

Chapter 2

Clinical and Basic Biology of Werner Syndrome, the Model Disease of Human Aging



Yoshiro Maezawa, Masaya Koshizaka, Hisaya Kato, and Koutaro Yokote

Abstract Werner syndrome is an autosomal recessive genetic disorder first described in 1904 by Otto Werner, a German ophthalmologist. It is considered the representative progeroid syndrome because various signs of aging, such as gray hair, cataracts, diabetes, and skin ulcers, appear after puberty. The onset of the disease begins in the 20s or 30s, leading to diabetes and atherosclerosis, and death in mid-50s due to myocardial infarction or malignant tumors. The number of patients in Japan is estimated to be between 700 and 2000, and 60% of the world's reports are from Japan, suggesting that this accelerated aging disease is more common in Japan. The cause of Werner syndrome was identified in 1996 as a mutation in the WRN gene, a RECQ helicase located in chromosome 8. Since then, various studies have shown that the syndrome is associated with decreased DNA damage repair and genomic instability, shortened telomeres, chronic inflammation due to cellular senescence- and senescence-associated secretory phenotype (SASP), decreased mitochondrial function and accumulation of oxidative stress, stem cell senescence, and epigenetic changes. While most premature aging syndromes occur in childhood and involve a growth and developmental disorder, only Werner syndrome occurs after normal growth and puberty, suggesting that this syndrome is a model of human aging. The elucidation of the pathogenesis and molecular mechanisms of this disease and the development of a treatment strategy are expected to lead to the elucidation of the pathogenesis of general human aging and of aging-related diseases such as diabetes and malignant tumors.

Keywords Werner syndrome · Adult progeria · Segmental aging · Skin ulcer · Genome instability · Cellular senescence

Y. Maezawa · M. Koshizaka · H. Kato · K. Yokote (✉)
Department of Endocrinology, Hematology and Gerontology, Chiba University Graduate School of Medicine, Chiba, Japan
e-mail: yoshiromaezawa@chiba-u.jp; kyokote@faculty.chiba-u.jp

2.1 Clinical Features and Pathogenesis of Werner Syndrome

2.1.1 Introduction

Werner syndrome (WS) is a rare autosomal recessive disorder, resulting from genetic instability (Gray et al. 1997), and is sometimes referred to as “accelerated aging syndrome” because beginning in puberty, it presents with symptoms such as cataracts and loss and graying of hair, which makes individuals look relatively old for their age. It is estimated that there are approximately 700–2000 patients in Japan (Matsumoto et al. 1997a; Satoh et al. 1999; Yokote et al. 2017; Yamaga et al. 2017). Of the world’s reported cases to date, 60–80% are from Japan. The life expectancy of people with WS is shorter than that of healthy individuals because of its frequent comorbidities, such as diabetes, arteriosclerosis, and malignant tumors (Onishi et al. 2012). The characteristics of patients with WS include a bird-like face and a high-pitched voice. Moreover, patients with WS have a high incidence of intractable ulcers in the legs and feet, resulting in pain, infections, and even amputation of the lower limbs, which can have a major negative impact on the person’s quality of life and vital prognosis.

In Japan, a guide for the diagnosis of WS was created in 1984. In 1996, a mutation in the DNA helicase Werner syndrome protein (WRN) encoded by the RecQ genes on chromosome 8 was identified as the cause of the disease. Although no fundamental treatment has been developed for the disease, many studies are beginning to suggest that patient life expectancy could potentially be extended with appropriate treatment interventions. The diagnostic criteria for WS were revised, and the first guideline for treatment was developed in 2012. WS was designated as an intractable disease in Japan in 2015. A nationwide survey in 2017 identified 116 patients diagnosed with WS (Koshizaka et al. 2020). The Werner Syndrome Registry (case registration system) was established in 2017 (Koshizaka et al. 2020). Treatments for WS were standardized, and the first guideline for its diagnosis and treatment in English were developed in 2020 (Takemoto and Yokote 2021).

2.1.2 Diagnostic Criteria

Based on a nationwide epidemiological study carried out from 2009 to 2011, the diagnostic criteria for WS were revised (Takemoto et al. 2013). From the results of the aforementioned study, progeroid faces, bilateral cataracts, skin atrophy, clavus and callus, flat feet, bird-like face, and abnormal voice were found in >85% of the genetically confirmed cases of WS. Calcification in the Achilles tendon is a frequent symptom of WS, and 80% of people with WS presented with this symptom. Furthermore, the segmental and flame-like patterns of calcification were highly specific to WS (Fig. 2.1) (Takemoto et al. 2013).



Fig. 2.1 Calcification in the Achilles tendon seen in Werner syndrome patients. (a) Segmental and (b) flame-like calcifications in the Achilles tendon

Table 2.1 Revised diagnostic criteria for Werner syndrome

I. Cardinal signs and symptoms (onset over 10 until 40 years of age)	
1. Progeroid changes of hair	Gray hair, baldness, etc.
2. Cataract	Bilateral
3. Changes of skin and intractable skin ulcers	Atrophic skin, tight skin, clavus, callus
4. Soft tissue calcification	Achilles tendon, etc.
5. Bird-like face	
6. Abnormal voice	High-pitched, squeaky, hoarse voice
II. Other signs and symptoms	
1. Abnormal glucose and/or lipid metabolism	
2. Deformation and abnormality of the bone	Osteoporosis, etc.
3. Malignant tumors	Non-epithelial tumors, thyroid cancer, etc.
4. Parental consanguinity	
5. Premature atherosclerosis	Angina pectoris, myocardial infarction
6. Hypogonadism	
7. Short stature and low bodyweight	
III. Genetic testing	

On the basis of these results and the extensive clinical experience with Japanese cases of WS accumulated over many years and with unanimous agreement from the Japanese Werner Syndrome Working Committee, new diagnostic criteria (Table 2.1) (Takemoto et al. 2013) were formulated.

Addendum: Mental retardation is seldomly found in WS, and cognitive function is often appropriate for the patient's age. Confirmed: All cardinal signs are present or a gene mutation in addition to at least three cardinal signs. Suspected: One or two of the cardinal signs (hair changes and cataract) plus at least two more from all the signs.

Because the incidence of bird-like face is high (>93%), it is used as a cardinal sign. The characteristic WS face (Fig. 2.2) (Takemoto et al. 2013) includes a pinched nasal bridge and diminished subcutaneous tissue. The patient's voice might be high-pitched, squeaky, and/or hoarse; an example of the voice in a WS case is available on the committee website, with the patient's consent (<http://www.m.chiba-u.jp/class/clin-cellbiol/werner/index.html>).

Regarding hypogonadism, it has been reported that WS is associated with secondary sexual underdevelopment, decreased fertility, and testicular or ovarian atrophy. It is significant that fewer than 35% of patients in the present study had children [Takemoto. et al. 2013].

On the basis of extensive clinical experience and published medical literature, the cognitive function of WS patients is usually appropriate for their age.

Genetic analysis is now included in the diagnostic criteria. *WRN* is the only gene associated with classic WS, and mutations can be identified by DNA sequence analysis (Yu et al. 1996; Oshima et al. 1996; Friedrich et al. 2010) or Western blotting (Goto et al. 1999; Shimizu et al. 2002; Takada-Watanabe et al. 2012). However, these molecular genetic tests are not feasible at all institutions, and genetic testing is not essential to confirm the diagnosis of WS.

For differential diagnosis, atypical WS (Chen et al. 2003a); mandibuloacral dysplasia (Cavallazzi et al. 1960; Novelli et al. 2002); Hutchinson-Gilford progeria syndrome (Eriksson et al. 2003), caused by *LMNA* mutation; Rothmund-Thomson syndrome, caused by *RECQ4* mutation (Kitao et al. 1999); and Bloom syndrome, caused by *RECQ2* mutation (Ellis et al. 1995), are listed. These syndromes typically present with progeroid symptoms earlier than WS and are extremely rare in Japan.

2.1.3 *Werner Syndrome Registry*

The Werner Syndrome Registry in Japan was established in 2017. Forty-three patients were enrolled and registered in the registry. Table 2.2 shows the major signs of WS observed in registered patients. Almost all patients exhibited some of the major signs, such as graying hair, hair loss, cataracts, skin atrophy changes, and soft tissue calcification. Approximately 90% of patients had a characteristic bird-like face and high-pitched voice. Over half of the patients had diabetes, impaired glucose tolerance (67.5%), dyslipidemia (65.0%), and fatty liver (52.5%). The major signs and clinical symptom at the time registered to the registry are shown in Table 2.2. A small percentage of patients in the registry had a history of atherosclerosis (0%), angina pectoris or myocardial infarction (2.5%), or arteriosclerosis obliterans (15.0%). Angina pectoris or myocardial infarction significantly decreased

Fig. 2.2 A bird-like face is typically seen in Werner syndrome patients. The nasal bridge of a 57-year-old woman appears pinched, and subcutaneous tissue is diminished. (a) front of face, (b) side of face



(2.5% vs. 14.8%, $P = 0.049$) compared with the previous survey conducted in 2009 (Takemoto et al. 2013). This result may have arisen because of improved comprehensive control of diabetes, dyslipidemia, and hypertension with new treatments such as HMG-CoA reductase inhibitors (statins) or peroxisome proliferator-activated receptor gamma agonists (Yokote and Saito 2008; Yokote et al. 2004a;

Table 2.2 The frequency of major signs and clinical symptoms of and medications administered to patients with Werner syndrome at the time registered to the registry

