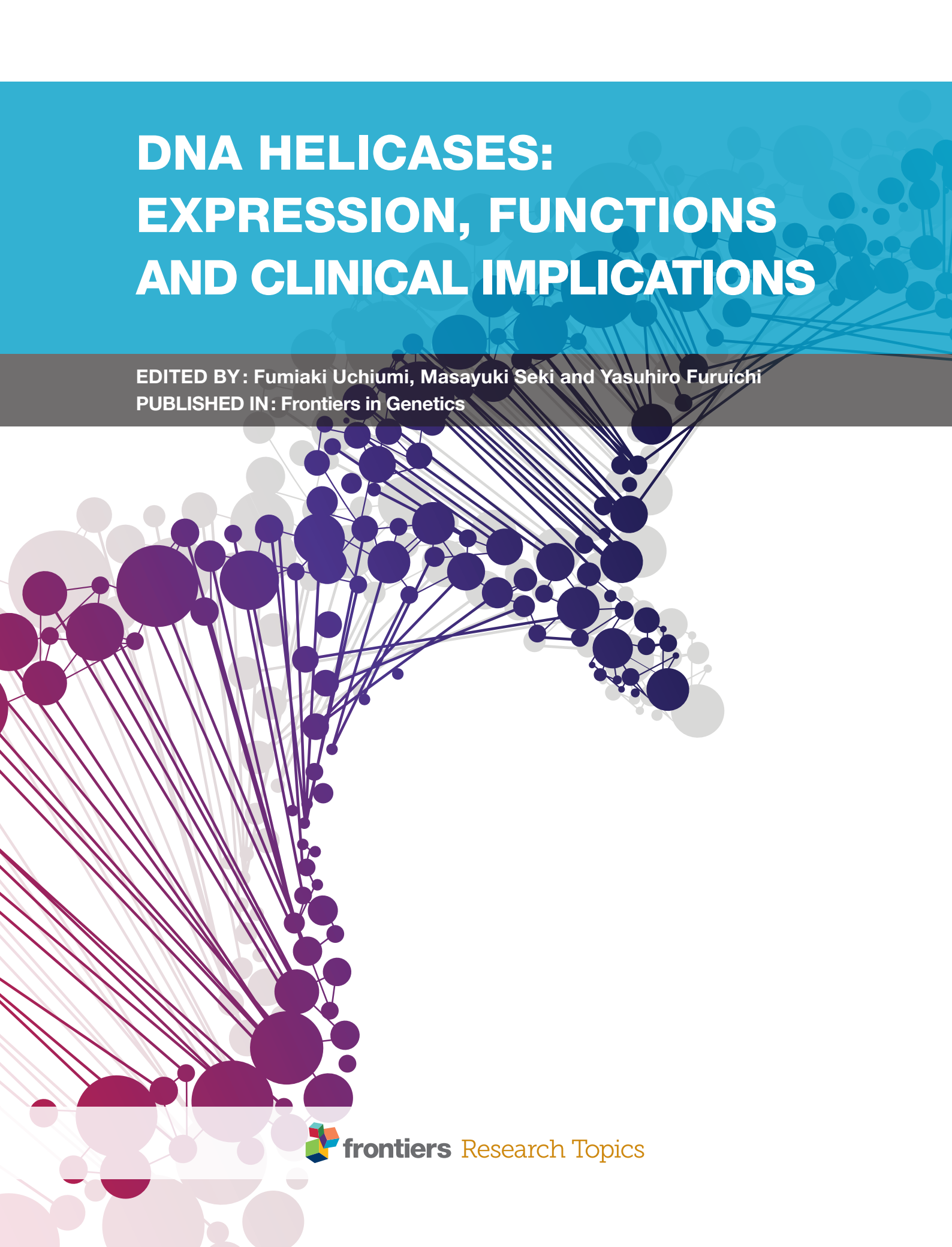
DNA HELICASES: EXPRESSION, FUNCTIONS AND CLINICAL IMPLICATIONS

EDITED BY: Fumiaki Uchiumi, Masayuki Seki and Yasuhiro Furuichi
PUBLISHED IN: Frontiers in Genetics



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88919-575-6
DOI 10.3389/978-2-88919-575-6

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

DNA HELICASES: EXPRESSION, FUNCTIONS AND CLINICAL IMPLICATIONS

Topic Editors:
Fumiaki Uchiumi, Tokyo University of Science, Japan
Masayuki Seki, Tohoku Pharmaceutical University, Japan
Yasuhiro Furuichi, Genecare Research Institute Co., Ltd., Japan

Helicases are the proteins that bind to double- or single-stranded DNA and/or RNA chains to unwind higher order structures, usually consuming energy from the hydrolysis of ATP molecules. The biological roles of helicases are associated with a variety of DNA and/or RNA metabolisms, including DNA-replication, -repair, -recombination, RNA processing, and transcription. Dysfunctions of helicases cause various diseases, such as xeroderma pigmentosum (XP), premature aging syndrome, cancer and immunodeficiency, in humans. Moreover, recent genetic analyses revealed that mutations in helicase-encoding genes are frequently found in patients of specific diseases. Some helicases regulate cellular senescence by controlling integrity of genomes, and others play a role in neuromuscular functions presumably by modulating processing of mRNAs. However, the molecular mechanisms of how helicases are regulated in order to maintain our health are not yet fully understood. In this research topic, we will focus on the expression and functions of helicases and their encoding genes, reviewing recent research progresses that provide new insights into development of clinical and pharmaceutical treatments targeting helicases.

Citation: Uchiumi, F., Seki, M., Furuichi, Y., eds. (2015). DNA Helicases: Expression, Functions and Clinical Implications. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-575-6

Table of Contents

frontiers in GENETICS

EDITORIAL
published: 12 February 2015
doi: 10.3389/fgene.2015.00039

Helicases and human diseases

Fumiaki Uchiumi[1]*, Masayuki Seki[2] and Yasuhiro Furuichi[3]

[1] *Department of Gene Regulation, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Japan*
[2] *Department of Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, Sendai, Japan*
[3] *GeneCare Research Institute Co., Ltd., Kamakura, Japan*
**Correspondence: uchiumi@rs.noda.tus.ac.jp*

Edited and reviewed by:
Blanka Rogina, University of Connecticut Health Center, USA

Keywords: helicasees, genetic diseases, RecQ helicases, *Fanconi Anemia*, premature aging, cancer, RNA helicases

Recent progress in pharmaceutical sciences has made it possible for us to live longer and longer. For example, antibiotics and vaccines have been developed that were successfully administered to patients with infectious diseases. A number of effective drugs for specific diseases could be purified from natural resources or created by chemical synthesis, and recent recombinant DNA technologies have brought about antibody-drugs. It seems increasingly possible that a treatment for every disease could be established in the near future. Nevertheless, prevention or remedies for inherited age-related diseases, including cancer, have not yet been completely established. However, recent progresses in human genetics and molecular biology revealed that premature aging is caused by mutations on DNA helicase encoding genes (Bernstein et al., 2010). These exciting findings have encouraged scientists to research mechanisms of the age-related diseases.

DNA/RNA helicases are enzymes that unwind DNA/DNA, DNA/RNA, and RNA/RNA duplexes to execute and regulate DNA replication, recombination, repair, and transcription (Patel and Donmez, 2006). To date, numerous genes have been identified to encode helicases. Importantly, genetic studies have revealed that mutations in some of these genes are associated with certain human diseases, including *Xeroderma Pigmentosum* (XP), *Cockayne Syndrome* (CS), and *Werner Syndrome* (WS) (Puzianowska-Kuznicka and Kuznicki, 2005). Given that helicases play an important role in the regulation and maintenance of chromosomal DNAs, it might not be so difficult to understand that their dysfunction leads to unfavorable states. Nuclear events, such as nucleotide excision repair (NER), transcription coupled repair (TCR), and telomere maintenance, are thought to be individually affected by XPB/XPD, CSA/CSB and WRN helicases, respectively (**Table 1**). Because epigenetic changes and disruption of chromosomal integrity have been strongly suggested to correlate with cellular senescence, these helicases may be important factors to regulate aging and age-related diseases.

Despite great efforts being made to elucidate the properties of helicases on a molecular and cellular level, it seems that the gap from molecule to patient is still distant. In this research topic, authors have described and discussed the forefront of the helicase studies. It is very important to establish a molecular model of how helicases interact with DNA repair machinery. In the research topic, the properties of the FANCJ (BRIP1) that affect cancer and *Fanconi Anemia* (FA) development have been summarized (Brosh and Cantor, 2014). In order to assess the mechanisms of diseases, including cancer, which are caused by dysfunctions of helicases, several approaches could be applied. Genetic and expression analyses of samples from patients will enable us to discuss the alterations in both the quality of DNA and the quantity of RNA. Therefore, diagnosis/prognosis of cancer or age-related diseases will be possible by analyzing the *RECQ1* (*RECQL*) gene expression (Sharma, 2014). Based on the concept that helicases play important roles in the maintenance of chromosomal DNAs, novel therapeutics will be applicable for cancer therapy with siRNAs

Table 1 | Helicases that associate with human diseases.

Helicase (*GENE ID*)	Disease	References
BLM (*BLM*)	BS[a,b]	Ellis et al., 1995
CSA (*ERCC8*), CSB (*ERCC6*)	CS[a,d]	Henning et al., 1995
DDX11 (*DDX11*)	Warsaw breakage syndrome[d]	van der Lelij et al., 2010
FANCJ (*BRIP1*)	FA[b,c]	Levitus et al., 2005
IGHMBP2 (*IGHMBP2*)	SMARD1[d], CMT2[d]	Grohmann et al., 2001; Cottenie et al., 2014
IFIH1 (*IFIH1*)	SLE[e]	Robinson et al., 2011
MCM4 (*MCM4*)	NKGCD, cancer	Hughes et al., 2012; Jackson et al., 2014
RECQ1/RECQL1 (*RECQL*)	Cancer	Sharma and Brosh, 2008
RECQL4 (*RECQL4*)	RTS[a,b]	Kitao et al., 1999
RTEL1 (*RTEL1*)	HHS[b,c,f]	Ballew et al., 2013
SETX (*SETX*)	ALS4[d]	Chen et al., 2004
TWINKLE (*c10orf2*)	MDS7[d]	Spelbrink et al., 2001
WRN (*WRN*)	WS[a,b,f]	Oshima et al., 1996
XPB (*ERCC3*), XPD (*ERCC2*)	XP[b], CS[a,d]	Sung et al., 1993; Hwang et al., 1996

[a] *Premature aging.*
[b] *Cancer or risk of cancer.*
[c] *Bone marrow failure.*
[d] *Impaired development of nervous system or deficiencies in neuromuscular junctions.*
[e] *Autoimmune disease.*
[f] *Telomere shortening.*
ALS, amyotrophic lateral sclerosis; BS, Bloom syndrome; CMT, Charcot-Marie-Tooth disease; CS, Cockayne syndrome; FA, Fanconi anemia; HHS, Hoyeraal Hreidarsson syndrome (Dyskeratosis congenita); MDS, Mitochondrial DNA depletion syndrome; NKGCD, Natural killer cell and glucocorticoid deficiency with DNA repair defect; SLE, systemic lupus erythematosus; RTS, Rothmund-Thomson syndrome; SMARD1, spinal muscular atrophy with respiratory distress type 1; WS, Werner syndrome; XP, Xeroderma pigmentosum.

of the RECQL1 (RECQL) and WRN DNA helicase-encoding genes (Futami and Furuichi, 2015). The therapy is supported by experimental results showing that siRNA of the *RECQL* could be effectively applied for ovarian cancer treatment by inducing apoptosis (Matsushita et al., 2014). Structural analyses of the helicase protein molecules will provide their precise function in the process of DNA repair. The precise molecular structure models of the WRN and BLM helicases will contribute for a development of rational design of specific drugs to prevent aging and cancer (Kitano, 2014). Moreover, establishment of iPSCs from helicase deficient cells will contribute to the clinical tests to develop novel drugs that delay aging and age-related diseases (Shimamoto et al., 2015). Furthermore, studies on RNA helicases, especially those that are involved in immune responses, will contribute to developing strategies against viral infections. It was shown that DDX3 could be a novel therapeutic target for HIV-1 and HCV replication (Ariumi, 2014). Importantly, IFIH1, which controls anti-viral responses, will be a molecular target of diagnosis and treatment for systemic lupus erythematosus (SLE) (Oliveira et al., 2014). All these articles provide new insights into the molecular pathology of the helicase-associated diseases. Further studies on various helicases will not only contribute to diagnoses and treatment of specific diseases (**Table 1**) but also to prevention and next generation-therapeutics on cancer and age-related diseases.

REFERENCES

Ariumi, Y. (2014). Multiple functions of DDX3 RNA helicase in gene regulation, tumoligenesis, and viral infection. *Front. Genet.* 5:423. doi: 10.3389/fgene.2014.00423

Ballew, B. J., Yeager, M., Jacobs, K., Giri, N., Boland, J., Burdett, L., et al. (2013). Germline mutations of regulator of telomere elongation helicase 1, RTEL1, in dyskeratosis congenita. *Hum. Genet.* 132, 473–480. doi: 10.1007/s00439-013-1265-8

Bernstein, K. A., Gangloff, S., and Rothstein, R. (2010). The RecQ DNA helicases in DNA repair. *Annu. Rev. Genet.* 44, 393–417. doi: 10.1146/annurev-genet-102209-163602

Brosh, R. M. Jr., and Cantor, S. B. (2014). Molecular and cellular functions of the FANCJ DNA helicase defective in cancer and in *Fanconi anemia*. *Front. Genet.* 5:372. doi: 10.3389/fgene.2014.00372

Chen, Y. Z., Bennett, C. L., Huynh, H. M., Blair, I. P., Puls, I., Irobi, J., et al. (2004). DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am. J. Hum. Genet.* 74, 1128–1135. doi: 10.1086/421054

Cottenie, E., Kochanski, A., Jordanova, A., Bansagi, B., Zimon, M., Horga, A., et al. (2014). Truncating and missense mutations in IGHMBP2 cause Charcot-Marie tooth disease type 2. *Am. J. Hum. Genet.* 95, 590–601. doi: 10.1016/j.ajhg.2014.10.002

Ellis, N. A., Groden, J., Ye, T. Z., Straughen, J., Lennon, D. J., Ciocci, S., et al. (1995). The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83, 655–666. doi: 10.1016/0092-8674(95)90105-1

Futami, K., and Furuichi, Y. (2015). RECQL1 and WRN DNA repair helicases: potential therapeutic targets and proliferative markers against cancers. *Front. Genet.* 5:441. doi: 10.3389/fgene.2014.00441

Grohmann, K., Schuelke, M., Diers, A., Hoffmann, K., Lucke, B., Adams, C., et al. (2001). Mutations in the gene encoding immunoglobulin mu-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. *Nat. Genet.* 29, 75–77. doi: 10.1038/ng703

Henning, K. A., Li, L., Iyer, N., McDaniel, L. D., Reagan, M. S., Legerski, R., et al. (1995). The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. *Cell* 82, 555–564. doi: 10.1016/0092-8674(95)90028-4

Hughes, C. R., Guasti, L., Meimaridou, E., Chuang, C.-H., Schimenti, J. C., King, P. J., et al. (2012). MCM4 mutation causes adrenal failure, short stature, and natural killer cell deficiency in humans. *J. Clin. Invest.* 122, 814–820. doi: 10.1172/JCI60224

Hwang, J. R., Moncollin, V., Vermeulen, W., Seroz, T., van Vuuren, H., Hoeijmakers, J. H., et al. (1996). A 3′ –> 5′ XPB helicase defect in repair/transcription factor TFIIH of xeroderma pigmentosum group B affects both DNA repair and transcription. *J. Biol. Chem.* 271, 15898–15904. doi: 10.1074/jbc.271.27.15898

Jackson, A. P., Laskey, R. A., and Coleman, N. (2014). "Replication proteins and human disease," in *DNA Replication*, eds S. D. Bell, M. Mechali, and M. L. DePamphilis (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), 327–342.

Kitano, K. (2014). Structural mechanisms of human RecQ helicases WRN and BLM. *Front. Genet.* 5:366. doi: 10.3389/fgene.2014.00366

Kitao, S., Shimamoto, A., Goto, M., Miller, R. W., Smithson, W. A., Lindor, N. M., et al. (1999). Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* 22, 82–84. doi: 10.1038/8788

Levitus, M., Waisfisz, Q., Godthelp, B. C., de Vries, Y., Hussain, S., Wiegant, W. W., et al. (2005). The DNA helicase BRIP1 is defective in *Fanconi anemia* complementation group J. *Nat. Genet.* 37, 934–935. doi: 10.1038/ng1625

Matsushita, Y., Yokoyama, Y., Yoshida, H., Osawa, Y., Mizunuma, M., Shigeto, T., et al. (2014). The level of RECQL1 expression is a prognostic factor for epithelial ovarian cancer. *J. Ovarian. Res.* 7:107. doi: 10.1186/s13048-014-0107-1

Oliveira, L., Sinicato, N. A., Postal, M., Appenzeller, S., and Niewold, T. B. (2014). Dysregulation of antiviral helicase pathways in systemic lupus erythematosus. *Front. Genet.* 5:418. doi: 10.3389/fgene.2014.00418

Oshima, J., Yu, C. E., 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, 1909–1913. doi: 10.1093/hmg/5.12.1909

Patel, S. S., and Donmez, I. (2006). Mechanisms of helicases. *J. Biol. Chem.* 281, 18265–18268. doi: 10.1074/jbc.R600008200

Puzianowska-Kuznicka, M., and Kuznicki, J. (2005). Genetic alterations in accelerated ageing syndromes. Do they play a role in natural ageing? *Int. J. Biochem. Cell Biol.* 37, 947–960. doi: 10.1016/j.biocel.2004.10.011

Robinson, T., Kariuki, S.N., Franek, B.S., Kumabe, M., Kumar, A.A., Badaracco, M., et al. (2011). Autoimmune disease risk variant of IFIH1 is associated with increased sensitivity to IFN-α and serologic autoimmunity in lupus patients. *J. Immunol.* 187, 1298–1303. doi: 10.4049/jimmunol.1100857

Sharma, S. (2014). An appraisal of RECQ1 expression in cancer progression. *Front. Genet.* 5:426. doi: 10.3389/fgene.2014.00426

Sharma, S., and Brosh, R. M. Jr. (2008). Unique and important consequences of RECQ1 deficiency in mammalian cells. *Cell Cycle* 7, 989–1000. doi: 10.4161/cc.7.8.5707

Shimamoto, A., Yokote, K., and Tahara, H. (2015). Werner syndrome-specific induced pluripotent stem cells: recovery of telomere function by reprogramming. *Front. Genet.* 6:10. doi: 10.3389/fgene.2015.00010

Spelbrink, J. N., Li, F. Y., Tiranti, V., Nikali, K., Yuan, Q. P., Tariq, M., et al. (2001). Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat. Genet.* 28, 223–231. doi: 10.1038/90058

Sung, P., Bailly, V., Weber, C., Thompson, L. H., Prakash, L., and Prakash, S. (1993). Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature* 365, 852–855. doi: 10.1038/365852a0

van der Lelij, P., Chrzanowska, K. H., Godthelp, B. C., Rooimans, M. A., Oostra, A. B., Stumm, M., et al. (2010). Warsaw breakage syndrome, a cohesinopathy associated with mutations in the XPD helicase family member DDX11/ChIR1. *Am. J. Hum. Genet.* 86, 262–266. doi: 10.1016/j.ajhg.2010.01.008

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 January 2015; accepted: 26 January 2015; published online: 12 February 2015.

Citation: Uchiumi F, Seki M and Furuichi Y (2015) Helicases and human diseases. Front. Genet. ***6****:39. doi: 10.3389/fgene.2015.00039*

This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.

frontiers in
GENETICS

REVIEW ARTICLE
published: 29 January 2015
doi: 10.3389/fgene.2015.00010

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.

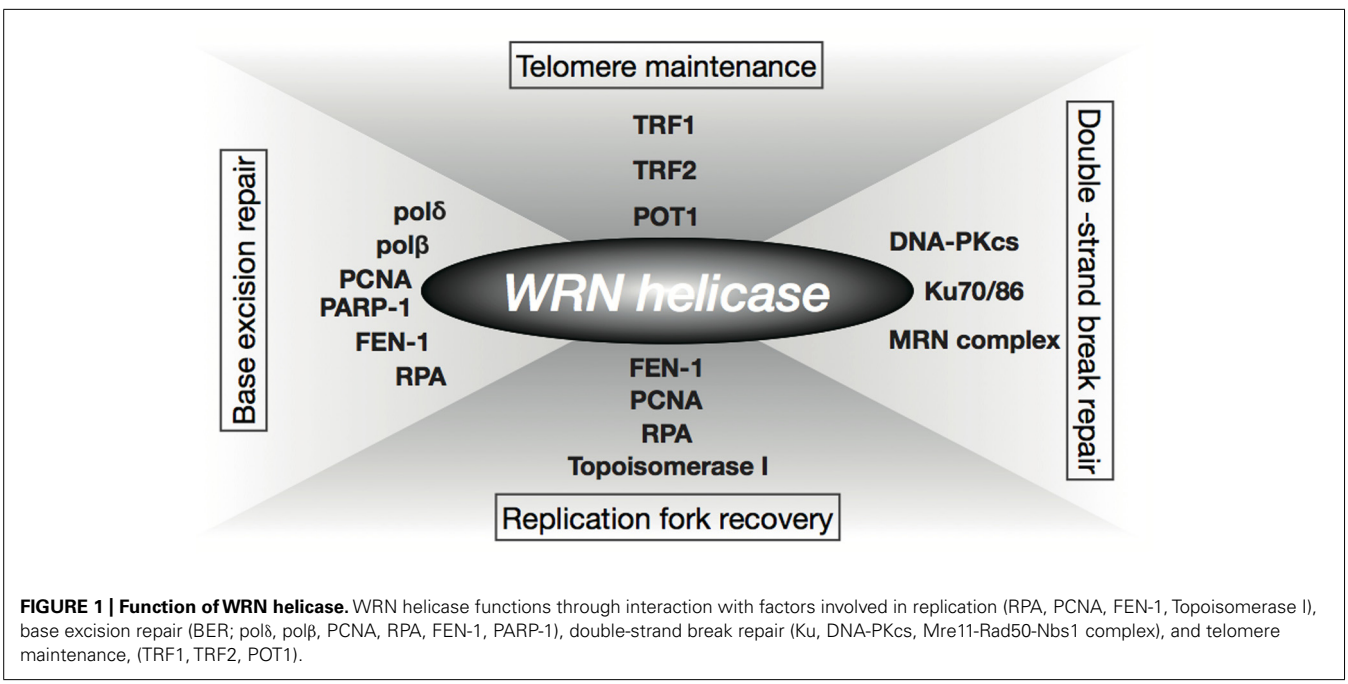


FIGURE 1 | Function of WRN helicase. WRN helicase functions through interaction with factors involved in replication (RPA, PCNA, FEN-1, Topoisomerase I), base excision repair (BER; polδ, polβ, PCNA, RPA, FEN-1, PARP-1), double-strand break repair (Ku, DNA-PKcs, Mre11-Rad50-Nbs1 complex), and telomere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

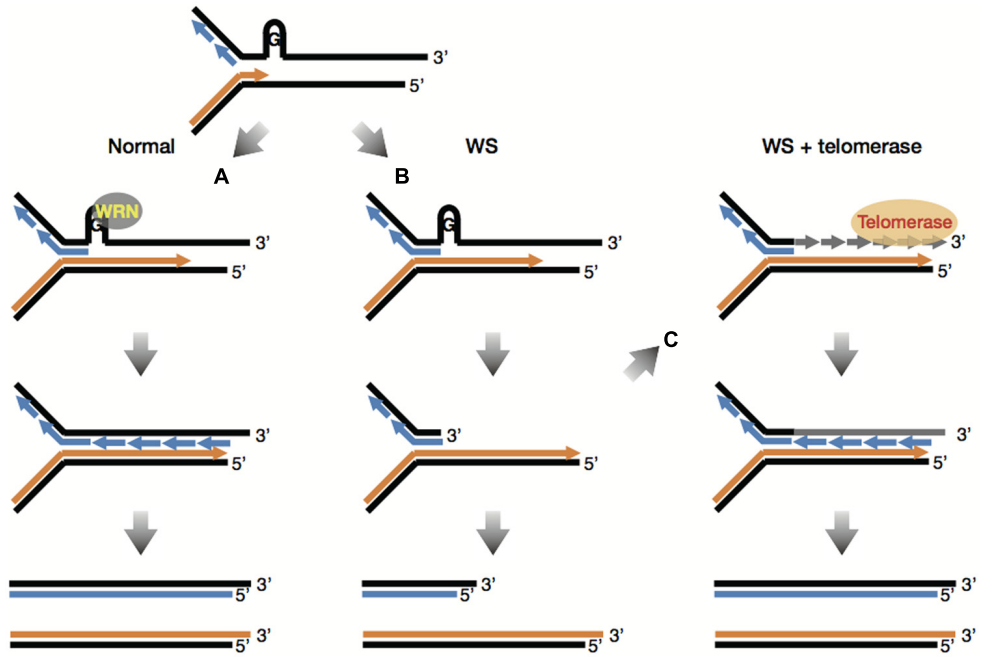


FIGURE 2 | Telomerase bypasses premature replicative senescence in WS fibroblasts. **(A)** G-quadruplexes at the lagging telomere are normally unwound by WRN helicase, making it possible to complete replication of lagging strand G-rich telomeres. **(B)** The absence of WRN causes stalled replication 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

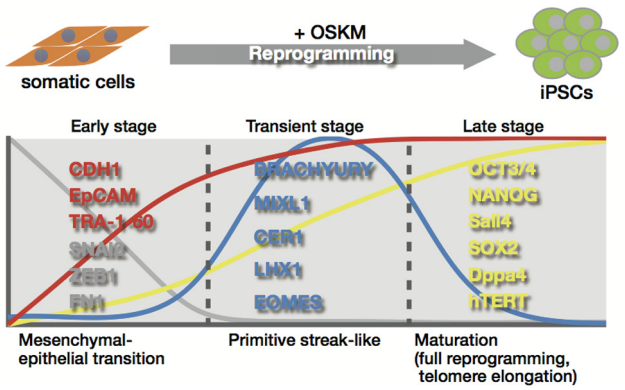


FIGURE 3 | Telomere rejuvenation by reprogramming. The reprogramming process is divided into three distinct stages; early stage, transient stage, and late stage. Telomerase activation and telomere elongation occur in the late stage by induction of telomerase reverse transcriptase subunit and the telomerase 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

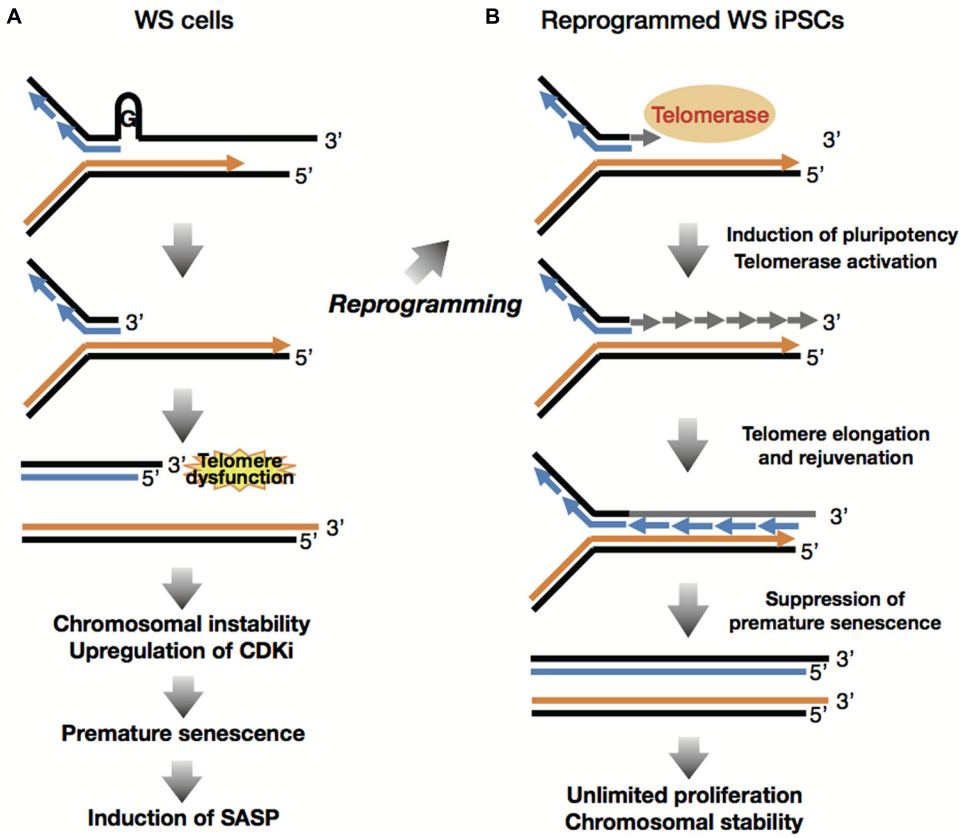


FIGURE 4 | Reprogramming suppresses premature senescence phenotypes. (A) Accelerated telomere shortening during replication induces telomere dysfunction and DNA damage response at the lagging telomere, leading to induction of premature senescence and SASP following chromosomal instability and upregulation 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

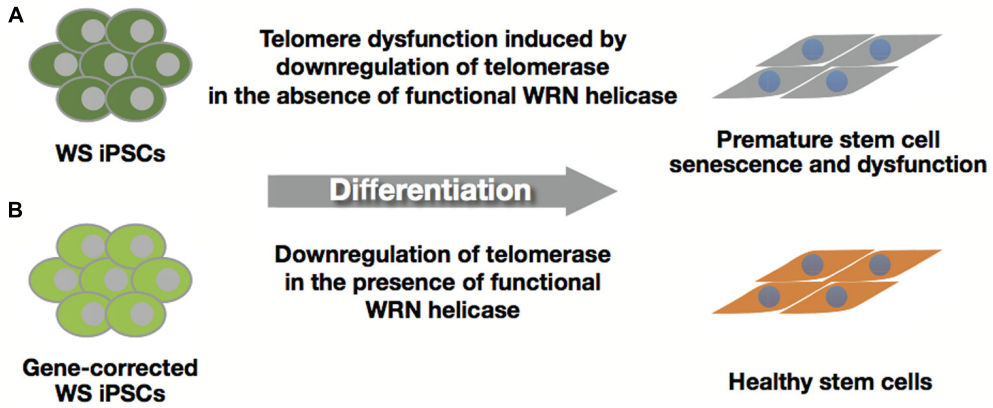


FIGURE 5 | Healthy stem cells from gene-corrected WS iPSCs. (A) WS iPSC-derived stem cells with insufficient levels of telomerase activity induce premature senescence and stem cell dysfunction. **(B)** Gene-corrected WS iPSCs can produce healthy stem cells by suppressing premature senescence due to functional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

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Challenging Exploratory Research No. 25670030 (to Akira Shimamoto), for Scientific Research No. 26115002 on Innovative Areas "Stem Cell Aging and Disease" (to Koutaro Yokote), and for Scientific Research No. 20014015 (to Hidetoshi Tahara) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This work was also supported by a Health and Labor Sciences Research Grant from the Ministry of Health Labor and Welfare of Japan (to Akira Shimamoto and Koutaro Yokote). The authors would like to thank Enago (www.enago.jp) for the English language review.

