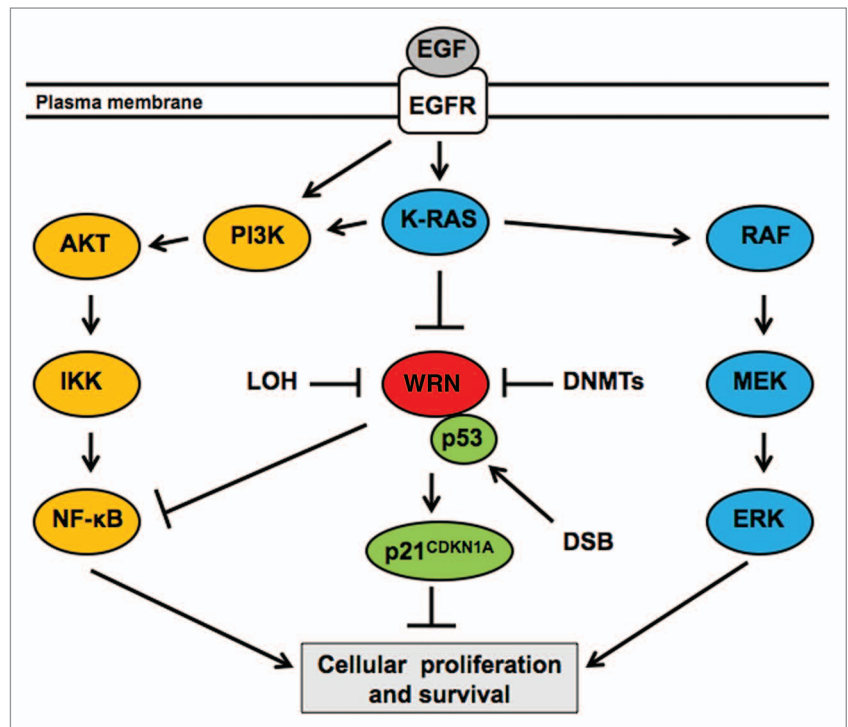
**Figure 3.** A working model for the role of WRN in the multiple steps of pancreatic carcinogenesis. We propose that suppression of *WRN* expression by K-RAS activation contributes to telomere shortening and genomic instability that is observed in PanINs. *WRN* may also be silenced in PanINs through loss-of-heterozygosity, methylation of its CpG-promoter region, and the loss-of-function mutations seen in WS. DPC4, deleted in pancreatic cancer 4; BRCA2, the DNA repair enzyme causing hereditary breast and ovarian cancer syndrome. The illustrated diagram is modified and reprinted from Am J Pathol 2000, 156: 1821-1825 with permission from the American Society for Investigative Pathology. →, activates; -, inhibits.

WRN-deficient mice, suggesting that the interaction of WRN with telomeres provides a key mechanism by which WRN prevents senescence and genomic instability. While the initiation of tumorigenesis is complex, evidence suggests that WRN acts in a pleiotropic fashion to suppress tumor initiation in humans and the myriad of cellular events that accompanies aging.

Recent evidence has suggested that WRN may act as a TSG in the progression and maintenance of established tumors in cancer cell lines in vivo.<sup>37</sup> This finding is supported by the observation of frequent loss-of-heterozygosity (LOH) at

the *WRN* loci located at chromosome 8p11.2-p12,<sup>29,38</sup> as well as by the epigenetic silencing of *WRN* in multiple tumor types.<sup>37</sup> In a panel of tumor specimens with epigenetically silenced *WRN* through methylation of CpG-islands of its promoter, forced

**Figure 4.** A working model for the role of WRN in the signaling pathways involved in pancreatic cancer cells. The epidermal growth factor receptor (EGFR) pathway is a key mediator of cellular proliferation and survival in exocrine pancreas and pancreatic adenocarcinoma. We propose that downregulation of WRN expression by oncogenic K-RAS signaling, DNA methyltransferases (DNMTs) or a loss-of-heterozygosity (LOH), contributes to cellular proliferation and survival in pancreatic neoplasia by preventing p53-mediated transcription of *p21<sup>CDKN1A</sup>* and promoting the NF-κB signaling cascade. EGFR, receptor dimer; AKT, a serine/threonine-specific protein kinase; DSB, double-stranded DNA break; ERK, extracellular-signal related kinases; IKK, IκB kinase; MEK, mitogen-activated protein kinase; RAF, a protein serine/threonine protein kinase. →, activates; -, inhibits.



ectopic overexpression of wild-type *WRN* reduced colony formation in vitro and growth of mouse tumor xenografts.<sup>37</sup> However, in cancers with intact *WRN* signaling, *WRN* has been found to promote cell cycle progression and oncogenicity.<sup>36</sup> These conflicting findings suggest that the role of *WRN* in neoplastic cells is context-dependent and that analysis of *WRN* expression may be necessary to develop patient-tailored therapy especially when using epigenetic modulators that affect *WRN* expression.