	%	<i>n</i>	<i>N</i>
Major signs			
Graying of hair, hair loss	97.5	39	40
Cataracts	100	40	40
Skin changes	97.5	39	40
Intractable skin ulcers	67.5	27	40
Soft tissue calcification	87.5	35	40
Bird-like face	90	36	40
High-pitched voice	87.5	35	40
Clinical symptoms			
Diabetes, IGT	67.5	27	40
Dyslipidemia	65	26	40
Hypertension	42.5	17	40
Fatty liver	52.5	21	40
Cerebral bleeding	0	0	40
Cerebral infarction	0	0	40
AP or MI	2.5	1	40
ASO	15	6	40
Amputation	15	6	40
Malignant tumor	20	8	40
Medications			
Diabetes, IGT			
DPP-4 inhibitor	37.0	10	27
Biguanide	33.3	9	27
Thiazolidine	48.1	13	27
Alpha GI	7.4	2	27
Sulfonylurea	11.1	3	27
SGLT2 inhibitor	3.7	1	27
Glinide	0	0	27
GLP-1 analog	3.7	1	27
Insulin	14.8	4	27
Dyslipidemia			
Statin	65.4	17	26
Fibrate	3.8	1	26
Ezetimibe	0	0	26
EPA	11.5	3	26
Resin	0.0	0	26
Nicotinic acid	19.2	5	26
Probucol	0	0	26
Hypertension, among others			
Ca blocker	47.1	8	17
ARB	35.3	6	17
ACE inhibitor	0.0	0	17

(continued)

Table 2.2 (continued)

	%	<i>n</i>	<i>N</i>
Alpha1 blocker	0.0	0	17
Beta blocker	11.8	2	17
Diuretics	0.0	0	17
Antiplatelet	5.0	2	40
Anticoagulant	12.5	5	40

The percentages of medications administered to treat abnormal glucose metabolism, dyslipidemia, and hypertension are shown for the total number of patients with each disorder. *N* number of patients, *n* number of patients with symptoms or treatment drugs, *IGT* impaired glucose tolerance, *AP* angina pectoris, *MI* myocardial infarction, *ASO* arteriosclerosis obliterans, *DPP-4* dipeptidyl peptidase-4, *alpha GI* alpha glucosidase inhibitor, *SGLT2* sodium-glucose cotransporter-2, *GLP-1* glucagon-like peptide-1, *EPA* eicosapentaenoic acid, *Ca* calcium, *ARB* angiotensin II receptor blocker, *ACE* angiotensin-converting enzyme inhibitor

Yasuda et al. 2010). These treatments appeared to have ameliorated the arteriosclerotic outcomes in the patients with WS.

Two thirds of patients with WS had an intractable ulcer. The nationwide survey in 2017 revealed that a higher percentage of patients with WS were reported by plastic surgeons (7.7%) and dermatology specialties (7.9%) than by other departments. It is speculated that patients with WS visited the hospital for the treatment of ulcers or to receive more specialized treatments in dermatology and plastic surgery departments. Limb amputation was observed in 15.0% of patients.

It is noteworthy that approximately 30% of the patients' parents had a consanguineous marriage.

2.1.3.1 General Information

Age at Onset and Diagnosis

The patients' average age at registration was 50.1 ± 7.5 years. The average age at WS onset was 26.1 ± 9.5 years; however, the age at diagnosis was 42.5 ± 8.6 years (Table 2.3). There was a delay between the age at onset and the age at diagnosis. This tendency was also reported in 2006 in the international WS Registry (Huang et al. 2006). In the Japanese registry, the age at onset of cataracts was 31 years, and the age at diagnosis or referral was 43 years. These results suggest that it is necessary to consider measures for early diagnosis and early intervention. WS onset is usually marked by bilateral cataracts or gray hair and hair loss, which are the first symptoms (Takemoto et al. 2013). Patients undergo an ophthalmologic operation for cataracts around their third decade of life. However, around their fourth decade, patients tend to have intractable ulcers and visit dermatologists or plastic surgeons. The national survey showed that many patients were reported by dermatologists or plastic surgeons, rather than ophthalmologists. Improvements in earlier diagnostic methods are needed. As one possible solution, calcification in the Achilles tendon is easily

Table 2.3 Patient background, physical findings, body composition, and physical function at the time registered to the registry

	Total				Men				Women			
	Mean	±	SD	<i>n</i>	Mean	±	SD	<i>n</i>	Mean	±	SD	<i>n</i>
<i>Patients' backgrounds</i>												
Age (years)	50.1	±	7.5	40	49.4	±	7.6	22	50.9	±	7.5	18
Onset age (years)	26.1	±	9.5	30	28.2	±	8.5	16	23.7	±	10.2	14
Diagnosed age (years)	42.5	±	8.6	39	42.0	±	6.4	21	43.2	±	10.8	18
<i>Physical findings</i>												
Height (cm)	154.0	±	10.7	40	159.7	±	8.6	22	147.2	±	9.0	18
Body weight (kg)	44.1	±	9.5	40	49.0	±	9.3	22	38.1	±	5.4	18
BMI (kg/m ²)	18.5	±	3.1	40	19.2	±	3.5	22	17.6	±	2.5	18
Waist circumference (cm)	77.3	±	12.0	24	80.4	±	12.2	14	73.0	±	10.8	10
Visceral fat area (cm ²)	102.3	±	61.4	10	112.4	±	81.5	4	95.6	±	51.7	6
SMI (kg/m ²)	4.3	±	0.8	9	4.5	±	0.9	5	4.1	±	0.6	4
<i>Physical function</i>												
Mean grip strength (right) (kg)	17.1	±	8.7	23	20.8	±	8.6	13	12.3	±	6.3	10
Mean grip strength (left) (kg)	16.0	±	7.6	23	19.5	±	7.3	13	11.4	±	5.3	10
Mean walking speed (m/s)	0.8	±	0.6	13	0.9	±	0.6	6	0.8	±	0.6	7

BMI body mass index, *SMI* skeletal muscle mass index, *SD* standard deviation

examined on routine X-ray and is highly indicative of WS, and proactive X-ray images are recommended when WS is suspected. However, Achilles tendon calcification might not be present in young patients, and minute calcifications might require diagnosis by a specialist, such as an orthopedic surgeon (Takemoto et al. 2013).

Physique

In the registry, the patients' average height, body weight, and body mass index (BMI) (159.7 cm, 49.0 kg, and BMI 19.2 kg/m² in men and 147.2 cm, 38.1 kg, and BMI 17.6 kg/m² in women, respectively) (Table 2.3) were lower than those of the average Japanese individual in the fifth decade of life (169.2 cm, 68.1 kg, and BMI 23.5 kg/m² in men and 156.6 cm, 55.0 kg, and BMI 22.2 kg/m² in women, respectively). Patients with WS present with central obesity; the average abdominal circumference was 80.4 ± 12.2 cm in men and 73.0 ± 10.8 cm in women. The average abdominal circumference was large, although the respective BMIs were low (Table 2.3). In other words, patients with WS have lipodystrophy.

Life Expectancy

Previously, it was reported that patients with WS die around the fifth decade of life (Goto and Matsuura 2008). However, the life expectancy for Japanese patients with WS has steadily increased compared with that two decades (Goto 2000) or even one decade ago (Koshizaka et al. 2020; Takemoto et al. 2013).

Laboratory Test

Regarding laboratory tests, on average according to the registry, patients had more than twice the levels of gamma-glutamyl transpeptidase than the upper normal limit [Koshizaka et al. 2020]. The levels of glycated hemoglobin, plasma glucose, and low-density lipoprotein cholesterol (LDL-C) levels were well controlled.

2.1.3.2 Symptoms

Patients with WS display various signs of aging that appear from the second decade of life. Gray hair and hair loss appear at 20 years of age, and bilateral cataracts appear at 30 years of age. They exhibit lipodystrophy and sarcopenic obesity and often have the following symptoms: sarcopenia, diabetes, dyslipidemia, fatty liver, osteoporosis, foot skin ulcers, ulcer infection, and calcification in tendons. They also often have myocardial infarctions, and malignant tumors appear at 40 years of age [Goto et al. 2000; Takemoto et al. 2013]. However, these symptoms in patients with WS are different from those of simple aging in that the incidence of some common aspects of aging, such as dementia, are rare.

Sarcopenia

Sarcopenia is defined as a condition that combines decreased skeletal muscle mass with weakness or decreased physical function. Patients with WS are characterized by visceral fat accumulation and thin limbs. A decrease in skeletal muscle mass frequently occurs in patients with WS before 40 years of age.

According to the registry, the average of the total limb skeletal mass index, identified using dual-energy X-ray absorptiometry, was 4.5 ± 0.9 kg/m² for men and 4.1 ± 0.6 kg/m² for women. Grip strengths were (right) 20.8 ± 8.6 kg and (left) 19.5 ± 7.3 kg for men and (right) 12.3 ± 6.3 kg and (left) 11.4 ± 5.3 kg for women. Walking speed was 0.8 ± 0.6 m/s on average (Table 2.3). The average grip strength, walking speed, and skeletal muscle mass index met the diagnostic criteria for sarcopenia. Therefore, most patients aged over 40 years had sarcopenia.

Although the mechanism is still unclear, various potential factors including aged skeletal muscle, metabolic abnormality, and inflammation, or a decreased amount of activity due to low physical function, have been considered. Resistance exercise may

prevent the appearance of sarcopenia, and early intervention is required in patients with WS (Kuzuya et al. 2021). Sarcopenia appears early in most patients with WS; therefore, sarcopenia may be prevented by early intervention with strength training and treatments including amino acids such as leucine, whey protein, calcium, and vitamin D (Cruz-Jentoft et al. 2020; Martinez-Arnau et al. 2019).

Diabetes

More than 60% of patients with WS have diabetes (Koshizaka et al. 2020). Diabetes associated with WS is classified as “accompanied with other diseases and conditions and the one occurring mainly in association with other genetic syndromes.” The patients used to require high amount of insulin and were still on poor glucose control. Diabetes due to WS is marked by accumulated visceral fat and high insulin resistance, despite low BMI (Takemoto et al. 2021). Therefore, thiazolidine derivatives and metformin (Yasuda et al. 2010) are effective for glycemic control. There have been many reports on the effectiveness of a thiazolidine derivative, an agonist, of peroxisome proliferator-activated receptor γ , an insulin sensitizer (Yokote et al. 2004a, b; Honjo et al. 2008; Takino et al. 1994; Izumino et al. 1997; Imano et al. 1997; Hattori et al. 2004; Yamamoto et al. 2007). Dipeptidyl peptidase-4 (DPP-4) inhibitors (Watanabe et al. 2013; Kitamoto et al. 2012) and glucagon-like peptide-1 receptor agonists (Ide et al. 2016) are also beneficial for patients with WS. More than 30% of patients with diabetes were treated with a DPP-4 inhibitor, biguanide, or thiazolidine in the registry (Koshizaka et al. 2020).