REFERENCES

Agarwal, S., Loh, Y. H., McLoughlin, E. M., Huang, J., Park, I. H., Miller, J. D., et al. (2010). Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. *Nature* 464, 292–296. doi: 10.1038/nature08792

Ahn, B., Harrigan, J. A., Indig, F. E., Wilson, D. M. III, and Bohr, V. A. (2004). Regulation of WRN helicase activity in human base excision repair. *J. Biol. Chem.* 279, 53465–53474. doi: 10.1074/jbc.M409624200

Allsopp, R. C., Cheshier, S., and Weissman, I. L. (2001). Telomere shortening accompanies increased cell cycle activity during serial transplantation of hematopoietic stem cells. *J. Exp. Med.* 193, 917–924. doi: 10.1084/jem.193.8.917

Allsopp, R. C., Morin, G. B., DePinho, R., Harley, C. B., and Weissman, I. L. (2003). Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood* 102, 517–520. doi: 10.1182/blood-2002-07-2334

Aoi, T., Yae, K., Nakagawa, M., Ichisaka, T., Okita, K., Takahashi, K., et al. (2008). Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 321, 699–702. doi: 10.1126/science.1154884

Apostolou, E., and Hochedlinger, K. (2013). Chromatin dynamics during cellular reprogramming. *Nature* 502, 462–471. doi: 10.1038/nature12749

Araki, R., Uda, M., Hoki, Y., Sunayama, M., Nakamura, M., Ando, S., et al. (2013). Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature* 494, 100–104. doi: 10.1038/nature11807

Batista, L. F., Pech, M. F., Zhong, F. L., Nguyen, H. N., Xie, K. T., Zaug, A. J., et al. (2011). Telomere shortening and loss of self-renewal in dyskeratosis congenita induced pluripotent stem cells. *Nature* 474, 399–402. doi: 10.1038/nature10084

Ben-David, U., and Benvenisty, N. (2011). The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat. Rev. Cancer* 11, 268–277. doi: 10.1038/nrc3034

Blasco, M. A. (2007). Telomere length, stem cells and aging. *Nat. Chem. Biol.* 3, 640–649. doi: 10.1038/nchembio.2007.38

Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., et al. (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349–352. doi: 10.1126/science.279.5349.349

Bohr, V. A. (2008). Rising from the RecQ-age: the role of human RecQ helicases in genome maintenance. *Trends Biochem. Sci.* 33, 609–620. doi: 10.1016/j.tibs.2008.09.003

Brem, H., and Lyder, C. (2004). Protocol for the successful treatment of pressure ulcers. *Am. J. Surg.* 188, 9–17. doi: 10.1016/S0002-9610(03)00285-X

Brouilette, S. W., Moore, J. S., McMahon, A. D., Thompson, J. R., Ford, I., Shepherd, J., et al. (2007). Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. *Lancet* 369, 107–114. doi: 10.1016/S0140-6736(07)60071-3

Buganim, Y., Faddah, D. A., Cheng, A. W., Itskovich, E., Markoulaki, S., Ganz, K., et al. (2012). Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. *Cell* 150, 1209–1222. doi: 10.1016/j.cell.2012.08.023

Buganim, Y., Faddah, D. A., and Jaenisch, R. (2013). Mechanisms and models of somatic cell reprogramming. *Nat. Rev. Genet.* 14, 427–439. doi: 10.1038/nrg3473

Chan, S. R., and Blackburn, E. H. (2004). Telomeres and telomerase. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 359, 109–121. doi: 10.1098/rstb.2003.1370

Chang, J. T., Chen, Y. L., Yang, H. T., Chen, C. Y., and Cheng, A. J. (2002). Differential regulation of telomerase activity by six telomerase subunits. *Eur. J. Biochem.* 269, 3442–3450. doi: 10.1046/j.1432-1033.2002.03025.x

Cheung, H. H., Liu, X., Canterel-Thouennon, L., Li, L., Edmonson, C., and Rennert, O. M. (2014). Telomerase protects werner syndrome lineage-specific stem cells from premature aging. *Stem Cell Reports* 2, 534–546. doi: 10.1016/j.stemcr.2014.02.006

Crabbe, L., Jauch, A., Naeger, C. M., Holtgreve-Grez, H., and Karlseder, J. (2007). Telomere dysfunction as a cause of genomic instability in Werner syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2205–2210. doi: 10.1073/pnas.0609410104

Crabbe, L., Verdun, R. E., Haggblom, C. I., and Karlseder, J. (2004). Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. *Science* 306, 1951–1953. doi: 10.1126/science.1103619

d'Adda di Fagagna, F., Reaper, P. M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., et al. (2003). A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426, 194–198. doi: 10.1038/nature02118

David, L., and Polo, J. M. (2014). Phases of reprogramming. *Stem Cell Res.* 12, 754–761. doi: 10.1016/j.scr.2014.03.007

Davis, T., Baird, D. M., Haughton, M. F., Jones, C. J., and Kipling, D. (2005). Prevention of accelerated cell aging in Werner syndrome using a p38 mitogen-activated protein kinase inhibitor. *J. Gerontol. A Biol. Sci. Med. Sci.* 60, 1386–1393. doi: 10.1093/gerona/60.11.1386

Davis, T., and Kipling, D. (2006). Werner Syndrome as an example of inflamm-aging: possible therapeutic opportunities for a progeroid syndrome? *Rejuvenation Res.* 9, 402–407. doi: 10.1089/rej.2006.9.402

De Luca, M., Pellegrini, G., and Green, H. (2006). Regeneration of squamous epithelia from stem cells of cultured grafts. *Regen. Med.* 1, 45–57. doi: 10.2217/17460751.1.1.45

Deng, Y., Chan, S. S., and Chang, S. (2008). Telomere dysfunction and tumour suppression: the senescence connection. *Nat. Rev. Cancer* 8, 450–458. doi: 10.1038/nrc2393

Dunham, M. A., Neumann, A. A., Fasching, C. L., and Reddel, R. R. (2000). Telomere maintenance by recombination in human cells. *Nat. Genet.* 26, 447–450. doi: 10.1038/82586

Egan, E. D., and Collins, K. (2012). Biogenesis of telomerase ribonucleoproteins. *RNA* 18, 1747–1759. doi: 10.1261/rna.034629.112

Ellis, N. A., Groden, J., Ye, T. Z., Straughen, J., Lennon, D. J., Ciocci, S., et al. (1995). The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83, 655–666. doi: 10.1016/0092-8674(95)90105-1

Epstein, C. J., Martin, G. M., Schultz, A. L., and Motulsky, A. G. (1966). Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine (Baltimore)* 45, 177–221. doi: 10.1097/00005792-196605000-00001

Ferron, S., Mira, H., Franco, S., Cano-Jaimez, M., Bellmunt, E., Ramirez, C., et al. (2004). Telomere shortening and chromosomal instability abrogates proliferation of adult but not embryonic neural stem cells. *Development* 131, 4059–4070. doi: 10.1242/dev.01215

Flores, I., Benetti, R., and Blasco, M. A. (2006). Telomerase regulation and stem cell behaviour. *Curr. Opin. Cell Biol* 18, 254–260. doi: 10.1016/j.ceb.2006.03.003

Flores, I., Cayuela, M. L., and Blasco, M. A. (2005). Effects of telomerase and telomere length on epidermal stem cell behavior. *Science* 309, 1253–1256. doi: 10.1126/science.1115025

Freund, A., Patil, C. K., and Campisi, J. (2011). p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J.* 30, 1536–1548. doi: 10.1038/emboj.2011.69

Gebhart, E., Bauer, R., Raub, U., Schinzel, M., Ruprecht, K. W., and Jonas, J. B. (1988). Spontaneous and induced chromosomal instability in Werner syndrome. *Hum. Genet.* 80, 135–139. doi: 10.1007/BF00702855

Gensch, C., Clever, Y. P., Werner, C., Hanhoun, M., Bohm, M., and Laufs, U. (2007). The PPAR-gamma agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells. *Atherosclerosis* 192, 67–74. doi: 10.1016/j.atherosclerosis.2006.06.026

Golipour, A., David, L., Liu, Y., Jayakumaran, G., Hirsch, C. L., Trcka, D., et al. (2012). A late transition in somatic cell reprogramming requires regulators distinct from the pluripotency network. *Cell Stem Cell* 11, 769–782. doi: 10.1016/j.stem.2012.11.008

Gore, A., Li, Z., Fung, H. L., Young, J. E., Agarwal, S., Antosiewicz-Bourget, J., et al. (2011). Somatic coding mutations in human induced pluripotent stem cells. *Nature* 471, 63–67. doi: 10.1038/nature09805

Goto, M. (1997). Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. *Mech. Ageing Dev.* 98, 239–254. doi: 10.1016/S0047-6374(97)00111-5

Goto, M. (2000). Werner's syndrome: from clinics to genetics. *Clin. Exp. Rheumatol.* 18, 760–766.

Goto, M. (2008). Inflammaging (inflammation + aging): A driving force for human aging based on an evolutionarily antagonistic pleiotropy theory? *Biosci Trends* 2, 218–230.

Goto, M., Imamura, O., Kuromitsu, J., Matsumoto, T., Yamabe, Y., Tokutake, Y., et al. (1997). Analysis of helicase gene mutations in Japanese Werner's syndrome patients. *Hum. Genet.* 99, 191–193. doi: 10.1007/s004390050336

Goto, M., Ishikawa, Y., Sugimoto, M., and Furuichi, Y. (2013). Werner syndrome: a changing pattern of clinical manifestations in Japan (1917∼2008). *Biosci. Trends* 7, 13–22.

Guenou, H., Nissan, X., Larcher, F., Feteira, J., Lemaitre, G., Saidani, M., et al. (2009). Human embryonic stem-cell derivatives for full reconstruction of the pluristratified epidermis: a preclinical study. *Lancet* 374, 1745–1753. doi: 10.1016/S0140-6736(09)61496-3

Hanaoka, F., Takeuchi, F., Matsumura, T., Goto, M., Miyamoto, T., and Yamada, M. (1983). Decrease in the average size of replicons in a Werner syndrome cell line by Simian virus 40 infection. *Exp. Cell Res.* 144, 463–467. doi: 10.1016/0014-4827(83)90425-1

Haq, R., Brenton, J. D., Takahashi, M., Finan, D., Finkielsztein, A., Damaraju, S., et al. (2002). Constitutive p38HOG mitogen-activated protein kinase activation induces permanent cell cycle arrest and senescence. *Cancer Res.* 62, 5076–5082.

Harley, C. B., Futcher, A. B., and Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature* 345, 458–460. doi: 10.1038/345458a0

Herbig, U., Jobling, W. A., Chen, B. P., Chen, D. J., and Sedivy, J. M. (2004). Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol. Cell.* 14, 501–513. doi: 10.1016/S1097-2765(04)00256-4

Hoffmeyer, K., Raggioli, A., Rudloff, S., Anton, R., Hierholzer, A., Del Valle, I., et al. (2012). Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* 336, 1549–1554. doi: 10.1126/science.1218370

Hong, H., Takahashi, K., Ichisaka, T., Aoi, T., Kanagawa, O., Nakagawa, M., et al. (2009). Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature* 460, 1132–1135. doi: 10.1038/nature08235

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, 173–174. doi: 10.1111/j.1532-5415.2007.01484.x

Hussein, S. M., Batada, N. N., Vuoristo, S., Ching, R. W., Autio, R., Narva, E., et al. (2011). Copy number variation and selection during reprogramming to pluripotency. *Nature* 471, 58–62. doi: 10.1038/nature09871

Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., et al. (2004). Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* 431, 997–1002. doi: 10.1038/nature02989

Iwasa, H., Han, J., and Ishikawa, F. (2003). Mitogen-activated protein kinase p38 defines the common senescence-signalling pathway. *Genes Cells* 8, 131–144. doi: 10.1046/j.1365-2443.2003.00620.x

Ji, G., Ruan, W., Liu, K., Wang, F., Sakellariou, D., Chen, J., et al. (2013). Telomere reprogramming and maintenance in porcine iPS cells. *PLoS ONE* 8:e74202. doi: 10.1371/journal.pone.0074202

Jiang, X. R., Jimenez, G., Chang, E., Frolkis, M., Kusler, B., Sage, M., et al. (1999). Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. *Nat. Genet.* 21, 111–114. doi: 10.1038/5056

Kawamura, T., Suzuki, J., Wang, Y. V., Menendez, S., Morera, L. B., Raya, A., et al. (2009). Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* 460, 1140–1144. doi: 10.1038/nature08311

Kim, H., and Kim, J. S. (2014). A guide to genome engineering with programmable nucleases. *Nat. Rev. Genet.* 15, 321–334. doi: 10.1038/nrg3686

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, e83. doi: 10.2337/dc12-1179

Kitao, S., Ohsugi, I., Ichikawa, K., Goto, M., Furuichi, Y., and Shimamoto, A. (1998). Cloning of two new human helicase genes of the RecQ family: biological significance of multiple species in higher eukaryotes. *Genomics* 54, 443–452. doi: 10.1006/geno.1998.5595

Kitao, S., Shimamoto, A., Goto, M., Miller, R. W., Smithson, W. A., Lindor, N. M., et al. (1999). Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* 22, 82–84. doi: 10.1038/8788

Kiuru, M., Boyer, J. L., O'Connor, T. P., and Crystal, R. G. (2009). Genetic control of wayward pluripotent stem cells and their progeny after transplantation. *Cell Stem Cell* 4, 289–300. doi: 10.1016/j.stem.2009.03.010

Kobayashi, J., Murano, S., Yokote, K., Mori, S., Matsunaga, A., Sasaki, J., et al. (2000). Marked decrease in plasma apolipoprotein A-I and high density lipoprotein-cholesterol in a case with Werner syndrome. *Clin. Chim. Acta* 293, 63–73. doi: 10.1016/S0009-8981(99)00219-3

Kuilman, T., Michaloglou, C., Mooi, W. J., and Peeper, D. S. (2010). The essence of senescence. *Genes Dev.* 24, 2463–2479. doi: 10.1101/gad.1971610

Kumar, S., Vinci, J. M., Millis, A. J., and Baglioni, C. (1993). Expression of interleukin-1 alpha and beta in early passage fibroblasts from aging individuals. *Exp. Gerontol.* 28, 505–513. doi: 10.1016/0531-5565(93)90039-G

Levy, M. Z., Allsopp, R. C., Futcher, A. B., Greider, C. W., and Harley, C. B. (1992). Telomere end-replication problem and cell aging. *J. Mol. Biol.* 225, 951–960. doi: 10.1016/0022-2836(92)90096-3

Li, H., Collado, M., Villasante, A., Strati, K., Ortega, S., Canamero, M., et al. (2009). The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature* 460, 1136–1139. doi: 10.1038/nature08290

Li, M., Suzuki, K., Kim, N. Y., Liu, G. H., and Izpisua Belmonte, J. C. (2014). A cut above the rest: targeted genome editing technologies in human pluripotent stem cells. *J. Biol. Chem.* 289, 4594–4599. doi: 10.1074/jbc.R113.488247

Lin, H. T., Otsu, M., and Nakauchi, H. (2013). Stem cell therapy: an exercise in patience and prudence. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368, 20110334. doi: 10.1098/rstb.2011.0334

Lister, R., Pelizzola, M., Kida, Y. S., Hawkins, R. D., Nery, J. R., Hon, G., et al. (2011). Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* 471, 68–73. doi: 10.1038/nature09798

Liu, G. H., Barkho, B. Z., Ruiz, S., Diep, D., Qu, J., Yang, S. L., et al. (2011). Recapitulation of premature ageing with iPSCs from Hutchinson-Gilford progeria syndrome. *Nature* 472, 221–225. doi: 10.1038/nature09879

Machwe, A., Xiao, L., Lloyd, R. G., Bolt, E., and Orren, D. K. (2007). Replication fork regression in vitro by the Werner syndrome protein (WRN): holliday junction formation, the effect of leading arm structure and a potential role for WRN exonuclease activity. *Nucleic Acids Res.* 35, 5729–5747. doi: 10.1093/nar/gkm561

Marion, R. M., Strati, K., Li, H., Murga, M., Blanco, R., Ortega, S., et al. (2009a). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* 460, 1149–1153. doi: 10.1038/nature08287

Marion, R. M., Strati, K., Li, H., Tejera, A., Schoeftner, S., Ortega, S., et al. (2009b). Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell* 4, 141–154. doi: 10.1016/j.stem.2008.12.010

Martins-Taylor, K., Nisler, B. S., Taapken, S. M., Compton, T., Crandall, L., Montgomery, K. D. et al. (2011). Recurrent copy number variations in human induced pluripotent stem cells. *Nat. Biotechnol.* 29, 488–491. doi: 10.1038/nbt.1890

Matsumoto, T., Imamura, O., Yamabe, Y., Kuromitsu, J., Tokutake, Y., Shimamoto, A., et al. (1997). Mutation and haplotype analyses of the Werner's syndrome gene based on its genomic structure: genetic epidemiology in the Japanese population. *Hum. Genet.* 100, 123–130. doi: 10.1007/s004390050477

Mikkelsen, T. S., Hanna, J., Zhang, X., Ku, M., Wernig, M., Schorderet, P., et al. (2008). Dissecting direct reprogramming through integrative genomic analysis. *Nature* 454, 49–55. doi: 10.1038/nature07056

Mohaghegh, P., Karow, J. K., Brosh, R. M., Jr., Bohr, V. A., and Hickson, I. D. (2001). The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases. *Nucleic Acids Res.* 29, 2843–2849. doi: 10.1093/nar/29.13.2843

Morales, C. P., Holt, S. E., Ouellette, M., Kaur, K. J., Yan, Y.,Wilson, K. S., et al. (1999). Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. *Nat. Genet.* 21, 115–118. doi: 10.1038/5063

Mori, S., Murano, S., Yokote, K., Takemoto, M., Asaumi, S., Take, A., et al. (2001). Enhanced intra-abdominal visceral fat accumulation in patients with Werner's syndrome. *Int. J. Obes. Relat. Metab. Disord.* 25, 292–295. doi: 10.1038/sj.ijo.0801529

Multani, A. S., and Chang, S. (2007). WRN at telomeres: implications for aging and cancer. *J. Cell Sci.* 120, 713–721. doi: 10.1242/jcs.03397

Murano, S., Nakazawa, A., Saito, I., Masuda, M., Morisaki, N., Akikusa, B., et al. (1997). Increased blood plasminogen activator inhibitor-1 and intercellular adhesion molecule-1 as possible risk factors of atherosclerosis in Werner syndrome. *Gerontology* 43(Suppl. 1), 43–52. doi: 10.1159/000213885

Nakayama, J., Tahara, H., Tahara, E., Saito, M., Ito, K., Nakamura, H., et al. (1998). Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. *Nat. Genet.* 18, 65–68. doi: 10.1038/ng0198-65

Niida, H., Matsumoto, T., Satoh, H., Shiwa, M., Tokutake, Y., Furuichi, Y., et al. (1998). Severe growth defect in mouse cells lacking the telomerase RNA component. *Nat. Genet.* 19, 203–206. doi: 10.1038/580

Niida, H., Shinkai, Y., Hande, M. P., Matsumoto, T., Takehara, S., Tachibana, M., et al. (2000). Telomere maintenance in telomerase-deficient mouse embryonic stem cells: characterization of an amplified telomeric DNA. *Mol. Cell. Biol.* 20, 4115–4127. doi: 10.1128/MCB.20.11.4115-4127.2000

Noda, S., Asano, Y., Masuda, S., Miyagawa, T., Sugita, M., Yamamoto, M., et al. (2011). Bosentan: a novel therapy for leg ulcers in Werner syndrome. *J. Am. Acad. Dermatol.* 65, e54–e55. doi: 10.1016/j.jaad.2011.03.013

O'Malley, J., Skylaki, S., Iwabuchi, K. A., Chantzoura, E., Ruetz, T., Johnsson, A., et al. (2013). High-resolution analysis with novel cell-surface markers identifies routes to iPS cells. *Nature* 499, 88–91. doi: 10.1038/nature12243

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, 997–998. doi: 10.1111/j.1532-5415.2012.03944.x

Okada, M., Goto, M., Furuichi, Y., and Sugimoto, M. (1998). Differential effects of cytotoxic drugs on mortal and immortalized B-lymphoblastoid cell lines from normal and Werner's syndrome patients. *Biol. Pharm. Bull.* 21, 235–239. doi: 10.1248/bpb.21.235

Okano, H., Nakamura, M., Yoshida, K., Okada, Y., Tsuji, O., Nori, S., et al. (2013). Steps toward safe cell therapy using induced pluripotent stem cells. *Circ. Res.* 112, 523–533. doi: 10.1161/CIRCRESAHA.111.256149

Okita, K., and Yamanaka, S. (2011). Induced pluripotent stem cells: opportunities and challenges. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 366, 2198–2207. doi: 10.1098/rstb.2011.0016

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. doi: 10.1007/s00592-012-0424-z

Opresko, P. L., 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, 763–774. doi: 10.1016/j.molcel.2004.05.023

Oshima, J., Yu, C. E., 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, 1909–1913. doi: 10.1093/hmg/5.12.1909

Palm, W., and de Lange, T. (2008). How shelterin protects mammalian telomeres. *Annu. Rev. Genet.* 42, 301–334. doi: 10.1146/annurev.genet.41.110306.130350

Papp, B., and Plath, K. (2013). Epigenetics of reprogramming to induced pluripotency. *Cell* 152, 1324–1343. doi: 10.1016/j.cell.2013.02.043

Pellegrini, G., and Luca, M. D. (2009). Human Embryonic Stem Cell-Derived Keratinocytes: how Close to Clinics? *Cell Stem Cell* 6, 8–9. doi: 10.1016/j.stem.2009.12.002

Pera, M. F. (2011). Stem cells: the dark side of induced pluripotency. *Nature* 471, 46–47. doi: 10.1038/471046a

Poot, M., Gollahon, K. A., and Rabinovitch, P. S. (1999). Werner syndrome lymphoblastoid cells are sensitive to camptothecin-induced apoptosis in S-phase. *Hum. Genet.* 104, 10–14. doi: 10.1007/s004390050903

Poot, M., Hoehn, H., Runger, T. M., and Martin, G. M. (1992). Impaired S-phase transit of Werner syndrome cells expressed in lymphoblastoid cell lines. *Exp. Cell Res.* 202, 267–273. doi: 10.1016/0014-4827(92)90074-I

Reardon, S., and Cyranoski, D. (2014). Japan stem-cell trial stirs envy. *Nature* 513, 287–288. doi: 10.1038/513287a

Robinton, D. A., and Daley, G. Q. (2012). The promise of induced pluripotent stem cells in research and therapy. *Nature* 481, 295–305. doi: 10.1038/nature10761

Rossi, M. L., Ghosh, A. K., and Bohr, V. A. (2010). Roles of Werner syndrome protein in protection of genome integrity. *DNA Repair (Amst.)* 9, 331–344. doi: 10.1016/j.dnarep.2009.12.011

Rubin, C. D., Zerwekh, J. E., Reed-Gitomer, B. Y., and Pak, C. Y. (1992). Characterization of osteoporosis in a patient with Werner's syndrome. *J. Am. Geriatr. Soc.* 40, 1161–1163.

