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2. 学会発表

The 10th International Association of Gerontology and Geriatrics-Asia/Oceania
(IAGG 2015) タイ、チェンマイ、平成 27 年 10 月 19 日
Submitted symposium04: Recent progress in studies on progeroid syndrome

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G. 知的財産権の出願・登録状況 (予定を含む)

1. 特許取得
該当なし
2. 実用新案登録
該当なし
3. その他

ウェルナー症候群患者・家族の会 関東地区集会
2015年7月25日 千葉県 (千葉大学病院)

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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IMPROVED GLYCEMIC CONTROL AND VASCULAR FUNCTION AND REDUCTION OF ABDOMINAL FAT ACCUMULATION WITH LIRAGLUTIDE IN A CASE OF WERNER SYNDROME WITH DIABETES MELLITUS

To the Editor: Werner syndrome (WS) is classified as a progeroid syndrome associated with symptoms of premature physical aging.[1] Approximately 60% to 70% of individuals with WS develop diabetes mellitus, and they have a high prevalence of atherosclerotic vascular disease at an early age.[2] Herein, a case of an individual with WS is reported in whom liraglutide was effective not only for glycemic control, but also for reducing abdominal fat accumulation and improving vascular function.

CASE PRESENTATION

A 51-year-old Japanese man was admitted to the hospital for glycemic control. He had begun to develop gray hair when he was still in high school and developed diabetes mellitus and bilateral cataracts at the age of 36 and a refractory lower leg skin ulcer at the age of 42. On admission, he was 164 cm tall, weighed 51.8 kg, and had a body mass index of 19.3 kg/m^2. His visceral fat area on computed tomography (CT) was 141.5 cm^2. He underwent genetic testing and was diagnosed with WS (mutation 4/7, compound heterozygote) and was prescribed 18, 22, and 16 U of insulin aspart three times a day before meals and 44 U of insulin degludec at night, in addition to a daily dose of sitagliptin 100 mg. Initial test results were fasting blood glucose 108 mg/dL, 2-hour postprandial blood glucose 152 mg/dL, and glycosylated hemoglobin 6.1%. Although his insulin level decreased after admission from 100 U (1.9 U/kg) to 87 U (1.7 U/kg), 0.9 mg of liraglutide was administered instead of 100 mg of sitagliptin to further reduce the amounts of insulin. Liraglutide dramatically reduced his requirement for insulin from a total of 87 U with sitagliptin to a total of 16 U (0.31 U/kg), and no adverse effects such as appetite loss were observed. Mean glucose ± standard deviation detected by using continuous glucose monitoring was 112 ± 24.8 mg/dL before the administration of liraglutide and 113 ± 18.9 mg/dL after. To understand the mechanism of the effectiveness of liraglutide in this case, changes in serum glucose, insulin, and glucagon and area under the curve for each of these parameters were analyzed 0, 15, 30, 60, and 120 minutes after consumption of a test meal (Calorie Mate 500 kcal; carbohydrate 60%, fat 26%, protein 14%) before (sitagliptin 100 mg, insulin total 100 U) and after (liraglutide 0.9 mg, insulin 16 U) administration of liraglutide (Table 1). After 1 month of liraglutide administration, visceral fat area on CT decreased from 141.5 to 92.4 cm^2. High-sensitivity C-reactive protein decreased from 3,010 to 1,490 ng/mL, tumor necrosis factor alpha decreased from 9.4 to 5.8 pg/mL, adiponectin increased from 6.2 to 6.8 μg/mL, and flow-mediated vasodilation improved from 4.7% to 10.5% (normal >6%).

DISCUSSION

Reports have suggested that there is a higher prevalence of metabolic disorders and vascular diseases in individuals with WS,[2] and the accumulation of visceral fat tissue in these individuals has been attributed to the development of metabolic syndrome.[3,4] In this report, liraglutide was shown to be effective not only for glycemic control (e.g., reducing glucose fluctuation, as shown in the standard deviation for continuous glucose monitoring) but also in reducing abdominal fat accumulation and improving vascular function. Liraglutide reduced the need for exogenous administration of insulin, with increases in the amount of endogenous insulin (Table 1), which may have led to a decrease in visceral fat. Liraglutide has recently been reported to stimulate brown adipose tissue thermogenesis and browning through hypothalamic adenosine monophosphate–activated protein kinase.[5] This mechanism may also contribute to a decrease in visceral fat. Liraglutide also improved flow-mediated vasodilation. Glucagon-like peptide (GLP)-1 analogues are reported to activate endothelial nitric oxide synthesis directly and inhibit the nuclear factor kappa-B pathway.[6] Evidence indicates that GLP-1 analogues mediate antiatherogenic effects through various mechanisms.[7] Considering that the incidence of atherosclerotic diseases is high in individuals with WS[2], liraglutide might be effective for the prevention of atherogenesis in these individuals.

Table 1. Changes in Laboratory Parameters After Consumption of a Test Meal (Calorie Mate 500 kcal; carbohydrate 60%, fat 26%, protein 14%) Before and After 1 Month of Liraglutide Administration

Parameter	0 Minutes	15 Minutes	30 Minutes	60 Minutes	120 Minutes	Area Under the Curve
Glucose, mg/dL						
Before	112	142	185	195	153	20,497.5
After	117	127	180	200	122	29,492.5
Insulin, IU/mL						
Before	9.3	20.6	34.8	73.6	112.9	7,860.8
After	32	27	33	127.9	108.4	10,395
Glucagon, pg/mL						
Before	77	121	128	91	94	12,187.5
After	99	120	122	111	96	13,162.5

CONCLUSION

Even though several alternatives exist for the treatment of diabetes mellitus in individuals with WS,[4,8,9] the current findings suggest that liraglutide is an effective treatment option.

Shintaro Ide, MD
Masashi Yamamoto, MD
Minoru Takemoto, MD, PhD
Masanori Fujimoto, MD
Kana Ide, MD
Kazuki Kobayashi, MD, PhD
Koutaro Yokote, MD, PhD
Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan

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Conflict of interest: All authors have no conflict of interests.

Author Contributions: Ide, Yamamoto, Fujimoto: data analysis and interpretation, acquisition of subjects and data. Takemoto: data interpretation, manuscript preparation. Ide, Kobayashi: data acquisition. Yokote: discussion, review and editing of manuscript.

Sponsor's Role: The sponsor had no role in this study.

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INCARCERATED INCISIONAL HERNIA: STRANGULATED TRANSVERSE COLON WITH PERFORATION ASSOCIATED WITH ABSCESS FORMATION

To the Editor: An obese 78-year-old woman with a medical history of hypertension was sent to the emergency department with a mass that had been protruding from her abdomen for a few days. Her pulse was 96 beats per minute, blood pressure was 119/60 mmHg, and body temperature was 36.8°C. She had had bariatric surgery 10 years before. On physical examination, an irreducible, tender mass over the upper abdominal region was found to have grown from her previous incision site. Blood tests showed leukocytosis with a left shift, hemoglobin 9.4 g/dL, platelet count 45,000/mL, and serum creatinine 0.86 mg/dL. Emergency abdominal computed tomography (CT) showed a ventral incisional hernia of a segment of the transverse colon (T-colon) with strangulation and perforation leading to abscess formation with subcutaneous emphysema (Figure 1). Considering all clinical evidence and imaging findings, incarcerated ventral incisional hernia with T-colon strangulation and perforation was suspected, and the woman underwent emergency surgery. Laparotomy showed that a segment of the T-colon had herniated through a 3-cm defect in the previous ventral incisional wound at the upper midline of the abdomen into the anterior abdominal wall. A herniated segment of the T-colon found to be strangulated and perforated and was associated with the abscess formation. The surgeon resected 18 cm of the T-colon with primary anastomosis, drainage, and hernia repair, followed by appropriate antibiotics. Persistent wound leakage and epidermal fistula formation complicated her clinical condition, but her condition improved with treatment and she was discharged in stable condition 33 days after admission.

DISCUSSION

Incisional hernias, the protrusion of abdominal viscera at the site of a previous surgical incision, occur in approximately 10% of individuals after laparotomy and in 0.1% to 3% after a laparoscopic procedure.[1] A previous study found that individuals with an incisional hernia were significantly older than those with umbilical or epigastric hernias and more likely to be female. Although most incisional hernias were at the sites of previous midline or paramedian incisions, 10% were port-site hernias. Twenty-two percent of individuals with umbilical hernias presented with incarceration or strangulation, compared with 8% for incisional hernias and 0% for epigastric hernias.[2]

For a variety of reasons, including loss of muscle strength in the anterior abdominal wall and the prevalence of comorbidities that lead to high intraabdominal pressure, elderly adults have a higher incidence of incisional ventral hernias than of other types of umbilical or epigastric hernias.[3] Defects in the abdominal wall can range from a few centimeters to extensive evisceration. Bowel loops often enter these defects, but strangulation and ischemia occur only rarely.[4]