Dyslipidemia

Arteriosclerosis is one of the two leading causes of death in patients with WS, along with malignancy (Goto 1997). Among the various forms of arteriosclerosis that patients with WS develop, coronary artery diseases and peripheral arterial disease have a high incidence, and the latter plays a role in causing skin ulcers in patients with WS to become refractory (Takemoto and Yokote 2012). Disorders of carbohydrate metabolism and lipid metabolism associated with WS act as promoting factors for atherosclerosis (Tsukamoto et al. 2021). Previous guidelines showed that hypercholesterolemia occurred in 53% of patients with WS (Takemoto and Yokote 2012). The incidence of dyslipidemia in patients with WS is high, at 85%. The most common type of dyslipidemia is hypertriglyceridemia, occurring in 76% of patients, followed by hyper-LDL cholesterol/non-high-density lipoprotein (HDL) cholesterol in 68% of patients and hypo-HDL cholesterol in 32% of patients. Patients with WS and dyslipidemia develop diabetes at a high rate ($\geq 90\%$). The mean BMI of patients with WS and hypertriglyceridemia was 18.2, showing a lack of association with obesity. The rates of achieving the lipid control target values among patients with WS are high, at 91% for LDL cholesterol, 91% for HDL cholesterol, and 82% for triglycerol. Strong statin dosage is mainly used as an

antidyslipidemic drug and contributes to the achievement of the control target values (Tsukamoto et al. 2021). Two-thirds of patients in the registry with dyslipidemia were treated with statins (Koshizaka et al. 2020).

Fatty Liver

WS patients with fatty liver had a mean BMI of 18.8 and a maximum BMI of 22.6, and 83% of these patients were underweight. The L/S ratio showed a positive correlation with HDL cholesterol levels and a negative correlation with triglyceride levels. It did not correlate with liver enzyme levels (Tsukamoto et al. 2021). Insulin resistance associated with a fatty liver (nonalcoholic fatty liver disease) and accumulation of visceral fat has been considered to play a major role in metabolic abnormalities (Kitade et al. 2017; Kahn and Flier 2000; Hardy et al. 2012). There is evidence regarding treatments with pioglitazone (Belfort et al. 2006; Aithal et al. 2008), vitamin E (Sanyal et al. 2010), and ursodeoxycholic acid (Leuschner et al. 2010) in the general population, and Takemoto et al. reported that astaxanthin, a carotenoid, improved fatty liver in patients with WS (Takemoto et al. 2015).

Atherosclerosis

Early-onset atherosclerosis occurs in WS patients, and the incidence of ischemic heart disease and arteriosclerosis obliterans is particularly high. Because of the high possibility of silent myocardial ischemia, proactive and regular tests for arteriosclerosis are recommended for confirmed cases of WS. In contrast, the prevalence of morbidity because of stroke in WS patients is similar to that found in the general population of the same age in Japan (Okabe et al. 2012).

Malignancy

Malignant tumors were observed in 20.0% of patients in the registry. The morbidity of malignant tumors is still high in patients with WS. Reportedly, age at cancer diagnosis in patients with WS advanced by 20 years when compared with that of the general Japanese population (Lauper et al. 2013). Because neoplastic lesions start developing from a young age, regular screening for malignancies is necessary for confirmed cases of WS. Epithelial and non-epithelial tumors are equally common in WS cases, in contrast to the 10:1 (epithelial-to-non-epithelial) ratio observed in the general population. Cancer was significantly more prevalent in WS patients with diabetes. Therefore, routine cancer screening is especially important in this particular subgroup. Epithelial tumors with high frequency include thyroid cancer, lung cancer, gastric cancer, hepatic cancer, and pancreatic cancer. Non-epithelial tumors with high frequency were malignant fibrous histiocytoma, melanoma, meningioma, and myelodysplastic syndrome (Onishi et al. 2012).

Osteoporosis

Osteoporosis has been observed in approximately 41% of WS patients (Murata and Nakashima 1982). Although osteoporosis was relatively rare in younger patients, almost all patients who were at least 40 years of developed osteoporosis. It is likely to be more severe in the femur than in the lumbar spine (Mori et al. 2017, 2021). Osteoporosis is considered to occur because bone formation is inhibited, while bone resorption is normal in WS (Rubin et al. 1992). Research showing the relation between the *WRN* gene polymorphism and osteoporosis suggests that genetic factors might also be involved in osteoporosis associated with Werner syndrome (Ogata et al. 2001; Zhou et al. 2015). No clear evidence to date regarding treatment for osteoporosis associated with WS has been found. Therefore, the treatment of osteoporosis according to the guidelines for this purpose is considered appropriate (HO osteoporosis prevention and treatment guideline 2015).

Skin Ulcers

Approximately 40% of patients with WS have skin ulcers (Kubota et al. 2021). WS is characterized by symptoms such as atrophy of subcutaneous tissues, decreased blood flow (Okabe et al. 2012), and lower activity of fibroblast cells (Hatamochi et al. 1994) due to metabolic disorders in connective tissues (Muftuoglu et al. 2008), which may easily cause refractory skin ulcers (Yeong and Yang 2004). Skin ulcers in patients with WS often arise from hyperkeratotic lesions and trauma to pressure points such as the plantar region and are more difficult to treat than wound healing in healthy individuals. The ulcers are often located at the distal one-third of the lower legs (Kubota et al. 2021). Skin ulcers lead to reduced quality of life of patients. Callosities in the foot also often form. A callosity in WS is an important therapeutic target for the prevention of ulcers.

Macroscopic evaluations of ulcers are important. Plain radiography and computed tomography are helpful for examining the shape of the entire foot and the conditions of the individual bones of the foot. Vascular evaluation is necessary. Magnetic resonance imaging examination is useful for suspected osteomyelitis.

Treatment includes topical application of a keratolytic agent for keratosis around the ulcer. The treatment of skin ulcers is the same as for normal ulcers, and if the ulcer is associated with infection and necrotic tissue, surgical debridement with a scalpel or scissors should be performed as much as possible after washing with saline or mildly warm water or with an antibacterial agent. Topical medications that promote softening and debridement of the necrotic tissue can be used with careful control of moisture in the wound. Topical agents that promote granulation should be used in wounds where necrotic tissue has been removed without infection. Dressings to maintain a moist environment in the wound may also be useful. If the wound does not improve with conservative treatment, surgical treatment should be considered (Motegi et al. 2021). The combination of surgical treatment and wound bed preparation is important in the treatment of skin ulcers (Kubota et al. 2021).

Infection

Generally, foot skin ulcers may often become severe, leading to the failure of conservative treatment and necessitating surgical excision of the infected site. The goal in the treatment of an infection caused by refractory skin ulcers in patients with WS is to minimize the exacerbation of the ulcerated skin lesion by detecting signs of infection early and treating it. It is important to identify the bacterial etiology causing an infection in the skin ulcer and treat with an effective antimicrobial. For poorly controlled infection, debridement and surgical excision are needed at an appropriate time (Taniguchi et al. 2021a).

Calcification in Tendons

It can be presumed that a considerable number of Japanese individuals with WS are never correctly diagnosed. Achilles tendon calcification was observed in 76.1% of patients with WS, whereas it was observed in only 0.88% of patients without WS, accompanied by 1–4 calcified masses with a maximum diameter ranging from 9.7 mm to 63.2 mm. The frequency of Achilles tendon calcification in patients with WS is far higher than that in patients without WS. Achilles tendon calcification could contribute to the diagnosis of WS (Taniguchi et al. 2021b).

2.2 Basic Research and Molecular Mechanisms of Werner Syndrome

2.2.1 *Werner Gene and Protein*

The causative gene of WS, WRN, was cloned in 1996 (WRN gene: OMIM 604611) (Yu et al. 1996; Oshima et al. 1996; Matsumoto et al. 1997b). It is a RecQ-type DNA helicase and consists of 1432 amino acids (Gray et al. 1997). To date, 83 different mutations have been reported, and additional novel mutations have been identified (Yokote et al. 2017). Among them, the type 4 mutation (the mutation of the base immediately before exon 26 from G to C results in the formation of a truncation mutant protein: c.3139-1G > C) is found in approximately 70% of Japanese WS patients and is considered to be the founder mutation (Oshima et al. 2017).

The accelerated aging mechanism of WS involves the loss of function of the RecQ-type DNA helicase, which plays an important role in various nuclear functions such as DNA repair, replication, recombination, and transcription (Oshima et al. 2017). In the presence of ATP, WRN protein converts DNA double strands into a single strand in the 3' → 5' direction. In addition, the N-terminus of WRN protein contains an exonuclease domain that removes bases one by one in the 3' → 5' direction. As a result of structural analysis using the large synchrotron radiation

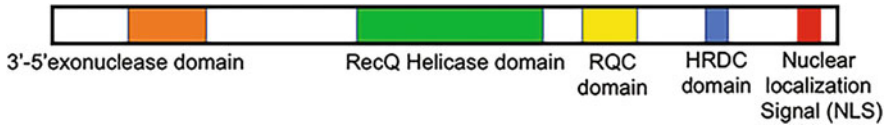


Fig. 2.3 A scheme of WRN protein. WRN has exonuclease domain, RecQ helicase domain, and nuclear localization signal at C-terminus

facility Spring 8, it has recently been reported that the winged-helix motif of the WRN protein acts as a “molecular knife” to unravel the double-stranded DNA at G quadruples, holiday junctions, or other unusual complex structures of DNA (Kitano et al. 2010; Gilson and Geli 2007; Huang et al. 2000). The C-terminus of WRN protein contains a nuclear translocation signal. The mutant WRN protein reported so far is a truncated protein that does not form a C-terminus and lacks the nuclear translocation signal, which inhibits nuclear translocation. This may be the reason why there is no obvious correlation between mutations and symptoms in WS. Indeed, mutant WRN protein that lacks C-terminal 30 amino acid residues cannot localize in the nucleolus where WRN usually resides (Suzuki et al. 2001). WRN contains two other domains, namely, RecQ helicase-conserved (RQC) region and the helicase, RNase D, C-terminal-conserved (HRDC) region (Fig. 2.3).