Sakamoto, S., Nishikawa, K., Heo, S. J., Goto, M., Furuichi, Y., and Shimamoto, A., (2001). Werner helicase relocates into nuclear foci in response to DNA damaging agents and co-localizes with RPA and Rad51. *Genes Cells* 6, 421–430. doi: 10.1046/j.1365-2443.2001.00433.x

Salama, R., Sadaie, M., Hoare, M., and Narita, M. (2014). Cellular senescence and its effector programs. *Genes Dev.* 28, 99–114. doi: 10.1101/gad.235184.113

Salk, D., Au, K., Hoehn, H., and Martin, G. M. (1981a). Effects of radical-scavenging enzymes and reduced oxygen exposure on growth and chromosome abnormalities of Werner syndrome cultured skin fibroblasts. *Hum. Genet.* 57, 269–275. doi: 10.1007/BF00278942

Salk, D., Au, K., Hoehn, H., Stenchever, M. R., and Martin, G. M. (1981b). Evidence of clonal attenuation, clonal succession, and clonal expansion in mass cultures of aging Werner's syndrome skin fibroblasts. *Cytogenet. Cell Genet.* 30, 108–117. doi: 10.1159/000131597

Samavarchi-Tehrani, P., Golipour, A., David, L., Sung, H. K., Beyer, T. A., Datti, A., et al. (2010). Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell* 7, 64–77. doi: 10.1016/j.stem.2010.04.015

Seki, M., Miyazawa, H., Tada, S., Yanagisawa, J., Yamaoka, T., Hoshino, S., et al. (1994). Molecular cloning of cDNA encoding human DNA helicase Q1 which has homology to Escherichia coli Rec Q helicase and localization of the gene at chromosome 12p12. *Nucleic Acids Res.* 22, 4566–4573. doi: 10.1093/nar/22.22.4566

Sharma, S., Otterlei, M., Sommers, J. A., Driscoll, H. C., Dianov, G. L., Kao, H. I., et al. (2004). WRN helicase and FEN-1 form a complex upon replication arrest and together process branchmigrating DNA structures associated with the replication fork. *Mol. Biol. Cell* 15, 734–750. doi: 10.1091/mbc.E03-08-0567

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:e112900. doi: 10.1371/journal.pone.0112900

Shimamoto, A., Nishikawa, K., Kitao, S., and Furuichi, Y. (2000). Human RecQ5beta, a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3alpha and 3beta. *Nucleic Acids Res.* 28, 1647–1655. doi: 10.1093/nar/28.7.1647

Shimamoto, A., Sugimoto, M., and Furuichi, Y. (2004). Molecular biology of Werner syndrome. *Int. J. Clin. Oncol.* 9, 288–298. doi: 10.1007/s10147-004-0426-0

Soldner, F., Laganiere, J., Cheng, A. W., Hockemeyer, D., Gao, Q., Alagappan, R., et al. (2011). Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. *Cell* 146, 318–331. doi: 10.1016/j.cell.2011.06.019

Sorensen, J. L., Jorgensen, B., and Gottrup, F. (2004). Surgical treatment of pressure ulcers. *Am. J. Surg.* 188, 42–51. doi: 10.1016/S0002-9610(03)00290-3

Spyridopoulos, I., Haendeler, J., Urbich, C., Brummendorf, T. H., Oh, H., Schneider, M. D., et al. (2004). Statins enhance migratory capacity by upregulation of the telomere repeat-binding factor TRF2 in endothelial progenitor cells. *Circulation* 110, 3136–3142. doi: 10.1161/01.CIR.0000142866.50300.EB

Stadtfeld, M., and Hochedlinger, K. (2010). Induced pluripotency: history, mechanisms, and applications. *Genes Dev.* 24, 2239–2263. doi: 10.1101/gad.1963910

Stadtfeld, M., Maherali, N., Breault, D. T., and Hochedlinger, K. (2008). Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. *Cell Stem Cell* 2, 230–240. doi: 10.1016/j.stem.2008.02.001

Sugimoto, M. (2014). A cascade leading to premature aging phenotypes including abnormal tumor profiles in Werner syndrome (review). *Int. J. Mol. Med.* 33, 247–253. doi: 10.3892/ijmm.2013.1592

Suzuki, K., Yu, C., Qu, J., Li, M., Yao, X., Yuan, T., et al. (2014). Targeted gene correction minimally impacts whole-genome mutational load in human-disease-specific induced pluripotent stem cell clones. *Cell Stem Cell* 15, 31–36. doi: 10.1016/j.stem.2014.06.016

Taapken, S. M., Nisler, B. S., Newton, M. A., Sampsell-Barron, T. L., Leonhard, K. A., McIntire, E. M., et al. (2011). Karotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. *Nat. Biotechnol.* 29, 313–314. doi: 10.1038/nbt.1835

Tahara, H., Tokutake, Y., Maeda, S., Kataoka, H., Watanabe, T., Satoh, M., et al. (1997). Abnormal telomere dynamics of B-lymphoblastoid cell strains from

Werner's syndrome patients transformed by Epstein-Barr virus. *Oncogene* 15, 1911–1920. doi: 10.1038/sj.onc.1201377

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872. doi: 10.1016/j.cell.2007.11.019

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Sasaki, A., Yamamoto, M., et al. (2014). Induction of pluripotency in human somatic cells via a transient state resembling primitive streak-like mesendoderm. *Nat. Commun.* 5, 3678. doi: 10.1038/ncomms4678

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676. doi: 10.1016/j.cell.2006.07.024

Takahashi, K., and Yamanaka, S. (2013). Induced pluripotent stem cells in medicine and biology. *Development* 140, 2457–2461. doi: 10.1242/dev.092551

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, 475–481. doi: 10.1111/j.1447-0594.2012.00913.x

Takubo, K., Ohmura, M., Azuma, M., Nagamatsu, G., Yamada, W., Arai, F., et al. (2008). Stem cell defects in ATM-deficient undifferentiated spermatogonia through DNA damage-induced cell-cycle arrest. *Cell Stem Cell* 2, 170–182. doi: 10.1016/j.stem.2007.10.023

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145–1147. doi: 10.1126/science.282.5391.1145

Utikal, J., Polo, J. M., Stadtfeld, M., Maherali, N., Kulalert, W., and Walsh, R. M., et al. (2009). Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* 460, 1145–1148. doi: 10.1038/nature08285

Vaziri, H., and Benchimol, S. (1998). Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr. Biol.* 8, 279–282. doi: 10.1016/S0960-9822(98)70109-5

Vaziri, H., Dragowska, W., Allsopp, R. C., Thomas, T. E., Harley, C. B., and Lansdorp, P. M. (1994). Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc. Natl. Acad. Sci. U.S.A.* 91, 9857–9860. doi: 10.1073/pnas.91.21.9857

Wang, F., Yin, Y., Ye, X., Liu, K., Zhu, H., Wang, L., et al. (2012). Molecular insights into the heterogeneity of telomere reprogramming in induced pluripotent stem cells. *Cell Res.* 22, 757–768. doi: 10.1038/cr.2011.201

Wang, J., Xie, L. Y., Allan, S., Beach, D., and Hannon, G. J. (1998). Myc activates telomerase. *Genes Dev.* 12, 1769–1774. doi: 10.1101/gad.12.12.1769

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, e119. doi: 10.2337/dc13-0709

Wollina, U., Gruner, M., Koch, A., Kostler, E., Hubl, W., Hanson, N. B., et al. (2004). Topical PDGF-BB results in limited healing in a patient with Werner's syndrome and chronic leg ulcers. *J. Wound Care* 13, 415–416.

Wong, C. W., Hou, P. S., Tseng, S. F., Chien, C. L., Wu, K. J., Chen, H. F., et al. (2010). Kruppel-like transcription factor 4 contributes to maintenance of telomerase activity in stem cells. *Stem Cells* 28, 1510–1517. doi: 10.1002/stem.477

Wong, K. K., Maser, R. S., Bachoo, R. M., Menon, J., Carrasco, D. R., Gu, Y., et al. (2003). Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. *Nature* 421, 643–648. doi: 10.1038/nature01385

Wu, K. J., Grandori, C., Amacker, M., Simon-Vermot, N., Polack, A., Lingner, J., et al. (1999). Direct activation of TERT transcription by c-MYC. *Nat. Genet.* 21, 220–224. doi: 10.1038/6010

Wyllie, F. S., Jones, C. J., Skinner, J. W., Haughton, M. F., Wallis, C., Wynford-Thomas, D., et al. (2000). Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts. *Nat. Genet.* 24, 16–17. doi: 10.1038/71630

Yeong, E. K., and Yang, C. C. (2004). Chronic leg ulcers in Werner's syndrome. *Br. J. Plast. Surg.* 57, 86–88. doi: 10.1016/j.bjps.2003.10.011

Yokote, K., Hara, K., Mori, S., Kadowaki, T., Saito, Y., and Goto, M. (2004a). Dysadipocytokinemia in werner syndrome and its recovery by treatment with pioglitazone. *Diabetes Care* 27, 2562–2563. doi: 10.2337/diacare.27.10.2562

Yokote, K., Honjo, S., Kobayashi, K., Fujimoto, M., Kawamura, H., and Mori, S., et al. (2004b). Metabolic improvement and abdominal fat redistribution in Werner syndrome by pioglitazone. *J. Am. Geriatr. Soc.* 52, 1582–1583. doi: 10.1111/j.1532-5415.2004.52430_4.x

Yokote, K., and Saito, Y. (2008). Extension of the life span in patients with Werner syndrome. *J. Am. Geriatr. Soc.* 56, 1770–1771. doi: 10.1111/j.1532-5415.2008.01817.x

Yu, C. E., Oshima, J., Fu, Y. H., Wijsman, E. M., Hisama, F., Alisch, R., et al. (1996). Positional cloning of the Werner's syndrome gene. *Science* 272, 258–262. doi: 10.1126/science.272.5259.258

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917–1920. doi: 10.1126/science.1151526

Yusa, K., Rashid, S. T., Strick-Marchand, H., Varela, I., Liu, P. Q., Paschon, D. E., et al. (2011). Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. *Nature* 478, 391–394. doi: 10.1038/nature10424

Zhang, J., Lian, Q., Zhu, G., Zhou, F., Sui, L., Tan, C., et al. (2011). A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects. *Cell Stem Cell* 8, 31–45. doi: 10.1016/j.stem.2010.12.002

Zhang, Y., Toh, L., Lau, P., and Wang, X. (2012). Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/beta-catenin pathway in human cancer. *J. Biol. Chem.* 287, 32494–32511. doi: 10.1074/jbc.M112.368282

Zhao, T., Zhang, Z. N., Rong, Z., and Xu, Y. (2011). Immunogenicity of induced pluripotent stem cells. Nature 474, 212–215. doi: 10.1038/nature10135

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 24 October 2014; accepted: 10 January 2015; published online: 29 January 2015.

*Citation: Shimamoto A, Yokote K and Tahara H (2015) Werner Syndrome-specific induced pluripotent stem cells: recovery of telomere function by reprogramming. Front. Genet. **6**:10. doi: 10.3389/fgene.2015.00010*

This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.

frontiers in
GENETICS

REVIEW ARTICLE
published: 09 January 2015
doi: 10.3389/fgene.2014.00441

RECQL1 and WRN DNA repair helicases: potential therapeutic targets and proliferative markers against cancers

Kazunobu Futami and Yasuhiro Furuichi*

GeneCare Research Institute Co., Ltd., Kamakura, Japan

Edited by:
Alexey Moskalev, Russian Academy of Sciences, Russia

Reviewed by:
Taru Deva, BioSymfonix, Denmark
Jie Shen, Hangzhou Dianzi University, China

Correspondence:
Yasuhiro Furuichi, GeneCare Research Institute Co., Ltd., 19-2 Kajiwara, Kamakura, Kanagawa 247-0063, Japan
e-mail: furuichi@genecare.co.jp

RECQL1 and WRN helicases in the human RecQ helicase family participate in maintaining genome stability, DNA repair, replication, and recombination pathways in the cell cycle. They are expressed highly in rapidly proliferating cells and tumor cells, suggesting that they have important roles in the replication of a genome. Although mice deficient in these helicases are indistinguishable from wild-type mice, their embryonic fibroblasts are sensitive to DNA damage. In tumor cells, silencing the expression of RECQL1 or WRN helicase by RNA interference induces mitotic catastrophe that eventually kills tumor cells at the mitosis stage of the cell cycle. By contrast, the same gene silencing by cognate small RNA (siRNA) never kills normal cells, although cell growth is slightly delayed. These findings indicate that RECQL1 and WRN helicases are ideal molecular targets for cancer therapy. The molecular mechanisms underlying these events has been studied extensively, which may help development of anticancer drugs free from adverse effects by targeting DNA repair helicases RECQL1 and WRN. As expected, the anticancer activity of conventional genotoxic drugs is significantly augmented by combined treatment with RECQL1- or WRN-siRNAs that prevents DNA repair in cancer cells. In this review, we focus on studies that clarified the mechanisms that lead to the specific killing of cancer cells and introduce efforts to develop anticancer RecQ-siRNA drugs free from adverse effects.

Keywords: RecQ helicases, genome instability, mitotic catastrophe, RECQL1-siRNA, WRN-siRNA, anticancer drug candidates, chemotherapy, DDS

INTRODUCTION

The RecQ helicase family is conserved in all organisms and participates in maintaining the genomic integrity of cells. Microorganisms, such as *Escherichia coli* and *Saccharomyces cerevisiae*, contain only one RecQ helicase, but higher eukaryotes have more than one RecQ helicase. In human cells, the RecQ helicase family comprises five DNA helicases: RECQL1 (also known as RECQL or RECQ1), Bloom syndrome (BLM), Werner syndrome (WRN), Rothmund–Thomson syndrome (RTS; also known as RECQL4) and RECQL5 (Furuichi, 2001; Hickson, 2003; Shimamoto et al., 2004). These RecQ helicases are expressed in the nucleus, participate in DNA repair during cell proliferation and are up-regulated at the time of DNA replication (Kawabe et al., 2000; Brosh, 2013). BLM, WRN, and RTS syndromes are recessive genetic disorders of humans caused by loss of function by BLM (Ellis et al., 1995), WRN (Yu et al., 1996), and RTS helicases (Kitao et al., 1998, 1999, 2002; Lindor et al., 2000), respectively, and are characterized by genomic instability in patient cells, predisposition to various cancers and accelerated onset of aging phenotypes. RECQL1 and RECQL5 helicases, however, have no association with mutations of human disorders.

The biochemical function of DNA helicase is to unwind double-stranded DNA to single-stranded DNAs, responding to cellular DNA metabolisms, such as DNA replication and repair of DNA damage (Sharma et al., 2006; Bernstein et al., 2010). Cells in the process of replication have various endogenous DNA damage in the chromosomes resulting from base-mismatch, deletion, DNA double-strand break (DSB) or from replication errors caused by stalled replication forks and other anomalous DNA structures, such as G-quadruplex (G4) DNA. DNA damage also includes damages generated exogenously by environmental mutagens, such as ionic radiation, reactive oxygen, and genotoxic compounds. Thus, the process of DNA replication associates with many errors that expose growing cells to a risk of acquiring mutations. In proliferating cells, however, cellular DNA damage checkpoints coordinate an arrest at G1, intra-S, and G2 of the cell-cycle to allow the DNA repair process to eventually avoid mitotic catastrophe or mitosis of cells having unrepaired DNA damage. Thus, the cellular systems responsible for genome stabilization, such as DNA repair pathways and cell-cycle checkpoint functions, are indispensable for survival and proliferation of cells (Zhou and Elledge, 2000; Nyberg et al., 2002; Helleday et al., 2008). The DNA repair system contains various reactions, such as nucleotide excision repair, base excision repair, homologous recombination (HR), and non-homologous end joining (NHEJ) recombination pathways, mismatch repair pathways, and telomere maintenance for genomic integrity, in all of which RecQ helicases are considered to participate. Each member of the RecQ helicase family may achieve specific tasks by interaction with DNA repair pathway proteins (Hickson, 2003; Sharma et al., 2006)

to cope with various types of DNA damage. Perhaps, accumulation of DNA damages is the most serious problem for cells because it causes cell mutagenesis, cell lethality, carcinogenesis, aging, and various other disorders. In this context, studies with retrovirus integration into host chromosomes and computational simulation have clearly shown that a single unrepaired DSB is sufficient to kill a cell deficient in DNA repair (Daniel et al., 2001). Regulation of the cell cycle and genomic integrity has also an important role in preventing carcinogenesis and in acquiring chemo-resistant malignancy in cancer cells; consequently, inhibition of these pathways in cancer cells provide a viable approach to manage cancer therapy (Helleday et al., 2008; Kelly and Fishel, 2008).

Among cellular DNA damage-responding elements, RecQ helicases directly participate in a specific DNA repair pathway and have important roles in DNA damage signaling (Frei and Gasser, 2000; Opresko et al., 2004; Pichierri et al., 2011). Based on the clarified roles of DNA helicases in the pathway of DNA repair in cancer cells, a number of strategies are suggested to fight against cancers by modulation of helicase activity (Gupta and Brosh, 2008; Brosh, 2013).

In this review, we pay special attention to recent studies on RECQL1-siRNA and WRN-siRNA that down-regulate the expression of RECQL1 and WRN helicases, respectively, and exert a tumor-specific killing effect on a wide range of tumor cells.

INCREASED EXPRESSION OF RecQ HELICASES IN CANCER CELLS

The expression of all RecQ helicases, except RECQL5, increases when resting B cells of humans are transformed to rapidly growing B-lymphoblastoid cell line (LCL) cells by Epstein–Barr virus (EBV) infection (**Table 1**). This high expression of RecQ helicases seems to be coordinated with an increased growth rate or with augmented malignancy of tumor cells (Kawabe et al., 2000), and provides a basis for searching specific inhibitors of individual RecQ helicases. The copy number of RECQL1 and WRN helicases increases nearly 10-fold when B-LCLs are transformed from a resting state to a rapidly growing state by EBV infection, and then it is further increased several-fold after the cells are transformed to an immortalized cell state in association with a simultaneous expression of telomerase activity (**Table 1**). This continuous up-regulation of RecQ protein expression in malignant B-LCLs (up to nearly 100-fold) may reflect the need of an increased DNA damage response to resolve replicative lesions that arise in highly proliferative cell states, which may occur in other cellular systems as a consequence of oncogene-activated growth signals. Indeed, Slupianek et al. (2011) showed that BCR-ABL induces WRN mRNA and protein expression by c-MYC-mediated activation of transcription in chronic myeloid leukemia cells. Alternatively, the increased expression of DNA helicase may overcome the impaired checkpoint function of cancer cells that results from inactivation of relevant proteins by mutation or are down-regulated by selection of cells in favor of proliferation. Accordingly, inhibitors of helicase activity or RNA interference (RNAi)-mediated acute depletion of helicase should act as an intervention to increase replication lesions and also leave DNA damage unrepaired to selectively kill cancer cells. In fact, both specific small molecular weight inhibitors of RecQ helicases (Aggarwal et al., 2011, 2013; Rosenthal et al., 2013; Shadrick et al., 2013) and RNAi-mediated gene silencing with siRNA suppress the growth of cancer cells in various model systems. In the following sections, we introduce both cases, while placing a greater emphasis on the recently established RNAi-mediated strategy that proved to act specifically on cancer cells that contain impaired checkpoint functions and represent high expressions of RecQ helicases (Futami et al., 2008a,c, 2010, Arai et al., 2011; Mendoza-Maldonado et al., 2011, Sanada et al., 2013; Tao et al., 2014).

Table 1 | Copy number of RecQ helicases in resting B cells, EBV-transformed LCL cells and telomerase-positive immortal LCL cells.

Cells	WRN	BLM	RTS	RECQL1	RECQL5
Resting B cells (N0008R)	1,200	330	120	2,100	340
Pre-immortal LCL cells (N0008T)	94,000	27,000	1,100	15,000	330
Post-immortal LCL cells (N68031M)	200,000	33,000	1,000	21,000	330

The copy number of each RecQ helicase was determined by quantitative immunoblotting versus standard recombinant RecQ helicases. Each numeral represents a mean of duplicated samples. Reprinted from the paper by Kawabe et al. (2000).

MITOTIC CATASTROPHE IN CANCER CELLS INDUCED BY RNAi-MEDIATED SILENCING OF RecQ HELICASES

In cancer chemotherapy, mitotic catastrophe is an important mechanism required to induce cell death by anticancer genotoxic agents (Roninson et al., 2001; Nitta et al., 2004). Cancer cells with an impaired G2 checkpoint function, including deficiencies in p53, p21 and 14-3-3 activities, show increased sensitivity to DNA-damaging agents, while normal cells that contain competent G2 checkpoint activity are resistant to DNA damage (Chan et al., 1999). Most cancer cells have a defective checkpoint function, and so the cell cycle of cancer cells that have DNA damage is transiently arrested at the metaphase without segregation of chromosomes, and the arrested cells subsequently undergo mitotic death. Attenuation of DNA repair capacity in cancer cells is, therefore, an effective approach to selectively kill cancer cells by using the differential capacity of checkpoint function between normal and cancer cells (**Figure 1**). Thus, inhibition of DNA repair may provide an opportunity to selectively kill cancer cells by increasing the cytotoxicity of DNA-damaging agents (and ionizing radiation) that is used for chemotherapeutic intervention. Conventional cancer chemotherapy drugs are used to induce excessive DNA damage beyond the capacity of repair in cancer cells so that cancer cells undergo cell death due to mitotic catastrophe caused by DNA damage that remains unrepaired (Nitta et al., 2004). Mitotic catastrophe occurs in replicating cells that carry DNA damage through cell cycle-mediated checkpoint regulation, and actively replicating

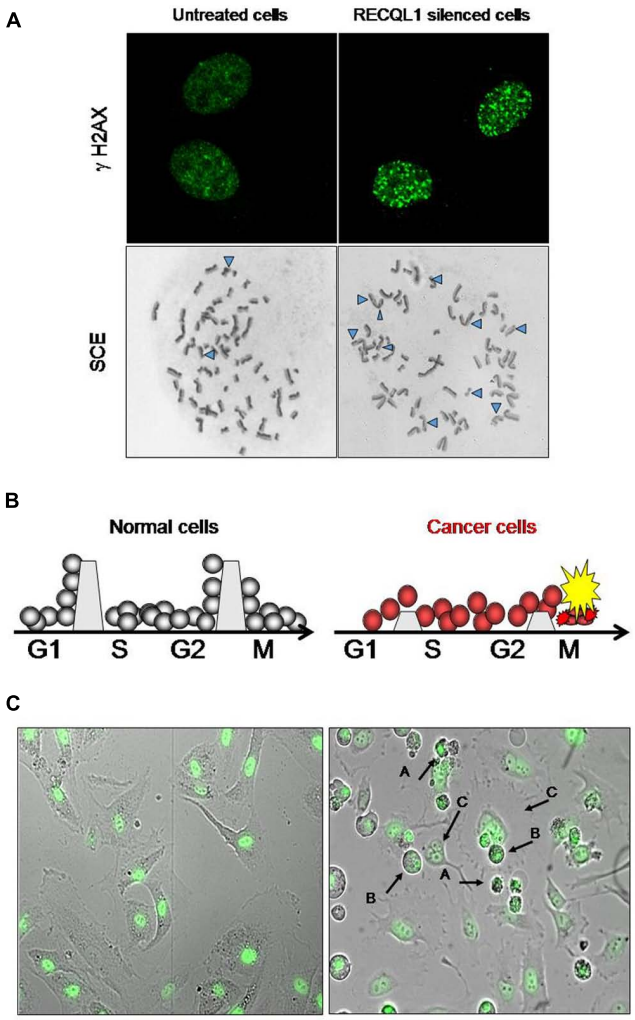


FIGURE 1 | Increase in DNA damage in cancer cells by RECQL1 silencing. (A) Accumulation of DNA damage in RECQL1-siRNA treated cells. Control siRNA(GL3-siRNA) treated HeLa cells (left panel) and RECQL1-silenced HeLa cells (right panel) are shown. γH2AX, Detection of DSB by staining with γH2AX antibody; SCE, sister chromatid exchange regions shown by Δ. **(B)** Schematic representation of the mechanism behind cancer cell-specific mitotic death by RECQL1 silencing with RECQL1-siRNA. The boundaries set between the stage of cell cycles, which are high in normal cells and low in cancer cells, represent an image of the potency of checkpoint ability. G1, S, G2, and M are the stages of the cell cycle. **(C)** Cancer cell-specific mitotic cell death induced by RECQL1-siRNA. RECQL1 silencing *in vitro* was done in normal cells (ARPE19 cells, left panel) and cancer cells (HeLa cells, right panel) by treatment with RECQL1-siRNA. Arrow heads show abnormal chromosomes induced by RECQL1-siRNA transfection. In the right panel **(A–C)** indicate the cells that died by mitotic catastrophe, M phase-arrested cells and multinucleated cells, respectively. Parts of the data are reprinted from the paper by Futami et al. (2008a).

cells are likely to be more sensitive to DNA damage than non-replicating cells (Erenpreisa and Cragg, 2001; Vogel et al., 2007). DNA damage formed endogenously during DNA replication and additional DNA damage resulting from cancer chemotherapy are all subjected to the cellular DNA repair system and are removed if the repair system is not down-regulated. Inhibitors of DNA repair pathways should augment the anticancer activity of chemotherapeutic drugs when such inhibitors are given in combination with DNA-damaging drugs. As mentioned in a later section, Futami et al. (2007) showed consistently that WRN-siRNA that silences the expression of WRN helicase and inhibits DNA repair increases the chemotherapeutic activity of camptothecin (CPT) in HeLa cells. DNA lesions that persist in the S-phase of the cell cycle inhibit progression of replication forks, resulting eventually in the formation of replication-associated DSBs of DNA (**Figure 1A**). Thus, the RecQ helicase-mediated repair mechanism must help replicating cells to remove DSB stress and prevent cells from chromosomal instability, permitting cell survival, and proliferation.

APPLICATION OF siRNA FOR DEVELOPMENT OF INNOVATIVE ANTICANCER THERAPEUTICS

Many conventional anti-cancer chemotherapeutic drugs are small molecular weight compounds and are designed to cause DNA damage in cancer cells, including 5-FU fluorouracil nucleotides (DNA modifying antimetabolite agents), CPT derivatives (topoisomerase inhibitors), platinum derivatives (DNA crosslinking agents), Adriamycin or Doxorubicin (DNA intercalation), and cyclophosphamide (DNA alkylation), bleomycin (DNA cleavage), and mitomycin C (DNA crosslinking). Radiation therapy with either X-ray or heavy ion irradiation causes cancer cells to have DSBs by direct collision with DNA or by DNA oxidation with hydroxyl radical ions derived from irradiated water molecules. However, radiation therapy and DNA damaging chemicals used in cancer chemotherapy have drawbacks by which such treatments affect normal cells as well, often giving rise to severe cytotoxic adverse effects. Long-term chemotherapy or radiotherapy also induces cancer cells to become resistant to DNA damage by increasing the ability to repair damaged DNA (Bouwman and Jonkers, 2012).

As alternatives, siRNA drugs and micro-RNA (miRNA) drugs, which use the principle of RNAi (Novina and Sharp, 2004), have been considered as a new area of promise in anti-cancer therapy (Duxbury et al., 2004; Zhou et al., 2006). The extremely high specificity of siRNA to silence the expression of specified mRNA targets has not only been reliably applied in a wide variety of bio-medical research for gene silencing, but also has led to its emergence as a novel class of pharmaceutical drugs. RNAi is a powerful approach that can expand the anti-cancer strategy because RNAi is a natural process that is not thought to perturb normal cell physiology. Moreover, siRNA has outstanding pharmacological properties (Vaishnaw et al., 2010) by which it can catalyze the degradation of hybridized target mRNA, and, unlike other low molecular weight compounds, it is stable in the cytoplasm of transfected cells, existing in its intact structure for 1 week or more. Thus, siRNA has the properties of (i) a high specificity in binding to target mRNA, (ii) a unique catalytic profile in the mRNA degradation reaction and (iii) a stable biochemical nature in the cytoplasm that permits effective siRNA concentrations as low as nanomolar or less for more than a week, and (iv) siRNA transfection is not too harmful to cells, such that siRNA-transfected cells could be used to continue cell biological experiments. These excellent properties of siRNA predict that siRNA therapeutics would

not exert serious adverse effects on cancer-peripheral normal cells.

We and others have tested candidate siRNA drugs that effectively silence the expression of RecQ helicases to attenuate cellular DNA repair activity. We studied the activity *in vitro* with cultured cancer cells and *in vivo* with human cancer-bearing xenograft animal models (Futami et al., 2007, 2008a,c, 2010; Arai et al., 2011; Mendoza-Maldonado et al., 2011; Tao et al., 2014). Recently, a first-in-man phase 1 trial was completed in the oncology field for a siRNA anti-hepatic cancer drug designed to silence two different targets of vascular endothelial growth factor-A (VEGF-A) and kinesin spindle protein (KSP) simultaneously (Tabernero et al., 2013). In that study, siRNAs were formulated with lipid nanoparticles and were administered by intravenous injection. The results indicated that the siRNA-liposome complex is tolerated in humans, is incorporated in both hepatic cells and tumor cells, and siRNA directs siRNA-sequence-matched cleavage of VEGF and KSP mRNAs in the cytoplasm of cells. This pioneering phase1 clinical study provided pharmacodynamics data that confirmed a safe siRNA-liposome complex and target mRNA-specific down-regulation in cancer cells. All these studies paved the way to make siRNA drugs truly realistic in the near future.

In the initial studies with a drug-oriented siRNA application, siRNAs were recognized to activate innate immune cells by Toll-like receptors, resulting in potent cytokine induction and immunotoxicity (Judge et al., 2005). This immune-stimulatory effect, generally associated with RNA, was thought to impair the development of RNA therapeutics. However, subsequent efforts clarified that immune recognition of siRNA is sequence-specific and is moderated by facilitating sequence design or by appropriate chemical modification of 2′-*O*-methylation at ribose moiety or both that ameliorates the immune response (Hamm et al., 2010).