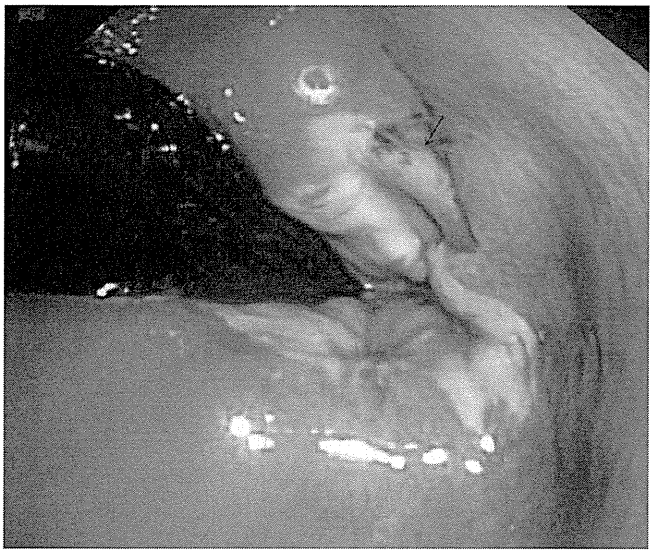

Figure 1. Endoscopic view of solitary rectal ulcer.

normal electrolyte panel values. Abdominal ultrasound was normal. Colonoscopy revealed a well-demarcated rectal ulceration 4 cm from the anal verge (Figure 1). Histopathological examination of the biopsy specimen revealed a chronic ulcer and fibromuscular hyperplasia of the lamina propria. There was no evidence of malignancy. Mesalazine suppository and lactulose suspension, twice daily, was given as treatment. Her symptoms were alleviated after 3 weeks. Mesalazine was discontinued after 2 months. SRUS is a rare lesion of the rectal mucosa, and to the knowledge of the authors, no case in an elderly adult has been published in the literature.[4] The pathogenesis of SRUS is multifactorial and not precisely understood. It is thought that ischemia and trauma of the rectal mucosa cause it.[5] SRUS is diagnosed according to endoscopy, clinical features, and histopathological findings and should be considered in elderly adults presenting with anorectal pain and rectal bleeding.

Hakan Demirci, MD
Kadir Ozturk, MD
Murat Kantarcioglu, MD
Ahmet Uygun, MD
Sait Bagci, MD
Department of Gastroenterology, Gulhane Military Medical Academy, Ankara, Turkey

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Author Contributions: All authors contributed to this paper.

Sponsor's Role: None.

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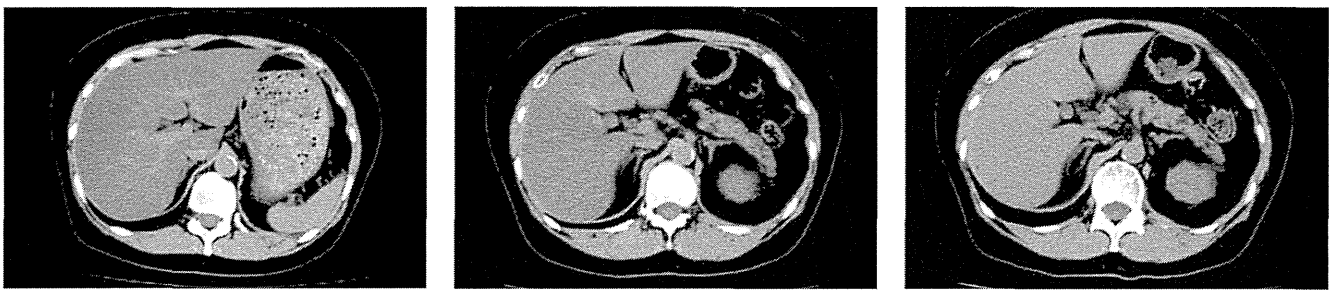
ASTAXANTHIN IMPROVES NONALCOHOLIC FATTY LIVER DISEASE IN WERNER SYNDROME WITH DIABETES MELLITUS

To the Editor: Otto Werner first described Werner syndrome (WS), an adult progeria syndrome with autosomal-recessive inheritance, in 1904. Individuals with WS have a high prevalence of vascular diseases and metabolic disorders, including nonalcoholic fatty liver disease (NAFLD).[1] Herein is reported a case in which astaxanthin, a xanthophyll carotenoid, improved NAFLD in individuals with WS and diabetes mellitus.

A 58-year-old woman was referred to the Department of Medicine, Division of Diabetes, Metabolism and Endocrinology, Chiba University Hospital for close examination and treatment of a skin ulcer. At age 36, she had developed bilateral cataracts; at age 55, an intractable ulcer emerged on her left leg; at age 57, she had surgery for a brain meningioma; and at age 58 she had developed diabetes mellitus. Her parents' marriage was not consanguineous. On examination, her height was 148.7 cm, weight was 42.5 kg, and body mass index was 19.2 kg/m^2. Because she had progeroid facies, she underwent genetic testing and was diagnosed with WS (mutation 4/4). X-ray revealed flame-like calcifications in the Achilles tendon—a phenomenon highly characteristic of WS.[2] She was prescribed a daily dose of 40 mg of telmisartan and 5 mg of atorvastatin. Initial examination results were fasting blood glucose 84 mg/dL, glycosylated hemoglobin 5.6%, immunoreactive insulin 35.1 IU/mL, total cholesterol 222 mg/dL, triglyceride 219 mg/dL, and high-density lipoprotein cholesterol 60 mg/dL. Computed tomography (CT) revealed a visceral fat area of 110.9 cm^2 and severe fatty liver (Figure 1). Astaxanthin 12 mg/d was initiated, and no adverse effects were observed. CT 3 and 6 months after initiation revealed that the severity of fatty liver had gradually but significantly improved (Figure 1). The results of blood transaminase concentrations before astaxanthin initiation and 3 and 6 months after initiation were aspartate aminotransferase 40 IU/L, 41 IU/L, and 20 IU/L; alanine aminotransferase 69 IU/L, 62 IU/L, and 34 IU/L; gamma-glutamyl transferase 38 IU/L, 41 IU/L, and 35 IU/L; and cholinesterase 360 IU/L, 366 IU/L, and 331 IU/L, respectively. Liver-to-spleen Hounsfield units on CT were 0.41 before astaxanthin initiation, 0.71 at 3 months, and 0.94 at 6 months. There were no significant changes after astaxanthin initiation in hyaluronic acid, a marker of liver fibrosis; high-sensitivity C-reactive protein, a marker of inflammation; and malondialdehyde-modified low-density lipoprotein, a marker of oxidative stress.



Before | 3 months after astaxanthin initiation | 6 months after astaxanthin initiation

Figure 1. Computed tomography revealed severe fatty liver, which improved significantly with astaxanthin.

DISCUSSION

Reports suggest that 50% of individuals with WS have complicated fatty liver disease[1,3] and a high prevalence of nonalcoholic steatohepatitis (NASH).[4] Although the exact etiology of NALFD and NASH has not been elucidated, the "two-hit theory" was proposed to explain the pathogenesis of these diseases. Insulin resistance seems to occur first, followed by oxidative stress. Moreover, it has been observed that individuals with WS exhibit high insulin resistance because of the accumulation of visceral fat tissue;[5,6] high oxidative stress in the fibroblasts of these individuals due to the instability of mitochondrial functions and low copper- and zinc-containing superoxide dismutase and manganese-containing superoxide dismutase activities have also been observed.[7] Therefore, individuals with WS may have a genetic predisposition to NAFLD and NASH. There are a few treatment options for NAFLD and NASH, including thiazolidine and vitamin E. Astaxanthin is a strong antioxidant, which *Haematococcus pluvialis*, a microalgae, produces and refines; it has been reported to possess several biological functions, including stabilizing biological membranes, improving mitochondrial functions, decreasing deoxyribonucleic acid damage, and anti-inflammatory effects. In murine obesity models, it has been recently reported that astaxanthin reduces liver endoplasmic reticulum stress and activation of nuclear factor kappa B–mediated inflammation.[8]

It has been reported that NASH, which can be one-third of NAFLD, progresses to liver cirrhosis and hepatocellular carcinoma. Because individuals with WS have a high prevalence of malignant tumors,[9] including liver tumors,[10] it is essential to treat the fatty liver disease frequently found with WS. Because levels of neither oxidative stress markers nor inflammation markers, which were selected, changed after astaxanthin initiation, it is unclear how astaxanthin improved NAFLD in this woman. Nevertheless, to the best of the knowledge of the authors, this is the first report revealing that astaxanthin improves fatty liver disease in humans. Therefore, astaxanthin may become the primary treatment option for individuals with fatty liver disease, especially those with WS, although longer, large-scale, population-based, randomized controlled trials are necessary to determine the benefits of astaxanthin in the future.

Minoru Takemoto, MD, PhD
Masaya Yamaga, MD
Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan
Division of Diabetes, Metabolism and Endocrinology, Department of Medicine, Chiba University Hospital, Chiba, Japan

Yasuhiro Furuichi, PhD
GeneCare Research Institute Co., Ltd., Kamakura, Kanagawa, Japan

Koutaro Yokote, MD, PhD
Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan
Division of Diabetes, Metabolism and Endocrinology, Department of Medicine, Chiba University Hospital, Chiba, Japan

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Author Contributions: Takemoto: analysis and interpretation of data, acquisition of subjects and data, preparation of manuscript. Yamaga: acquisition of subjects and data. Furuichi, Yokote: discussion, review, and editing of manuscript.

Sponsor's Role: None.