The ability of *WRN* to influence expression of genes involved in cell cycle progression such as inflammatory signaling cascades have recently been implicated as a potential mechanism by which it acts as a TSG.<sup>40</sup> While expression of *WRN* potentially blocks cell cycle progression by enhancing p53-dependent transcription of *p21<sup>CDKN1A</sup>*,<sup>32</sup> short interfering RNA (siRNA)-induced knockdown of *WRN* expression alternatively promotes cell cycle progression by inducing transcription of E2F family members.<sup>40</sup> Consistent with a TSG role in neoplasia, expression of *WRN* can be suppressed by activation of RAS and the RB/E2F pathway.<sup>41</sup> Furthermore, silencing of *WRN* with siRNA also induces the expression of other pro-oncogenic cascades such as MYC, IL-6 and NFκB signaling.<sup>40</sup>

The mechanism by which *WRN* suppresses oncogenesis may also involve cellular responses to free radical reactive oxygen species (ROS). Suppressing the formation of the free radical scavenger-reduced glutathione using 2-deoxy-glucose (2-DG) causes the up-regulation of *WRN* expression.<sup>42</sup> This event is consistent with the observation that fibroblasts derived from patients with WS are resistant to hydrogen peroxide induced cell cycle arrest.<sup>43</sup> Moreover, pre-clinical studies have shown the anti-tumor efficacy of 2-DG in combination with 5-fluorouracil, and ionizing radiation in pancreatic cancer.<sup>44</sup> Further analysis of *WRN* signaling in the context of free radical biology will be necessary to clearly delineate its role in ROS-induced senescence,<sup>45</sup> tumorigenesis and response to therapeutic small molecules and radiation.

Other genetic syndromes with mutations in enzymes involving DNA damage and repair have been implicated in premature aging and/or various human cancers (Table 2).<sup>46-57</sup> Collectively, the loss of their enzymatic functions impairs the normal activities of these enzymes, resulting in genomic instability and cancer predisposition.<sup>58</sup> Although population studies of heterozygous *WRN* loss-of-function mutations have not clearly indicated a risk of tumorigenesis, it has been observed that families of WS patients have a high rate of cancer.<sup>56</sup> Further study of individuals carrying heterozygous loss-of-function *WRN* mutations will determine whether they can benefit from early cancer screening, as with other DNA repair disorders.

### Potential Role of *WRN* in Pathogenesis and Treatment of Pancreatic Adenocarcinoma

Several lines of evidence suggest a contributory role of *WRN* silencing in malignant transformation of pancreatic ductal epithelia. First, chromosome 8p12 exhibits deletions and LOH in pancreatic neoplasia,<sup>59</sup> implicating the loss of functional *WRN* in pancreatic adenocarcinoma. Second, activation of RAS and the RB/E2F pathway suppresses the expression of *WRN*,<sup>41</sup> suggesting

*WRN* is a downstream effector of RAS signaling. Third, genetic instability and telomere shortening occurs in greater than 90% of PanINs,<sup>6</sup> and *WRN* may be involved in telomere maintenance and neoplastic transformation of pancreatic epithelia. Fourth, cigarette smoking is an environmental risk factor for developing tumors of the exocrine pancreas,<sup>60,61</sup> and provided that cigarette smoke downregulates *WRN* in respiratory epithelia,<sup>61</sup> a similar molecular phenomenon could occur in the exocrine pancreas. Taken together, these lines of evidence along with this clinical case series have provided rationale for our rigorous examination of the functional role of *WRN* in the pathogenesis of pancreatic neoplasia.