RECQL1 HELICASE AS THE TARGET OF ANTICANCER THERAPY

BIOLOGICAL FUNCTIONS OF RECQL1 HELICASE

Of the five human RecQ helicase members, RECQL1 helicase was characterized for the first time after isolation from human cancer cells and was named RECQL1 after *E. coli* RecQ (i.e., RECQ-like human helicase number 1; Seki et al., 1994). Biochemical and cell biological data show that RECQL1 helicase unwinds DNA *in vitro* ATP-dependently, catalyzes base-matching ATP-independently (Cui et al., 2003, 2004) and resolves Holliday junctions during DNA replication in cell proliferation (Doherty et al., 2005; LeRoy et al., 2005). RECQL1 is assumed to have a role in DNA mismatch repair together with the human EXO1 and MSH2-MSH6 mismatch repair recognition complex (Doherty et al., 2005). Popuri et al. (2014) recently found that human RECQL1 participates in telomere maintenance.

RECQL1 is expressed ubiquitously in a wide variety of cells and tissues participating in maintaining the genomic integrity of cells. It is highly up-regulated in rapidly proliferating cells, particularly in carcinoma cells originating from various organs, including lung, liver, pancreas, colon, brain, ovary, and head-and-neck cancers (Futami et al., 2008a,c, 2010; Arai et al., 2011; Mendoza-Maldonado et al., 2011; Sanada et al., 2013; Tao et al., 2014). Acute depletion of human RECQL1 by RNAi renders cells sensitive to DNA damage and results in spontaneous increase in DSB-mediated gamma-H2AX foci and increased sister chromatid exchanges (SCEs), suggesting an abrogation of DNA repair (**Figure 1A**; Sharma et al., 2007; Futami et al., 2008a). Growth arrest in cancer cells by RECQL1 depletion is characterized by accumulation of unrepaired DNA damage and arrested cells at the G2 or M cell cycle phases, resulting in mitotic cell death and eventual decreased proliferation (**Figures 1A,C**).

As expected, RECQL1-silencing by RNAi technology also made cancer cells sensitive to genotoxic drugs *in vitro* (Arai et al., 2011). Mendoza-Maldonado et al. (2011) showed that human RECQL1 is highly expressed in biopsied glioblastoma tissues paralleled by a lower expression of perilesional control cells in non-dividing tissues. They showed that acute depletion of RECQL1 by RNAi results in a significant reduction of cell proliferation, perturbation of S-phase progression and an increase in spontaneous gamma-H2AX foci formation in glioblastoma cells, which become hypersensitive to anti-brain tumor drug HU or temozolomide treatment. Berti et al. (2013) showed that RECQL1 helicase has a specific role not shared with other DNA helicases in the restart of stalled replication forks induced by CPT, the reaction of which could be utilized as a new target in the search for anti-cancer drugs. Although RECQL1-deficient mice are indistinguishable from wild-type mice, their embryonic fibroblasts are sensitive to ionizing radiation (Sharma and Brosh, 2007, 2008). The function of RECQL1 helicase seems to be non-essential and complementary with other DNA repair helicases, because no human disease is known to correlate with mutations in the RECQL1 gene.

Individual variations in DNA repair capacity affects the clinical response to cytotoxic cancer therapy and overall survival of patients. Li et al. (2006a,b) and Cotton et al. (2009) found that polymorphism of A159C SNP at the 3′-untranslated region in RECQL1 genes significantly affect the overall survival of patients with resectable pancreatic cancer who were treated with adjuvant or neoadjuvant chemoradiation using gemcitabine as the radiosensor. Why the polymorphism at the non-coding region of mRNA affects the clinical progonosis of patients is not clear, but A159C SNP may function as a binding site for regulatory molecules that affect either splicing, translation efficiency, or stability of RECQL1 mRNA. In this case, the gemcitabine and radiation-induced DNA damage is likely to be repaired by the RECQL1-mediated pathway; patients with the AA genotype with A159C polymorphism showed a significantly longer survival time than those with the CC genotype, suggesting that cancer cell species having the AA genotype are sensitive to DNA damage stress, perhaps due to a reduced expression of RECQL1 helicase.

DNA repair has been termed a double-edged sword because decreased DNA repair may increase the risk of developing cancer, but, on the other hand, the decreased DNA repair of cancer cells improves survival in patients when treated with DNA-damaging agents. Generally, however, RECQL1 and other helicase genes are not mutated or are epigenetically down-regulated in tumor cells, and instead expression is most often up-regulated (Kawabe et al., 2000; Futami et al., 2008a,b,c, 2010; Sanada et al., 2013). Thus, RecQ helicases may be important survival factors for many

tumors. The role of RecQ helicases in response to replication stress suggests an ideal molecular target to selectively eliminate malignant tumor cells by combined therapy with siRNA and cancer chemotherapeutic agents.

IMMUNOHISTOCHEMICAL ANALYSIS OF RECQL1 EXPRESSIONS IN CANCERS AND ITS RELATIONSHIP TO CLINICOPATHOLOGICAL FEATURES OF CANCERS

The expression level of RECQL1 protein in hepato-cellular carcinoma (HCC) cells obtained clinically was investigated to know if the expression level of RECQL1 helicase is directly related to the differentiation stage of HCC cells, malignancy of cancer cells and prognosis of patients (Futami et al., 2010). In the relationship between RECQL1 expression and clinicopathological features, RECQL1 expression (i) significantly correlates with histological grade and MIB-1 indices of HCC, (ii) is significantly higher in simple nodular type HCC than extranodular type HCC, (iii) is significantly higher in HCC with portal vein invasion than in HCC without portal vein invasion, and (iv) is higher in HCC nodules of diameter $\geq$2.0 cm than in HCC nodules of diameter $<$2.0 cm. These data showed that RECQL1 could be used as a molecular marker to evaluate the malignancy of HCC, although RECQL1 expression and other clinicopathological features in ovary cancers do not seem to relate to prognosis, including recurrence, and survival time of patients.

In the case of ovarian cancers, an immunohistochemical study was done to test if RECQL1 expression in cancer cells can diagnose the type of cancer, such as serous type, endometrioid type, clear cell type, and mucinous type (Sanada et al., 2013). The data of that study showed that RECQL1 expression cannot distinguish type of ovarian cancer, although a trend suggests that a high expression associates with serous type and endometrical type cancers rather than with clear cell type and mucinous type cancers.

ANTICANCER EFFECT OF RECQL1-siRNA/DDS *IN VITRO* AND *IN VIVO*

RECQL1-targeted siRNA suppresses the growth of a wide range of cultured tumor cells at low concentrations around 10 nM. Microscopic analysis showed that growth suppression is mainly due to mitotic cell death after the cell cycling is arrested at the M phase (Futami et al., 2008a, 2010). A few populations of cancer cells that survive mitotic death undergo morphological changes to non-dividing large cells that resemble the morphology of senescent cells. Notably, this same treatment with RECQL1-siRNA does not kill normal cells, such as ARPE-19, TIG3, WI38, and HUVEC cells that grow rapidly, although normal cells treated with RECQL1-siRNA slightly slow the growth rate. Thus, these experiments showed that cancer cells are extremely sensitive to depletion of RECQL1 and that cell growth is severely inhibited, as if the growth of cancer cells is dependent on a high expression of RECQL1, but non-cancerous cells and all normal cells tested are unaffected by RNAi-mediated acute depletion of RECQL1 helicase. This event was characterized as mitotic catastrophe that occurs during the cell cycle of cancer cells deficient in the checkpoint system due to accumulation of DNA damage caused by the absence of RECQL1 helicase (Futami et al., 2008a,c, 2010).

To apply this specific killing of cancer cells for clinical benefit in anticancer chemotherapy, a series of experiments were carried out with model mice inoculated with various human cancer cells and RECQL1-siRNA formulated with cationic liposome LIC-101 (Yano et al., 2004). The results showed that RECQL1-targeted siRNA can suppress tumor growth in many mouse xenograft models, including lung, liver, pancreatic, colorectal cancer models and in orthotropic hepatic cancer models (**Figure 2A**), as well as in nude mice that have head and neck carcinoma (Futami et al., 2008a,c, 2010; Arai et al., 2011). Here, RECQL1-siRNA formulated with cationic liposome LIC-101 did not show any noticeable adverse effects.

In addition, RECQL1-siRNA increases the anticancer activity of *cis*-platinum (II) diamine dichloride (CDDP), a DNA-damaging cancer therapeutic, when they are administered together in a murine model of hypopharyngeal xenografts that had been inoculated with notorious FaDu cancer cells. This co-treatment of RECQL1-siRNA with CDDP increases DNA damage and cancer cell death (**Figure 2B**; Arai et al., 2011). Thus, the anticancer strategy using siRNA to attenuate the DNA repair function in cancer cells is not only superb by itself in suppressing cancer cell growth through mitotic catastrophe, but also the simultaneous treatment of genotoxic agents increases DNA damage and thus increases the efficacy of anticancer chemotherapy. Recently, Tao et al. (2014) confirmed that RECQL1 silencing by overproduction of miR-203, an miRNA that suppresses the expression of RECQL1, also leads to a strong anticancer effect on squamous carcinoma cells both *in vitro* and *in vivo*.

All these results clearly showed that RECQL1 helicase protein and RECQL1 mRNA are excellent targets for cancer chemotherapy, and that RECQL1-siRNA formulated with liposome, such as LIC-101 liposome as a drug delivery system (DDS), is a promising anticancer drug candidate. Selection of DDS, however, remains to be a key issue in the development of potential siRNA drugs, because it helps protect siRNA from degradation by ribonucleases and delivers siRNA to specified tissues, organs, and cells.

WRN HELICASE AS THE TARGET FOR ANTICANCER THERAPY

BIOLOGICAL FUNCTION OF WRN AS DEDUCED FROM ABERRANT DNA METABOLISM IN CELLS FROM WS PATIENTS

Werner syndrome is an autosomal recessive disease characterized by premature aging and a high incidence of malignant neoplasms. The causative gene *WRN* was identified as coding for DNA helicase belonging to the RecQ helicase family (Yu et al., 1996). The WRN protein (WRN) contains 3′->5′ exonuclease at the N-terminal region in addition to 3′->5′ DNA unwinding helicase activity, implicating its biological function in maintaining genomic stability by DNA repair. The biological function of each helicase in the RecQ family can be deduced from the outcome of aberrant DNA metabolism seen in patient cells that lack a particular RecQ helicase. In the case of WRN helicase, chromosomal instability associated with increased somatic mutation, large DNA deletions (Fukuchi et al., 1989) and unusual dynamics in telomere shortening (Tahara et al., 1997; Sugimoto, 2014) have been found in cells from WS patients, suggesting that WRN may participate in the repair of DSB through HR and NHEJ repair pathways. Because WS patients are highly predisposed to tumorigenesis, showing a particularly higher incidence of non-epithelial tumors such as soft-tissue sarcoma and benign meningioma than epithelial carcinoma

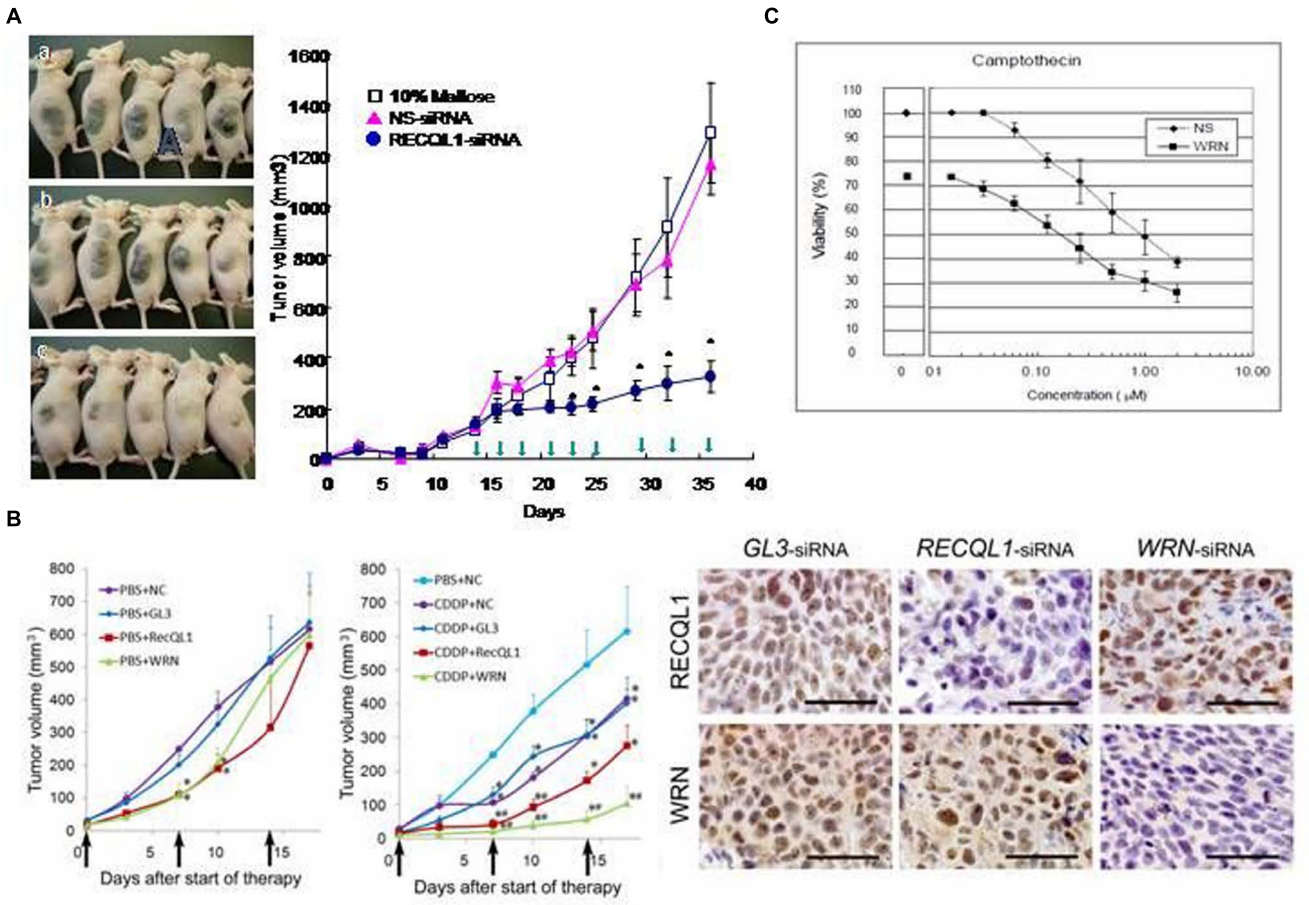


FIGURE 2 | Anticancer effects of RECQL1-siRNA and WRN-siRNA. **(A)** Time course experiments measuring the inhibitory effect of the RECQL1-siRNA-LIC101 complex on the growth of Hep3B cancer nodules. Open squares, red triangles and closed circles show mice treated with 10% maltose solution as a control, NS(non-silencing)-siRNA/LIC101 complex and RECQL1-siRNA/LIC101 complex, respectively. Arrow heads represent injections made subcutaneously into cancer-bearing mice. The anticancer effect of RECQL1-siRNA/LIC101 on Hep3B cancer-bearing mice is represented visually. Panels **(A–C)** represent the size of cancer nodules grown until 42 days after inoculation. Reprinted from the paper by Futami et al. (2008b). **(B)** Anticancer effects of locally injected WRN-siRNA and RECQL1-siRNA on head and neck squamous cell carcinoma, and the synergistic effect made by the simultaneously intravenously injected genotoxic platinum drug CDDP. Therapeutic effects of WRN-siRNA and RECQL1-siRNA, and the combined effect with CDDP were examined in human FaDu carcinoma-bearing mouse xenografts. NC, no siRNA addition; GL3, GL3-siRNA; RECQL1, RECQL1-siRNA; WRN,WRN-siRNA. The right panel shows immunohistochemical evaluations of WRN and RECQL1 proteins expressed in surgically isolated cancerous regions of a human FaDu carcinoma-bearing xenograft mouse model after treatment with injections of the siRNA-atelocollagen complex. GL3-siRNA represents control siRNA that does not inhibit the expression of WRN or RECQL1 helicases. WRN-siRNA and RECQL1-siRNA treatments reduced the expression of WRN and RECQL1 proteins, respectively, stained by specific antibodies. Reprinted from the paper by Arai et al. (2011) by courtesy of those authors. **(C)** Increased cytotoxicity of anticancer drug camptothecin (CPT) in cancer cells by co-treatment with WRN-siRNA. HeLa cells were transfected with a sub-lethal concentration of WRN-siRNA and were treated with a serial dilutions of CPT added after transfection. After 48 h of culture, the cell viability was monitored. Reprinted from the paper by Futami et al. (2007). The half-lethal dose (LD 50%) of CPT was reduced by nearly ten-fold by treatment with WRN-siRNA (10 μM). Reprinted from the paper by Futami et al. (2007).

that are seen frequently in normal subjects (Goto et al., 1996), we hypothesized that WRN helicase could be a tumor suppressor in normal subjects that prevents tumorigenesis of non-epithelial cells and is required for the growth of carcinoma of epithelial origin (Futami et al., 2008b). Earlier studies have clarified that most mutations in WS patients generate the premature termination codon in WRN mRNA that explains the basis of the loss of function of the *WRN* gene in WS patients (review by Shimamoto et al., 2004).

In the case of the expression of RecQ helicases, five RecQ helicase genes appear to be transcribed to mRNAs differentially depending on the organ or tissue in normal subjects. WRN seems to be expressed more or less tissue-specifically in pancreas, testis and ovary, but RECQL1 is expressed ubiquitously in all tissues (Kitao et al., 1998). Therefore, genomic instability in WS patients caused by the loss of WRN function is predicted to occur severely in tissues where WRN protein is most efficiently expressed in normal subjects. The characteristic clinical symptoms of WS patients, such as diabetes mellitus and early hypogonadism in both male and female WS patients, correspond well to organs in which WRN helicases are expressed highly in normal subjects. In those tissues or organs, DNA damage generated endogenously during

DNA replication is not repaired in WS patients due to lack of the functional WRN protein. A functional relationship seems to exist between WRN and the replication checkpoint in normal cells, but an inability of this crosstalk might contribute to induce genomic instability, a common feature in senescent cells and cancer cells. In the absence of functional WRN, a series of correlated outcomes occurs, including replication arrest during cell cycling, slow growth in cell proliferation, and incomplete DNA repair resulting in many genomic deletions, all of which result in a decrease in number of cells leading to deterioration of specified tissues representing premature aging phenotypes referred to as segmental progeroid syndrome (Martin, 1985).

ROLE OF WRN HELICASE IN TUMORIGENESIS

Transcription of the *WRN* gene is directed by a house-keeping SP1 promotor. An *in vitro* promotor analysis showed that WRN expression is attenuated by tumor suppressor p53 protein, suggesting that WRN protein, which shows a putative tumor suppressor function, decreases in p53-enriched senesced cells (Yamabe et al., 1998). In this context, Agrelo et al. (2006) showed that WRN function is abrogated in human colorectal cancer cells by transcriptional silencing associated with CpG island-promoter hypermethylation. Restoration of WRN expression by *in vitro* demethylation of promotor or by reintroduction of WRN into cancer cells induces tumor-suppressor-like features, such as reduced density of colony formation and inhibition of tumor growth, in mouse xenograft models. Analysis of many human primary tumors showed that hypermethylation of the WRN promotor is a common event in various tumor cells and is indicative of tumorigenesis by the absence of WRN expression. Agrelo et al. (2006) concluded that these findings underline the significance of WRN as a caretaker of a genome that has tumor-suppressor activity and they identify epigenetic silencing of WRN as a key step to cancer development. Decreased WRN expression may cause genomic instability in normal cells and induce initial tumorigenesis, but malignant tumor cells would acquire increased levels of WRN expression to protect their own genomic stability. Malignant tumor cell lines from various sources show a high expression of WRN helicase. In the case of colorectal cancer, the presence of aberrant hypermethylation of the WRN promotor in cancer cells predicts improved survival in patients treated with a CPT derivative irinotecan, a topoisomerase inhibitor used in therapeutics of this neoplasm, because decreased WRN makes neoplasmic cells sensitive to a DSB-inducing genotoxic agent (Agrelo et al., 2006). WS patients have a high frequency of having non-epithelial tumors than epithelial carcinoma, but the underlying reason remains to be clarified. However, a high WRN expression may possibly be a prerequisite to maintain genomic integrity in rapidly growing carcinoma cells in normal subjects.

Futami et al. (2007) showed that WRN-siRNA abrogates the DNA repair activity of HeLa cells and increases the sensitivity to topoisomerase inhibitor CPT. In this case, the silencing of WRN helicase prevents the repair of CPT-induced DNA damage and permits accumulation of unrepaired DNA damage that explains increased cytotoxicity of CPT in the combined treatment of CPT and WRN-siRNA (**Figure 2C**). The data also explained why WS patient cells also show increased sensitivity to other genotoxic reagents, including CPT, etoposide, 4NQO, and bleomycin (Sakamoto et al., 2001). Recently, Aggarwal et al. (2011) used small molecular weight compound NSC19630 that inhibits WRN activity in anti-cancer cell biology experiments. Exposure of human cancer cells to NSC 19630 dramatically impairs growth and proliferation, induces apoptosis WRN-dependently and results in increased γ-H2AX reactive chromosomal DSB, similar to the results obtained by Futami et al. (2007) by silencing WRN with siRNA treatment. NSC 19630 exposure leads to delayed S-phase progression, consistent with accumulation of stalled replication forks, and to DNA damage WRN-dependently. Exposure to NSC 19630 sensitizes cancer cells to the G4-binding compound telomestatin or to a poly(ADP-ribose) polymerase (PARP) inhibitor. Sublethal dosage of NSC 19630 and the chemotherapy drug topotecan acts synergistically to inhibit cell proliferation and induce DNA damage (Aggarwal et al., 2011). The use of this WRN helicase inhibitor (NSC 19630) may provide insight into the importance of WRN-mediated pathways important for DNA repair and the replicational stress response.

ANTICANCER EFFECT OF WRN-siRNA/DDS *IN VITRO* AND *IN VIVO*

Both WRN and RECQL1 helicases are expressed highly in head and neck squamous carcinoma cells (HNSCCs; **Figure 2B**), and siRNA-mediated silencing of either gene suppresses growth of HNSCC *in vitro* (Arai et al., 2011). Similarly, local injections of WRN-siRNA and RECQL1-siRNA formulated with atelocollagen into a mouse zenograft model of hypopharyngeal carcinoma markedly inhibits tumor growth. A combination of either siRNA with CDDP, a genotoxic drug commonly used in HNSCC treatment, significantly augments the *in vivo* anticancer effect of CDDP (**Figure 2B**). Notably, no recurrence was observed for some tumors after siRNA and CDDP treatment in this model. These findings offer a preclinical proof of WRN and RECQL1 helicase as novel therapeutic targets to treat aggressive HNSCC and possibly other cancers.

Several lines of evidence suggest that WRN helicase has an important role in DNA replication and S-phase progression (review by Croteau et al., 2014). Loss of WRN markedly extends the time of cell cycles after genotoxic treatments. This result comfirmed the importance of WRN during genomic replication, and indicated that WRN acts to facilitate fork progression after DNA damage or replication arrest. Recent studies showed that conditional gene silencing of WRN expression in non-small-cell lung cancer xenografts that are over-expressed with c-MYC inhibits tumor growth, suggesting that targeting WRN protein inhibits growth of c-MYC-associated cancers (Moser et al., 2012). A growing body of evidence shows that WRN functions in close connection with the replication checkpoint that includes both ataxia telangiectasia mutated (ATM) protein and ATM-Rad3-related (ATR) kinase activities and maintains fork integrity and re-establishment of fork progression. Recent findings also support the view that ATR and ATM kinases modulate WRN function at defined steps of the response to replication fork arrest. Or *vice versa*, WRN may be required to activate ATM kinase and the intra-S-phase checkpoint in response to DNA interstrand crosslink-induced DSBs

and other forms of fork arrest (Cheng et al., 2008; Pichierri et al., 2011). WRN is speculated to be a DNA helicase that efficiently unwinds G4 DNA and other forms of alternative DNA structures, such as telomeric displacement loops. These data indicate that DNA repair helicases, such as RECQL1 and WRN, represent a novel class of important targets required for anticancer therapy. Inhibition of their activities either by siRNA or by small molecular weight compounds in cancer cells having checkpoint mutation should give rise to a new type of anticancer therapeutic drug that also permits strong synergistic anticancer effects when combined with genotoxic drugs as depicted in **Figure 2B** (with CDDP) and **Figure 2C** (with CPT; Futami et al., 2007; Arai et al., 2011).

BLM, RTS, AND RECQL5 HELICASES ARE POTENTIAL TARGETS OF ANTICANCER THERAPY

Patients of BLM and a subset of patients with RTS who lack functional BLM and RTS helicases, respectively, manifest clinical phenotypes of genomic instability and a high incidence of cancers (Ellis et al., 1995; Lindor et al., 2000). Like *WRN*, helicase genes *BLM* and *RTS* are suggested to function as tumor suppressor genes in normal individuals. Both BLM and RTS are expressed highly in rapidly growing EBV-infected LCL cells from normal individuals, and the expression of BLM is, like WRN, further augmented when EBV-transformed cells are differentiated to telomerase-positive immortal cells (**Table 1**). In a pioneering effort to find an antiproliferative drug, Nguyen et al. (2013) discovered small molecular weight compound ML216 from a screening of a chemical compound library that inhibits the DNA binding activity of BLM *in vitro* (IC50% ∼3 uM) and abrogates the unwinding activity of BLM on a forked DNA duplex. As expected, ML216 increased the rate of SCE in cultured human fibroblasts and increased the sensitivity to aphidicolin, an inhibitor of replicative DNA polymerases (Banerjee et al., 2013; Rosenthal et al., 2013). However, ML216 does not seem to be highly specific to BLM helicase, because it also inhibits DNA unwinding activity of WRN (Banerjee et al., 2013). Further, pharmacological studies may be needed to improve the specificity and efficacy before ML216 is tested for clinical application.

Mao et al. (2010) showed that depletion of BLM by RNAi-mediated shRNA gene silencing suppresses the growth of osteosarcoma U2OS cell line with an increase in DNA damage detected by gamma-H2AX, suggesting that BLM is a potential target for anticancer therapeutics. They also showed depletion of BLM and WRN by shRNA from U2OS and normal fibroblast sensitized cells to dose-dependent killing by CPT, CDDP and 5-FU, consistent with previous observations by Futami et al. (2007) in which WRN-siRNA treatment sensitized HeLa cells to CPT and lowered the cytotoxic dose by nearly 10-fold.

Few reports exist of RTS and RECQL5 helicases as possible cancer targets. However, Su et al. (2010) elegantly showed that RECQL4 (RTS) helicase has critical roles in prostate carcinogenesis, and that RNAi-mediated gene suppression reduces the growth and survival of metastatic prostate cancer cells. In addition, RECQL4-suppressed cells treated with siRNA show increased apoptotic death after treatment with ultraviolet C and γ-ray radiation. Thus, Su et al. (2010) conclude that RECQL4 protects the genomic integrity of prostate cancer cells from endogenous and exogenous DNA damage, similar to our discussion above with RECQL1. In fact, RECQL4-depleted prostate cancer cells undergo extensive apoptotic death in PARP -1-dependently *in vitro* and show reduced tumorigenicity in nude mice *in vivo*. Because the expression of RECQL4 increased highly in metastatic prostate cancer cells and in tumor tissue, RECQL4 protein could be used as new tumor marker.

While much insight has been gained into the function of WRN and BLM, little is known about RECQL5. Three isomers result from differential splicing from the RecQ gene transcript. The largest molecule, RECQL5, localizes to the nucleus, whereas the two smaller molecules are found in the cytoplasm (Shimamoto et al., 2000). Nuclear RECQL5 has a role in maintaining genomic stability by cooperating with topoisomerase II(alpha) in DNA decatenation and cell cycle progression (Ramamoorthy et al., 2012). Because RECQL5- depleted cells show slow proliferation, G2 and M cell cycle arrests and late S-phase cycling defects, resulting in entangled abnormal chromosomes at the M phase, possibly RECQL5 could be challenged, like RECQL1, as a new cancer therapeutic target.