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REVERSIBLE METHOTREXATE-INDUCED DEMENTIA: A CASE REPORT

To the Editor: Low-dose oral methotrexate is an established and highly effective treatment for severe psoriasis and rheumatoid arthritis, but its mechanism of action for these indications remains unclear.[1] Folate antagonism and inhibition of polyamines are known to contribute to the antiproliferative effects.[2] In patients with hematological cancer, methotrexate is known for its central neurotoxic side effects after long-term intrathecal administration, ranging from acute aseptic meningitis to delayed toxicities comprising cognitive deficits and progressive dementia, but all these cases concerned long-term intrathecal administration in children.[3,4] A case with reversible dementia after withholding low-dose oral methotrexate for the treatment of rheumatoid arthritis is reported.

CASE

A 78-year-old man was treated with 15 mg of methotrexate once a week in combination of 2.5 mg of folic acid 6 days a week after a diagnosis of rheumatoid arthritis. His medical history included periods of major depression. Eleven months after starting methotrexate, he was hospitalized on a psychiatric ward because of mood and behavioral disorders and cognitive disturbances. Methotrexate was continued. After 3 weeks of clinical observation (including comprehensive neuropsychologic testing and magnetic resonance imaging (MRI) of the brain) and treatment with nortriptyline 75 mg once a day, a diagnosis of dementia syndrome, probably symptomatic frontal lobe, was made, with a Clinical Dementia Rating (CDR) severity of 1. The cognitive domain of the CDR was 2.

Four months later, he visited the memory clinic for a second opinion. Cognitive disturbances (Montreal Cognitive Assessment 20/30) and symptoms of frontal lobe dysfunction such as agitation, lack of empathy, and inappropriate and rude reactions were seen. After consulting the rheumatologist, methotrexate was withdrawn. Four weeks later, the neuropsychological tests started to improve (e.g., Mini–Mental State Examination (MMSE) score 26), and his symptoms of frontal lobe dysfunction decreased. He had no symptoms of depression. One year after withdrawal of methotrexate, his MMSE score was 30, with a clinical diagnosis of multidomain mild cognitive impairment. The behavioral disturbances no longer existed.

DISCUSSION

This case-report describes a 78-year-old mean with a dementia syndrome, symptomatic frontal lobe, after the use of low-dose oral methotrexate that was reversed upon withdrawal of the methotrexate. In the literature, reversible cognitive disturbances after start and rechallenge with low-dose methotrexate were described 25 years ago in older adults with mild renal insufficiency,[5] but a case of methotrexate-induced dementia is not described in the literature. In the Netherlands, seven cases (aged 52–77) with cognitive disturbances after the start (latency time 1–24 months) of low-dose oral methotrexate (5–32.5 mg once a week) were reported to the Netherlands Pharmacovigilance Centre Lareb, which is responsible for the collection and analysis of spontaneously reported adverse drug reactions in the Netherlands. The cognition of three of these individuals recovered after withdrawal of methotrexate. Like the case described herein, improvement of an adverse reaction after withdrawal of a suspected drug without additional treatment points to a causal relationship between the suspected drug and the adverse reaction.

The reason methotrexate affects cognition is not clear. There may be several mechanisms. One explanation is depletion of folic acid. Methotrexate is a folic acid antagonist, and the addition of folic acid with the use of methotrexate is standard care. Older people are especially vulnerable to cognitive problems caused by folic acid deficiencies, and the administration of methotrexate may enhance this.[6] Furthermore, folate deficiency induces several pathophysiological changes that are supposed to be pathogenetic in Alzheimer's disease, such as mitochondrial dysfunction, loss of calcium regulation, neuronal and synaptic impairment, and accumulation of hyperphosphorylated tau and β-amyloid.[7] Another explanation for the effect of methotrexate on cognition is the effect of methotrexate on polyamines like putrescine, spermidine, and spermine seen in rodents.[8] Polyamines influence the aggregation of amyloid-beta (Aβ) peptides into fibrils, known as a risk for Alzheimer's disease.[9] Polyamines have recently been recognized as important elements in the development of many brain diseases.[10] Finally, the man describe herein had had several bouts of depression. Depression and frontal lobe dementia decrease the function of the prefrontal areas,[11] so this man might have been prone to the potential negative effect of methotrexate on cognition.

This case shows that in cases of vulnerability, older people or people known to have depressed periods, low-dose methotrexate can cause cognitive disturbances and even the clinical syndrome of dementia. If low-dose oral methotrexate is needed in these individuals, screening cognitive functions before and during methotrexate treatment

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Werner Syndrome-specific induced pluripotent stem cells: recovery of telomere function by reprogramming

***Akira Shimamoto*[1]*, *Koutaro Yokote*[2] *and *Hidetoshi Tahara*[1]**

[1] *Department of Cellular and Molecular Biology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan*
[2] *Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan*

Edited by:
Fumiaki Uchiumi, Tokyo University of Science, Japan

Reviewed by:
Antonella Sgura, Roma Tre University, Italy
In-Hyun Park, Yale University, USA

***Correspondence:**
Akira Shimamoto, Department of Cellular and Molecular Biology, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan
e-mail: shim@hiroshima-u.ac.jp

Werner syndrome (WS) is a rare human autosomal recessive premature aging disorder characterized by early onset of aging-associated diseases, chromosomal instability, and cancer predisposition. The function of the DNA helicase encoded by WRN, the gene responsible for WS, has been studied extensively. WRN helicase is involved in the maintenance of chromosome integrity through DNA replication, repair, and recombination by interacting with a variety of proteins associated with DNA repair and telomere maintenance. The accelerated aging associated with WS is reportedly caused by telomere dysfunction, and the underlying mechanism of the disease is yet to be elucidated. Although it was reported that the life expectancy for patients with WS has improved over the last two decades, definitive therapy for these patients has not seen much development. Severe symptoms of the disease, such as leg ulcers, cause a significant decline in the quality of life in patients with WS. Therefore, the establishment of new therapeutic strategies for the disease is of utmost importance. Induced pluripotent stem cells (iPSCs) can be established by the introduction of several pluripotency genes, including *Oct3/4*, *Sox2*, *Klf4*, and *c-myc* into differentiated cells. iPSCs have the potential to differentiate into a variety of cell types that constitute the human body, and possess infinite proliferative capacity. Recent studies have reported the generation of iPSCs from the cells of patients with WS, and they have concluded that reprogramming represses premature senescence phenotypes in these cells. In this review, we summarize the findings of WS patient-specific iPSCs (WS iPSCs) and focus on the roles of telomere and telomerase in the maintenance of these cells. Finally, we discuss the potential use of WS iPSCs for clinical applications.

Keywords: Werner syndrome (WS), accelerated aging, chromosomal instability, telomere dysfunction, induced pluripotent stem cells (iPSCs), reprogramming, telomerase, premature senescence phenotypes

INTRODUCTION

Werner syndrome (WS) is a rare human autosomal recessive disorder characterized by early onset of aging-associated diseases, chromosomal instability, and cancer predisposition (Goto, 1997, 2000). Fibroblasts from patients with WS exhibit premature replicative senescence (Salk et al., 1981b). *WRN*, the gene responsible for the disease, encodes a RecQ-type DNA helicase (Oshima et al., 1996; Yu et al., 1996; Goto et al., 1997; Matsumoto et al., 1997) that is involved in the maintenance of chromosome integrity during DNA replication, repair, and recombination (Shimamoto et al., 2004; Rossi et al., 2010).

WRN is a member of the RecQ helicase gene family, and other members of the family include BLM and RTS/RECQL4, which are mutated in Bloom syndrome (BS) and Rothmund–Thomson syndrome (RTS), respectively (Ellis et al., 1995; Kitao et al., 1999). BS and RTS, along with WS, are characterized by chromosomal instability, due to which RecQ helicases are considered to be the guardian angels of the genome (Shimamoto et al., 2004; Bohr, 2008). There are five members in the RecQ helicase gene family, including RECQL1 (Seki et al., 1994) and RECQL5 (Kitao et al., 1998; Shimamoto et al., 2000), the mutations of which have yet to be identified in human diseases.

Major clinical symptoms of WS include common age-associated diseases, such as insulin-resistant diabetes mellitus, and atherosclerosis. Recent advances in drug therapy for these diseases are available and are known to increase the lifespan of patients with WS. However, there is no effective therapy for intractable features, such as severe skin ulcers leading to a decrease in quality of life (QOL), which is a serious problem in patients with WS. Thus, there is an urgent need to develop a new treatment strategy for this syndrome. Regenerative medicine, such as autologous cell transplantation, could be considered as one of the therapeutic strategies for WS, and a potential choice is the use of patient-specific iPSCs.

Somatic cell reprogramming follows the introduction of several pluripotency genes, including *Oct3/4*, *Sox2*, *Klf4*, *c-myc*, *Nanog*, and *Lin-28*, into differentiated cells such as dermal fibroblasts, blood cells, and others (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yu et al., 2007; Aoi et al., 2008; Stadtfeld and Hochedlinger, 2010; Okita and Yamanaka, 2011). During reprogramming, somatic cell-specific genes are suppressed, while embryonic stem cell (ESC)-specific pluripotency genes are induced, leading to the generation of induced pluripotent stem cells (iPSCs) with undifferentiated states and pluripotency

(Stadtfeld et al., 2008). Somatic cell reprogramming generates iPSCs characterized by pluripotency and infinite proliferative potential similar to the ESCs, and this technology opens up new possibilities for tailor-made regenerative medicine (Stadtfeld and Hochedlinger, 2010; Okita and Yamanaka, 2011).