Currently, we are examining the expression level of *WRN* in human primary pancreatic cancer tissue, with the aim of determining whether *WRN* could serve as a clinical biomarker and a therapeutic target. Analysis of epigenetic silencing of *WRN* through mRNA expression and CpG-promoter methylation may yield prognostic information by identifying pancreatic tumors that are sensitive to DNA damaging agents.<sup>63</sup> Cells deficient in *WRN* signaling have been proposed to be sensitive to DNA alkylating agents and γ-irradiation,<sup>29,64</sup> and determination of relative *WRN* expression could provide valuable information to select patients whose tumors would respond to such therapies.<sup>63</sup> Given a potential tumor suppressor role in pancreatic adenocarcinoma, *WRN* also represents a “druggable” therapeutic target as its expression is regulated by the DNA methyltransferase (DNMT) inhibitor 5-azacytidine,<sup>37</sup> and potentially with histone deacetylase (HDAC) inhibitors.<sup>65</sup> Our ongoing studies will clarify the role of *WRN* as a biomarker that may be useful in the development patient-tailored interventions for pancreatic adenocarcinoma.

In summary, numerous lines of evidence strongly suggest a contributory role of *WRN* in the initiation, progression and maintenance of pancreatic adenocarcinoma. Using pancreatic cancer models, we aim to delineate the molecular mechanisms by which *WRN* mediates its role. A pancreatic cancer cell line established from the hepatic metastasis of the 43-year-old patient with WS (Fig. 1, Table 1) will serve as a novel translational tool and provide an experimental platform to interrogate the milieu of inter-related mechanisms involving cancer and aging.<sup>25</sup>

### Conclusion and Prospective

There are a few known risk factors associated with development of pancreatic adenocarcinoma, including aging, cigarette smoking and certain hereditary syndromes.<sup>66-84</sup> As shown in the clinical case series in this report, pancreatic adenocarcinoma in patients with WS is rare, but increasingly being recognized (Table 1). We propose that WS is a heritable disease that predisposes affected individuals to develop pancreatic adenocarcinoma (Table 3). Moreover, we hypothesize that epigenetic silencing of *WRN* or its loss-of-function causes genomic instability that leads to neoplastic transformation in pancreatic ductal epithelia (Fig. 3), as well as the promotion of cellular proliferation in pancreatic neoplasia (Fig. 4). Analyzing the role of *WRN* silencing in PanINs is expected to help determine whether *WRN* is a feasible

**Table 3.** Genetic syndromes with inherited predisposition to pancreatic cancer

Familial syndrome	Gene product (locus)	References
Werner syndrome	WRN RecQ helicase/exonuclease 8p11.2-p12	23–25
Peutz-Jeghers syndrome	STK11/LKB1 (19p13.3)	10, 66
Familial pancreatitis	PRSS/TRY1 (7q35)	67, 68
Familial atypical multiple mole melanoma	CDKN2A/p16/MTS1 (9p21)	69
Li-Fraumeni syndrome	p53 (17p13)	70, 71
Hereditary non-polyposis colorectal cancer (Lynch syndrome)	HMSH2 (2p22-21), hMLH1 (3p21.3)	72
Ataxia-telangiectasia	ATM (11q22-23)	73, 74
Hereditary breast-ovarian cancer syndrome	BRCA2 (13q12)	75–78
Cystic fibrosis carrier	CFTR1 (7q31)	79, 80
Familial adenomatous polyposis	APC (5q21)	81, 82
Family X of pancreatic insufficiency and diabetes mellitus	not identified (4q32-34)	83, 84

Multiple DNA repair disorders cause a hereditary predisposition to the development of exocrine pancreatic cancer including Werner syndrome, Lynch syndrome, ataxia-telangiectasia and hereditary breast-ovarian cancer syndrome.

biomarker and/or target for intervention prior to their evolution to invasive adenocarcinoma for the goal of early detection and treatment.

Pancreatic cancer remains one of the greatest oncologic challenges and novel therapeutic targets and an understanding of its molecular basis is desperately needed to advance care in this deadly malignancy. Currently, our research is actively focusing on the role of WRN in the pathogenesis of pancreatic neoplasia and how these findings can be translated into screening and therapeutic approaches for pancreatic adenocarcinoma. Results of these studies will have important clinical implications. First, relatively young patients with WS and early stage pancreatic adenocarcinoma are eligible for potentially curative surgical intervention. Second, if WRN acts as a TSG in pancreatic adenocarcinoma, it is a feasible pharmacologic target for epigenetic therapy with DNMT and HDAC inhibitors, 2-DG or other small molecules. Clarification of its precise molecular roles in pancreatic

adenocarcinoma, as well as analysis of *WRN* expression in patient tumors, will form the basis to guide such approaches.

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