PERSPECTIVE

The relation between helicases and DNA damage response and genomic stability suggests that DNA repair helicases may be suitable targets for cancer chemotherapy (Helleday et al., 2008). Most cancer cells are deficient in G1 and G2 checkpoint function and fail to arrest the cell cycle at G1 and G2 phases to engage in DNA repair. Instead, cells that proceed in the cell cycle to the M phase, where DNA repair is no longer permitted, eventually undergo cell death as they enter mitosis (Nitta et al., 2004). Several studies have reported a greater efficacy in the therapeutic application of siRNA against malignant tumors in combination with other chemotherapeutic agents, such as with gemcitabine together with ribonucleotide reductase-siRNA (Duxbury et al., 2004), CPT together with WRN helicase-siRNA (Futami et al., 2007) and Adriamycin together with survivin-siRNA (Yonesaka et al., 2006). Such approaches of targeting RecQ helicases with a strict specificity, such as by gene silencing with siRNA, would be most conspicuous in cooperation with DNA damaging agents or radiation. Thus, DNA repair helicases expressed highly in most malignant and rapidly proliferating cancer cells not only provide new proliferative protein markers, such as WRN and RECQL1 proteins (**Figure 2**), but also provide logical consequences for combination therapy to fight against drug-resistant cancer cells.

In conclusion, DNA repair helicases are ideal molecular targets for anticancer drug discovery, and, in particular, RECQL1-siRNA and WRN-siRNA and perhaps BLM-siRNA and RTS-siRNA are promising new therapeutic interventions against malignant cancers of various histological and clinical characteristics, including, for example, those of the platinum-resistant, CPT-resistant, and 5-FU-resistant subtypes.

ACKNOWLEDGMENT

We thank Dr. Kazuhiro Kataoka for kind assistance with preparation of manuscript.

REFERENCES

Aggarwal, M., Banerjee, T., Sommers, J. A., and Brosh, R. M. Jr. (2013). Targeting an Achilles' heel of cancer with a WRN helicase inhibitor. *Cell Cycle* 12, 3329–3335. doi: 10.4161/cc.26320

Aggarwal, M., Sommers, J. A., Shoemaker, R. H., and Brosh, R. M. Jr. (2011). Inhibition of helicase activity by a small molecule impairs Werner syndrome helicase (WRN) function in the cellular response to DNA damage or replication stress. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1525–1530. doi: 10.1073/pnas.1006423108

Agrelo, R., Cheng, W. H., Setien, F., Ropero, S., Espada, J., Fraga, M. F., et al. (2006). Epigenetic inactivation of the premature aging Werner syndrome gene in human cancer. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8822–8827. doi: 10.1073/pnas.0600645103

Arai, A., Chano, T., Futami, K., Furuichi, Y., Ikebuchi, K., Inui, T., et al. (2011). RECQL1 and WRN proteins are potential therapeutic targets in head and neck squamous cell carcinoma. *Cancer Res.* 71, 4598–4607. doi: 10.1158/0008-5472

Banerjee, T., Aggarwal, M., and Brosh, R. M. Jr. (2013). A new development in DNA repair modulation: discovery of a BLM helicase inhibitor. *Cell Cycle* 12, 713–714. doi: 10.4161/cc.23953

Bernstein, K. A., Gangloff, S., and Rothstein, R. (2010). The RecQ DNA helicases in DNA repair. *Annu. Rev. Genet.* 44, 393–417. doi: 10.1146/annurev-genet-102209-163602

Berti, M., Ray Chaudhuri, A., Thangavel, S., Gomathinayagam, S., Kenig, S., Vujano Vic, M., et al. (2013). Human RECQ1 promotes restart of replication forks reversed by DNA topoisomerase I inhibition. *Nat. Struct. Mol. Biol.* 20, 347–354. doi: 10.1038/nsmb.2501

Bouwman, P., and Jonkers, J. (2012). The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. *Nat. Rev. Cancer* 12, 587–598. doi: 10.1038/nrc3342

Brosh, R. M. Jr. (2013). DNA helicases involved in DNA repair and their roles in cancer. *Nat. Rev. Cancer* 13, 542–558. doi: 10.1038/nrc3560

Chan, T. A., Hermeking, H., Lengauer, C., Kinzler, K. W., and Vogelstein, B. (1999). 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. *Nature* 401, 616–620.

Cheng, W. H., Muftic, D., Muftuoglu, M., Dawut, L., Morris, C., and Helleday, T. (2008). WRN is required for ATM activation and the S-phase checkpoint in response to interstrand cross-link-induced DNA double-strand breaks. *Mol. Biol. Cell* 19, 3923–3933. doi: 10.1091/mbc.E07-07-0698

Cotton, R. T., Li, D., Scherer, S. E., Muzny, D. M., Hodges, S. E., Catania, R. L., et al. (2009). Single nucleotide polymorphism in RECQL and survival in resectable pancreatic adenocarcinoma. *HPB (Oxford)* 11, 435–444. doi: 10.1111/j.1477-2574

Croteau, D. L., Popuri, V., Opresko, P. L., and Bohr, V. A. (2014). Human RecQ helicases in DNA repair, recombination, and replication. *Annu. Rev. Biochem.* 83, 519–552. doi: 10.1146/annurev-biochem-060713-035428

Cui, S., Arosio, D., Doherty, K. M., Brosh, R. M. Jr., Falaschi, A., and Vindigni, A. (2004). Analysis of the unwinding activity of the dimeric RECQ1 helicase in the presence of human replication protein A. *Nucleic Acids Res.* 32, 2158–2170. doi: 10.1093/nar/gkh540

Cui, S., Klima, R., Ochem, A., Arosio, D., Falaschi, A., and Vindigni, A. (2003). Characterization of the DNA-unwinding activity of human RECQ1, a helicase specifically stimulated by human replication protein A. *J. Biol. Chem.* 278, 1424–1432. doi: 10.1074/jbc.M209407200

Daniel, R., Litwin, S., Katz, R. A., and Skalka, A. M. (2001). Computational analysis of retrovirus-induced scid cell death. *J. Virol.* 75, 3121–3128. doi: 10.1128/JVI.75.7.3121-3128.2001

Doherty, K. M., Sharma, S., Uzdilla, L. A., Wilson, T. M., Cui, S., Vindigni, A., et al. (2005). RECQ1 helicase interacts with human mismatch repair factors that regulate genetic recombination. *J. Biol. Chem.* 280, 28085–28094. doi: 10.1074/jbc.M500265200

Duxbury, M. S., Ito, H., Zinner, M. J., Ashley, S. W., and Whang, E. E. (2004). RNA interference targeting the M2 subunit of ribonucleotide reductase enhances pancreatic adenocarcinoma chemosensitivity to gemcitabine. *Oncogene* 23, 1539–1548. doi: 10.1038/sj.onc.1207272

Ellis, N. A., Groden, J., Ye, T. Z., Straughen, J., Lennon, D. J., Ciocci, S., et al. (1995). The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83, 655–666. doi: 10.1016/0092-8674(95)90105-1

Erenpreisa, J., and Cragg, M. S. (2001). Mitotic death: a mechanism of survival? A review. *Cancer Cell Int.* 1, 1. doi: 10.1186/1475-2867-1-1

Frei, C., and Gasser, S. M. (2000). RecQ-like helicases: the DNA replication checkpoint connection. *J. Cell Sci.* 113, 2641–2546.

Fukuchi, K., Martin, G. M., and Monnat, R. J. Jr. (1989). Mutator phenotype of Werner syndrome is characterized by extensive deletions. *Proc. Natl. Acad. Sci. U.S.A.* 86, 5893–5897. doi: 10.1073/pnas.86.15.5893

Furuichi, Y. (2001). Premature aging and predisposition to cancers caused by mutations in RecQ family helicases. *Ann. N. Y. Acad. Sci.* 928, 121–131. doi: 10.1111/j.1749-6632.2001.tb05642.x

Futami, K., Kumagai, E., Makino, H., Goto, H., Takagi, M., Shimamoto, A., et al. (2008a). Induction of mitotic cell death in cancer cells by small interference RNA suppressing the expression of RecQL1 helicase. *Cancer Sci.* 99, 71–80.

Futami, K., Ishikawa, Y., Goto, M., Furuichi, Y., and Sugimoto, M. (2008b). The role of WRN helicase in carcinogenesis and in resistance by cancer cells to genotoxins. *Cancer Sci.* 99, 843–848. doi: 10.1111/j.1349-7006.2008.00778.x

Futami, K., Kumagai, E., Makino, H., Sato, A., Takagi, M., Shimamoto, A., et al. (2008c). Anticancer activity of RecQL1 helicase siRNA in mouse xenograft models. *Cancer Sci.* 99, 1227–1236. doi: 10.1111/j.1349-7006.2008.00794

Futami, K., Ogasawara, S., Goto, H., Yano, H., and Furuichi, Y. (2010). RecQL1 DNA repair helicase: a potential tumor marker and therapeutic target against hepatocellular carcinoma. *Int. J. Mol. Med.* 25, 537–545.

Futami, K., Takagi, M., Shimamoto, A., Sugimoto, M., and Furuichi, Y. (2007). Increased chemotherapeutic activity of camptothecin in cancer cells by siRNA-induced silencing of WRN helicase. *Biol. Pharm. Bull.* 30, 1958–1961. doi: 10.1248/bpb.30.1958

Goto, M., Miller, R. W., Ishikawa, Y., and Sugano, H. (1996). Excess of rare cancers in Werner syndrome (adult progeria). *Cancer Epidemiol. Biomarkers Prev.* 5, 239–246.

Gupta, R., and Brosh, R. M. Jr. (2008). Helicases as prospective targets for anti-cancer therapy. *Anticancer Agents Med. Chem.* 8, 390–401. doi: 10.2174/187152008784220339

Hamm, S., Latz, E., Hangel, D., Müller, T., Yu, P., Golenbock, D., et al. (2010). Alternating 2′-O-ribose methylation is a universal approach for generating non-stimulatory siRNA by acting as TLR7 antagonist. *Immunobiology* 215, 559–569. doi: 10.1016/j.imbio.2009.09.003

Helleday, T., Petermann, E., Lundin, C., Hodgson, B., and Sharma, R. A. (2008). DNA repair pathways as targets for cancer therapy. *Nat. Rev. Cancer* 8, 193–204. doi: 10.1038/nrc2342

Hickson, I. D. (2003). RecQ helicases: caretakers of the genome. *Nat. Rev. Cancer* 3, 169–178. doi: 10.1038/nrc1012

Judge, A. D., Sood, V., Shaw, J. R., Fang, D., McClintock, K., and MacLachlan, I. (2005). Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. *Nat. Biotechnol.* 23, 457–462. doi: 10.1038/nbt1081

Kawabe, T., Tsuyama, N., Kitao, S., Nishikawa, K., Shimamoto, A., Shiratori, M., et al. (2000). Differential regulation of human RecQ family helicases in cell transformation and cell cycle. *Oncogene* 19, 4764–4772. doi: 10.1038/sj.onc.1203841

Kelly, M. R., and Fishel, M. L. (2008). DNA repair proteins as molecular targets for cancer therapeutics. *Anticancer Agents Med. Chem.* 8, 417–425. doi: 10.2174/187152008784220294

Kitao, S., Ohsugi, I., Ichikawa, K., Goto, M., Furuichi, Y., and Shimamoto, A. (1998). Cloning of two new human helicase genes of the RecQ family:biological significance of multiple species in higher eukaryote. *Genomics* 54, 443–452. doi: 10.1006/geno.1998.5595

Kitao, S., Shimamoto, A., and Furuichi, Y. (2002). "Molecular biology of Rothmund-Thomson syndrome," in *Chromosomal Instability and Aging: Basic Science and Clinical Implication*, eds F. M. Hisama, S. M. Weisman, and G. M. Martin (New York: Marcel Dekker), 223–224.

Kitao, S., Shimamoto, A., Goto, M., Miller, R. W., Smithson, W. A., Lindor, N. M., et al. (1999). Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* 22, 82–84. doi: 10.1038/8788

LeRoy, G., Carroll, R., Kyin, S., Seki, M., and Cole, M. D. (2005). Identification of RecQL1 as a holliday junction processing enzyme in human cell lines. *Nucleic Acids Res.* 33, 6251–6257. doi: 10.1093/nar/gki929

Li, D., Frazier, M., Evans, D. B., Hess, K. R., Crane, C. H., Jia, L., et al. (2006a). Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. *J. Clin. Oncol.* 24, 1720–1728. doi: 10.1200/JCO.2005.04.4206

Li, D., Liu, H., Jiao, L., Chang, D. Z., Beinart, G., Wolff, R. A., et al. (2006b). Significant effect of homologous recombination DNA repair gene polymorphisms

on pancreatic cancer survival. *Cancer Res.* 66, 3323–3330. doi: 10.1158/0008-5472.CAN-05-3032

Lindor, N. M., Furuichi, Y., Kitao, S., Shimamoto, A., Arndt, C., and Jalal, S. (2000). Rothmund-Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome. *Am. J. Med. Genet.* 90, 223–228. doi: 10.1002/(SICI)1096-8628(20000131)90:3<223::AID-AJMG7>3.0.CO;2-Z

Mao, F. J., Sidorova, J. M., Lauper, J. M., Emond, M. J., and Monnat, R. J. (2010). The human WRN and BLM RecQ helicases differentially regulate cell proliferation and survival after chemotherapeutic DNA damage. *Cancer Res.* 70, 6548–6555. doi: 10.1158/0008-5472.CAN-10-0475

Martin, G. M. (1985). Genetics and aging; the Werner syndrome as a segmental progeroid syndrome. *Adv. Exp. Med. Biol.* 190, 161–170. doi: 10.1007/978-1-4684-7853-2_5

Mendoza-Maldonado, R., Faoro, V., Bajpai, S., Berti, M., Odreman, F., Vindigni, M., et al. (2011). The human RECQ1 helicase is highly expressed in glioblastoma and plays an important role in tumor cell proliferation. *Mol. Cancer* 10, 83. doi: 10.1186/1476-4598-10-83.

Moser, R., Toyoshima, M., Robinson, K., Gurley, K. E., Howie, H. L., Davison, J., et al. (2012). MYC-driven tumorigenesis is inhibited by WRN syndrome gene deficiency. *Mol. Cancer Res.* 10, 535–545. doi: 10.1158/1541-7786.MCR-11-0508

Nguyen, G. H., Dexheimer, T. S., Rosenthal, A. S., Chu, W. K., Singh, D. K., Mosedale, G., et al. (2013). A small molecule inhibitor of the BLM helicase modulates chromosome stability in human cells. *Chem. Biol.* 20, 55–62. doi: 10.1016/j.chembiol.2012.10.016

Nitta, M., Kobayashi, O., Honda, S., Hirota, T., Kuninaka, S., Marumoto, T., et al. (2004). Spindle checkpoint function is required for mitotic catastrophe induced by DNA-damaging agents. *Oncogene* 23, 6548–6558. doi: 10.1038/sj.onc.1207873

Novina, C. D., and Sharp, P. A. (2004). The RNAi revolution. *Nature* 430, 161–164. doi: 10.1038/430161a

Nyberg, K. A., Michelson, R. J., Putnam, C. W., and Weinert, T. A. (2002). Toward maintaining the genome: DNA damage and replication checkpoints. *Annu. Rev. Genet.* 36, 617–656. doi: 10.1146/annurev.genet.36.060402.113540

Opresko, P. L., Cheng, W. H., and Bohr, V. A. (2004). Junction of RecQ helicase biochemistry and human disease. *J. Biol. Chem.* 279, 18099–18102. doi: 10.1074/jbc.R300034200

Pichierri, P., Ammazzalorso, F., Bignami, M., and Franchitto, A. (2011). The Werner syndrome protein: linking the replication checkpoint response to genome stability. *Aging* 3, 311–318.

Popuri, V., Hsu, J., Khadka, P., Horvath, K., Liu, Y., Croteau, D. L., et al. (2014). Human RECQL1 participates in telomere maintenance. *Nucleic Acids Res.* 42, 5671–5688. doi: 10.1093/nar/gku200

Ramamoorthy, M., Tadokoro, T., Rybanska, I., Ghosh, A. K., Wersto, R., and May, A. (2012). RECQL5 cooperates with Topoisomerase II alpha in DNA decatenation and cell cycle progression. *Nucleic Acids Res.* 40, 1621–1635. doi: 10.1093/nar/gkr844

Roninson, I. B., Broude, E. V., and Chang, B. D. (2001). If not apoptosis, then what? Treatment induced senscence and mitotic catastrophe in tumor cells. *Drug Resist. Updat.* 4, 303–313.

Rosenthal, A. S., Dexheimer, T. S., Gileadi, O., Nguyen, G. H., Chu, W. K., Hickson, I. D., et al. (2013). Synthesis and SAR studies of 5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine derivatives as potent inhibitors of Bloom helicase. *Bioorg. Med. Chem. Lett.* 23, 5660–5666. doi: 10.1016/j.bmcl.2013.08.025

Sakamoto, S., Nishikawa, K., Heo, S. J., Goto, M., Furuichi, Y., and Shimamoto, A. (2001). Werner helicase relocates into nuclear foci in response to DNA damaging agents and co-localizes with RPA and Rad51. *Genes Cells* 6, 421–430. doi: 10.1046/j.1365-2443.2001.00433.x

Sanada, S., Futami, K., Terada, A., Yonemoto, K., Ogasawara, S., Akiba, J., et al. (2013). RECQL1 DNA repair helicase: a potential therapeutic target and a proliferative marker against ovarian cancer. *PLoS ONE* 8:e72820. doi: 10.1371/journal.pone.0072820

Seki, M., Miyazawa, H., Tada, S., Yanagisawa, J., Yamaoka, T., Hoshino, S., et al. (1994). Molecular cloning of cDNA encoding human DNA helicase Q1 which has homology to *Escherichia coli* Rec Q helicase and localization of the gene at chromosome 12p12. *Nucleic Acids Res.* 22, 4566–4573. doi: 10.1093/nar/22.22.4566

Shadrick, W. R., Ndjomou, J., Kolli, R., Mukherjee, S., Hanson, A. M., and Frick, D. N. (2013). Discovering new medicines targetting helicases: challenges and recent progress. *J. Biomol. Screen.* 18, 761–781. doi: 10.1177/1087057113482586

Sharma, S., and Brosh, R. M. Jr. (2007). Human RECQ1 is a DNA damage responsive protein required for genotoxic stress resistance and suppression of sister chromatid exchanges. *PLoS ONE* 2:e1297. doi: 10.1371/journal.pone.0001297

Sharma, S., and Brosh, R. M. Jr. (2008). Unique and important consequences of RECQ1 deficiency in mammalian cells. *Cell Cycle* 7, 989–1000. doi: 10.4161/cc.7.8.5707

Sharma, S., Doherty, K. M., and Brosh, R. M. Jr. (2006). Mechanisms of RecQ helicases in pathways of DNA metabolism and maintenance of genomic stability. *Biochem. J.* 398, 319–337. doi: 10.1042/BJ20060450

Sharma, S., Stumpo, D. J., Balajee, A. S., Bock, C. B., Lansdorp, P. M., Brosh, R. M. Jr., and Blackshear, P. J. (2007). RECQL, a member of the RecQ family of DNA helicases, suppresses chromosomal instability. *Mol. Cell. Biol.* 27, 1784–1794. doi: 10.1128/MCB.01620-06

Shimamoto, A., Nishikawa, K., Kitao, S., and Furuichi, Y. (2000). Human RecQ5beta, a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3alpha and 3beta. *Nucleic Acids Res.* 28, 1647–1655. doi: 10.1093/nar/28.7.1647

Shimamoto, A., Sugimoto, M., and Furuichi, Y. (2004). Molecular biology of Werner syndrome. *Int. J. Clin. Oncol.* 9, 288–298. doi: 10.1007/s10147-004-0426-0

Slupianek, A., Poplawski, T., Jozwiakowski, S. K., Cramer, K., Pytel, D., Stoczynska, E., et al. (2011). BCR/ABL stimulates WRN to promote survival and genomic instability. *Cancer Res.* 71, 842–851. doi: 10.1158/0008-5472.CAN-10-1066

Su, Y., Meador, J. A., Calaf, G. M., Proietti De-Santis, L., Zhao, Y., Bohr, V. A., et al. (2010). Human RecQL4 helicase plays critical roles in prostate carcinogenesis. *Cancer Res.* 70, 9207–9217. doi: 10.1158/0008-5472.CAN-10-1743

Sugimoto, M. (2014). A cascade leading to premature aging phenotypes including abnormal tumor profiles in Werner syndrome. *Int. J. Mol. Med.* 33, 247–253. doi: 10.3892/ijmm.2013.1592

Tabernero, J., Shapiro, G. I., LoRusso, P. M., Cervantes, A., Schwartz, G. K., Weiss, G. J., et al. (2013). First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. *Cancer Discov.* 4, 406–417. doi: 10.1158/2159-8290.

Tahara, H., Tokutake, Y., Maeda, S., Kataoka, H., Watanabe, T., Satoh, M., et al. (1997). Abnormal telomere dynamics of B-lymphoblastoid cell strains from Werner's syndrome patients transformed by Epstein-Barr virus. *Oncogene* 15, 1911–1920. doi: 10.1038/sj.onc.1201377

Tao, J., Tao, S., Han, J., Zhou, Z., Zhang, X., Wang, H., et al. (2014). RECQL1 plays an important role in the development of tongue squamous cell carcinoma. *Cell Physiol. Biochem.* 33, 1579–1590. doi: 10.1159/000358721

Vaishnaw, A. K., Gollob, J., Gamba-Vitalo, C., Hutabarat, R., Sah, D., Meyers, R., et al. (2010). A status report on RNAi therapeutics. *Silence* 8, 1–14. doi: 10.1186/1758-907X-1-14

Vogel, C., Hager, C., and Bastians, H. (2007). Mechanism of mitotic cell death induced by chemotherapy-mediated G2 checkpoint abrogation. *Cancer Res.* 67, 339–345. doi: 10.1158/0008-5472.CAN-06-2548

Yamabe, Y., Shimamoto, A., Goto, M., Yokota, J., Sugawara, M., and Furuichi, Y. (1998). Sp1-mediated transcription of the Werner helicase gene is modulated by Rb and p53. *Mol. Cell. Biol.* 18, 6191–6200.

Yano, J., Hirabayashi, K., Nakagawa, S., Yamaguchi, T., Nogawa, M., Kashimori, I., et al. (2004). Antitumor activity of small interfering RNA/cationic liposome complex in mouse models of cancer. *Clin. Cancer Res.* 10, 7721–7726. doi: 10.1158/1078-0432.CCR-04-1049

Yonesaka, K., Tamura, K., Kurata, T., Satoh, T., Ikeda, M., Fukuoka, M., et al. (2006). Small interfering RNA targeting survivin sensitizes lung cancer cell with mutant p53 to adriamycin. *Int. J. Cancer* 118, 812–820. doi: 10.1002/ijc.21350

Yu, C. E., Oshima, J., Fu, Y. H., Wijsman, E. M., Hisama, F., Alisch, R., et al. (1996). Positional cloning of the Werner's syndrome gene. *Science* 272, 258–262. doi: 10.1126/science.272.5259.258

Zhou, B. B., and Elledge, S. J. (2000). The DNA damage response: putting checkpoints in perspective. *Nature* 408, 433–439. doi: 10.1038/35044005

Zhou, D., He, Q. S., Wang, C., Zhang, J., and Wong-Staal, F. (2006). RNA interference and potential applications. *Curr. Top. Med. Chem.* 6, 901–911. doi: 10.2174/156802606777303630

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 September 2014; accepted: 02 December 2014; published online: 09 January 2015.
Citation: Futami K and Furuichi Y (2015) RECQL1 and WRN DNA repair helicases: potential therapeutic targets and proliferative markers against cancers. Front. Genet. ***5****:441. doi: 10.3389/fgene.2014.00441*

This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.

frontiers in
GENETICS

REVIEW ARTICLE
published: 05 December 2014
doi: 10.3389/fgene.2014.00423

Multiple functions of DDX3 RNA helicase in gene regulation, tumorigenesis, and viral infection

Yasuo Ariumi*

Ariumi Project Laboratory, Center for AIDS Research – International Research Center for Medical Sciences, Kumamoto University, Kumamoto, Japan

Edited by:
Masayuki Seki, Tohoku Pharmaceutical University, Japan

Reviewed by:
Kaoru Tominaga, Jichi Medical University, Japan
Victoria V. Lunyak, Buck Institute for Research on Aging, USA

***Correspondence:**
Yasuo Ariumi, Ariumi Project Laboratory, Center for AIDS Research – International Research Center for Medical Sciences, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan
e-mail: ariumi@kumamoto-u.ac.jp

The DEAD-box RNA helicase DDX3 is a multifunctional protein involved in all aspects of RNA metabolism, including transcription, splicing, mRNA nuclear export, translation, RNA decay and ribosome biogenesis. In addition, DDX3 is also implicated in cell cycle regulation, apoptosis, Wnt-β-catenin signaling, tumorigenesis, and viral infection. Notably, recent studies suggest that DDX3 is a component of anti-viral innate immune signaling pathways. Indeed, DDX3 contributes to enhance the induction of anti-viral mediators, interferon (IFN) regulatory factor 3 and type I IFN. However, DDX3 seems to be an important target for several viruses, such as human immunodeficiency virus type 1 (HIV-1), hepatitis C virus (HCV), hepatitis B virus (HBV), and poxvirus. DDX3 interacts with HIV-1 Rev or HCV Core protein and modulates its function. At least, DDX3 is required for both HIV-1 and HCV replication. Therefore, DDX3 could be a novel therapeutic target for the development of drug against HIV-1 and HCV.

Keywords: DDX3, HCV, HIV-1, innate immunity, RNA helicases, stress granules, translation, tumor suppressor

INTRODUCTION

DDX3 belongs to the DEAD (D-E-A-D: Asp-Glu-Ala-Asp)-box RNA helicase family, which is an ATPase-dependent RNA helicase, is found in various organisms from yeast to human (Cordin et al., 2006; Linder and Lasko, 2006; Linder, 2008; Jankowsky, 2011). DDX3 has two homologs designated DDX3X (DBX) and DDX3Y (DBY), which were located on X and Y chromosomes, respectively (Lahn and Page, 1997; Park et al., 1998; Kim et al., 2001). DDX3X is ubiquitously expressed in most tissues, while the expression of DDX3Y protein is limited to the male germline (Ditton et al., 2004) and DDX3Y seems to be involved in male fertility (Leory et al., 1989; Mazeyrat et al., 1998; Foresta et al., 2000). DDX3 is involved in various RNA metabolism, including transcription, translation, RNA splicing, RNA transport, and RNA degradation (Chang and Liu, 2010; Schröder, 2010).

REGULATION OF GENE EXPRESSION BY DDX3

DDX3 regulates gene expression at different levels, such as transcription, splicing, mRNA export, and initiation of translation. First, DDX3 participates in transcriptional regulation of gene promoters. Indeed, DDX3 up-regulates the interferon (IFN) β promoter (Soulat et al., 2008) and the $p21^{waf1/cip1}$ promoter (Chao et al., 2006), respectively. DDX3 binds to the transcription factor Sp1 and enhance the $p21^{waf1/cip1}$ promoter. On the other hand, DDX3 down-regulates the E-cadherin promoter (Botlagunta et al., 2008). *In vivo* association of DDX3 with the E-cadherin or the IFNβ promoter was demonstrated by chromatin immunoprecipitation assay. Second, DDX3 seems to contribute to splicing. DDX3 associates with spliced mRNAs in an exon junction complex (EJC)-dependent manner (Merz et al., 2007) and DDX3 contains C-terminal RS-like domain, which is stretches of protein sequence rich in arginine and serine residues and is found in splicing factors. Third, DDX3 contributes to the nuclear export of RNA. DDX3 shuttles between the cytoplasm and the nucleus (Owsianka and Patel, 1999; Yedavalli et al., 2004; Lai et al., 2008; Schröder et al., 2008). Accordingly, DDX3 interacts with two nuclear export shuttle protein: CRM1 as a receptor for protein containing the nuclear export signal (NES) and tip-associated protein (TAP) as the major receptor for mRNA export (Yedavalli et al., 2004; Lai et al., 2008). DDX3 interacts with CRM1 and functions in the human immunodeficiency virus type 1 (HIV-1) Rev-dependent nuclear export of HIV-1 mRNA (Yedavalli et al., 2004). Depletion of TAP resulted in nuclear accumulation of DDX3, suggesting DDX3 exports along with messenger ribonucleoprotein (mRNP) to the cytoplasm via the TAP-mediated pathway (Lai et al., 2008).