Recently, two groups reported the generation of iPSCs from the cells of patients with WS and came to the similar conclusion that reprogramming repressed premature senescence phenotypes in WS cells (Cheung et al., 2014; Shimamoto et al., 2014). They demonstrated the successful reprogramming of cells from patients with WS into iPSCs with restored telomere function and stable karyotypes, suggesting that the induction of the gene encoding human telomerase reverse transcriptase (hTERT) during reprogramming suppresses telomere dysfunction in WS cells lacking WRN. In this review, we summarize the findings of WS patient-specific iPSCs (WS iPSCs) reported in the literature, and focus on the roles of telomere and telomerase in maintenance of these cells. We also review the recent progress in the clinical management of WS and explore stem cell therapy as a new strategy for WS treatment. WS iPSCs will provide opportunities not only for a better understanding of the pathogenic processes and modeling of the complex features of WS, but also for drug screening as well as the discovery and development of a new strategy for its treatment.

FUNCTION OF WRN HELICASE

Prolonged S-phase and reduction in frequency of DNA replication initiation observed in WS cells have implicated the role of WRN helicase in DNA replication (Hanaoka et al., 1983; Poot et al., 1992). The fact that WRN helicase interacts with several factors involved in DNA replication, including RPA, PCNA, FEN-1, and Topoisomerase I, supports this theory (**Figure 1**; Shimamoto et al., 2004; Rossi et al., 2010). WS cells are hypersensitive to a Topoisomerase I inhibitor, camptothecin (Okada et al., 1998; Poot et al., 1999), and WRN nuclear foci induced by the DNA damage caused by camptothecin are co-localized with RPA in the S-phase (Sakamoto et al., 2001). In addition, WRN helicase forms or unwinds the Holliday junction intermediate associated with a regressed replication fork (Sharma et al., 2004; Machwe et al., 2007). These observations suggest that the WRN helicase is involved in the re-initiation of a stalled replication fork. WS cells also show hypersensitivity to 4NQO that induces oxidative damage (Gebhart et al., 1988). Since accumulation of oxidative DNA damage is associated with aging, it is suggested that the WRN helicase is associated with one of the oxidative repair mechanisms, base excision repair (BER), and is known to interact with BER factors, polδ, polβ, PCNA, RPA, FEN-1, and PARP-1 (**Figure 1**; Rossi et al., 2010). Furthermore, the WRN helicase unwinds a BER substrate produced by uracil-DNA glycosylase and AP endonuclease (Ahn et al., 2004). It is also known that the helicase interacts with the double-strand break repair factors Ku, DNA-PKcs, and the Mre11-Rad50-Nbs1 complex, as well as the telomeric DNA protecting proteins, TRF1, TRF2, and POT1 (**Figure 1**; Shimamoto et al., 2004; Rossi et al., 2010). Additionally, Tahara et al. (1997) reported abnormal telomere dynamics in WS lymphoblastoid cell lines (LCLs) with weak or no telomerase activity. These findings suggest that the WRN helicase is involved in telomere metabolism. WRN helicase is shown to resolve Holliday junctions (Sharma et al., 2004), G-quadruplexes formed in telomere G-rich sequences (Mohaghegh et al., 2001), and higher-ordered DNA structures, such as the D-loop (Opresko et al., 2004). These DNA structures formed at telomere ends must be resolved during DNA replication to be accessible to DNA polymerases and telomerase, therefore, WRN helicase might function in the resolution of higher order structures in telomeric DNA.

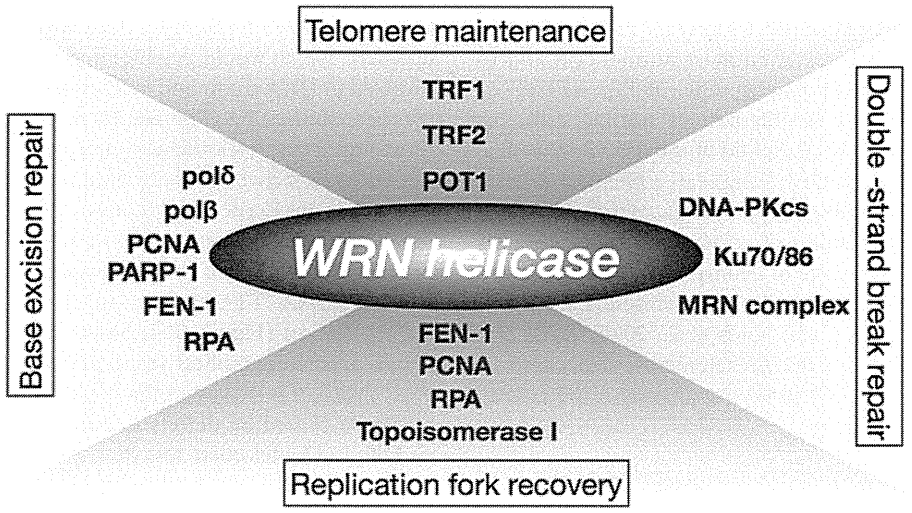

FIGURE 1 | Function of WRN helicase. WRN helicase functions through interaction with factors involved in replication (RPA, PCNA, FEN-1, Topoisomerase I), base excision repair (BER; polδ, polβ, PCNA, RPA, FEN-1, PARP-1), double-strand break repair (Ku, DNA-PKcs, Mre11-Rad50-Nbs1 complex), and telomere maintenance, (TRF1, TRF2, POT1).

ROLES OF TELOMERE IN REPLICATIVE LIFESPAN AND IMMORTALITY

Telomeres, the ends of linear chromosomes in eukaryotes, are ribonucleoprotein-containing specialized structures essential for the protection of chromosomes from a sensing mechanism of double-stranded DNA breaks (Chan and Blackburn, 2004). Mammalian telomeres are composed of TTAGGG repeat sequences, while their specific binding protein complex, shelterin, is composed of the six proteins TRF1, TRF2, RAP1, TIN2, POT1, and TPP1. The chromosome ends are capped by t-loop structures formed by the telomeric DNA and shelterin complex to protect them from DNA damage responses (Palm and de Lange, 2008). In normal human cells, progressive telomere shortening occurs with each successive cell division because of the "end replication problem," wherein regions of RNA primers involved in lagging strand DNA synthesis at most chromosome ends cannot be replaced with DNA during DNA replication (Harley et al., 1990; Levy et al., 1992). Most of the cells in the human body, such as terminally differentiated cells, have no detectable telomerase activity. Further, tissue stem cells such as hematopoietic stem cells (Vaziri et al., 1994; Allsopp et al., 2001, 2003), epidermal stem cells (Flores et al., 2005), and neural stem cells (Ferron et al., 2004) do not exhibit substantial telomerase activity that can add telomeric repeats sufficient to prevent their chromosomal ends from attrition with successive cell division, which is a major cause of human and other organismal aging (Blasco, 2007). On the other hand, germline stem cells and cancer cells express high levels of telomerase that maintains telomere length sufficient for their immortality (Flores et al., 2006). The human telomerase holoenzyme complex consists of a telomerase reverse transcriptase subunit, hTERT, and a template RNA, TERC, which are the basic components required for catalytic activity. (Egan and Collins, 2012) In addition, it also consists of other accessory proteins, including dyskerin, NHP2, NOP10, and NAF1 required for its assembly and stability (Egan and Collins, 2012). Introduction of hTERT is necessary and sufficient for the activation of telomerase in cells, as other components are already expressed in most normal cells and tissues (Nakayama et al., 1998; Chang et al., 2002). *hTERT* can elongate telomeres, extend the lifespan of normal cells, and immortalize cells such as dermal diploid fibroblasts (Bodnar et al., 1998; Vaziri and Benchimol, 1998; Jiang et al., 1999; Morales et al., 1999). Homologous recombination between telomeres, known as ALT (alternative lengthening of telomeres) is an alternative mechanism for the maintenance of telomere length, and has been observed in subsets of cancer cells, telomerase-deficient ESCs and iPSCs (Dunham et al., 2000; Niida et al., 2000; Wang et al., 2012). These findings indicate that the telomerase-dependent and -independent mechanisms of telomere maintenance are essential for cellular immortality.

WS FIBROBLASTS EXHIBIT PREMATURE REPLICATIVE SENESCENCE

Intrinsic DNA damage caused by the loss of WRN helicase could activate stress responses leading to cellular senescence. Senescence is defined as a state of permanent cell cycle arrest mediated by the p53-$p21^{Cip1/Waf1}$ and $p16^{INK4A}$-RB pathways. It is one of the tumor suppressor mechanisms exerted in cells that undergo replicative aging with telomere attrition, generation of reactive oxygen species, abnormal proliferation by oncogene activation, and DNA damage activated by DNA damaging agents such as ionizing radiation (Kuilman et al., 2010; Salama et al., 2014). Stress-associated p38 mitogen-activated protein kinase is constitutively activated in WS fibroblasts (Davis et al., 2005). Activation of p38 is known to mediate cellular senescence in the presence of elevated p21 levels (Haq et al., 2002; Iwasa et al., 2003), and p38 inhibitors can suppress premature senescence phenotypes of WS fibroblasts by reducing p21 expression (Davis et al., 2005). These observations indicate that p38 is a major mediator of the reduced replicative lifespan of WS fibroblasts. Meanwhile, activation of p38 also mediates induction of the senescence-associated secretory phenotype (SASP; Freund et al., 2011) that is the hallmark of aging. It is widely accepted that age-associated inflammatory responses contribute to human aging mechanisms (Goto, 2008). WS fibroblasts express inflammatory cytokines (Kumar et al., 1993), and WS is associated with inflammatory conditions responsible for common age-associated diseases, such as atherosclerosis, diabetes, and osteoporosis (Rubin et al., 1992; Murano et al., 1997; Yokote et al., 2004a; Davis and Kipling, 2006). Taken together, these findings suggest that premature replicative senescence with concomitant induction of p21 and SASP, mediated by the activation of p38, could be pathogenic hallmarks of WS.