2.2.2 WRN and DNA Damage Repair

The role of WRN gene in DNA damage repair, especially after double-strand break (DSB), has been extensively reported. WRN protein is usually located in the nucleolus and moves to DNA damage sites upon CBP/P300-mediated acetylation (Blander et al. 2002). Sirt1 deacetylates WRN and regulates its reentry to the nucleolus from the nucleoplasm after DNA damage response (Li et al. 2008). When exonuclease domain-deficient WRN protein, helicase domain-deficient WRN, and WRN protein lacking both domains were expressed in fibroblasts from WS patients, DNA damage repair was most improved when the WRN protein lacking both domains was expressed. Interestingly, DNA damage repair requires balanced activities of helicase and exonuclease domains (Chen et al. 2003b).

There are two types of repair systems after DSB: homologous recombination (HR) that produces the new strands using genomic homologous sequences as templates and nonhomologous end joining (NHEJ), which connects both damaged ends. In addition, NHEJ includes two pathways, classical NHEJ and alternative NHEJ, which utilize different mechanisms. HR is preferentially active in S and G2 as sister chromatid is available after DNA replication, and NHEJ is active in all the cell cycle phases. HR has a high fidelity, and NHEJ is more prone to produce errors because it does not refer to intact homology sequence. Ku70/80 heterodimer protein, along with DNA-dependent protein kinase catalytic subunit (DNA-PKcs), is the essential regulator of DNA damage response that initiates the cascades of classical

NHEJ (Walker et al. 2001). WRN has two putative Ku-binding motifs: one at the N-terminus next to the exonuclease domain and one at the C-terminus next to an XLF-like motif. The N-terminal Ku binding motif enhances exonuclease activity of WRN (Grundy et al. 2016). DNA-PK also interacts with WRN, thereby phosphorylating its serine 440 and 447 sites and regulating relocalization of WRN to the nucleoli (Kusumoto-Matsuo et al. 2014). WRN also interacts with XRCC4-DNA ligase IV complex (X4L4), and this binding promotes the exonuclease activity of WRN to generate DNA ends suitable for XRCC4-LIG4-mediated ligation (Kusumoto et al. 2008). In addition, it has recently been reported that WRN promotes classical NHEJ via helicase and exonuclease enzymatic activities and inhibits alternative NHEJ using nonenzymatic functions (Shamanna et al. 2016). Taken together, the enzymatic activity of WRN, especially the exonuclease activity to process damaged DNA ends, promotes classical NHEJ.

On the other hand, WRN is also involved in HR. The critical regulators of HR are MRE11/RAD50/NBS1 (MRN) complex in conjunction with CtIP (Garcia et al. 2011). The endonuclease and 3'-5' exonuclease activities of MRE11 are required for the initiation of HR. Following this, exonuclease 1 and/or the nuclease DNA2 with the RECQ helicase Bloom syndrome protein mediates extensive DNA resection (Nimonkar et al. 2011). WRN interacts with MRE11 and NBS1 (Cheng et al. 2004), and this results in the enhancement of WRN helicase activity. WRN also binds to DNA2 and facilitates extensive DNA resection (Sturzenegger et al. 2014). Moreover, cyclin-dependent kinase 1 (CDK1) phosphorylates at Ser1133 and promotes the DNA end resection by DNA2 at replication-related DSBs (Palermo et al. 2016). The Ser1133 phosphorylation of WRN is required for the interaction with the MRE11 complex. WRN also interacts with other HR proteins including RAD51, RAD54, RAD52, and BRCA1/BARD1 complex, and these interactions stimulate WRN helicase activity. These studies indicate the critical role of WRN also in HR (Lachapelle et al. 2011; Otterlei et al. 2006; Lu and Davis 2021) (Fig. 2.4).

2.2.3 *WRN and Telomeres*

WRN proteins are also involved in telomere maintenance. Telomeres are chromosome-terminating complexes composed of the characteristic repeating DNA sequence TTAGGG and a protein called the shelterin complex, which protects the DNA ends of chromosomes. Telomeres shorten with aging, and when telomeres exceed a certain length, cells irreversibly stop proliferating, leading to cellular senescence. The shelterin complex mainly consists of six proteins, TRF1, TRF2, RAP1, TIN2, TPP1, and POT1. WRN interacts with POT1 and TRF2 (Opresko et al. 2004) (Machwe et al. 2004), and this interaction facilitates WRN to resolve G4 quadruplex, holiday junctions formed at D- and T-loops of telomeres (Opresko 2008; Nora et al. 2010). WS patients aged 40–60 years show prominent telomere reductions compared to WS patients younger than 30 years or age-matched non-WS patients, indicating that the loss of WRN function leads to accelerated telomere

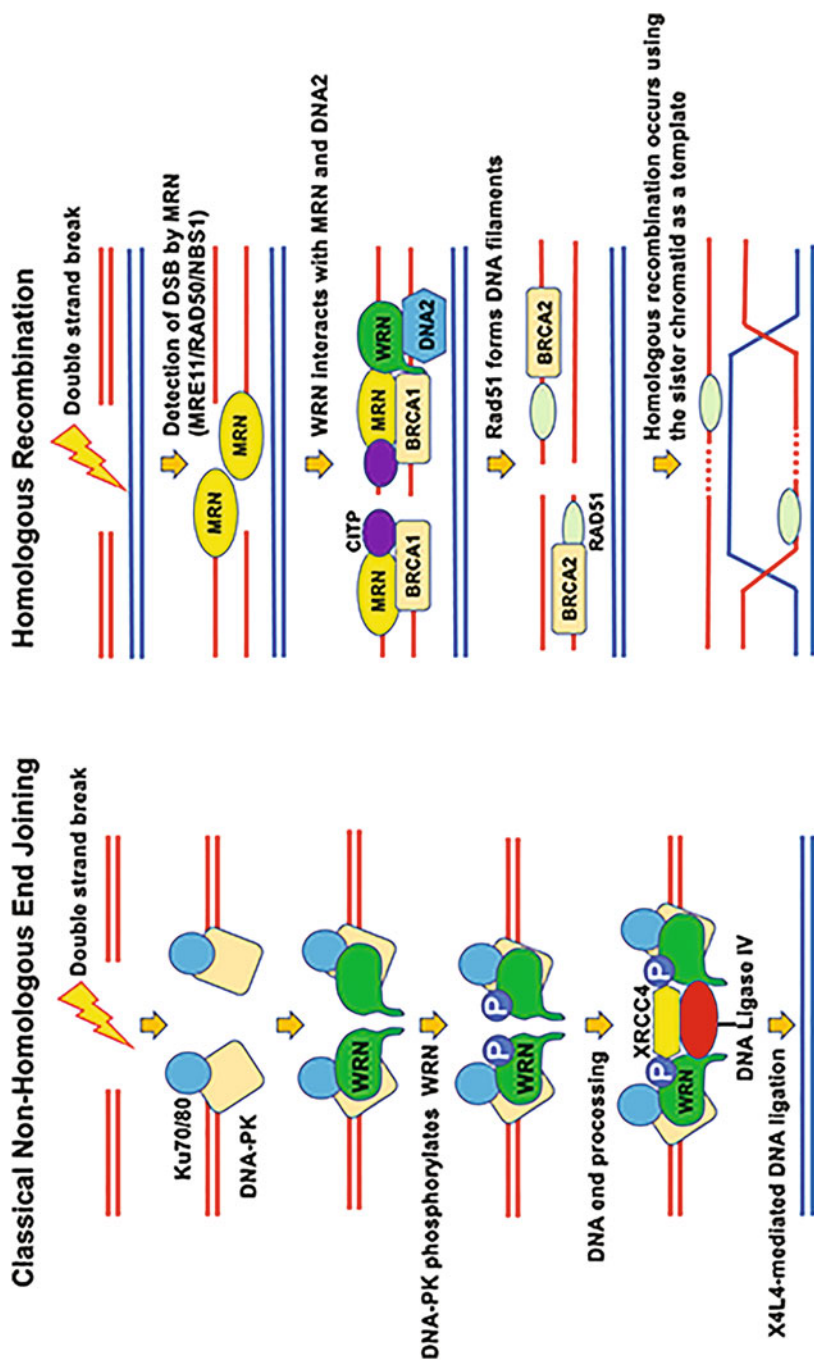


Fig. 2.4 A scheme of the roles of WRN in classical NHEJ and HR after DNA double-strand break

attrition (Ishikawa et al. 2011). In fibroblasts derived from WS patients and genetically created WRN-deficient cells, telomere loss leads to fusion, segregation defects, and instability of chromosomes (Laud et al. 2005; Crabbe et al. 2007). When WRN is mutated, the guanine tetramer produced during telomere replication cannot be unraveled, and the lagging strand of DNA synthesis is stopped, preventing replication and accelerating telomere shortening (Shimamoto et al. 2015). In fact, it has been reported that reintroduction of telomerase activity can inhibit telomere loss, new chromosomal aberrations, and cellular senescence in cells derived from WS patients. Therefore, at least part of the WS phenotype is thought to be due to telomere defects (Fig. 2.3) (Crabbe et al. 2007; Wyllie et al. 2000). At telomeres, SIRT6, one of the longevity genes, sirtuin, removes the acetylation of histone H3K9 in a nicotinamide adenine dinucleotide (NAD⁺)-dependent manner. SIRT6-mediated histone acetylation is required for WRN to bind to telomere chromatin (Michishita et al. 2008). On the other hand, shortening of telomeres was not observed in hepatocytes derived from WS patients, and keratinocytes in the skin retained telomerase activity and did not show replicative senescence (Ibrahim et al. 2016), suggesting that telomere damage caused by WRN deficiency may vary depending on the tissue (Tokita et al. 2016).