Forth, DDX3 plays a role in translational regulation. DDX3 localizes in cytoplasmic stress granules under stress conditions (Lai et al., 2008; Shih et al., 2012), suggesting a role for DDX3 in translational control. DDX3 represses the cap-dependent translation by trapping eIF4E in a translationally inactive complex to block an interaction with eIF4G (Shih et al., 2008), indicating that DDX3 acts as a translational suppressor. Since depletion of DDX3 does not significantly affect general translation, DDX3 may be dispensable for general mRNA translation (Lai et al., 2008). Indeed, DDX3 associates with eIF4E together with several translation initiation factors, including eIF4a, eIF4G, eIF2a, eIF3, and poly(A)-binding protein (PABP), and facilitates translation of mRNA containing structured 5′ untranslated region (UTR; Lai et al., 2008; Shih et al., 2012; Soto-Rifo et al., 2012). In contrast, others reported that primary function for DDX3 is in protein translation via an interaction with eIF3 (Lee et al., 2008). Accordingly, DDX3 interacts with eIF3 and 40S ribosome to support the assembly of functional 80S ribosome (Geissler et al., 2012). The yeast DDX3 homolog, Ded1, also modulates translation by the formation of a translation initiation factor eIF4F-mRNA complex (Hilliker et al., 2011). Taken together, DDX3 modulates the protein translation.

Finally, DDX3 interacts with Ago2, which is an essential factor in RNA interference (RNAi) pathway that cleaves target mRNA, and acts as an essential factor involved in RNAi pathway (Kasim et al., 2013).

DDX3 IN CELL CYCLE REGULATION AND TUMORIGENESIS

It has been indicated a role of DDX3 in cell cycle regulation, apoptosis, and tumorigenesis. In the temperature-sensitive DDX3 mutant hamster cell line tsET24 or the DDX3 knockdown cells, cell cycle was impedes transition from G_1 to S-phase (Fukumura et al., 2003; Lai et al., 2010). DDX3 enhances cyclin E1 during cell cycle by a translational regulation (Lai et al., 2010). On the other hand, DDX3 regulates the cell cycle by inhibiting cyclin D1 and causing cell cycle arrest (Chao et al., 2006). DDX3 is known to be phosphorylated by cyclin B/cdc2 at threonine 204 to inhibit the function (Sekiguchi et al., 2007). Furthermore, DDX3 interacts with DDX5, which colocalizes with it in the cytoplasm through the phosphorylation of both proteins during G_2/M phase of cell cycle (Choi and Lee, 2012), indicating the cell cycle-dependent regulation of DDX3 localization and the function. During mouse early embryonic development, DDX3 also regulates cell survival and cell cycle (Li et al., 2014b).

It has been indicated the oncogenic role of DDX3 in breast cancer (Botlagunta et al., 2008). Activation of DDX3 by benzo[a]pyrene diol epoxide (BPDE) present in tobacco smoke, can promote growth, proliferation and neoplastic transformation of breast epithelial cells. Consistent with this finding, overexpression of DDX3 induced an epithelial-mesenchymal-like transformation, exhibited increased motility and invasive properties, and formed colonies in soft agar assays. In addition, DDX3 is recruited to the E-cadherin promoter and represses the E-cadherin expression resulting the increased cell migration and metastasis (Botlagunta et al., 2008). Similarly, DDX3 also modulates cell adhesion, motility and cancer cell metastasis via Rac1-mediated signaling pathway (Chen et al., 2014). In fact, DDX3 knockdown reduces the cell migration, the invasive and metastatic activities, suggesting that DDX3 is required for metastasis and the oncogenic role of DDX3 in malignant cancers. The DDX3 knockdown also reduces the expression of levels of both Rac1 and β-catenin. DDX3 regulates Rac1 mRNA translation through an interaction with its 5′UTR and affects β-catenin protein stability in Rac1-dependent manner. In response to Wnt signaling, DDX3 binds to casein kinase (CK) 1ε and stimulates CK1ε-mediated phosphorylation of the Wnt effector disheveled and thereby activates β-catenin (Cruciat et al., 2013), indicating a role of DDX3 as a regulator of Wnt-β-catenin network. Moreover, DDX3 may aid cancer progression by promoting increased levels of the transcription factor Snail (Sun et al., 2011). Snail is known to repress the expression of cellular adhesion proteins, leading to increased cell migration and metastasis of many types of cancer. In addition, recent study reported that positive DDX3 expression is significantly associated with large tumor size and high TNM (Tumor, Node, and Metastasis) stage, invasion, lymph node metastasis in gallbladder cancers (Miao et al., 2013), suggesting that DDX3 is a biomarker for metastasis and poor prognosis of gallbladder cancers. TNM classification is an anatomically based staging system that records the primary and regional nodal extent of the tumor and the absence or presence of metastases.

Hypoxia is a major characteristic of solid tumors and affects gene expression, which greatly impacts cellular and tumor tissue physiology particularly respiration and metabolism. Expression of hypoxia-responsive genes is predominantly regulated by hypoxia inducible factors (HIFs). DDX3 is aberrantly expressed in breast cancer cells ranging from weakly invasive to aggressive phenotypes (Botlagunta et al., 2011). HIF-1 binds to the DDX3 promoter and enhances the DDX3 expression (Botlagunta et al., 2011), indicating a DDX3 as a hypoxia inducible gene.

In contrast, DDX3 has been proposed to be a tumor suppressor (McGivern and Lemon, 2009). In fact, DDX3 inhibits colony formation in various cell lines and down-regulates cyclin D1 and up-regulates the $p21^{waf1/cip1}$ promoter (Chao et al., 2006). DDX3 expression is deregulated in hepatocellular carcinoma (HCC; Chang et al., 2006; Chao et al., 2006). Loss of DDX3 leads to enhanced cell proliferation and reduced apoptosis (Chang et al., 2006). Similarly, loss of DDX3 by p53 inactivation promotes tumor malignancy via the MDM2/Slug/E-cadherin pathway and consequently results in poor patient outcome in non-small-cell lung cancer (Wu et al., 2014). In addition, DDX3 contributes to both antiapoptotic and proapoptotic actions. Death receptors are found to be capped by an antiapoptotic protein complex containing GSK3, DDX3 and cIAP-1 and DDX3 protects from apoptotic signaling (Sun et al., 2008). In contrast, DDX3 also associates with p53, increases p53 accumulation, and positively regulates DNA damage-induced apoptosis (Sun et al., 2013). Furthermore, reduced $p21^{waf1/cip1}$ via alteration of p53-DDX3 pathway is associated with poor relapse-free survival in early stage human papillomavirus-associated lung cancer (Wu et al., 2011). Thus, $p21^{waf1/cip1}$ is considered to act as a tumor suppressor. Since low/negative DDX3 expression in tumor cells is significantly associated with aggressive clinical manifestations, low/negative expression of DDX3 might predict poor prognosis in oral cancer patients (Lee et al., 2014).

Altogether, DDX3 has both tumor suppression and oncogenic properties. This may reflect on the cell type used in their experiments. Further studies are necessary to clarify the potential role of DDX3 in cell growth regulation. These studies may shed a light on the development of drugs for chemotherapy against cancer and viral infection described below.

DDX3 AS A TARGET OF VIRUSES

DDX3 has been implicated in a target of several viruses, including hepatitis C virus (HCV), HIV-1, hepatitis B virus (HBV), West Nile virus (WNV), Japanese encephalitis virus, norovirus, pestivirus, vaccinia virus, and cytomegalovirus (**Table 1**). DDX3 is required for several RNA viral replication such as HCV and HIV-1, while DDX3 restricts HBV replication. At least, DDX3 may be a therapeutic target for anti-viral drug against HCV and HIV-1.

REQUIREMENT OF DDX3 IN HCV LIFE CYCLE

Hepatitis C virus is a causative agent of chronic hepatitis, which progresses to liver cirrhosis and HCC. HCV is an enveloped virus with a positive single-stranded 9.6 kb RNA genome, which encodes

Table 1 | DDX3 as a target of viruses.

Virus	Effect of DDX3 on viral replication	Viral binding protein	Cellular function
HCV	Up-regulation	Core	Translational regulation
HIV-1	Up-regulation	Rev	Nuclear export of mRNA
		Tat	Translational regulation
HBV	Down-regulation	Pol	Inhibition of IFN induction
Vaccinia virus	?	K7	Inhibition of IFN induction
WNV	Up-regulation	?	?

DDX3 interacts with several RNA virus including hepatitis C virus (HCV), human immunodeficiency virus type 1 (HIV-1), hepatitis B virus (HBV), vaccinia virus, and West Nile virus (WNV). DDX3 is required for HCV, HIV-1, WNV replication, while DDX3 restricts HBV replication. Furthermore, these viral proteins suppress the DDX3-mediated type I IFN induction though an interaction with DDX3.

a large polyprotein precursor of ∼3,000 amino acid residues (Kato et al., 1990). This polyprotein is cleaved by a combination of the host and viral proteases into at least 10 proteins in the following order: core, envelope 1 (E1), E2, p7, non-structural 2 (NS2), NS3, NS4A, NS4B, NS5A, and NS5B (Hijikata et al., 1991, 1993). The HCV core protein is a viral structural protein, which forms the viral nucleocapsid, is targeted to lipid droplets (LDs). Recently, LDs have been found to be an important cytoplasmic organelle for HCV production (Miyanari et al., 2007). Budding is an essential step in the life cycle of enveloped viruses. HCV utilizes the endosomal sorting complex required for transport (ESCRT) system as the budding machinery (Ariumi et al., 2011b).

Several DEAD-box RNA helicases have been shown to interact with HCV proteins and regulate the HCV replication (Schröder, 2010; Upadya et al., 2014). DDX3 was identified as an HCV core-binding protein by yeast two-hybrid screening (Mamiya and Worman, 1999; Owsianka and Patel, 1999; You et al., 1999). HCV core protein was the first viral protein to be described as a DDX3-binding protein. HCV core binds to the C-terminal RS-like domain of DDX3 and the interaction is mediated by the N-terminal 59 amino acid residues of HCV core. DDX3 and HCV core colocalized in distinct spots in the perinuclear region of the cytoplasm. However, these studies lack evidence regarding the functional relevance of the DDX3-HCV core interaction in HCV replication and the HCV-associated liver diseases. Recent studies have demonstrated that DDX3 is required for HCV replication (Ariumi et al., 2007; Randall et al., 2007). The accumulation of both genome-length HCV RNA (HCV-O strain, genotype 1b; Ikeda et al., 2005) and its replicon RNA were significantly suppressed in the DDX3 knockdown cells. As well, HCV infection (JFH1 strain, genotype 2a; Wakita et al., 2005) was also suppressed in the DDX3 knockdown cells. Notably, HCV infection dynamically redistributes DDX3 to the HCV production site around LDs and colocalizes with HCV core (**Figure 1**; Ariumi et al., 2011a). However, the specific interactions between DDX3 and HCV core and the functional importance of these interactions for the HCV viral life cycle remain unclear. In this regard, Mutagenesis studies located a single amino acid in the N-terminal domain of JFH1 core that when changed to alanine significantly abrogated this interaction. Surprisingly, this mutation did not alter infectious virus production and RNA replication, indicating that the core-DDX3 interaction is dispensable in the HCV life cycle (Angus et al., 2010). On the other hand, there is a contradictory report that the inhibition of HCV replication due to expression of the green fluorescent protein (GFP) fusion to HCV core protein residues 16–36 can be reversed by overexpression of DDX3 (Sun et al., 2010). These results suggest that the protein interface on DDX3 that binds the HCV core protein is important for replicon maintenance. However, infection

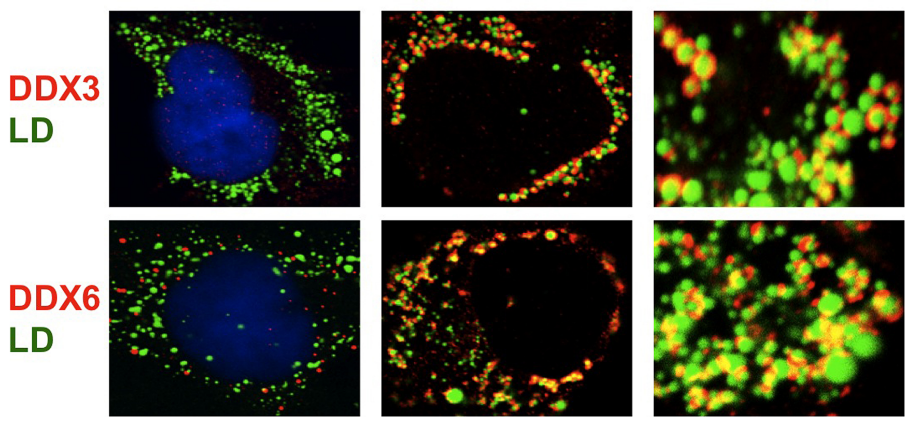


FIGURE 1 | Dynamic recruitment of DDX3 and DDX6 around lipid droplets (LDs) in response to HCV-JFH1 infection. Cells were fixed 60 h post-infection with HCV (JFH1 strain) and stained with either anti-DDX3 or anti-DDX6 antibody and were then visualized with Cy3 (red). Lipid droplets were specifically stained with fluorescent lipophilic dye BODIPY 493/503 (green; Listenberger and Brown, 2007) and nuclei were stained with DAPI (blue), respectively. Images were visualized using confocal laser scanning microscopy. The two-color overlay images are also exhibited (merged). Colocalization is shown in yellow. High magnification image is also shown.

of HuH-7 cells by HCV (JFH1) was not affected by expression of the GFP fusion protein. These results suggest that the role of DDX3 in HCV infection involves aspects of the viral life cycle that vary in importance between HCV genotypes. Therefore, the exact contribution of HCV core-DDX3 interaction remains to be determined.

In addition to DDX3, other DEAD-box RNA helicases DDX1, DDX5, and DDX6 have been involved in the HCV life cycle (Goh et al., 2004; Tingting et al., 2006; Jangra et al., 2010; Ariumi et al., 2011a; Kuroki et al., 2013). DDX1 bound to both the HCV 3′UTR and the HCV 5′UTR and DDX1 knockdown caused a marked reduction in the replication of subgenomic replicon RNA (Tingting et al., 2006). Furthermore, DDX5 was identified as an HCV NS5B RNA-dependent RNA polymerase-binding protein by yeast two-hybrid screening (Goh et al., 2004). Depletion of endogenous DDX5 correlated with a reduction in the transcription of negative strand HCV RNA, suggesting that DDX5 participates in the HCV RNA replication. Overexpression of HCV NS5B or the HCV infection redistributes DDX5 from the nucleus to the cytoplasm. Moreover, recent study reported that knockdown of DDX5 reduces HCV (JFH1) virus production in the supernatant, suggesting that DDX5 is important for a late stage of the HCV life cycle (Kuroki et al., 2013).

The microRNA miR122 and DDX6/Rck/p54, a microRNA effector, have been implicated in HCV replication (Jopling et al., 2005; Scheller et al., 2009; Jangra et al., 2010; Ariumi et al., 2011a). The liver-specific and abundant miR-122 interacts with the 5′UTR of the HCV RNA genome and facilitates the HCV replication (Jopling et al., 2005). DDX6 interacts with the eukaryotic initiation factor 4E (eIF-4E) to repress the translational activity of mRNP. Furthermore, DDX6 regulates the activity of the decapping enzymes DCP1 and DCP2 and interacts directly with Argonaute-1 (Ago1) and Ago2 in the microRNA-induced silencing complex (miRISC) and is involved in RNA silencing. DDX6 predominantly localizes in the discrete cytoplasmic foci termed processing (P)-body. Thus, the P-body may play a role in the translation repression and mRNA decay machinery (Parker and Sheth, 2007; Beckham and Parker, 2008). The knockdown of DDX6 was found to reduce the accumulation of intracellular HCV RNA and infectious HCV production, indicating that DDX6 is essential for the HCV RNA replication (Scheller et al., 2009; Jangra et al., 2010; Ariumi et al., 2011a). Notably, HCV (JFH1) infection disrupts the P-body formation of DDX3, DDX6, Lsm1, Xrn1, PATL1, and Ago2 and dynamically redistributes them to the HCV production site around LDs (**Figure 2**; Ariumi et al., 2011a), indicating that HCV hijacks the P-body components around LDs and regulates the HCV replication and translation. Recent studies suggested that DDX3 is also required for WNV, Japanese encephalitis virus, norovirus, and pestivirus (Vashist et al., 2012; Chahar et al., 2013; Jefferson et al., 2014; Li et al., 2014a; Tsai and Lloyd, 2014). Similarly, P-body components LSM1, GW182, DDX3, DDX6, and XRN1 are also recruited to WNV replication sites and positively regulate viral replication (Chahar et al., 2013).

On the other hand, recent studies have suggested a potential role of DDX3 and DDX5 in the pathogenesis of HCV-related liver diseases. DDX3 expression is deregulated in HCC (Chang et al., 2006; Chao et al., 2006) and single-nucleotide polymorphisms were identified in the DDX5 genes that were associated with an increased risk of advanced fibrosis in patients with chronic hepatitis C (Huang et al., 2006). DDX3 has been proposed to be a tumor suppressor (McGivern and Lemon, 2009). In fact, DDX3 inhibits colony formation in various cell lines, including human hepatoma HuH-7, and up-regulates the $p21^{waf1/cip1}$ promoter (Chao et al., 2006). Therefore, HCV core protein might overcome the DDX3-mediated cell growth arrest and down-regulate $p21^{waf1/cip1}$ through an interaction with DDX3, and it might be involved in the development of HCC.

DDX3 IS ESSENTIAL FOR HIV-1 REPLICATION

Human immunodeficiency virus type 1 is the causative agent of acquired immune deficiency syndrome (AIDS). HIV-1 is a retrovirus with a positive strand RNA genome of 9 kb which encodes nine polypeptides, structural proteins, Gag (group specific antigen), Pol (polymerase) and Env (envelope), the accessory proteins, Vif, Vpu, Vpr, and Nef, and the regulatory proteins, Tat and Rev. The gene expression of HIV-1 is regulated transcriptionally by Tat through its binding to a nascent viral *trans*-activation responsive (TAR) RNA (Berkhout et al., 1989; Jeang et al., 1999),

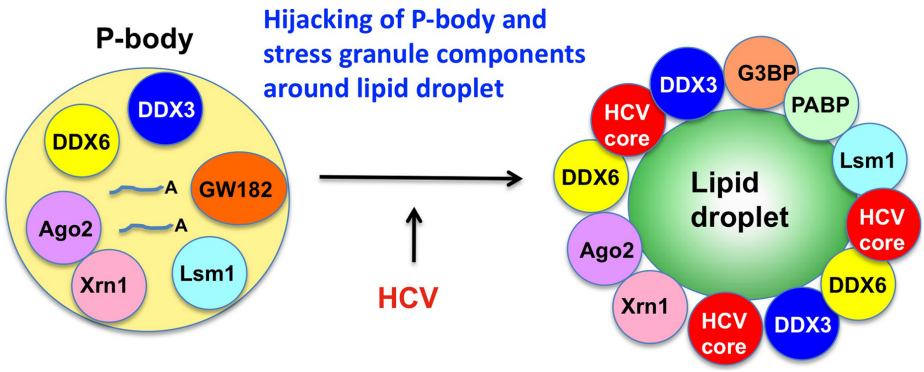


FIGURE 2 | Hijacking of P-body components around LD by HCV. HCV disrupts the P-body and hijacks the P-body components including DDX3, DDX6, Ago2, Xrn1, and Lsm1 around LD, an HCV production site.

and post-transcriptionally by Rev through its association with Rev-responsive element (RRE) in the *env* gene (Hope and Pomerantz, 1995; Pollard and Malim, 1998; Cullen, 2003). Since the intron-containing host RNA cannot leave the nucleus before it is completely spliced, HIV-1 needs to evade host surveillance system to export unspliced or partially spliced viral RNA into cytoplasm and produce HIV-1 structural proteins and accessory proteins. For this, Rev contains a leucine-rich NES that recruits nuclear export receptor CRM1 (Hope and Pomerantz, 1995; Pollard and Malim, 1998; Cullen, 2003). Upon binding to the RRE together with the GTP-bound form of Ran (Ran-GTP), CRM1 forms the nuclear export complex and Rev-CRM1-RRE-Ran-GTP complex exports unspliced or partially spliced HIV-1 RNA from the nucleus to the cytoplasm.

Several viruses are known to carry their own RNA helicases to facilitate the replication of their viral genome, including HCV, flavivirus, severe acute respiratory syndrome (SARS) coronavirus, rubella virus, and alphavirus, however, HIV-1 does not encode own RNA helicase (Utama et al., 2000; Kwong et al., 2005). Thus, host RNA helicases may be involved in HIV-1 replication at multiple stages, including the reverse transcription of HIV-1 RNA, HIV-1 mRNA transcription, the nucleus-to-cytoplasm transport of HIV-1 mRNA, and HIV-1 RNA packaging (Cochrane et al., 2006; Lorgeoux et al., 2012).

In fact, DDX3 was first found to involve in the Rev-dependent nuclear export of unspliced and partially spliced HIV-1 RNAs (**Figure 3**; Yedavalli et al., 2004). Over-expression of DDX3 enhanced the Rev-dependent nuclear export function. Conversely, knockdown of DDX3 or expression of dominant negative mutant of DDX3 significantly suppressed the Rev function as well as HIV-1 replication (Yedavalli et al., 2004; Ishaq et al., 2008). Rev is co-immunoprecipitated with DDX3. DDX3 is a nucleo-cytoplasmic shuttling protein, which binds CRM1 and localizes to nuclear membrane pores.

In addition to DDX3, another DEAD-box RNA helicase DDX1 also associates with Rev and promotes the Rev-dependent RNA nuclear export function (Fang et al., 2004). DDX1 interacts with Rev via the N-terminal domain, suggesting a role of DDX1 in initial complex assembly. DDX1 promotes Rev oligomerization on the RRE through this interaction (Robertson-Anderson et al., 2011). Thus, DDX1 and DDX3 act sequentially in the Rev-dependent RNA nuclear export. DDX1 first binds to Rev and promotes Rev oligomerization on the RRE. Then, the oligomerized Rev recruits the CRM1/DDX3 complex that subsequently exports the RRE-containing HIV-1 RNAs into the cytoplasm (Lorgeoux et al., 2012). In addition to DDX1 and DDX3, we and other group recently reported that other RNA helicases, including DDX5, DDX17, DDX21, DHX36, DDX47, DDX56, and RNA helicase A (RHA) associate with the Rev-dependent nuclear export function (**Figure 3**; Li et al., 1999; Naji et al., 2012; Yasuda-Inoue et al., 2013a; Zhou et al., 2013). Furthermore, DDX3 interacts with DDX5 and synergistically enhances the Rev-dependent nuclear export. As well, combination of other distinct DDX RNA helicases such as DDX1 and DDX3 also synergistically facilitates the Rev function (Yasuda-Inoue et al., 2013a) suggesting that a set of distinct Rev-interacting DEAD-box RNA helicases cooperate to modulate the HIV-1 Rev function.

On the other hand, HIV-1 Tat activates the HIV-1 RNA synthesis. Tat binds to the TAR RNA and recruits several host factors including p300/CREB-binding protein (p300/CBP), p300/CBP-associated factor (PCAF), SWI/SNF chromatin-remodeling complex, and positive transcription elongation factor b (P-TEFb) to stimulate both transcription initiation and elongation (Jeang et al., 1999; Ariumi et al., 2006; Lorgeoux et al., 2012). P-TEFb

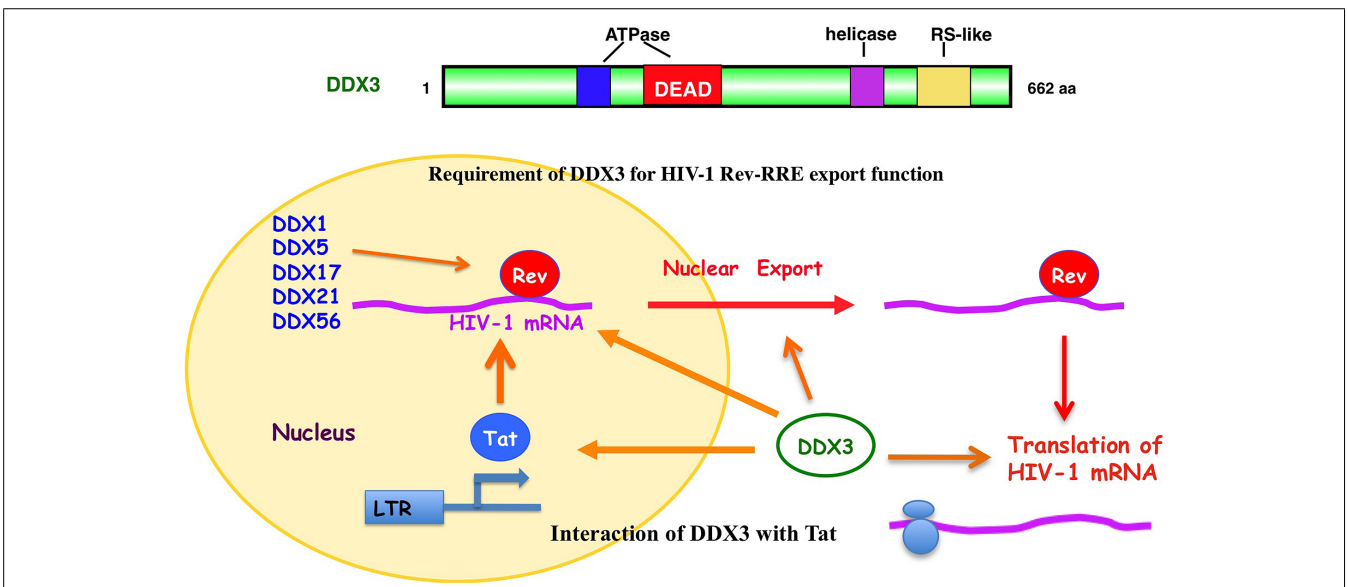


FIGURE 3 | Role of DDX3 in the HIV-1 gene expression. DDX3 interacts with HIV-1 Rev and facilitates the Rev-dependent nuclear export of HIV-1 mRNA. DDX3 interacts with Tat and contributes to the translation of HIV-1 mRNA. Other DEAD-box RNA helicases, including DDX1, DDX5, DDX17, DDX21, and DDX56, also interact with HIV-1 Rev and facilitate its function.

contains cyclin T1 and cyclin-dependent kinase 9 (CDK9). CDK9 hyperphosphorylates the C-terminal domain (CTD) of RNA Pol II and activates transcription elongation. The Werner syndrome (WRN) helicase and RHA were reported to act as co-factors of Tat and enhance the HIV-1 gene expression (Fujii et al., 2001; Sharma et al., 2007). In addition to WRN and RHA, DDX3 interacts with Tat (**Figure 3**; Lai et al., 2013; Yasuda-Inoue et al., 2013b). Tat is partially targeted to cytoplasmic stress granules upon DDX3 overexpression or cell stress conditions, suggesting a potential role of Tat/DDX3 complex in translation. Accordingly, Tat remains associated with translating mRNAs and facilitates translation of mRNAs containing the HIV-1 5′UTR. In this regard, DDX3 is essential for translation of HIV-1 genomic RNA (gRNA; **Figure 3**; Soto-Rifo et al., 2012). DDX3 directly binds to the HIV-1 5′UTR and interacts with eIF4G and PABP but lacking the major cap-binding proteins eIF4E in large cytoplasmic RNA granules (Soto-Rifo et al., 2013), indicating that DDX3 promotes the HIV-1 gRNA translation initiation in an eIF4E-independent manner.