TELOMERASE BYPASSES PREMATURE REPLICATIVE SENESCENCE IN WS FIBROBLASTS

As mentioned previously, WRN helicase might play an important role in telomere maintenance. This has been verified by Crabbe et al. (2004) wherein, defects in WRN helicase caused impairment of telomeric lagging-strand synthesis and accelerated telomere loss during DNA replication. Moreover, the telomere loss caused by mutation in the WRN gene involved telomere dysfunction such as chromosome end fusions (Crabbe et al., 2007). It is postulated that the absence of WRN causes stalled replication forks at the sites of unresolved G-quadruplexes at the lagging telomere, which would produce degradable substrates for factors involved in DNA repair and recombination, leading to accelerated telomere shortening (**Figures 2A,B**; Multani and Chang, 2007). More importantly, telomerase prevented sister telomere loss (STL) caused by defective telomeric lagging-strand synthesis and suppressed chromosome end fusions in *WRN*-deficient cells (Crabbe et al., 2004, 2007). These results demonstrate that telomerase can provide WS fibroblasts with a complementation effect by adding telomeric DNA "TTAGGG" to lagging telomeres that are lost during replication (**Figure 2C**). Since telomerase is also known to bypass premature replicative senescence in WS fibroblasts (Wyllie et al., 2000), it is suggested that premature senescence in WS cells might be caused by defects in telomeric lagging-strand synthesis followed by telomere loss during DNA replication (Sugimoto, 2014).

PATHOLOGY IN RECENT WS PATIENTS AND THEIR LIFESPAN

Although WS patients usually grow normally until they reach the late teens, they generally exhibit short stature during adulthood due to impaired maturation. In their 20s and 30s, WS patients start

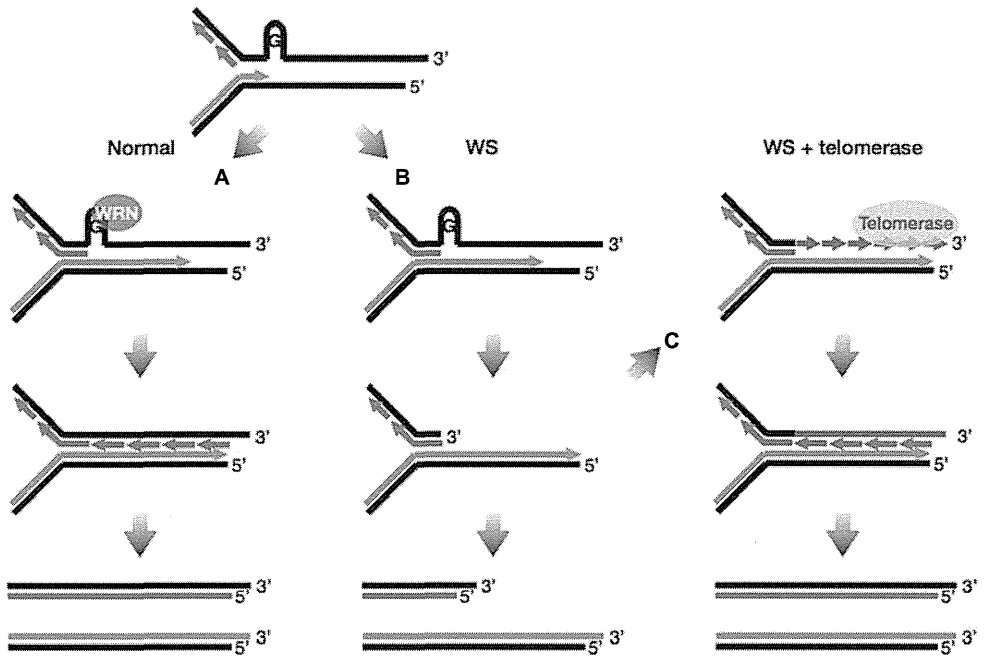

FIGURE 2 | Telomerase bypasses premature replicative senescence in WS fibroblasts. **(A)** G-quadruplexes at the lagging telomere are normally unwound by WRN helicase, making it possible to complete replication of lagging strand G-rich telomeres. **(B)** The absence of WRN causes stalled replication forks at the sites of unresolved G-quadruplexes at the lagging telomere, which would produce degradable substrates for factors involved in DNA repair and recombination, leading to accelerated telomere shortening. **(C)** Telomerase can add telomeric DNA "TTAGGG" to lagging telomeres that are lost during replication in WS cells, which overcomes the lack of WRN, enabling complete replication of lagging strand G-rich telomeres. This figure is based on reference (Multani and Chang, 2007).

to prematurely develop common age-associated diseases, including cataract, graying of hair and hair loss, atrophic skin, skin ulcers, abdominal fat accumulation, osteoporosis, insulin resistant diabetes mellitus, hypogonadism, atherosclerosis, and cancer (Epstein et al., 1966; Goto, 1997; Mori et al., 2001). Recent increase in life expectancy of patients with WS as well as normal individuals suggest that present-day environment, including diet and medical treatment, might have an effect in delaying and/or improving common age-associated diseases. The clinical review of recent WS case reports was updated in a recent study (Goto et al., 2013). In addition, a nation-wide epidemiological survey was conducted in Japan from 2009 to 2011 to elucidate the current clinical picture of WS, as most patients suffering from this disease are of Japanese origin (Takemoto et al., 2013).

It was found that from 2004 to 2008, patients with WS generally survived until their early 50s; this life expectancy is higher than that in 1966 (below the age of 40), with malignancy and cardiac infarction being the major causes of death (Goto et al., 2013). Several symptoms such as short stature with stocky trunk, bilateral cataracts, graying of hair and hair loss, osteoporosis, and atherosclerosis are still the hallmarks of WS. Skin abnormalities including atrophy, sclerosis, ulcers, pigmentation, and subcutaneous calcification have also been observed recently in most WS patients. Endocrine and metabolic diseases including insulin-resistant diabetes mellitus, hypogonadism, and hyperlipidemia are constantly reported, but not observed in all patients with this disease (Goto et al., 2013).

Our recent epidemiological survey revealed that progeroid changes of the hair, bilateral cataracts, soft-tissue calcifications, and skin abnormalities, including atrophy and intractable ulcers, are the most prominent diagnostic clinical features of WS (Takemoto et al., 2013). Bird-like face and abnormal voice are also the discriminating features of WS. The following features are not observed in all WS patients but are critical symptoms: endocrine and metabolic diseases, such as glucose and/or lipid metabolism abnormalities; bone diseases, such as osteoporosis; atherosclerosis; hypogonadism; short stature; and malignancy (Okabe et al., 2012; Onishi et al., 2012; Takemoto et al., 2013). These endocrine and metabolic symptoms are common age-associated diseases in normal individuals, and recent longevity in the general Japanese population could be attributed to recent advances in medicine. In the same way, the use of current medical care for the treatment of the several critical symptoms of patients with WS might increase their life expectancy (Yokote and Saito, 2008; Goto et al., 2013).

CURRENT STRATEGIES FOR TREATMENT OF WS

Recent advances in drug therapy for common age-associated diseases are also available for patients with WS. For example, in most of these patients, insulin-resistant diabetes improved by administration of the PPAR-γ agonist pioglitazone that is generally used for the treatment of type 2 diabetes mellitus. In these cases, pioglitazone ameliorated glycemic irregularities and hyperlipidemia as well as impaired insulin sensitivity (Yokote et al., 2004b;

Honjo et al., 2008). Insulin-resistant diabetes is also improved by treatment with the dipeptidyl peptidase-4 inhibitor sitagliptin in patients with WS (Kitamoto et al., 2012; Watanabe et al., 2013). Furthermore, hyperlipidemia is one of the predictors of coronary artery disease in WS, and statins have been shown to address this issue in patients with WS (Kobayashi et al., 2000). Since premature senescence in WS cells seem to be caused by accelerated telomere loss during DNA replication (Crabbe et al., 2007), the relationship between telomere and these drugs should be considered in the light of protection against aging. It was reported that a short telomere length is a risk factor for coronary heart disease, which is attenuated when combined with the intake of statins (Brouilette et al., 2007). This may be corroborated by a previous finding which suggests that statins prevent telomere dysfunction caused by the loss of telomere repeat-binding factor, TRF2, in cultured endothelial progenitor cells (Spyridopoulos et al., 2004). A PPAR-γ agonist was reported to increase the expression of TRF2 and prevent apoptosis of endothelial progenitor cells (Gensch et al., 2007). Thus, it is likely that improvement in the condition of patients with WS is associated with the effects of these drugs.