Concerning G4 quadruplex and WRN, in fibroblasts from WS patients, significant association was observed between G-quadruplex loci and the loci whose gene expressions were upregulated (Johnson et al. 2010). Significant enrichment of G4 motifs has also been observed at the transcription start site and 5' end of first introns of genes downregulated in WS fibroblasts. WS fibroblasts display senescence-associated gene expression programs, disease-associated miRNAs, and dysregulation of canonical pathways that regulate cell signaling, genome stability, and tumorigenesis. Together, WRN regulates transcription by binding to G4-DNA motifs (Fig. 2.5).

In addition, we recently reported the difference of telomeres between regions of the body. The differences of gene expression profiles were compared in fibroblasts between the limbs and trunk in WS patients, in whom the trunk is relatively plump, but the limbs are extremely atrophic, leading to intractable skin ulcers. We found increased cellular senescence and shortened telomeres in the periphery compared to the abdomen. Interestingly, fibroblasts in the periphery restored osteo-differentiation capacity and markedly reduced adipogenicity, reflecting the pathogenesis of WS (Kato et al. 2021a).

2.2.4 WRN and Mitochondria, mTOR, and Autophagy

Cells from WS patients and genetically modified WRN-deficient cells show phenotypes with mitochondrial dysfunction. WRN depletion leads to a global alteration in the gene expressions that regulates energy production and redox condition. This change attenuates antioxidative defenses and increases mitochondrial oxygen consumption leading to increased reactive oxygen species (ROS) and oxidative DNA

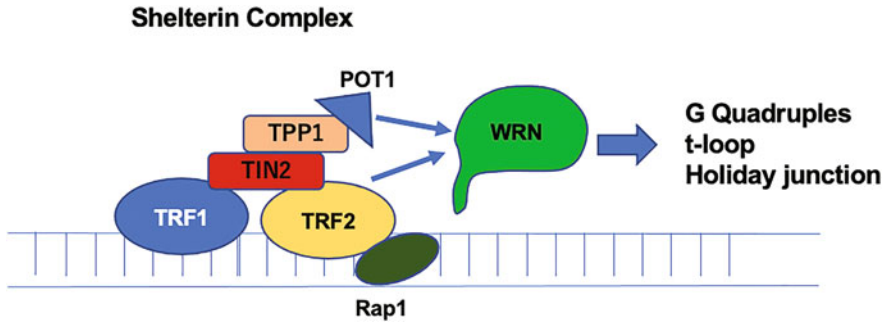


Fig. 2.5 The interaction between the shelterin complex and WRN: WRN plays a role in unraveling the G quadruples that occurs during telomere replication and the DNA loops called t-loop structures at telomeres. POT1 and TRF2, which are part of the telomeric shelterin complex, facilitate this function of WRN

damage. In cancer cells, this metabolic change counteracts the Warburg effect and results in the suppression of cell proliferation and senescence-like phenotype. This effect may be preferable in cancer settings, and a reason why WRN-deficient cells show the synthetic lethality that will be discussed later (Li et al. 2014). Cells that lack WRN accumulate oxidative bases including 8-oxoguanine, FapyG, and FapyA, and the double-strand break marker gamma H2AX, indicating the function of WRN in oxidative damage repair in the genome. NEIL1 is a mammalian DNA glycosylase required for repairing oxidatively damaged DNA bases. Upon exposure to oxidative stress, WRN binds to NEIL1 via the RQC region, colocalized in the nuclei, and functions to excise oxidative regions from bubble DNA substrates (Das et al. 2007). In 2019, Fang et al. reported impaired mitophagy, accumulation of ROS and depletion of NAD⁺, the co-enzyme of sirtuins, and many metabolic enzymes, in WS patient cells and WRN-deficient invertebrates. NAD⁺ repletion using nicotinamide riboside (NR) improves the transcriptome profile of the WRN-deficient worms and their mitochondrial quality through DCT and ULK1-dependent mitophagy. Moreover, NR extends the life span of *Caenorhabditis elegans* and *Drosophila melanogaster* models of WS. Thus, mitochondrial dysfunction is a critical mechanism for the accelerated aging phenotype of WS and simultaneously may be a potential therapeutic target (Fang et al. 2019).

The mammalian target of rapamycin (mTOR) is an essential signaling molecule that forms the hub of multiple metabolic pathways, and WRN may be involved in this pathway and autophagy. In WRN knockdown primary fibroblasts, increased autophagy was observed (Saha et al. 2014; Talaei et al. 2013). Short-term rapamycin treatment, which inhibits mTOR, increased autophagy activity, while long-term rapamycin treatment led to improved cell growth, reduced accumulation of DNA damage marker 53 BPI, improved nuclear morphology, and reduced autophagy markers LC3II and P62 (Saha et al. 2014). It is speculated that reduced protein aggregates by early enhancement of autophagy resulted in better cellular function.

2.2.5 Phenotype of WRN KO Mice

Although WS has a pronounced phenotype in humans, another feature of WRN is that there is almost no phenotype in deficient mice. The first WRN mutant mice were generated in 1998 by deleting the catalytic site of the helicase domain, but they did not show any signs of aging such as gray hair or short life span until 13 months of age. On the other hand, when heterozygotes were crossed, the birth rate of wild type: heterozygote:homozygote was 1:1.9:0.6, and the birth rate of KO mice was reduced. In addition, Embryonic stem cells derived from KO mice showed increased sensitivity to topoisomerase inhibitors such as camptothecin (Lebel and Leder 1998). Long-term observation of these mice showed mild shortening of life span after 20 months, the accumulation of ROS in the liver, and fatty liver (Massip et al. 2010). In contrast, Guarente's group created mice lacking exon 18 and later part of the WRN gene (most of the C-terminal side of the helicase domain), which resulted in no signs of aging. But, double KO mice of WRN KO with P53 KO, a molecule that protects the genome from DNA damage, showed shortened life span (Lombard et al. 2000). Interestingly, double KO of WRN KO mice with telomere RNA component-deficient mice, a noncoding RNA of the telomere maintenance complex, resulted in the development of aging phenotypes such as gray hair, cataracts, hunchback, and short life span after five to six generations (Chang et al. 2004). The mechanism of this minor phenotype of WRN KO mice is not clear, but it may be due to the redundancy of five subtypes of RecQ helicases that are complementary to the WRN deficiency, and the possibility that the DNA damage accumulation is insufficient because of the short life span. Intriguingly, telomerase activity is relatively high in mice. Because the phenotype of WS is telomere dependent, the relatively high telomerase activity in mice may mask the phenotype.

2.2.6 WRN and Stem Cell Senescence and Epigenome Regulation

WS is also involved in stem cell senescence and epigenetic regulation. As cells undergo senescence, the cytoplasm enlarges and the nucleus swells, and the traditional transcriptionally inactive heterochromatin structure loosens and becomes euchromatin dominant, a structure that facilitates transcription (Zhang et al. 2020). In 2015, Belmonte and colleagues at the Salk Institute generated WRN-deficient ES cells and found no abnormalities in their ability to differentiate into endoderm, mesoderm, and ectoderm, or in ES cell proliferation. Interestingly, when mesenchymal stem cells (MSCs) were induced from these WRN-deficient ES cells, they showed decreased proliferation; senescence-associated beta-Gal staining; expressions of p16 and p21 and other cell cycle-inhibitory senescence markers, IL6 and IL8, and other senescence-associated secretory phenotypes (SASPs). In addition, WRN protein interacts with histone methyltransferase complex proteins such as

SUV39H1, LAP2beta, and HP1alpha to regulate histone methylation H3K9me3 and H3K27me3. In WRN-deficient cells, this mechanism is impaired, and H3K9me3 and H3K27me3 are reduced, resulting in an aging phenotype (Zhang et al. 2015). WRN-deficient MSCs and MSCs derived from ES cells carrying the mutation of other early-onset progeroid syndrome, Hutchinson-Gilford progeria syndrome (HGPS), have been compared (Wu et al. 2018). HGPS is caused by a mutation of lamin A, shows symptoms of early-onset premature aging from infancy, and develops severe atherosclerosis leading to myocardial or cerebral infarction. Contrary to expectations, WRN-deficient MSCs show more early-onset cellular senescence than HGPS MSCs; however, the phenotype was much milder. The HGPS MSCs show late-onset acute premature aging phenotype, providing a useful tool that recapitulates the pathology of WS and HGPS. Using this system, a flavonoid, quercetin, and the antioxidative vitamin, vitamin C, were identified as compounds that retard cellular senescence of WS (Li et al. 2016; Geng et al. 2019).

In addition, CRISPR Cas9-based screen was performed using mesenchymal precursor cells that have pathogenic mutations for WS and Hutchinson-Gilford syndrome. The result identified KAT7, a histone acetyltransferase, as a factor whose deficiency alleviated cellular senescence. The lentivirus treatment encoding Cas9 and Kat7 targeting guide RNA improved hepatocyte senescence and liver aging and extended the life span in aged mice and progeroid model Zempste24 null mice, indicating that epigenetic modification may also be a target of antiaging interventions (Wang et al. 2021).

Epigenomic findings in WS patients have also been reported. Horvath et al. proposed the epigenetic clock, which estimates the aging of cells and tissues by analyzing DNA methylation in 391 CpG regions of the genome. Using this method to analyze DNA methylation in the whole blood of WS patients, the average age of methylation in WS is 6.4 years more than that in the healthy group (Maierhofer et al. 2017). However, further analysis showed that DNA methylation in WS did not show differences in retrotransposons such as LINE1 and ALU, which are reportedly altered during aging. This suggests that WS aging is partly different from the normal aging epigenome (Maierhofer et al. 2019).