Both HIV-1 and HCV have been shown to utilize DDX3 as a cofactor for viral genome replication. Therefore, DDX3 could be an important therapeutic target for development of anti-viral drug (Kwong et al., 2005). Indeed, small molecule inhibitors were used to inhibit ATPase activity of DDX3 with anti-HIV-1 activity (Maga et al., 2008, 2011; Yedavalli et al., 2008; Radi et al., 2012).

DDX3 RESTRICTS HBV REPLICATION

Hepatitis B virus is also the causative agent of chronic hepatitis, which progresses to liver cirrhosis and HCC worldwide. HBV belongs to hepadnavirus family and contains a small partially double-stranded circular DNA genome of 3.2 kb. Even though HBV is a DNA virus, HBV replicates its DNA genome via reverse transcription. Upon HBV infection, the HBV DNA is converted into covalently closed circular DNA (cccDNA) as the template for the viral transcription. Pregenomic RNA (pgRNA) of 3.5 kb is selectively packaged into nucleocapsid together with HBV Pol. The pgRNA is reverse transcribed by HBV Pol to generate relaxed circular (RC) DNA. The HBV reverse transcription occurs entirely within nucleocapsid following encapsidation.

Recently, it was shown that DDX3 specifically binds to the HBV Pol and is incorporated into nucleocapsid together with HBV Pol (Wang et al., 2009). However, unlike HIV-1 and HCV replication, which is enhanced by DDX3 (Yedavalli et al., 2004; Ariumi et al., 2007; Randall et al., 2007), HBV reverse transcription was inhibited by DDX3. In addition, recent study reported that DDX3 suppresses transcription from HBV promoter (Ko et al., 2014). The helicase activity is dispensable for this DDX3-mediated transcription suppression. Thus, DDX3 is identified as a new host restriction factor for HBV.

ROLE OF DDX3 IN ANTI-VIRAL INNATE IMMUNITY

Viral infection triggers host innate immune responses through activation of the transcription factors NF-κB and IFN regulatory factor (IRF)-3 leading to type I IFN production and anti-viral state in mammalian cells (Gale and Foy, 2005; Saito and Gale, 2007). Similar to NF-κB, IRF-3 is retained in cytoplasm in uninfected cells. After viral infection, IRF-3 is phosphorylated by IKKε and TBK1 and the phosphorylated IRF-3 then homodimerizes and translocates into the nucleus to activate type I IFN. Type I IFNs, such as IFN-α and IFN-β are essential for immune defense against viruses. These IFNs activate the JAK-STAT pathway to induce the IFN-stimulated genes (ISGs), which impact immune enhancing and antiviral action of host cells.

Double-stranded RNA (dsRNA) produced during viral replication is recognized by the host cell as pathogen-associated molecular patterns (PAMPs) by two major pathogen recognition receptor (PRR) proteins: the Toll-like receptors (TLRs; Akira and Takeda, 2004) and DEAD-box RNA helicases RIG-I and Mda5 (Andrejeva et al., 2004; Yoneyama et al., 2004). RIG-I contains two N-terminal caspase activation and recruitment domains (CARD) and a C-terminal RNA helicase domain that binds to dsRNA. Binding viral RNA to RIG-I lead to a conformational change that allows to interact with the RIG-I/Mda5 adaptor IPS-1/MAVS/Cardif/VISA (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005) leading to the activation of IRF-3 and NF-κB. Notably, RIG-I and Mda5 distinguish RNA viruses and are critical for host antiviral responses (Kato et al., 2006). RIG-I is essential for the production of IFN in response to RNA viruses including paramyxoviruses, influenza virus and Japanese encephalitis virus, while Mda5 is critical for picornavirus detection.

DDX3 was recently reported to be a component of anti-viral innate immune signaling pathway leading to type I IFN (**Figure 4**; Schröder et al., 2008; Soulat et al., 2008; Gu et al., 2013). Indeed, DDX3 contributes to enhance the induction of anti-viral mediators, IRF3 and type I IFN. DDX3 up-regulates the IFN-β induction through an interaction with IKKε (**Figure 4**; Schröder et al., 2008; Gu et al., 2013) or TBK1 (Soulat et al., 2008). Phosphorylation of

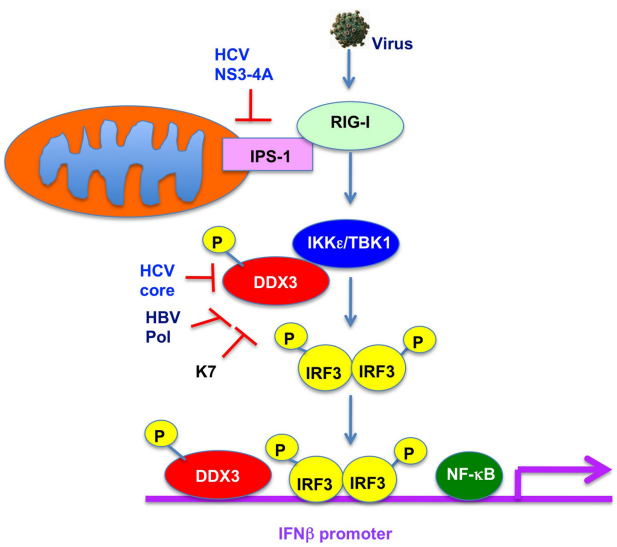


FIGURE 4 | Role of DDX3 in anti-viral innate immunity. DDX3 interacts with TBK1/IKKε and is phosphorylated by TBK1/IKKε. TBK1/IKKε then phosphorylates IRF3 and translocates into the nucleus leading to the activation of IFNβ promoter. DDX3 is also recruited on the IFNβ promoter and enhances the IFNβ production. In contrast, HCV core, HBV Pol, or vaccinia virus K7 interacts with DDX3 and suppresses the IFNβ induction.

DDX3 at serine 102 by IKKε was required for the recruitment of IRF-3 into the complex. Both IKKε and TBK1 are IRF-3-activating kinase to leading the NF-κB and IFN induction. Furthermore, DDX3 is recruited to the IFNβ promoter (**Figure 4**; Soulat et al., 2008), suggesting that DDX3 acts as a transcriptional regulator. In addition, DDX3 also forms a complex with RIG-I and Mda5 and binds to IPS-1 to facilitate IFNβ induction (Oshiumi et al., 2010b), suggesting that DDX3 acts as a viral RNA sensor and a scaffolding adaptor to link of viral RNA with the IPS-1 complex.

In contrast, viruses must overcome the host anti-viral innate immunity. HCV NS3-4A protease cleaves IPS-1/Cardif to block IFNβ induction (**Figure 4**; Meylan et al., 2005) In addition, HCV core protein can disrupt the DDX3-IPS-1/MAVS/Cardif/VISA interaction and act as a viral immune evasion protein preventing IFNβ induction (**Figure 4**; Oshiumi et al., 2010a). Furthermore, DDX3 is known to bind to HBV Pol and restrict the HBV replication (Wang et al., 2009). Conversely, HBV Pol acts as a viral immune evasion protein by disrupting the interaction of DDX3 with TBK1/ IKKε (**Figure 4**; Wang and Ryu, 2010; Yu et al., 2010). Similarly, vaccinia virus K7 protein targets DDX3 (Schröder et al., 2008; Kalverda et al., 2009; Oda et al., 2009) and inhibits the IFNβ induction by preventing TBK1/ IKKε-mediated IRF activation (**Figure 4**; Schröder et al., 2008). Moreover, DDX3 contributes the DNA sensor ZBP1/DAI-dependent IFN response after human cytomegalovirus infection (DeFilippis et al., 2010).

In conclusion, DDX3 participates in anti-viral innate immune signaling pathway leading to type I IFN induction. In contrast, viruses must target DDX3 and evolve mechanisms to overcome this host immune system. Indeed, several RNA viruses sequester and utilize DDX3 for their viral replication and prevent IFN induction.

ACKNOWLEDGMENTS

I thank Ms. Noriko Tokushige for secretarial assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS), by a Grant-in-Aid for Research on Hepatitis from the Ministry of Health, Labor, and Welfare of Japan, and by Takeda Science Foundation.

REFERENCES

Akira, S., and Takeda, K. (2004). Toll-like receptor signaling. *Nat. Rev. Immunol.* 4, 499–511. doi: 10.1038/nri1391

Andrejeva, J., Child, K. S., Young, D. F., Carlos, T. S., Stocks, N., Goodbourn, S., et al. (2004). The V protein of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN- promoter. *Proc. Natl. Acad. Sci. U.S.A.* 101, 17264–17269. doi: 10.1073/pnas.0407639101

Angus, A. G., Dalrymple, D., Boulant, S., McGivern, D. R., Clayton, R. F., Scott, M. J., et al. (2010). Requirement of cellular DDX3 for hepatitis C virus replication is unrelated to its interaction with the viral core protein. *J. Gen. Virol.* 91, 122–132. doi: 10.1099/vir.0.015909-0

Ariumi, Y., Kuroki, M., Abe, K., Dansako, H., Ikeda, M., Wakita, T., et al. (2007). DDX3 DEAD-box RNA helicase is required for hepatitis C virus RNA replication. *J. Virol.* 81, 13922–139226. doi: 10.1128/JVI.01517-07

Ariumi, Y., Kuroki, M., Kushima, Y., Osugi, K., Hijikata, M., Maki, M., et al. (2011a). Hepatitis C virus hijacks P-body and stress granule components around lipid droplets. *J. Virol.* 85, 6882–6892. doi: 10.1128/JVI02418-10

Ariumi, Y., Kuroki, M., Maki, M., Ikeda, M., Dansako, H., Wakita, T., et al. (2011b). The ESCRT system is required for hepatitis C virus production. *PLoS ONE* 6:e14517. doi: 10.1371/journal.pone.0014517

Ariumi, Y., Serhan, F., Turelli, P., Telenti, A., and Trono, D. (2006). The integrase interactor 1 (INI1) proteins facilitate Tat-mediated human immunodeficiency virus type 1 transcription. *Retrovirology* 3, 47. doi: 10.1186/1742-4690-3-47

Beckham, C. J., and Parker, R. (2008). P bodies, stress granules, and viral life cycle. *Cell Host Microbe* 3, 206–212. doi: 10.1016/j.chom.2008.03.004

Berkhout, B., Silverman, R. H., and Jeang, K. T. (1989). Tat *trans*-activates the human immunodeficiency virus through a nascent RNA target. *Cell* 59, 273–282. doi: 10.1016/0092-8674(89)90289-4

Botlagunta, M., Krishnamachary, B., Vesuna, F., Winnard, P. T. Jr., Bol, G. M., Patel, A. H., et al. (2011). Expression of DDX3 is directly modulated by hypoxia inducible factor-1 alpha in breast epithelial cells. *PLoS ONE* 6:e17563. doi: 10.1371/journal.pone.0017563

Botlagunta, M., Vesuna, F., Mironchik, Y., Raman, A., Lisok, A., Winnard, P. Jr., et al. (2008). Oncogenic role of DDX3 in breast cancer biogenesis. *Oncogene* 27, 3912–3922. doi: 10.1038/onc.2008.33

Chahar, H. S., Chen, S., and Manjunath, N. (2013). P-body components LSM1, GW182, DDX3, DDX6 and XRN1 are recruited to WNV replication sites and positively regulate viral replication. *Virology* 436, 1–7. doi: 10.1016/j.virol.2012.09.041

Chang, P. C., Chi, C. W., Chau, G. Y., Li, F. Y., Tsai, Y. H., Wu, J. C., et al. (2006). DDX3, a DEAD box RNA helicase, is deregulated in hepatitis virus-associated hepatocellular carcinoma and is involved in cell growth control. *Oncogene* 25, 1991–2003. doi: 10.1038/sj.onc.1209239

Chang, T. C., and Liu, W. S. (2010). The molecular evolution of PL10 homologs. *BMC Evol. Biol.* 10:127. doi: 10.1186/1471-2148-10-127

Chao, C. H., Chen, C. M., Cheng, P. L., Shih, J. W., Tsou, A. P., and Lee, Y. H. (2006). DDX3, a DEAD box RNA helicase with tumor growth-suppressive property and transcriptional regulation activity of the p21waf1/cip1 promoter, is a candidate tumor suppressor. *Cancer Res.* 66, 6579–6588. doi: 10.1158/0008-5472.CAN-05-2415

Chen, H. H., Yu, H. I., Cho, W. C., and Tarn, W. Y. (2014). DDX3 modulates cell adhesion and mobility and cancer cell metastasis via Rac1-mediated signaling pathway. *Oncogene* doi: 10.1038/onc.2014.190 [Epub ahead of print].

Choi, Y. J., and Lee, S. G. (2012). The DEAD-box RNA helicase DDX3 interacts with DDX5, co-localizes with it in the cytoplasm during the G2/M phase of the cycle, and affects its shuttling during mRNP export. *J. Cell Biochem.* 113, 985–996. doi: 10.1002/jcb.23428

Cochrane, A. W., McNally, M. T., and Mouland, A. J. (2006). The retrovirus RNA trafficking granule: from birth to maturity. *Retrovirology* 3, 18. doi: 10.1186/1742-4690-3-18

Cordin, O., Banroques, J., Tanner, N. K., and Linder, P. (2006). The DEAD-box protein family of RNA helicases. *Gene* 367, 17–37. doi: 10.1016/j.gene.2005.10.019

Cruciat, C. M., Dolde, C., de Groot, R. E., Ohkawara, B., Reinhard, C., Korswagen, H. C., et al. (2013). RNA helicase DDX3 is a regulatory subunit of casein kinase 1 in Wnt-β-catenin signaling. *Science* 339, 1436–1441. doi: 10.1126/science.1231499

Cullen, B. R. (2003). Nuclear mRNA export: insights from virology. *Trends Biochem. Sci.* 28, 419–424. doi: 10.1016/S0968-0004(03)00142-7

DeFilippis, V. R., Alvarado, D., Sali, T., Rothenburg, S., and Früh, K. (2010). Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1. *J. Virol.* 84, 585–598. doi: 10.1128/JVI.01748-09

Ditton, H. J., Zimmer, J., Kamp, C., Rajpert-De Meyts, E., and Vogt, P. H. (2004). The AZFa gene DBY (DDX3Y) is widely transcribed but the protein is limited to the male germ cells by translation control. *Hum. Mol. Genet.* 13, 2333–2341. doi: 10.1093/hmg/ddh240

Fang, J., Kubota, S., Yang, B., Zhou, N., Zhang, H., Godbout, R., et al. (2004). A DEAD box protein facilitates HIV-1 replication as a cellular co-factor of Rev. *Virology* 330, 471–480. doi: 10.1016/j.virol.2004.09.039

Foresta, C., Ferlin, A., and More, E. (2000). Deletion and expression analysis of AZFa genes on the human chromosome revealed a major role for DBY in male infertility. *Hum. Mol. Genet.* 9, 1161–1169. doi: 10.1093/hmg/9.8.1161

Fujii, R., Okamoto, M., Aratani, S., Oishi, T., Ohshima, T., Taira, K., et al. (2001). A role of RNA helicase A in *cis*-acting transactivation response element-mediated transcriptional regulation of human immunodeficiency virus type 1. *J. Biol. Chem.* 276, 5445–5451. doi: 10.1074/jbc.M006892200

Fukumura, J., Noguchi, E., Sekiguchi, T., and Nishimoto, T. (2003). A temperature-sensitive mutant of the mammalian RNA helicase, DEAD-box isoform, DBX, defective in the transition from G1 to S phase. *J. Biochem.* 134, 71–82. doi: 10.1093/jb/mvg126

Gale, M. Jr., and Foy, E. M. (2005). Evasion of intracellular host defence by hepatitis C virus. *Nature* 436, 939–945. doi: 10.1038/nature04078

Geissler, R., Golbik, R. P., and Behrens, S. E. (2012). The DEAD-box helicase DDX3 supports the assembly of functional 80S ribosomes. *Nucleic Acids Res.* 40, 4998–5011. doi: 10.1093/nar/gks070

Goh, P. Y., Tan, Y. J., Lim, S. P., Tan, Y. H., Lim, S. G., Fuller-Pace, F., et al. (2004). Cellular RNA helicase p68 relocalization and interaction with the hepatitis C virus (HCV) NS5B protein and the potential role of p68 in HCV RNA replication. *J. Virol.* 78, 5288–5298. doi: 10.1128/JVI.7810.5288-5298.2004

Gu, L., Fullam, A., Brennan, R., and Schröder, M. (2013). Human DEAD box helicase 3 couples IκB kinase ε to interferon regulatory factor 3 activation. *Mol. Cell. Biol.* 33, 2004–2015. doi: 10.1128/MCB.01603-12

Hijikata, M., Kato, N., Ootsuyama, Y., Nakagawa, M., and Shimotohno, K. (1991). Gene mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis. *Proc. Natl. Acad. Sci. U.S.A.* 88, 5547–5551. doi: 10.1073/pnas.88.13.5547

Hijikata, M., Mizushima, H., Tanji, Y., Komoda, Y., Hirowatari, Y., Akagi, T., et al. (1993). Proteolytic processing and membrane association of putative nonstructural proteins of hepatitis C virus. *Proc. Natl. Acad. Sci. U.S.A.* 90, 10773–10777. doi: 10.1073/pnas.90.22.10773

Hilliker, A., Gao, Z., Jankowsky, E., and Parker, R. (2011). The DEAD-box protein Ded1 modulates translation by the formation and resolution of an eIF4F-mRNA complex. *Mol. Cell* 43, 962–972. doi: 10.1016/j.molcel.2011.08.008

Hope, T. J., and Pomerantz, R. J. (1995). The human immunodeficiency virus type 1 Rev protein: a pivotal protein in the viral life cycle. *Curr. Top. Microbiol. Immunol.* 193, 91–105. doi: 10.1007/978-3-642-78929-8_5

Huang, H., Shiffman, M. L., Cheung, R. C., Layden, T. J., Friedman, S., Abar, O. T., et al. (2006). Identification of two gene variants associated with risk of advanced fibrosis in patients with chronic hepatitis C. *Gastroenterology* 130, 1679–1687. doi: 10.1053/j.gastro.2006.02.032

Ikeda, M., Abe, K., Dansako, H., Nakamura, T., Naka, K, and Kato, N. (2005). Efficient replication of a full-length hepatitis C virus genome, strain O, in cell culture, and development of a luciferase reporter system. *Biochem. Biophys. Res. Commun.* 329, 1350–1359. doi: 10.1016/j.bbrc.2005.02.138

Ishaq, M., Hu, J., Wu, X., Fu, Q., Yang, Y., Liu, Q., et al. (2008). Knockdown of cellular RNA helicase DDX3 by short hairpin RNAs suppresses HIV-1 replication without inducing apoptosis. *Mol. Biotechnol.* 39, 231–238. doi: 10.1007/s12033-008-9040-0

Jangra, R. K., Yi, M., and Lemon, S. M. (2010). DDX6 (Rck/p54) is required for efficient hepatitis C virus replication but not for internal ribosome entry site-directed translation. *J. Virol.* 84, 6810–6824. doi: 10.1128/jvi.00397-10

Jankowsky, E. (2011). RNA helicases at work: binding and rearranging. *Trends Biochem. Sci.* 36, 19–29. doi: 10.1016/j.tibs.2010.07.008

Jeang, K. T., Xiao, H., and Rich, E. A. (1999). Multifaceted activities of the HIV-1 transactivator of transcription, Tat. *J. Biol. Chem.* 274, 28837–28840. doi: 10.1074/jbc.274.41.28837

Jefferson, M., Donaszi-Ivanov, A., Pollen, S., Dalmary, T., Saalbach, G., and Powell, P. P. (2014). Host factors interacting with the pestivirus N terminal protease, Npro are components of the ribonucleoprotein complex. *J. Virol.* 88, 10340–1053. doi: 10.1128/JVI.00984-14

Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M., and Sarnow, P. (2005). Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science* 309, 1577–1581. doi: 10.1126/science.1113329

Kalverda, A. P., Thompson, G. S., Vogel, A., Schröder, M., Bowie, A. G., Khan, A. R., et al. (2009). Poxvirus K7 protein adopts a Bcl-2 fold: biochemical mapping of its interactions with human DEAD box RNA helicase DDX3. *J. Mol. Biol.* 385, 843–853. doi: 10.1016/j.jmb.2008.09.048

Kasim, V., Wu, S., Taira, K., and Miyagishi, M. (2013). Determination of the role of DDX3 a factor involved in mammalian RNAi pathway using an shRNA-expression library. *PLoS ONE* 8:e59445. doi: 10.1371/journal.pone.0059445

Kato, N., Hijikata, M., Ootsuyama, Y., Nakagawa, M., Ohkoshi, S., and Shimotohno, K. (1990). Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc. Natl. Acad. Sci. U.S.A.* 87, 9524–9528. doi: 10.1073/pnas.87.24.9524

Kato, N., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., et al. (2006). Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 441, 101–105. doi: 10.1038/nature04734

Kawai, T., Takahashi, K., Sato, S., Coban, C., Kumar, H., Kato, H., et al. (2005). IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat. Immunol.* 6, 981–988. doi: 10.1038/ni1243

Kim, Y. S., Lee, S. G., Park, S. H., and Song, K. (2001). Gene structure of the human DDX3 and chromosome mapping of its related sequences. *Mol. Cells* 12, 209–214.

Ko, C., Lee, S., Windisch, M. P., and Ryu, W. S. (2014). DDX3 DEAD-box RNA helicase is a host factor that restricts hepatitis B virus replication at the transcriptional level. *J. Virol.* 88, 13689–13698. doi: 10.1128/JVI.02035-14

Kuroki, M., Ariumi, Y., Hijikata, M., Ikeda, M., Dansako, H., Wakita, T., et al. (2013). PML tumor suppressor protein is required for HCV production. *Biochem. Biophys. Res. Commun.* 430, 592–597. doi: 10.1016/j.bbrc.2012.11.108

Kwong, A. D., Rao, B. G., and Jeang, K. T. (2005). Viral and cellular RNA helicases as antiviral targets. *Nat. Rev. Drug Discov.* 4, 845–853. doi: 10.1038/nrd1853

Lahn, B. T., and Page, D. C. (1997). Functional coherence of the human Y chromosome. *Science* 278, 675–680. doi: 10.1126/science.278.5338.675

Lai, M. C., Chang, W. C., Shieh, S. Y., and Tarn, W. Y. (2010). DDX3 regulates cell growth through translational control of cyclin E1. *Mol. Cell Biol.* 30, 5444–5453. doi: 10.1128/MCB.00560-10

Lai, M. C., Lee, Y. H., and Tarn, W. Y. (2008). The DEAD-box RNA helicase DDX3 associates with export messenger ribonucleoproteins as well as tip-associated protein and participates in translational control. *Mol. Biol. Cell* 19, 3847–3858. doi: 10.1091/mbc.E07-12-1264

Lai, M. C., Wang, S. W., Cheng, L., Tarn, W. Y., Tsai, S. J., and Sun, H. S. (2013). Human DDX3 interacts with the HIV-1 Tat protein to facilitate viral mRNA translation. *PLoS ONE* 8:e68665. doi: 10.1371/journal.pone.0068665

Lee, C. H., Lin, S. H., Yang, S. F., Yang, S. M., Chen, M. K., Lee, H., et al. (2014). Low/negative expression of DDX3 might predict poor prognosis in non-smoker patients with oral cancer. *Oral Dis.* 20, 76–83. doi: 10.1111/odi.12076

Lee, C. S., Dias, A. P., Jedrychowski, M., Patel, A. H., Hsu, J. L., and Reed, R. (2008). Human DDX3 functions in translation and interacts with the translation initiation factor eIF3. *Nucleic Acids Res.* 36, 4708–4718. doi: 10.1093/nar/gkn454

Leory, P., Alzari, P., Sassoon, D., Wolgemuth, D., and Fellous, M. (1989). The protein encoded by a murine male germ cell-specific transcript is a putative ATP-dependent RNA helicase. *Cell* 57, 549–559. doi: 10.1016/0092-8674(89)90125-6

Li, C., Ge, L. L., Li, P. P., Wang, Y., Dai, J. J., Sun, M. X., et al. (2014a). Cellular DDX3 regulates Japanese encephalitis virus replication by interacting with viral un-translated regions. *Virology* 449, 70–81. doi: 10.1016/j.virol.2013.11.008

Li, Q., Zhang, P., Zhang, C., Wang, Y., Wan, R., Yang, Y., et al. (2014b). DDX3X regulates cell survival and cell cycle during mouse early embryonic development. *J. Biomed. Res.* 28, 282–291. doi: 10.7555/JBR.27.20130047

Li, J., Tang, H., Mullen, T. M., Westberg, C., Reddy, T. R., Rose, D. W., et al. (1999). A role for RNA helicase A in post-transcriptional regulation of HIV type 1. *Proc. Natl. Acad. Sci. U.S.A.* 96, 709–714. doi: 10.1073/pnas.96.2.709

Linder, P. (2008). mRNA export: RNP remodeling by DEAD-box proteins. *Curr. Biol.* 18, R297–R299. doi: 10.1016/j.cub.2008.02.027

Linder, P., and Lasko, P. (2006). Bent out of shape: RNA unwinding by the DEAD-box helicase Vasa. *Cell* 125, 219–221. doi: 10.1016/j.cell.2006.03.030

Listenberger, L. L., and Brown, D. A. (2007). Fluorescent detection of lipid droplets and associated proteins. *Curr. Protoc. Cell Biol.* 35, 24.2.1–24.2.11. doi: 10.1002/0471143030.cb2402s35

Lorgeoux, R. P., Guo, F., and Liang, C. (2012). From promoting to inhibiting: diverse roles of helicases in HIV-1 replication. *Retrovirology* 9, 79. doi: 10.1186/1742-4690-9-79

Maga, G., Falchi, F., Garbelli, A., Belfiore, A., Witvrouw, M., Manetti, F., et al. (2008). Pharmacophore modeling and molecular docking led to the discovery of inhibitors of human immunodeficiency virus-1 replication targeting the human cellular aspartic acid-glutamic acid-alanine-aspartic acid box polypeptide 3. *J. Med. Chem.* 51, 6635–6638. doi: 10.1021/jm8008844

Maga, G., Falchi, F., Radi, M., Botta, L., Casaluce, G., Bernardini, M., et al. (2011). Toward the discover of novel anti-HIV drug. Second-generation inhibitors of the cellular ATPase DDX3 with improved anti-HIV activity: synthesis, structure-activity relationship analysis, cytotoxicity studies, and target validation. *Chem. Med. Chem.* 6, 1371–1389. doi: 10.1002/cmdc.201100166

Mamiya, N., and Worman, H. J. (1999). Hepatitis C virus core protein binds to a DEAD box RNA helicase. *J. Biol. Chem.* 274, 15751–15756. doi: 10.1074/jbc.274.22.15751

Mazeyrat, S., Saut, N., Sargent, C. A., Grimmond, S., Longepied, G., Ehrmann, I. E., et al. (1998). The mouse Y chromosome interval necessary for spermatogonial

proliferation is gene dense with syntenic homology to the human AZFa region. *Hum. Mol. Genet.* 7, 1713–1724. doi: 10.1093/hmg/7.11.1713

McGivern, D. R., and Lemon, S. M. (2009). Tumor suppressors, chromosomal instability, and hepatitis C virus-associated liver cancer. *Annu. Rev. Pathol.* 4, 399–415. doi: 10.1146/annurev.pathol.4.110807.092202

Merz, C., Urlaub, H., Will, C. L., and Lührmann, R. (2007). Protein composition of human mRNPs spliced in vitro and differential requirement for mRNP protein recruitment. *RNA* 13, 116–128. doi: 10.1261/rna.336807

Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R., et al. (2005). Cardif is an adaptor protein in the RIG-1 antiviral pathway and is targeted by hepatitis C virus. *Nature* 437, 1167–1172. doi: 10.1038/nature 04193

Miao, X., Yang, Z. L., Xiong, L., Zou, Q., Yuan, Y., Li, J., et al. (2013). Nectin-2 and DDX3 are biomarkers for metastasis and poor prognosis of squamous cell/adenosquamous carcinomas and adenocarcinoma of gallbladder. *Int. J. Clin. Exp. Pathol.* 6, 179–190.