INCREASED LONGEVITY AND QOL IN WS PATIENTS

As mentioned earlier, recent protocols for drug therapy in patients with WS have led to an improvement in their lifespan. The average life expectancy of patients with WS at the Chiba University hospital has increased by more than 10 years from 1987 to 2007 (Yokote and Saito, 2008), and a most recent record reported that the longest-living patient had survived until the age of 64 (Yokote, Personal communication). This retrospective study revealed that 7 of the 11 living patients with WS after 1997 had a history of taking statins and/or pioglitazone, suggesting that medical procedures, possibly improved by drug development, as well as early detection and early intervention may increase the life expectancy of patients with WS. However, improvement in the QOL of these patients is also imperative, as skin ulcers are known to have a negative effect on it (Goto et al., 2013).

Excruciatingly painful skin ulcers in patients with WS are considered to be caused by multiple factors, including dermal fragility caused by a decrease in connective and fat tissues, a delay in wound healing caused by impaired proliferative ability of dermal cells, and poor circulation associated with diabetes and arteriosclerotic lesions, and are extremely difficult to treat (Yeong and Yang, 2004; Takemoto et al., 2013). Severe ulcers are commonly found in heels, ankles, elbows, and other areas subject to pressure, and can be surgically treated in some cases only (Yeong and Yang, 2004). However, drug therapy including basic fibroblast growth factor spray, hydrocolloid dressing, and PGE1 preparation have little effect on the ulcers in WS, although it is reported that topical platelet-derived growth factor-BB and the endothelin receptor antagonist bosentan has shown some beneficial effects (Wollina et al., 2004; Noda et al., 2011). Most deep and severe leg ulcers with necrosis require amputations (Yeong and Yang, 2004; Goto et al., 2013). In spite of the increase in the average of life expectancy in WS patients due to the recent improvement in drug therapy for common age-associated diseases, the decrease in QOL caused by excruciatingly painful ulcers is still a major problem that needs to be addressed in these patients.

Intractable skin ulcers also include diabetic ulcers, stasis ulcers, arterial ulcers associated with arteriosclerotic obliteration and Buerger's disease, ulcers associated with connective tissue disease, and radiation-induced ulcers. These ulcers might be treated with debridement ointment under infection control for the enhancement of granulation tissue with vascularization and connective tissue repair (Brem and Lyder, 2004; Sorensen et al., 2004). If the affected area contains necrotic tissue, surgical debridement would be performed followed by skin grafting or flap as required (Sorensen et al., 2004). However, as described above, ulcers in patients with WS heal poorly because of atrophic connective and fat tissues, impaired proliferative ability of dermal fibroblasts, and poor circulation, leading to limited healing of skin grafts and flap as a result of defective granulation tissue formation. At present, there is an urgent need to develop an effective therapeutic strategy for the treatment of severe ulcers in patients with WS.

TELOMERE REJUVENATION IN iPSCs BY REPROGRAMMING

Induced pluripotent stem cells are similar to ESCs, which are generated from individual somatic cells such as dermal fibroblasts, blood cells, and other cell types by the introduction of several pluripotency genes, including *Oct3/4*, *Sox2*, *Klf4*, *c-myc*, *Nanog*, and *Lin-28* (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yu et al., 2007; Aoi et al., 2008; Stadtfeld and Hochedlinger, 2010; Okita and Yamanaka, 2011). Because of their ability to differentiate into various cell types as well as their unlimited proliferative potential, iPSCs, like ESCs, are expected to contribute to regenerative medicine (Takahashi et al., 2007; Stadtfeld and Hochedlinger, 2010). However, unlike ESCs, iPSCs are generated from individual patients, therefore, they can be applied to tailor-made medicine based on syngeneic cell transplantation without allograft rejection (Robinton and Daley, 2012; Lin et al., 2013; Takahashi and Yamanaka, 2013). Moreover, disease-specific iPSCs that can differentiate into multiple cell types can be used to resolve the pathogenic processes of several diseases where cell types available from patients are usually limited to patient-derived lymphocytes and/or fibroblasts.

The reprogramming process includes several key events that define the mechanism of reprogramming of somatic cells into an ES-like state. One proposed idea separates the process into three distinct phases in human and mouse (**Figure 3**; Samavarchi-Tehrani et al., 2010; Golipour et al., 2012; David and Polo, 2014). In the *Oct3/4*, *Sox2*, *Klf4*, and *c-Myc* (OSKM)-driven reprogramming of mouse embryonic fibroblasts, changes in the expression of genes related to the mesenchymal-to-epithelial transition (MET) are observed in the initiation phase (Mikkelsen et al., 2008; Samavarchi-Tehrani et al., 2010; David and Polo, 2014), along with the loss of mesenchymal cell surface markers, CD44 and Thy1, and a gain of the pluripotency markers, alkaline phosphatase activity, and ESC markers (Stadtfeld et al., 2008; Samavarchi-Tehrani et al., 2010; O'Malley et al., 2013; David and Polo, 2014). MET is also observed in reprogramming of human fibroblasts. Using Tra-1-60 positive intermediate reprogrammed cells, similar events are observed during reprogramming of human fibroblasts (**Figure 3**; Takahashi et al., 2014). MET-associated gene expression change occurs in early phage, where induction of epithelial

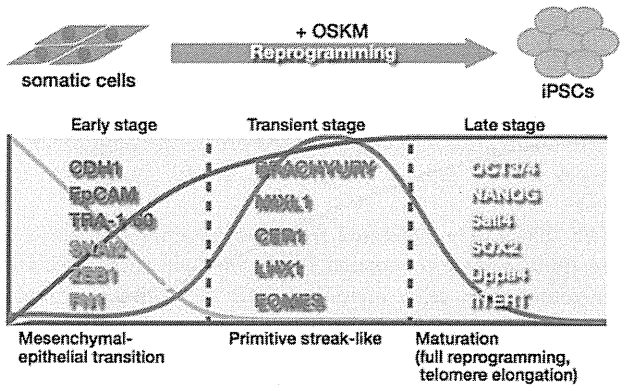

FIGURE 3 | Telomere rejuvenation by reprogramming. The reprogramming process is divided into three distinct stages; early stage, transient stage, and late stage. Telomerase activation and telomere elongation occur in the late stage by induction of telomerase reverse transcriptase subunit and the telomerase RNA (TERC) component, which induce telomere rejuvenation similar in length and their epigenetic state to those of ESCs.

marker genes such as *CDH1* and *EpCAM*, and suppression of mesenchymal genes including *SNAI2*, *ZEB1*, and *FN1* is observed. In transient stage, intermediate cells transiently express genes related to the primitive streak, including *BRACHYURY*, *MIXL1*, *CER1*, *LHX1*, and *EOMES* (**Figure 3**; Takahashi et al., 2014). These events, along with the later phases, are directly or indirectly regulated though the OSKM transcription network by which chromatin decondensation, loss of suppressive histone modification, DNA demethylation, and gain of active histone modification are directed in the genes to be activated, while a concomitantly opposite regulation is observed in the lineage-specific genes to be inactivated (Apostolou and Hochedlinger, 2013; Buganim et al., 2013; Papp and Plath, 2013). During late stage, activation of the first pluripotency-associated genes, including endogenous *Oct3/4*, *Nanog*, *Sall4*, and *Esrrb*, followed by subsequent activation of *Sox2* and *Dppa4* is essentially required to initiate the transformation into pluripotent cells (**Figure 3**; Stadtfeld et al., 2008; Samavarchi-Tehrani et al., 2010; Buganim et al., 2012; Golipour et al., 2012; David and Polo, 2014; Takahashi et al., 2014). The cells activating the first pluripotency genes successfully shift into late stage from transient stage, leading to the accomplishment of a full reprogramming state that ensures sustained self-renewal ability and differentiation potential. In late stage, successive passages are required to eliminate small differences in gene expression profiles between human iPSCs and hESCs, and to erase epigenetic memory derived from somatic cells used in reprogramming (David and Polo, 2014).

Late stage also involves telomerase activation and telomere elongation that provide somatic cells with infinite proliferative potential (**Figure 3**; Takahashi et al., 2007; Stadtfeld et al., 2008; Marion et al., 2009b; Wang et al., 2012). During the reprogramming process, telomerase is activated by induction of telomerase reverse transcriptase subunit (hTERT in humans) and the telomerase RNA component (TERC; Takahashi et al., 2007; Agarwal et al., 2010; Ji et al., 2013), which are likely to be regulated in stem cells by Wnt/β-catenin signaling with KLF4 and/or TCF4 and OCT3/4 and NANOG, respectively (Agarwal et al., 2010; Wong et al., 2010; Hoffmeyer et al., 2012; Zhang et al., 2012). Although c-Myc is known to induce telomerase activity through direct activation of the hTERT gene (Wang et al., 1998; Wu et al., 1999), it might be less involved in telomerase activation during reprogramming, because OSK of the Yamanaka 4 factors without c-Myc is shown to generate iPSCs with enough telomerase activity (Marion et al., 2009b). hESCs have much longer telomeres with higher expression levels of hTERT and stronger telomerase activity than the differentiated cells (Thomson et al., 1998), and telomerase-dependent telomere maintenance is critical for the growth of mammalian ESCs (Niida et al., 1998). Telomere elongation accompanied by telomerase activation occurs during reprogramming, leading to acquisition of telomeres similar in length to those of ESCs after reprogramming (Marion et al., 2009b). iPSC generation also involves epigenetic alterations to ESC-like states with reduced histone codes associated with heterochromatin, and enhanced transcription at the telomere loci. Elevated frequencies in telomeric sister chromatid exchanges and telomere elongation were observed even when old cells with shortened telomeres were used (Marion et al., 2009b). These observations indicate that telomeres are rejuvenated toward an ESC-like state during reprogramming (**Figure 3**).