2.2.7 WS Patient-Derived iPS Cells

As mentioned above, ES cells lacking WRN do not show an obvious phenotype, but then what about iPS cells created from WS patient cells that have accumulated DNA damage over a lifetime? In 2014, Shimamoto et al. established iPS cells from skin fibroblasts of WS patients, which had infinite proliferative potential for more than 2 years, maintained an undifferentiated state, and showed no increase in chromosomal aberrations during the culture period. On the other hand, when the cells were induced to differentiate into somatic cells, signs of premature senescence were observed. Furthermore, it was speculated that part of this phenotype was due to the extension of telomeres by reprogramming (Shimamoto et al. 2014). At the same

time, WS-specific iPS cells were also established in the National Institute of Health in the USA, and early senescence was observed when induced into MSCs, but not when differentiated into neural progenitor cells in the ectoderm. Recently, genomic gene correction was performed on iPS cells generated from a WS patient's blood using CRISPR/Cas9 technique and enabled the isogenic comparison between patient-derived iPS cells and normal iPS cells (Kato et al. 2021b). MSCs derived from WS-specific iPS cells showed decreased expression of various growth factors and reduced ability to differentiate into cartilage, bone, and fat (Tu et al. 2020). These abnormalities in MSCs and the relative preservation of other germ-derived tissues are consistent with the symptoms of WS, in which abnormalities are concentrated in mesenchymal tissues, resulting in subcutaneous tissue atrophy, visceral fat accumulation, myocardial infarction, and mesenchymal sarcoma, but not cognitive decline. In recent years, MSCs have shown promise as a cell therapy for a variety of conditions, including skin ulcers and diabetes, and may be a potential therapeutic strategy for WS.

2.2.8 *Malignancy and WRN*

Patients with WS have a high incidence of malignancy, as described in the previous section. The mechanism is considered to be genomic instability caused by the loss of WRN, leading to the accumulation of DNA damage and tumorigenesis. On the other hand, WRN is attracting attention as a synthetic lethal gene in cancer therapy, although it appears to be quite the opposite. Synthetic lethality refers to a phenomenon in which the loss of a single gene is not lethal to cells, but the simultaneous loss or disruption of two or more pathways causes cell death. In 2019, a CRISPR- or RNAi-based screen showed that tumors exhibiting microsatellite instability (MSI) caused by defective DNA mismatch repair showed a significant decrease in cell survival when WRN was deleted or suppressed. In these cells, the loss of WRN leads to DNA breaks and apoptosis. On the other hand, the survival of cancer cells without MSI remains unchanged even when WRN is reduced (van Wietmarschen et al. 2020; Chan et al. 2019). Furthermore, in 2021, a study using 60 preclinical cancer models with MSI reported that the phenomenon of cancer cell death after loss of WRN was widely observed. This means that WRN-targeted therapy may be effective in a wide range of settings, such as initially or after the acquisition of anticancer drug resistance in malignancies with MSI (Picco et al. 2021). These studies may suggest that WRN deficiency relatively suppress epithelial cancers. Indeed, increased chemotherapeutic activity of camptothecin in cancer cells by siRNA-induced silencing of WRN helicase has been reported in 2007 (Futami et al. 2007). Although these findings are about the function of the WRN gene in tumors, not in patients with WS, the fact that there is a biological advantage to suppressing the WRN gene may indicate the significance of the existence of a substantial population with heterozygous WRN mutations.

2.3 Conclusion

Although more than 20 years has passed since the identification of the WRN gene, it has been difficult to elucidate the pathogenesis of WS because of the lack of phenotype in WRN-deficient mice and the difficulty in culturing cells due to premature aging. In recent years, however, advances in genetic modification technology, material delivery systems, and stem cell biology have gradually revealed the molecular mechanisms of WS. On the other hand, the average life expectancy of patients with WS has extended due to the improved management of metabolic complications such as diabetes and dyslipidemia and advances in the treatment of intractable skin ulcers. The combined knowledge of clinical and basic medicine will lead to further improvement in the prognosis of WS, as well as to a better understanding of the mechanisms of aging and aging-related diseases.

References

- Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I et al (2008) Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* 135(4):1176–1184
- Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J et al (2006) A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 355(22): 2297–2307
- Blander G, Zalle N, Daniely Y, Taplick J, Gray MD, Oren M (2002) DNA damage-induced translocation of the Werner helicase is regulated by acetylation. *J Biol Chem* 277(52): 50934–50940
- Cavallazzi C, Cremoncini R, Quadri A (1960) [On a case of cleidocranial dysostosis]. *Riv Clin Pediatr* 65:312–26
- Chan EM, Shibue T, McFarland JM, Gaeta B, Ghandi M, Dumont N et al (2019) WRN helicase is a synthetic lethal target in microsatellite unstable cancers. *Nature* 568(7753):551–556
- Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D et al (2004) Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet* 36(8):877–882
- Chen L, Lee L, Kudlow BA, Dos Santos HG, Sletvold O, Shafeghati Y et al (2003a) LMNA mutations in atypical Werner's syndrome. *Lancet* 362(9382):440–445
- Chen L, Huang S, Lee L, Davalos A, Schiestl RH, Campisi J et al (2003b) WRN, the protein deficient in Werner syndrome, plays a critical structural role in optimizing DNA repair. *Aging Cell* 2(4):191–199
- Cheng WH, von Kobbe C, Opresko PL, Arthur LM, Komatsu K, Seidman MM et al (2004) Linkage between Werner syndrome protein and the Mre11 complex via Nbs1. *J Biol Chem* 279(20): 21169–21176
- Crabbe L, Jauch A, Naeger CM, Holtgreve-Grez H, Karlseder J (2007) Telomere dysfunction as a cause of genomic instability in Werner syndrome. *Proc Natl Acad Sci U S A* 104(7):2205–2210
- Cruz-Jentoft AJ, Dawson Hughes B, Scott D, Sanders KM, Rizzoli R (2020) Nutritional strategies for maintaining muscle mass and strength from middle age to later life: a narrative review. *Maturitas* 132:57–64
- Das A, Boldogh I, Lee JW, Harrigan JA, Hegde ML, Piotrowski J et al (2007) The human Werner syndrome protein stimulates repair of oxidative DNA base damage by the DNA glycosylase NEIL1. *J Biol Chem* 282(36):26591–26602

- Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, Ciocci S et al (1995) The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83(4):655–666
- Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L et al (2003) Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423(6937):293–298
- Fang EF, Hou Y, Lautrup S, Jensen MB, Yang B, SenGupta T et al (2019) NAD(+) augmentation restores mitophagy and limits accelerated aging in Werner syndrome. *Nat Commun* 10(1):5284
- Friedrich K, Lee L, Leistritz DF, Nurnberg G, Saha B, Hisama FM et al (2010) WRN mutations in Werner syndrome patients: genomic rearrangements, unusual intronic mutations and ethnic-specific alterations. *Hum Genet* 128(1):103–111
- Futami K, Takagi M, Shimamoto A, Sugimoto M, Furuichi Y (2007) Increased chemotherapeutic activity of camptothecin in cancer cells by siRNA-induced silencing of WRN helicase. *Biol Pharm Bull* 30(10):1958–1961
- Garcia V, Phelps SE, Gray S, Neale MJ (2011) Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. *Nature* 479(7372):241–244
- Geng L, Liu Z, Zhang W, Li W, Wu Z, Wang W et al (2019) Chemical screen identifies a geroprotective role of quercetin in premature aging. *Protein Cell* 10(6):417–435
- Gilson E, Geli V (2007) How telomeres are replicated. *Nat Rev Mol Cell Biol* 8(10):825–838
- Goto M (1997) Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. *Mech Ageing Dev* 98(3):239–254
- Goto M (2000) Werner's syndrome: from clinics to genetics. *Clin Exp Rheumatol* 18(6):760–766
- Goto M, Matsuura M (2008) Secular trends towards delayed onsets of pathologies and prolonged longevity in Japanese patients with Werner syndrome. *Biosci Trends* 2(2):81–87
- Goto M, Yamabe Y, Shiratori M, Okada M, Kawabe T, Matsumoto T et al (1999) Immunological diagnosis of Werner syndrome by down-regulated and truncated gene products. *Hum Genet* 105(4):301–307
- Gray MD, Shen JC, Kamath-Loeb AS, Blank A, Sopher BL, Martin GM et al (1997) The Werner syndrome protein is a DNA helicase. *Nat Genet* 17(1):100–103
- Grundy GJ, Rulten SL, Arribas-Bosacoma R, Davidson K, Kozik Z, Oliver AW et al (2016) The Ku-binding motif is a conserved module for recruitment and stimulation of non-homologous end-joining proteins. *Nat Commun* 7:11242
- Hardy OT, Czech MP, Corvera S (2012) What causes the insulin resistance underlying obesity? *Curr Opin Endocrinol Diabetes Obes* 19(2):81–87
- Hatamochi A, Arakawa M, Takeda K, Ueki H (1994) Activation of fibroblast proliferation by Werner's syndrome fibroblast-conditioned medium. *J Dermatol Sci* 7(3):210–216
- Hattori S, Kasai M, Namatame T, Hattori Y, Kasai K (2004) Pioglitazone treatment of insulin resistance in a patient with Werner's syndrome. *Diabetes Care* 27(12):3021–3022
- HO osteoporosis prevention and treatment guideline (2015) Edition (in Japanese), Committee for making osteoporosis prevention and treatment guidelines
- Honjo S, Yokote K, Fujishiro T, Maezawa Y, Sato S, Koshizaka M et al (2008) Early amelioration of insulin resistance and reduction of interleukin-6 in Werner syndrome using pioglitazone. *J Am Geriatr Soc* 56(1):173–174
- Huang S, Beresten S, Li B, Oshima J, Ellis NA, Campisi J (2000) Characterization of the human and mouse WRN 3'→5' exonuclease. *Nucleic Acids Res* 28(12):2396–2405
- Huang S, Lee L, Hanson NB, Lenaerts C, Hoehn H, Poot M et al (2006) The spectrum of WRN mutations in Werner syndrome patients. *Hum Mutat* 27(6):558–567
- Ibrahim B, Sheerin AN, Jennert-Burston K, Bird JL, Massala MV, Illsley M et al (2016) Absence of premature senescence in Werner's syndrome keratinocytes. *Exp Gerontol* 83:139–147
- Ide S, Yamamoto M, Takemoto M, Fujimoto M, Ide K, Kobayashi K et al (2016) Improved glycemic control and vascular function and reduction of abdominal fat accumulation with liraglutide in a case of Werner syndrome with diabetes mellitus. *J Am Geriatr Soc* 64(3):687–688