However, telomerase-deficient cells with critically shortened telomeres fail to be reprogrammed, suggesting that iPSC generation requires a minimum telomere length to be reprogrammed (Marion et al., 2009b). Critically shortened telomeres resulting from the progression of replicative aging in normal human cells lose the protective function of the chromosomal ends and are recognized as endogenous DNA damage. As a result, dysfunctional telomeres induce DNA damage responses including the activation of ataxia telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), and downstream CHK1 and CHK2 kinases, as well as the phosphorylation of p53, inducing cellular senescence via stimulation of the expression of the cyclin-dependent kinase inhibitor (CDKI) p21 (d'Adda di Fagagna et al., 2003; Herbig et al., 2004; Deng et al., 2008). It has been shown that the activation of p53 significantly suppresses reprogramming efficiency, known as the reprogramming barrier (Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009; Utikal et al., 2009), while suppression of p53 improves the reprogramming efficiency in cells with critically shortened telomeres (Marion et al., 2009a). These findings demonstrate that activation of telomerase during reprogramming plays a pivotal role not only in telomere elongation with chromatin state characteristic of ESCs, but also in the restoration and maintenance of the protective functions of the telomere at the chromosomal ends, in order to suppress DNA damage responses.

iPSCs AS A POTENTIAL STRATEGY FOR WS TREATMENT

As described above, skin ulcers in patients with WS heal poorly, and so far no effective therapy has been developed to treat them or the other symptoms associated with WS. Thus, there is an urgent need to develop a new treatment strategy in order to improve the health and QOL of patients with WS. Understanding

the molecular basis and development of therapeutics requires an appropriate disease modeling system. Primary cells from affected tissues of these patients are required for better understanding of the pathogenic processes and complex features involved with this disease. However, their use is usually limited to patient-derived lymphocytes and/or fibroblasts, which are difficult to propagate in culture for extended periods of time. Thus, regenerative medicine such as autologous cell transplantation could be used as a therapeutic strategy for WS, which provides cells with high proliferative ability and differentiation potential in large quantities over a long period.

The skin, composed of epidermis and dermis, is one of the main affected tissues in WS. A recent study demonstrated that hESCs can generate a homogeneous population of epithelial cells expressing postnatal keratinocyte markers in squamous epithelia, and these hESC-derived keratinocytes could reconstitute a functional pluri-stratified epithelium (Guenou et al., 2009). On the other hand, human epidermal keratinocytes can reconstitute stratified epithelium in culture, and it is known that expanded culture of epidermal stem cells from a tiny skin biopsy can cover the whole body surface of an individual, because of the high proliferative potential of these cells (De Luca et al., 2006), thus raising the argument as to whether pluripotent cell-derived epithelium can be used for clinical purposes (Pellegrini and Luca, 2009). In the case of WS, as the regenerative potential of adult skin cells is expected to be hampered due to their impaired proliferative ability, the concerns over premature senescence phenotype in cells from these patients might be eliminated by the development of rejuvenated resources. Patient-specific iPSCs might be a potential candidate that can meet these requirements. The epoch-making invention of iPSCs has the potential to bring innovation to regenerative medicine as well as drug discovery, as these cells are known to possess the ability to differentiate into all cell types, including those belonging to the skin, hair root, blood vessel, bone, and pancreatic islets.

GENERATION OF iPSCs FROM WS PATIENT CELLS

During the reprogramming process, both telomere and telomerase play protective roles at chromosomal ends against DNA damage responses, causing a reprogramming barrier (Marion et al., 2009a,b). Fibroblasts from patients with WS exhibit premature senescence caused by accelerated telomere loss during DNA replication (Salk et al., 1981b; Crabbe et al., 2007). Thus, it is interesting to note that forced expression of the telomerase catalytic gene hTERT in WS fibroblasts bypassed the phenotype, raising the question as to whether WS fibroblasts can be reprogrammed into iPSCs. In addition, there are doubts as to whether WS iPSCs, if successfully generated, can maintain hESC-like characteristics during long-term culture. Inconsistent consequences of the generation of patient-specific iPSCs from dyskeratosis congenita (DKC), another disease involving telomere abnormalities, have been reported (Agarwal et al., 2010; Batista et al., 2011). Batista et al. demonstrated that DKC iPSCs presented with progressive telomere shortening and loss of self-renewal ability in long-term culture (Batista et al., 2011). Therefore, it is important to evaluate the properties of iPSCs derived from the cells of the patient with telomere dysfunction over the long term. The findings from a recent study by Cheung et al. demonstrating the successful generation of disease-specific iPSCs from cells of patients with WS were in accordance with one of our current works, leading us to believe that reprogramming repressed premature senescence phenotypes in WS cells (Cheung et al., 2014; Shimamoto et al., 2014).

WS iPSCs were generated from the patient's fibroblasts and were quite similar to normal iPSCs in their characteristics as pluripotent stem cells, including their hESC-like morphology, expression of pluripotency genes, and hESC-specific surface markers, global gene expression profiles, embryoid body (EB) formation and subsequent differentiation into three embryonic germ layers, and teratoma formation. The WS iPSCs maintained their telomeres with reactivation of endogenous telomerase by induction of hTERT as well as other components of telomerase, such as TERC and DKC1, and were sustained in culture for more than 35 (Cheung et al., 2014) and 150 passages (Shimamoto et al., 2014) without morphological changes and loss of growth capacity. These observations indicate that induction levels of telomerase activity during reprogramming are sufficient for generation and subsequent cloning and maintenance of iPSCs from WS fibroblasts (**Figure 4**).

REPROGRAMMING SUPPRESSES PREMATURE SENESCENCE PHENOTYPES AND GENOMIC INSTABILITY OF WS FIBROBLASTS

Expression levels of senescence-associated genes including the CDKIs as well as the SASP factors, were compared between WS fibroblasts and WS iPSCs, because it is widely accepted that age-associated inflammatory responses, including SASP, contribute to human aging mechanisms (Goto, 2008). The results demonstrated that in addition to the CDKI genes $p21^{Cip1/Waf1}$ and $p16^{INK4A}$, the SASP genes such as *IL-6*, *gp130*, *IGFBP5*, *IGFBP7*, *ANGPTL2*, and *TIMP1* were highly expressed in cells of patients with WS as compared with PDL-matched normal fibroblasts. However, the expression levels of the same genes were suppressed in their iPSC derivatives to the level generally seen in normal iPSCs. These observations revealed that reprogramming suppresses and rejuvenates the premature aging phenotypes of WS fibroblasts (**Figure 4**; Shimamoto et al., 2014).

WS is characterized by genomic instability and chromosomal aberrations, including translocations, inversions, and deletions that have been observed during culture of patient-derived cells (Salk et al., 1981a). As the generation and subsequent maintenance of iPSCs involve extensive cell division, WS iPSCs may acquire additional chromosomal abnormalities during the process. In one of our recent works, we performed a chromosomal profiling analysis which indicated karyotype stability in WS iPSCs in long-term culture (Shimamoto et al., 2014). We performed G-banding stain and multicolor fluorescence *in situ* hybridization, and showed that 3 of 6 WS iPSC clones had the same karyotypes as their parental cells after approximately 100 passages, suggesting that karyotypes of WS cells are stabilized following reprogramming (**Figure 4**; Shimamoto et al., 2014). Normal human iPSCs are known to acquire genomic instability with high incidence

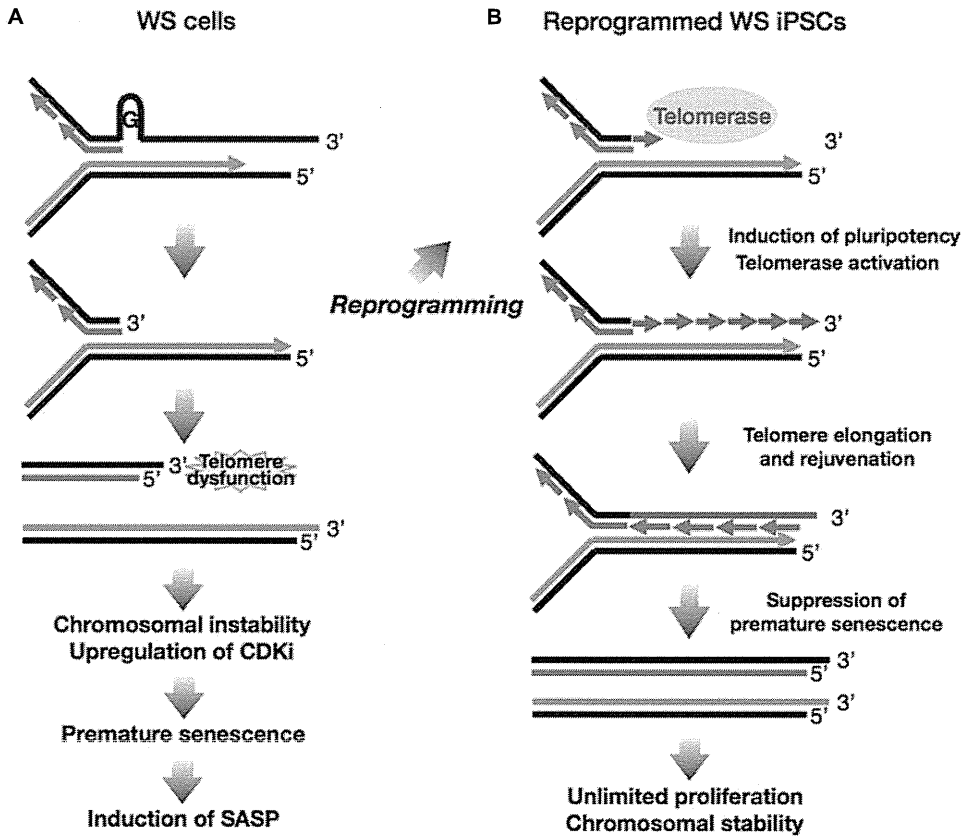

FIGURE 4 | Reprogramming suppresses premature senescence phenotypes. (A) Accelerated telomere shortening during replication induces telomere dysfunction and DNA damage response at the lagging telomere, leading to induction of premature senescence and SASP following chromosomal instability and upregulation of CDK inhibitors. **(B)** Reprogramming involves induction of pluripotency and global epigenetic alteration toward ESC-like state, which suppress senescence phenotypes including CDKi and SASP. Reprogramming also overcomes the lack of WRN through activation of telomerase, consequently inducing telomere elongation and cellular immortality.