- Imano E, Kanda T, Kawamori R, Kajimoto Y, Yamasaki Y (1997) Pioglitazone-reduced insulin resistance in patient with Werner syndrome. *Lancet* 350(9088):1365
- Ishikawa N, Nakamura K, Izumiyama-Shimomura N, Aida J, Ishii A, Goto M et al (2011) Accelerated in vivo epidermal telomere loss in Werner syndrome. *Aging (Albany NY)* 3(4): 417–429
- Izumino K, Sakamaki H, Ishibashi M, Takino H, Yamasaki H, Yamaguchi Y et al (1997) Troglitazone ameliorates insulin resistance in patients with Werner's syndrome. *J Clin Endocrinol Metab* 82(8):2391–2395
- Johnson JE, Cao K, Ryvkin P, Wang LS, Johnson FB (2010) Altered gene expression in the Werner and Bloom syndromes is associated with sequences having G-quadruplex forming potential. *Nucleic Acids Res* 38(4):1114–1122
- Kahn BB, Flier JS (2000) Obesity and insulin resistance. *J Clin Invest* 106(4):473–481
- Kato H, Maezawa Y, Takayama N, Ouchi Y, Kaneko H, Kinoshita D et al (2021a) Fibroblasts from different body parts exhibit distinct phenotypes in adult progeria Werner syndrome. *Aging (Albany NY)* 13(4):4946–4961
- Kato H, Maezawa Y, Ouchi Y, Takayama N, Sone M, Sone K et al (2021b) Generation of disease-specific and CRISPR/Cas9-mediated gene-corrected iPSCs from a patient with adult progeria Werner syndrome. *Stem Cell Res* 53:102360
- Kitade H, Chen G, Ni Y, Ota T (2017) Nonalcoholic fatty liver disease and insulin resistance: new insights and potential new treatments. *Nutrients* 9(4):387
- Kitamoto T, Takemoto M, Fujimoto M, Ishikawa T, Onishi S, Okabe E et al (2012) Sitagliptin successfully ameliorates glycemic control in Werner syndrome with diabetes. *Diabetes Care* 35(12):e83
- Kitano K, Kim SY, Hakoshima T (2010) Structural basis for DNA strand separation by the unconventional winged-helix domain of RecQ helicase WRN. *Structure* 18(2):177–187
- Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM et al (1999) Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat Genet* 22(1):82–84
- Koshizaka M, Maezawa Y, Maeda Y, Shoji M, Kato H, Kaneko H et al (2020) Time gap between the onset and diagnosis in Werner syndrome: a nationwide survey and the 2020 registry in Japan. *Aging (Albany NY)* 12(24):24940–24956
- Kubota Y, Takemoto M, Taniguchi T, Motegi SI, Taniguchi A, Nakagami H et al (2021) Management guideline for Werner syndrome 2020. 6. Skin ulcers associated with Werner syndrome: prevention and non-surgical and surgical treatment. *Geriatr Gerontol Int* 21(2):153–159
- Kusumoto R, Dawut L, Marchetti C, Wan Lee J, Vindigni A, Ramsden D et al (2008) Werner protein cooperates with the XRCC4-DNA ligase IV complex in end-processing. *Biochemistry* 47(28):7548–7556
- Kusumoto-Matsuo R, Ghosh D, Karmakar P, May A, Ramsden D, Bohr VA (2014) Serines 440 and 467 in the Werner syndrome protein are phosphorylated by DNA-PK and affects its dynamics in response to DNA double strand breaks. *Aging (Albany NY)* 6(1):70–81
- Kuzuya M, Takemoto M, Kubota Y, Taniguchi T, Motegi SI, Taniguchi A et al (2021) Management guideline for Werner syndrome 2020. 2. Sarcopenia associated with Werner syndrome. *Geriatr Gerontol Int* 21(2):139–141
- Lachapelle S, Gagne JP, Garand C, Desbiens M, Coulombe Y, Bohr VA et al (2011) Proteome-wide identification of WRN-interacting proteins in untreated and nuclease-treated samples. *J Proteome Res* 10(3):1216–1227
- Laud PR, Multani AS, Bailey SM, Wu L, Ma J, Kingsley C et al (2005) Elevated telomere-telomere recombination in WRN-deficient, telomere dysfunctional cells promotes escape from senescence and engagement of the ALT pathway. *Genes Dev* 19(21):2560–2570
- Laufer JM, Krause A, Vaughan TL, Monnat RJ Jr (2013) Spectrum and risk of neoplasia in Werner syndrome: a systematic review. *PLoS One* 8(4):e59709
- Lebel M, Leder P (1998) A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. *Proc Natl Acad Sci U S A* 95(22):13097–13102

- Leuschner UF, Lindenthal B, Herrmann G, Arnold JC, Rossle M, Cordes HJ et al (2010) High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. *Hepatology* 52(2):472–479
- Li K, Casta A, Wang R, Lozada E, Fan W, Kane S et al (2008) Regulation of WRN protein cellular localization and enzymatic activities by SIRT1-mediated deacetylation. *J Biol Chem* 283(12):7590–7598
- Li B, Iglesias-Pedraz JM, Chen LY, Yin F, Cadenas E, Reddy S et al (2014) Downregulation of the Werner syndrome protein induces a metabolic shift that compromises redox homeostasis and limits proliferation of cancer cells. *Aging Cell* 13(2):367–378
- Li Y, Zhang W, Chang L, Han Y, Sun L, Gong X et al (2016) Vitamin C alleviates aging defects in a stem cell model for Werner syndrome. *Protein Cell* 7(7):478–488
- Lombard DB, Beard C, Johnson B, Marciniak RA, Dausman J, Bronson R et al (2000) Mutations in the WRN gene in mice accelerate mortality in a p53-null background. *Mol Cell Biol* 20(9):3286–3291
- Lu H, Davis AJ (2021) Human RecQ Helicases in DNA double-strand break repair. *Front Cell Dev Biol* 9:640755
- Machwe A, Xiao L, Orren DK (2004) TRF2 recruits the Werner syndrome (WRN) exonuclease for processing of telomeric DNA. *Oncogene* 23(1):149–156
- Maierhofer A, Flunkert J, Oshima J, Martin GM, Haaf T, Horvath S (2017) Accelerated epigenetic aging in Werner syndrome. *Aging (Albany NY)* 9(4):1143–1152
- Maierhofer A, Flunkert J, Oshima J, Martin GM, Poot M, Nanda I et al (2019) Epigenetic signatures of Werner syndrome occur early in life and are distinct from normal epigenetic aging processes. *Aging Cell* 18(5):e12995
- Martinez-Arnau FM, Fonfria-Vivas R, Cauli O (2019) Beneficial effects of leucine supplementation on criteria for sarcopenia: a systematic review. *Nutrients* 11(10):2504
- Massip L, Garand C, Paquet ER, Cogger VC, O'Reilly JN, Tworek L et al (2010) Vitamin C restores healthy aging in a mouse model for Werner syndrome. *FASEB J* 24(1):158–172
- Matsumoto T, Imamura O, Yamabe Y, Kuromitsu J, Tokutake Y, Shimamoto A et al (1997a) Mutation and haplotype analyses of the Werner's syndrome gene based on its genomic structure: genetic epidemiology in the Japanese population. *Hum Genet* 100(1):123–130
- Matsumoto T, Shimamoto A, Goto M, Furuichi Y (1997b) Impaired nuclear localization of defective DNA helicases in Werner's syndrome. *Nat Genet* 16(4):335–336
- Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M et al (2008) SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* 452(7186):492–496
- Mori S, Zhou H, Yamaga M, Takemoto M, Yokote K (2017) Femoral osteoporosis is more common than lumbar osteoporosis in patients with Werner syndrome. *Geriatr Gerontol Int* 17(5):854–856
- Mori S, Takemoto M, Kubota Y, Taniguchi T, Motegi SI, Taniguchi A et al (2021) Management guideline for Werner syndrome 2020. 4. Osteoporosis associated with Werner syndrome. *Geriatr Gerontol Int* 21(2):146–149
- Motegi SI, Takemoto M, Taniguchi T, Kubota Y, Taniguchi A, Nakagami H et al (2021) Management guideline for Werner syndrome 2020. 7. Skin ulcer associated with Werner syndrome: dermatological treatment. *Geriatr Gerontol Int* 21(2):160–162
- Muftuoglu M, Oshima J, von Kobbe C, Cheng WH, Leistriz DF, Bohr VA (2008) The clinical characteristics of Werner syndrome: molecular and biochemical diagnosis. *Hum Genet* 124(4):369–377
- Murata K, Nakashima H (1982) Werner's syndrome: twenty-four cases with a review of the Japanese medical literature. *J Am Geriatr Soc* 30(5):303–308
- Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, Wyman C et al (2011) BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev* 25(4):350–362
- Nora GJ, Buncher NA, Opresko PL (2010) Telomeric protein TRF2 protects Holliday junctions with telomeric arms from displacement by the Werner syndrome helicase. *Nucleic Acids Res* 38(12):3984–3998