of additions, deletions and translocations (Martins-Taylor et al., 2011; Taapken et al., 2011). Thus, given the genomic instability of WS cells, these data reveal the unexpected maintenance of chromosomal profiles in WS iPSC clones during long-term culture, indicating the possibility of its application clinically, although general risk factors, including genetic and epigenetic abnormalities (Gore et al., 2011; Hussein et al., 2011; Lister et al., 2011; Pera, 2011) and the potential for tumorigenicity (Kiuru et al., 2009; Ben-David and Benvenisty, 2011) and immunogenicity (Zhao et al., 2011; Araki et al., 2013) must be taken into consideration.

RECAPITULATION OF PREMATURE SENESCENCE PHENOTYPES IN DIFFERENTIATED CELLS

Differentiated cells including mesenchymal stem cells (MSCs) and other cell types derived from WS iPSCs were examined to determine their roles as models of WS, because the WS iPSCs do not exhibit any of the characteristic features of the syndrome. Cheung et al. demonstrated premature senescence of WS MSCs with elevated expression levels of p53, p21, and p16; accelerated telomere shortening; and impaired telomeric lagging-strand synthesis that causes telomere loss and dysfunction (Cheung et al., 2014). They also showed that WS iPSC-derived neural progenitor cells (NPCs) expressing telomerase activity maintained telomere and proliferative capacity with NPC phenotypes, and treatment with telomerase inhibitor was seen to decrease growth and increase the incidence of γH2AX in WS NPCs. These results, together with the fact that hTERT rescues premature senescence and telomere dysfunction, suggest that premature senescence in WS MSCs is due to insufficient levels of telomerase activity downregulated during differentiation (**Figure 5A**). Thus, adequate telomerase activity could maintain tissue stem cell function in WS (Cheung et al., 2014).

Differentiated cells derived from WS iPSC EBs were also examined for their growth defects. We found that the cells underwent premature senescence with a higher rate of SA-β-gal positive cells, upregulation of p21 concomitantly with downregulation of hTERT and induction of SASP genes (**Figure 5A**; Shimamoto et al., 2014). Since EB-derived differentiated cells include a variety of cell types originating from the three germ layers, these results suggest that EB-mediated iPSC differentiation could provide a simple and rapid method for the identification of cell lineages other than the MSCs in WS.

ATM, a causative gene for premature aging syndrome ataxia telangiectasia (AT) is required for the maintenance of stem cells

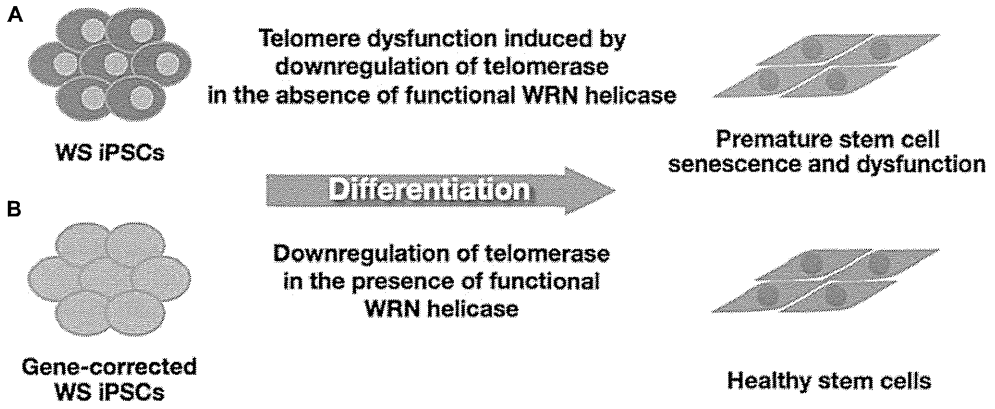

FIGURE 5 | Healthy stem cells from gene-corrected WS iPSCs. (A) WS iPSC-derived stem cells with insufficient levels of telomerase activity induce premature senescence and stem cell dysfunction. **(B)** Gene-corrected WS iPSCs can produce healthy stem cells by suppressing premature senescence due to functional WRN helicase.

including hematopoietic and spermatogonial stem cells (Ito et al., 2004; Takubo et al., 2008). Furthermore, the pathophysiology of AT might be associated with dysregulation of the reservoir of adult stem cell populations (Wong et al., 2003). Recent findings have shown dysfunction of vascular smooth muscle cells (VSMCs) and their progenitor SMCs in Hutchinson–Gilford Progeria syndrome (HGPS; Liu et al., 2011; Zhang et al., 2011). Taken together, these findings suggest that premature aging syndromes including WS, AT, and HGPS are stem cell dysfunction-associated diseases.

GENE-CORRECTED WS iPSCs AND ITS CLINICAL APPLICATION

Autologous cell transplantation can be selected as one of the therapeutic strategies for treating the intractable symptoms of WS, including skin ulcers. Clinical application of iPSCs requires unlimited proliferative ability and differentiation potential into various cell types with healthy conditions that could replace the affected area. Although WS iPSCs are almost indistinguishable from normal iPSCs in many aspects, differentiated cells from WS iPSCs manifest premature aging phenotypes (**Figure 5A**; Cheung et al., 2014; Shimamoto et al., 2014). Thus, gene-corrected WS iPSCs could offer a unique treatment strategy for patients with WS. Recent progress in gene therapy and genome engineering technology provides powerful tools for genome editing, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and RNA-guided engineered nucleases derived from the bacterial clustered regularly interspaced short palindromic repeat (CRISPR)-Cas system (Kim and Kim, 2014; Li et al., 2014). This technology could be used for correction of the disease-specific mutations in iPSCs by gene targeting (Yusa et al., 2011; Suzuki et al., 2014) and establishment of disease-specific iPSCs from wild-type iPSC lines (Soldner et al., 2011). Because WS is inherited in an autosomal recessive manner, a single gene-targeting event of specific mutations in WRN loci might be sufficient for recovery from WS, which could be confirmed by examining differentiated cells such as MSCs for the restoration of telomere dysfunction and premature growth defects (**Figure 5B**). In addition to the safety of iPSCs (Okano et al., 2013), evaluation of whole-genome sequencing and epigenomic analysis will be needed before their clinical application because WS patient cells are reported to have chromosomal aberrations including translocations, inversions, and deletions (Salk et al., 1981a). Furthermore, the differentiation potential and corrected phenotypes in gene-corrected WS iPSCs must be warranted for their clinical use. For example, reconstituted epithelium might be clinically applicable for skin ulcers after finding evidence that gene-corrected WS iPSC-derived keratinocytes could form functional pluri-stratified epithelium with fibroblast-containing fibrin dermal matrix *in vivo* (Guenou et al., 2009).

CONCLUSION

Recent findings including ours, demonstrate that reprogramming bypasses premature senescence and suppresses genomic instability in WS cells, leading to sustained undifferentiated states with the ability to differentiate into three embryonic germ layers over the long term. It is noteworthy that WS iPSCs exhibited stable chromosomal profiles, and this unexpected property might be achieved by the expression of the endogenous telomerase gene induced during reprogramming. As normal iPSCs exhibited higher expression levels of WRN protein than normal fibroblasts, WRN helicase might have a role in chromosomal stability as well as telomere maintenance in iPSCs. Thus, thorough safety tests, especially concerning genetic and epigenetic abnormalities and the potential for tumorigenicity, will be necessary before the clinical application of these cells. The use of WS iPSCs will enhance our understanding of the pathogenic processes and modeling of complex features associated with WS. In addition, it can provide opportunities for drug screening and the discovery and development of new strategies for the treatment this disease. Finally, although challenges and concerns remain regarding the general safety and risk of iPSCs as well as the WS-specific defects, a recent clinical trial using patient-specific iPSCs at the RIKEN Center for Developmental Biology (CDB) will encourage and promote stem cell research toward clinical application (Reardon and Cyranoski, 2014), at the same time, we need

to consider the results of the RIKEN CDB clinical trial in a calm manner.

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