- Novelli G, Muchir A, Sangiuolo F, Helbling-Leclerc A, D'Apice MR, Massart C et al (2002) Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. *Am J Hum Genet* 71(2):426–431
- Ogata N, Shiraki M, Hosoi T, Koshizuka Y, Nakamura K, Kawaguchi H (2001) A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. *J Bone Miner Metab* 19(5):296–301
- Okabe E, Takemoto M, Onishi S, Ishikawa T, Ishibashi R, He P et al (2012) Incidence and characteristics of metabolic disorders and vascular complications in individuals with Werner syndrome in Japan. *J Am Geriatr Soc* 60(5):997–998
- Onishi S, Takemoto M, Ishikawa T, Okabe E, Ishibashi R, He P et al (2012) Japanese diabetic patients with Werner syndrome exhibit high incidence of cancer. *Acta Diabetol* 49(Suppl 1): S259–S260
- Opresko PL (2008) Telomere ResQue and preservation--roles for the Werner syndrome protein and other RecQ helicases. *Mech Ageing Dev* 129(1–2):79–90
- Opresko PL, Otterlei M, Graakjaer J, Bruheim P, Dawut L, Kolvraa S et al (2004) The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2. *Mol Cell* 14(6):763–774
- Oshima J, Yu CE, Piussan C, Klein G, Jabkowski J, Balci S et al (1996) Homozygous and compound heterozygous mutations at the Werner syndrome locus. *Hum Mol Genet* 5(12): 1909–1913
- Oshima J, Sidorova JM, Monnat RJ Jr (2017) Werner syndrome: clinical features, pathogenesis and potential therapeutic interventions. *Ageing Res Rev* 33:105–114
- Otterlei M, Bruheim P, Ahn B, Bussen W, Karmakar P, Baynton K et al (2006) Werner syndrome protein participates in a complex with RAD51, RAD54, RAD54B and ATR in response to ICL-induced replication arrest. *J Cell Sci* 119(Pt 24):5137–5146
- Palermo V, Rinalducci S, Sanchez M, Grillini F, Sommers JA, Brosh RM Jr et al (2016) CDK1 phosphorylates WRN at collapsed replication forks. *Nat Commun* 7:12880
- Picco G, Cattaneo CM, van Vliet EJ, Crisafulli G, Rospo G, Consonni S et al (2021) Werner helicase is a synthetic-lethal vulnerability in mismatch repair-deficient colorectal cancer refractory to targeted therapies, chemotherapy and immunotherapy. *Cancer Discov* 11(8):1923–1937
- Rubin CD, Zerwekh JE, Reed-Gitomer BY, Pak CY (1992) Characterization of osteoporosis in a patient with Werner's syndrome. *J Am Geriatr Soc* 40(11):1161–1163
- Saha B, Cypio A, Martin GM, Oshima J (2014) Rapamycin decreases DNA damage accumulation and enhances cell growth of WRN-deficient human fibroblasts. *Aging Cell* 13(3):573–575
- Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM et al (2010) Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 362(18): 1675–1685
- Satoh M, Imai M, Sugimoto M, Goto M, Furuichi Y (1999) Prevalence of Werner's syndrome heterozygotes in Japan. *Lancet* 353(9166):1766
- Shamanna RA, Lu H, de Freitas JK, Tian J, Croteau DL, Bohr VA (2016) WRN regulates pathway choice between classical and alternative non-homologous end joining. *Nat Commun* 7:13785
- Shimamoto A, Kagawa H, Zensho K, Sera Y, Kazuki Y, Osaki M et al (2014) Reprogramming suppresses premature senescence phenotypes of Werner syndrome cells and maintains chromosomal stability over long-term culture. *PLoS One* 9(11):e112900
- Shimamoto A, Yokote K, Tahara H (2015) Werner Syndrome-specific induced pluripotent stem cells: recovery of telomere function by reprogramming. *Front Genet* 6:10
- Shimizu T, Tateishi Y, Furuichi Y, Sugimoto M, Kawabe T, Matsumoto T et al (2002) Diagnosis of Werner syndrome by immunoblot analysis. *Clin Exp Dermatol* 27(2):157–159
- Sturzenegger A, Burdova K, Kanagaraj R, Levikova M, Pinto C, Cejka P et al (2014) DNA2 cooperates with the WRN and BLM RecQ helicases to mediate long-range DNA end resection in human cells. *J Biol Chem* 289(39):27314–27326
- Suzuki T, Shiratori M, Furuichi Y, Matsumoto T (2001) Diverged nuclear localization of Werner helicase in human and mouse cells. *Oncogene* 20(20):2551–2558

- Takada-Watanabe A, Yokote K, Takemoto M, Fujimoto M, Irisuna H, Honjo S et al (2012) A case of Werner syndrome without metabolic abnormality: implications for the early pathophysiology. *Geriatr Gerontol Int* 12(1):140–146
- Takemoto M, Yokote K (2012) The guideline of Werner syndrome for diagnosis and clinical practice
- Takemoto M, Yokote K (2021) Preface to management guideline for Werner syndrome 2020. *Geriatr Gerontol Int* 21(2):131–132
- Takemoto M, Mori S, Kuzuya M, Yoshimoto S, Shimamoto A, Igarashi M et al (2013) Diagnostic criteria for Werner syndrome based on Japanese nationwide epidemiological survey. *Geriatr Gerontol Int* 13(2):475–481
- Takemoto M, Yamaga M, Furuichi Y, Yokote K (2015) Astaxanthin improves nonalcoholic fatty liver disease in Werner syndrome with diabetes mellitus. *J Am Geriatr Soc* 63(6):1271–1273
- Takemoto M, Kubota Y, Taniguchi T, Motegi SI, Taniguchi A, Nakagami H et al (2021) Management guideline for Werner syndrome 2020. 3. Diabetes associated with Werner syndrome. *Geriatr Gerontol Int* 21(2):142–145
- Takino H, Okuno S, Uotani S, Yano M, Matsumoto K, Kawasaki E et al (1994) Increased insulin responsiveness after CS-045 treatment in diabetes associated with Werner's syndrome. *Diabetes Res Clin Pract* 24(3):167–172
- Talaei F, van Praag VM, Henning RH (2013) Hydrogen sulfide restores a normal morphological phenotype in Werner syndrome fibroblasts, attenuates oxidative damage and modulates mTOR pathway. *Pharmacol Res* 74:34–44
- Taniguchi T, Takemoto M, Kubota Y, Motegi SI, Taniguchi A, Nakagami H et al (2021a) Management guideline for Werner syndrome 2020. 5. Infection associated with Werner syndrome. *Geriatr Gerontol Int* 21(2):150–152
- Taniguchi A, Tanaka Y, Takemoto M, Kubota Y, Taniguchi T, Motegi SI et al (2021b) Management guideline for Werner syndrome 2020. 8. Calcification in tendons associated with Werner syndrome. *Geriatr Gerontol Int* 21(2):163–165
- Tokita M, Kennedy SR, Risques RA, Chun SG, Pritchard C, Oshima J et al (2016) Werner syndrome through the lens of tissue and tumour genomics. *Sci Rep* 6:32038
- Tsukamoto K, Takemoto M, Kubota Y, Taniguchi T, Motegi SI, Taniguchi A et al (2021) Management guideline for Werner syndrome 2020. 1. Dyslipidemia and fatty liver associated with Werner syndrome. *Geriatr Gerontol Int* 21(2):133–138
- Tu J, Wan C, Zhang F, Cao L, Law PWN, Tian Y et al (2020) Genetic correction of Werner syndrome gene reveals impaired pro-angiogenic function and HGF insufficiency in mesenchymal stem cells. *Aging Cell* 19(5):e13116
- Walker JR, Corpina RA, Goldberg J (2001) Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412(6847):607–614
- Wang W, Zheng Y, Sun S, Li W, Song M, Ji Q et al (2021) A genome-wide CRISPR-based screen identifies KAT7 as a driver of cellular senescence. *Sci Transl Med* 13(575):eabd2655
- Watanabe K, Kobayashi K, Takemoto M, Ishibashi R, Yamaga M, Kawamura H et al (2013) Sitagliptin improves postprandial hyperglycemia by inhibiting glucagon secretion in Werner syndrome with diabetes. *Diabetes Care* 36(8):e119
- van Wietmarschen N, Sridharan S, Nathan WJ, Tubbs A, Chan EM, Callen E et al (2020) Repeat expansions confer WRN dependence in microsatellite-unstable cancers. *Nature* 586(7828):292–298
- Wu Z, Zhang W, Song M, Wang W, Wei G, Li W et al (2018) Differential stem cell aging kinetics in Hutchinson-Gilford progeria syndrome and Werner syndrome. *Protein Cell* 9(4):333–350
- Wyllie FS, Jones CJ, Skinner JW, Haughton MF, Wallis C, Wynford-Thomas D et al (2000) Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts. *Nat Genet* 24(1):16–17
- Yamaga M, Takemoto M, Takada-Watanabe A, Koizumi N, Kitamoto T, Sakamoto K et al (2017) Recent trends in WRN gene mutation patterns in individuals with Werner syndrome. *J Am Geriatr Soc* 65(8):1853–1856

- Yamamoto H, Kurebayashi S, Kouhara H, Yoshiuchi K, Matsuhisa M, Yamasaki Y et al (2007) Impacts of long-term treatments with testosterone replacement and pioglitazone on glucose and lipid metabolism in male patients with Werner's syndrome. *Clin Chim Acta* 379(1–2):167–170
- Yasuda H, Nagata M, Hara K, Moriyama H, Yokono K (2010) Biguanide, but not thiazolidinedione, improved insulin resistance in Werner syndrome. *J Am Geriatr Soc* 58(1): 181–182
- Yeong EK, Yang CC (2004) Chronic leg ulcers in Werner's syndrome. *Br J Plast Surg* 57(1):86–88
- Yokote K, Saito Y (2008) Extension of the life span in patients with Werner syndrome. *J Am Geriatr Soc* 56(9):1770–1771
- Yokote K, Honjo S, Kobayashi K, Fujimoto M, Kawamura H, Mori S et al (2004a) Metabolic improvement and abdominal fat redistribution in Werner syndrome by pioglitazone. *J Am Geriatr Soc* 52(9):1582–1583
- Yokote K, Hara K, Mori S, Kadowaki T, Saito Y, Goto M (2004b) Dysadipocytokinaemia in Werner syndrome and its recovery by treatment with pioglitazone. *Diabetes Care* 27(10):2562–2563
- Yokote K, Chanprasert S, Lee L, Eirich K, Takemoto M, Watanabe A et al (2017) WRN mutation update: mutation spectrum, patient registries, and translational prospects. *Hum Mutat* 38(1): 7–15
- Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R et al (1996) Positional cloning of the Werner's syndrome gene. *Science* 272(5259):258–262
- Zhang W, Li J, Suzuki K, Qu J, Wang P, Zhou J et al (2015) Aging stem cells. A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. *Science* 348(6239):1160–1163
- Zhang W, Qu J, Liu GH, Belmonte JCI (2020) The ageing epigenome and its rejuvenation. *Nat Rev Mol Cell Biol* 21(3):137–150
- Zhou H, Mori S, Tanaka M, Sawabe M, Arai T, Muramatsu M et al (2015) A missense single nucleotide polymorphism, V114I of the Werner syndrome gene, is associated with risk of osteoporosis and femoral fracture in the Japanese population. *J Bone Miner Metab* 33(6): 694–700