Miyanari, Y., Atsuzawa, K., Usuda, N., Watashi, K., Hishiki, T., Zayas, M., et al. (2007). The lipid droplet is an important organelle for hepatitis C virus production. *Nat. Cell Biol.* 9, 1089–1097. doi: 10.1038/ncb1631

Naji, S., Ambrus, G., Cimermančič, P., Reyes, J. R., Johnson, J. R., Filbrandt, R., et al. (2012). Host cell interactome of HIV-1 Rev includes RNA helicases involved in multiple facets of virus production. *Mol. Cell. Proteomics* 11, M111.015313. doi: 10.1074/mcp.M111.015313

Oda, S., Schröder, M., and Khan, A. R. (2009). Structural basis for targeting of human RNA helicase DDX3 by poxvirus protein K7. *Structure* 17, 1528–1537. doi: 10.1016/j.str.2009.09.005

Oshiumi, H., Ikeda, M., Matsumoto, M., Watanabe, A., Takeuchi, O., Akira, S., et al. (2010a). Hepatitis C virus core protein abrogates the DDX3 function that enhances IPS-1-mediated IFN-beta induction. *PLoS ONE* 5:e14258. doi: 10.1371/journal.pone.0014258

Oshiumi, H., Sakai, K., Matsumoto, M., and Seya, T. (2010b). DEAD/H BOX 3 (DDX3) helicase binds the RIG-I adaptor IPS-1 to up-regulate IFN-beta-inducing potential. *Eur. J. Iummunol.* 40, 940–948. doi: 10.1002/eji.200940203

Owsianka, A. M., and Patel, A. H. (1999). Hepatitis C virus core protein interacts with a human DEAD box protein DDX3. *Virology* 257, 330–340. doi: 10.1006/viro.1999.9659

Park, S. H., Lee, S. G., Kim, Y., and Song, K. (1998). Assignment of a human putative RNA helicase gene, DDX3, to human X chromosome bands p11.3−−> p11.23. *Cytogenet. Cell Genet.* 81, 178–179. doi: 10.1159/000015022

Parker, R., and Sheth, U. (2007). P bodies and the control of mRNA trabslation and degradation. *Mol. Cell* 25, 635–646. doi: 10.1016/j.molcel.2007.02.011

Pollard, V. W., and Malim, M. H. (1998). The HIV-1 Rev protein. *Annu. Rev. Microbiol.* 52, 491–532. doi: 10.1146/annurev.micro.52.1.491

Radi, M., Falchi, F., Garbelli, A., Samuele, A., Bernardo, V., Paolucci, S., et al. (2012). Discovery of the first small molecule inhibitor of human DDX3 specifically designed to target the RNA binding site: towards the next generation HIV-1 inhibitors. *Bioorg. Med. Chem. Lett.* 22, 2094–2098. doi: 10.1016/j.bmcl.2011.12.135

Randall, G., Panis, M., Cooper, J. D., Tellinghuisen, T. L., Sukhodolets, K. E., Pfeffer, S., et al. (2007). Cellular cofactors affecting hepatitis C virus infection and replication. *Proc. Natl. Acad. Sci. U.S.A.* 104, 12884–12889. doi: 10.1073/pnas.0704894104

Robertson-Anderson, R. M., Wang, J., Edgcomb, S. P., Carmel, A. B., Williamson, J. R., and Millar, D. P. (2011). Single-molecule studies reveal that DEAD box protein DDX1 promotes oligomerization of HIV-1 Rev on the Rev responsive element. *J. Mol. Biol.* 410, 959–971. doi: 10.1016/j.jmb.2011.04.026

Saito, T., and Gale, M. Jr. (2007). Principles of intracellular viral recognition. *Curr. Opin. Immunol.* 19, 17–23. doi: 10.1016/j.coi.2006.11.003

Scheller, N., Mina, L. B., Galão, R. P., Chari, A., Giménez-Barcons, M., Noueiry, A., et al. (2009). Translation and replication of hepatitis C virus genomic RNA dependes on ancient cellular proteins that control mRNA fates. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13517–13522. doi: 10.1073/pnas.0906413106

Schröder, M. (2010). Human DEAD-box protein 3 has multiple functions in gene regulation and cell cycle control and is a prime target for viral manipulation. *Biochem. Pharmcol.* 79, 297–306. doi: 10.1016/j.bcp.2009.08.032

Schröder, M., Baran, M., and Bowie, A. G. (2008). Viral targeting of DEAD box protein 3 reveals its role in TBK/IKKepsilon-mediated IRF activation. *EMBO J.* 27, 2147–2157. doi: 10.1038/emboj.2008.143

Sekiguchi, T., Kurihara, Y., and Fukumura, J. (2007). Phosphorylation of threonine 204 of DEAD-box RNA helicase DDX3 by cyclin B/cdc2 in vitro. *Biochem. Biophys. Res. Commun.* 356, 668–673. doi: 10.1016/j.bbrc.2007.03.038

Seth, R. B., Sun, L., Ea, C. K., and Chen, Z. J. (2005). Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-B and IRF3. *Cell* 122, 669–682. doi: 10.1016/j.cell.2005.08.012

Sharma, A., Awasthi, S., Harrod, C. K., Matlock, E. F., Khan, S., Xu, L., et al. (2007). The Werner syndrome helicase is a cofactor for HIV-1 long terminal repeat transactivation and retroviral replication. *J. Biol. Chem.* 282, 12048–12057. doi: 10.1074/jbc.M608104200

Shih, J. W., Tsai, T. Y., Chao, C. H., and Wu Lee, Y. H. (2008). Candidate tumor suppressor DDX3 RNA helicase specifically represses cap-dependent translation by acting as an eIF4E inhibitory protein. *Oncogene* 27, 700–714. doi: 10.1038/sj.onc.1210687

Shih, J. W., Wang, W. T., Tsai, T. Y., Kuo, C. Y., Li, H. K., and Wu Lee, Y. H. (2012). Critical roles of RNA helicase DDX3 and its interactions with eIF4E/PABP1 in stress granule assembly and stress response. *Biochem. J.* 441, 119–1129. doi: 10.1042/BJ20110739

Soto-Rifo, R., Rubilar, P. S., Limousin, T., de Breyne, S., Décimo, D., and Ohlmann, T. (2012). DEAD-box protein DDX3 associates with eIF4F to promote translation of selected mRNAs. *EMBO J.* 31, 3745–3756. doi: 10.1038/emboj.201 2.220

Soto-Rifo, R., Rubilar, P. S., and Ohlmann, T. (2013). The DEAD-box helicase DDX3 substitutes for the cap-binding protein eIF4E to promote compartmentalized translation initiation of the HIV-1 genomic RNA. *Nucleic Acids Res.* 41, 6286–6299. doi: 10.1093/nar/gkt306

Soulat, D., Burckstummer, T., Westermayer, S., Goncalves, A., Bauch, A., Stefanovic, A., et al. (2008). The DEAD-box helicase DDX3X is a critical component of the TANK-binding kinase 1-dependent innate immune response. *EMBO J.* 27, 2135–2146. doi: 10.1038/emboj.2008.126

Sun, C., Pager, C. T., Luo, G., Sarnow, P., and Cate, J. H. (2010). Hepatitis C virus core-derived peptides inhibit genotype 1b viral genome replication via interaction with DDX3X. *PLoS ONE* 5:e12826. doi: 10.1371/journal.pone.00 12826

Sun, M., Song, L., Li, Y., Zhou, T., and Jope, R. S. (2008). Identification of an antiapoptotic protein complex at death receptors. *Cell Death Differ.* 15, 1887–1900. doi: 10.1038/cdd.2008.124

Sun, M., Song, L., Zhou, T., Gillespie, G. Y., and Jope, R. S. (2011). The role of DDX3 in regulating Snail. *Biochim. Biophys. Acta* 1813, 438–447. doi: 10.1016/j.bbamcr.2011.01003

Sun, M., Zhou, T., Jonasch, E., and Jope, R. S. (2013). DDX3 regulates DNA damage-induced apoptosis and p53 stabilization. *Biochem. Biophys. Acta* 1833, 1489–1497. doi: 10.1016/j.bbamcr.2013.02.026

Tingting, P., Caiyun, F., Zhigang, Y., Pengyuan, Y., and Zhenghong, Y. (2006). Subproteomic analysis of the cellular proteins associated with the 3′ untranslated region of the hepatitis C virus genome in human liver cells. *Biochem. Biophys. Res. Commun.* 347, 683–691. doi: 10.1016/j.bbrc.2006.06.144

Tsai, W. C., and Lloyd, R. E. (2014). Cytoplasmic RNA granules and viral infection. *Annu. Rev. Virol.* 1, 147–170. doi: 10.1146/annurev-virology-031413-085505

Upadya, M. H., Aweya, J. J., and Tan, Y. J. (2014). Understanding the interaction of hepatitis C virus with host DEAD-box RNA helicases. *World J. Gastroenterol.* 20, 2913–2926. doi: 10.3748/wjg.v20.i11.2913

Utama, A., Shimizu, H., Hasebe, F., Morita, K., Igarashi, A., Shoji, I., et al. (2000). Role of the DExH motif of the Japanese encephalitis virus and hepatitis C virus NS3 proteins in the ATPase and RNA helicase activities. *Virology* 273, 316–324. doi: 10.1006/viro.2000.0417

Vashist, S., Urena, L., Chaudhry, Y., and Goodfellow, I. (2012). Identification of RNA-protein interaction networks involved in the norovirus life cycle. *J. Virol.* 86, 11977–11990. doi: 10.1128/JVI.00432-12

Wakita, T., Pietschmann, T., Kato, T., Date, T., Miyamoto, M., Zhao, Z., et al. (2005). Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat. Med.* 11, 791–796. doi: 10.1038/nm1268

Wang, H., Kim, S., and Ryu, W. S. (2009). DDX3 DEAD-Box RNA helicase inhibits hepatitis B virus reverse transcription by incorporation into nucleocapsids. *J. Virol.* 83, 5815–5824. doi: 10.1128/JVI.00011-09

Wang, H., and Ryu, W. S. (2010). Hepatitis B virus polymerase blocks pattern recognition receptor signaling via interaction with DDX3: implications for immune evasion. *PLoS Pathog.* 6:e1000986. doi: 10.1371/journal.ppat.1000986

Wu, D. W., Lee, M. C., Wang, J., Chen, C. Y., Cheng, Y. W., and Lee, H. (2014). DDX3 loss by p53 inactivation promotes tumor malignancy via the MDM2/Slug/E-cadherin pathway and poor patient outcome in non-small-cell lung cancer. *Oncogene* 33, 1515–1526. doi: 10.1038/onc.2013.107

Wu, D. W., Liu, W. S., Wang, J., Chen, C. Y., Cheng, Y. W., and Lee, H. (2011). Reduced p21(WAF1/CIP1) via alteration of p53-DDX3 pathway is associated with poor relapse-free survival in early-stage human papillomavirus-associated lung cancer. *Clin. Cancer Res.* 17, 1895–1905. doi: 10.1158/1078-0432.CCR-10-2316

Xu, L. G., Wang, Y. Y., Han, K. J., Li, L. Y., Zhai, Z., and Shu, H. B. (2005). VISA is an adaptor protein required for virus-triggered IFN- signaling. *Mol. Cell* 19, 727–740. doi: 10.1016/j.molcel.2005.08.014

Yasuda-Inoue, M., Kuroki, M., and Ariumi, Y. (2013a). Distinct DDX DEAD-box RNA helicases cooperate to modulate the HIV-1 Rev function. *Biochem. Biophys. Res. Commun.* 434, 803–808. doi: 10.1016/j.bbrc.2013.04.016

Yasuda-Inoue, M., Kuroki, M., and Ariumi, Y. (2013b). DDX3 RNA helicase is required for HIV-1 Tat function. *Biochem. Biophys. Res. Commun.* 441, 607–611. doi: 10.1016/j.bbrc.2013.10.107

Yedavalli, V. S., Neuveut, C., Chi, Y. H., Kleiman, L., and Jeang, K. T. (2004). Requirement of DDX3 DEAD box RNA helicase for HIV-1 Rev-RRE export function. *Cell* 119, 381–392. doi: 10.1016/j.cell.2004.09.029

Yedavalli, V. S., Zhang, N., Cai, H., Zhang, P., Starost, M. F., Hosmane, R. S., et al. (2008). Ring expanded nucleoside analogues inhibit RNA helicase and intracellular human immunodeficiency virus type 1 replication. *J. Med. Chem.* 51, 5043–5051. doi: 10.1021/jm800332m

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., et al. (2004). The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral response. *Nat. Immunol.* 5, 730–737. doi: 10.1038/ni1087

You, L. R., Chen, C. M., Yeh, T. S., Tsai, T. Y., Mai, R. T., Lin, C. H., et al. (1999). Hepatitis C virus core protein interacts with cellular putative RNA helicase. *J. Virol.* 73, 2841–2853.

Yu, S., Chen, J., Wu, M., Chen, H., Kato, N., and Yuan, Z. (2010). Hepatitis B virus polymerase inhibits RIG-I- and Toll-like receptor 3-mediated beta interferon induction in human hepatocytes through interference with interferon regulatory factor 3 activation and dampening of the interaction between TBK1/IKKepsilon and DDX3. *J. Gen. Virol.* 91, 2080–2090. doi: 10.1099/vir.0.020552-0

Zhou, X., Luo, J., Mills, L., Wu, S., Pan, T., Geng, G., et al. (2013). DDX5 facilitates HIV-1 replication as a cellular co-factor of Rev. *PLoS ONE* 8:e65040. doi: 10.1371/journal.pone.0065040

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 September 2014; paper pending published: 23 October 2014; accepted: 19 November 2014; published online: 05 December 2014.

*Citation: Ariumi Y (2014) Multiple functions of DDX3 RNA helicase in gene regulation, tumorigenesis, and viral infection. Front. Genet. **5**:423. doi: 10.3389/fgene.2014.00423*

This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.

frontiers in GENETICS

PERSPECTIVE ARTICLE
published: 05 December 2014
doi: 10.3389/fgene.2014.00426

An appraisal of RECQ1 expression in cancer progression

Sudha Sharma *

Department of Biochemistry and Molecular Biology, College of Medicine, Howard University, Washington, DC, USA

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

Reviewed by:
Srinivas Ayyadevara, Central Arkansas Veterans Healthcare System, USA
David Lombard, University of Michigan, USA

***Correspondence:**
Sudha Sharma, Department of Biochemistry and Molecular Biology, College of Medicine, Howard University, 520 W Street, Northwest, Washington, DC 20059, USA
e-mail: sudha.sharma@howard.edu

RECQ1 is the most abundant member of the human RecQ family of DNA helicases genetically linked with cancer predisposition syndromes and well known for their functions in genome stability maintenance through DNA repair. Despite being the first discovered RecQ homolog in humans, biological functions of RECQ1 have remained largely underappreciated and its relevance to cellular transformation is yet unclear. RECQ1 is overexpressed and amplified in many clinical cancer samples. *In silico* evaluation of RECQ1 mRNA expression across the NCI-60 cancer cell lines predicts an association of RECQ1 with cancer cell migration, invasion, and metastasis. Consistent with this, latest work implicates RECQ1 in regulation of gene expression, especially of those associated with cancer progression. Functionally, silencing RECQ1 expression significantly reduces cell proliferation, migration, and invasion. Collectively, these results propose that discerning the role of RECQ1 in conferring proliferative and invasive phenotype to cancer cells could be useful in developing therapeutic strategies to block primary tumor progression and metastasis.

Keywords: RecQ, helicase, gene expression, vimentin, metastasis, epithelial mesenchymal transition

INTRODUCTION

The RecQ helicase family is a group of highly conserved DNA unwinding enzymes described as caretakers of the genome (Hickson, 2003; Brosh, 2013; Croteau et al., 2014). In humans, RECQ1, also known as RECQL or RECQL1, is the most abundant member of the RecQ helicase family represented by five homologs: RECQ1, WRN, BLM, RECQL4, and RECQL5 (Sharma and Brosh, 2008). Loss of function of three of the five members of the RecQ family are genetically linked with rare cancer susceptibility syndromes (Werner syndrome, Bloom syndrome, and Rothmund–Thomson syndrome) but the association of RECQ1 with cellular transformation is yet unclear. The five human RecQ helicases are expected to exhibit some functional redundancy, but the fact that the functional loss of one homolog cannot be substituted by others clearly indicates critical specialized functions.

RECQ1 is an integral component of replication complex in unperturbed cells and implicated in maintaining replication fork progression (Thangavel et al., 2010). Mechanistically, RECQ1 helicase binds and preferentially unwinds structural intermediates of DNA replication and repair, such as forked duplexes, D-loops, and Holliday junctions; and also exhibits an intrinsic ability to promote annealing of complementary single strand DNA (Sharma et al., 2005; Popuri et al., 2008). RECQ1-deficient cells are characterized by spontaneously elevated sister chromatid exchanges (Sharma and Brosh, 2008) that represent non-productive attempts to restart replication (Helleday, 2003). Furthermore, RECQ1-deficient cells accumulate DNA damage, display increased sensitivity to DNA damaging agents that induce stalled and collapsed replication forks, and exhibit chromosomal instability (Sharma and Brosh, 2007, 2008; Sharma et al., 2007). Thus it is believed that RECQ1 acts to restore productive DNA replication following stress and prevents subsequent genomic instability (Sharma and Brosh, 2008; Popuri et al., 2012; Berti et al., 2013; Sami and Sharma, 2013). RECQ1-deficiency is tolerated by cells to repair I-Sce induced double strand breaks by homologous recombination (Sharma et al., 2012), but a role of RECQ1 in replication stress induced recombination is possible given the demonstrated activity of RECQ1 protein to dissociate native Rad51-bound D-loops by branch migration (Branzei and Foiani, 2007; Bugreev et al., 2008).

Human RECQ1 is ubiquitously expressed in all cell types regardless of cell cycle stage (Kawabe et al., 2000). Previous studies have shown that RECQ1 is upregulated in rapidly dividing cells and its expression is higher in many cancer cell lines as compared to normal cells (Kawabe et al., 2000; Futami et al., 2008a). A meta-analyses of gene expression pattern included RECQ1 among the common signature genes for cancer as defined by the Gene Ontology Consortium (Xu et al., 2007). We noted that RECQ1 is overexpressed and amplified in many clinical cancer samples[1]. Forty three out of 447 differential expression analyses included RECQ1 in the top 10% upregulation list while only two did in the top 10% downregulation list in cancer versus normal[2]. Indeed, overexpression of RECQ1 has been experimentally demonstrated in human glioblastoma (Mendoza-Maldonado et al., 2011), ovarian cancer (Sanada et al., 2013), and head and neck squamous cell carcinoma (Arai et al., 2011). Furthermore, RECQ1 protein levels correlated with histological grade and Ki-67 labeling index in hepatocellular carcinoma (Futami et al., 2010) and high proliferative potential in ovarian cancer (Sanada et al., 2013). A polymorphism

[1]www.cbioportal.org
[2]www.oncomine.org

in RECQ1, A159C, has been associated with faster tumor progression and significantly reduced survival of pancreatic adenocarcinoma patients that received gemcitabine and radiotherapy (Li et al., 2006; Cotton et al., 2009). The A159C SNP is located in the 3′UTR and, thus, may functionally alter RECQ1 expression and affect clinical outcome. Remarkably, RECQ1 silencing significantly reduced proliferation of cancer cells as compared to normal cells in cell culture models and also suppressed tumor growth in mouse *xenograft* models (Sharma and Brosh, 2007; Futami et al., 2008a,b, 2010; Arai et al., 2011; Mendoza-Maldonado et al., 2011). Tumor cell growth was significantly inhibited *in vitro* by silencing RECQ1 in hypopharyngeal carcinoma cells and the combination treatment of RECQ1 siRNA and *cis*-platinum (II) diammine dichloride significantly augmented the *in vivo* anticancer effects of the drug (Arai et al., 2011). Dysfunctional checkpoint status, e.g., p53 in cancer cells may be linked to mitotic catastrophe following RECQ1 silencing (Futami et al., 2008a) but the ability of RECQ1 to facilitate recovery from replication stress could also be especially important for cancer cells (Popuri et al., 2012; Berti et al., 2013; Lu et al., 2013). Although these studies imply an association of RECQ1 with tumor growth, progression or differentiation, the molecular mechanisms through which RECQ1 might support malignant progression are not understood.

Recent analysis of genome-wide changes in gene expression has revealed a novel involvement of RECQ1 in regulation of gene expression in addition to its role in DNA damage repair (Li et al., 2014). Besides cell proliferation, the top 10 over-represented processes also included cellular movement and cell morphology suggesting that RECQ1 enhances the expression of multiple genes that play key roles in cell migration, invasion, and metastasis, including EZR, ITGA2, ITGA3, ITGB4, SMAD3, and TGFBR2. Functionally, silencing RECQ1 significantly reduced migration and invasion of the highly invasive MDA-MB-231 breast cancer cells indicating that RECQ1 plays a role in enhancing cell migration and invasion (Li et al., 2014). Consistent with the results from *in vitro* cell culture based study, high RECQ1 expression significantly associated (P = 4.2E-06) with poor overall survival in breast cancer in TCGA dataset (Gyorffy et al., 2010). High expression of RECQL4 was also associated with poor survival in breast cancer but the expression levels of WRN, BLM, and RECQL5 did not correlate with survival (Li et al., 2014).

A putative role in regulation of gene expression has been suggested by studies in *Neurospora* where a RECQ1 homolog mediates posttranscriptional gene silencing (Cogoni and Macino, 1999). Remarkably, rat RECQ1 was identified in a piRNA protein complex important for gene silencing (Lau et al., 2006), raising the possibility that human RECQ1 may function in a conserved mechanism. Although the mechanism of gene regulation by RECQ1 is yet unknown, the promoters of genes downregulated upon RECQ1 silencing were significantly enriched for a potential G4 sequence motif which are associated with potential to form G-quadruplex structures in DNA (G4 DNA; Li et al., 2014). Chromatin immunoprecipitation experiments demonstrated RECQ1 binding to G4 sequence motifs in the promoters of select downregulated genes indicating that RECQ1 may modulate gene expression by regulating the *in vivo* stability of G4 DNA structures. Elegant studies from the Maizels (Gray et al., 2014), Monnat and Harris (Nguyen et al., 2014) labs have recently identified that gene regulatory functions of XPB, XPD, and BLM helicases also involve G4 DNA suggesting that the binding to G4 motifs may be a common mechanism of transcriptional regulation by these distinct DNA helicases. Importantly, similar to XPB, but unlike BLM, RECQ1 binds but does not unwind G4 DNA *in vitro* (Popuri et al., 2008; Sharma, 2011). It is likely that binding to specific G4 sequence motifs by each of these helicases may characterize specific signaling and regulatory pathways associated with cancer. In addition to gene regulatory regions, G4 sequence motifs are also present at chromatin regions preferentially bound by RECQ1 such as replication origins, fragile sites, and telomeres indicating that RECQ1 functions are especially critical at genomic loci having potential to form secondary structures (Thangavel et al., 2010; Lu et al., 2013; Maizels and Gray, 2013; Popuri et al., 2014).

RECQ1 EXPRESSION ACROSS THE NCI-60 HUMAN TUMOR CELL LINES

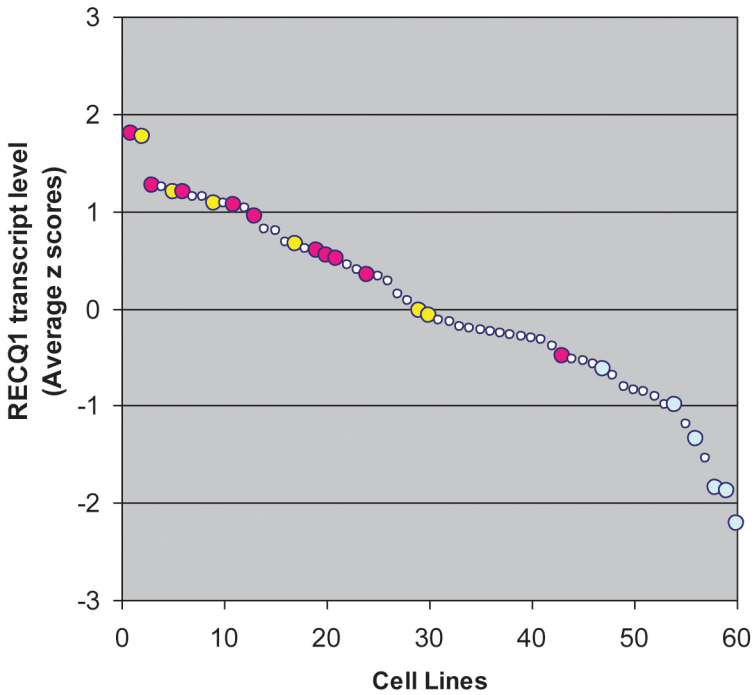
By virtue of its overexpression in cancer and its association with cell proliferation, migration, and invasion, RECQ1 can potentially serve as a predictive biomarker and attractive target for cancer therapy. To assess gene expression pattern of RECQ1 in various tumors, we utilized a panel of 60 human tumor cell lines derived from nine different tissues of origin (NCI-60) that has been extensively characterized for gene expression and copy-number variations, and commonly used for genetic analysis and screening of potential chemotherapeutic agents (Shoemaker, 2006; Weinstein, 2006; Liu et al., 2010). We conducted *in silico* analysis of RECQ1 expression pattern across the NCI-60 employing a publicly accessible webtool CellMiner[3] (Reinhold et al., 2012; **Figure 1**). RECQ1 mRNA expression in the NCI-60 panel is shown in **Figure 1A** both as average transcript intensity values and as the average *z* score that allows a better comparison of relative expression. Noticeably, the NCI-60 cell lines exhibit a wide spectrum of RECQ1 expression with transcript level values ranging from both lower and higher than the mean (**Figure 1B**). Cell lines melanoma LOXIMVI and central nervous system SF_268 showed the highest RECQ1 mRNA levels, whereas ovarian cancer cell lines OVCAR_4 and SK_OV_3 showed the lowest (**Figure 1B**). Nine out of the 10 melanoma cell lines and 4 out of 6 central nervous system cell lines expressed high RECQ1 mRNA (**Figure 1B**). SK_MEL_5 cells were the only melanoma cells within the NCI-60 panel with lower RECQ1 transcript levels. The lung and renal cell lines were most variable in RECQ1 expression and the ovarian cell lines expressed lowest levels of RECQ1 transcript. It remains to be tested whether RECQ1 transcript levels correlate with the protein level, but a statistically significant correlation with DNA copy number (Pearson's correlation $R = 0.38$; $P = 0.003$) was observed with the estimated RECQ1 DNA copy number ranging from 3.67 for lung cancer cell line NCI_H322M to 1.35 in ovarian cancer cell line SK_OV_3. Expression profiles of five known RecQ family genes across the NCI-60 panel revealed no significant correlation of RECQ1 mRNA expression with WRN, BLM, or RECQL5;

[3]http://discover.nci.nih.gov/cellminer/

A

	Cell lines	Avg. intensity	Avg. z scores
1	ME:LOXIMVI	7.26	1.8
2	CNS:SF_268	7.5	1.76
3	ME:M14	7.99	1.27
4	RE:CAKI_1	8.05	1.25
5	CNS:SF_539	7.7	1.2
6	ME:MALME_3M	8.93	1.19
7	LC:NCI_H226	8.47	1.15
8	LC:NCI_H23	7.76	1.15
9	CNS:SF_295	7.75	1.08
10	CO:SW_620	8.22	1.08
11	ME:UACC_62	7.79	1.06
12	LC:HOP_62	7.56	1.02
13	ME:SK_MEL_2	7.48	0.95
14	LE:SR	7.25	0.81
15	RE:UO_31	7.6	0.79
16	LC:NCI_H322M	7.46	0.67
17	CNS:SNB_75	7.04	0.66
18	RE:786_0	8.47	0.61
19	ME:MDA_MB_435	7.59	0.59
20	ME:MDA_N	7.27	0.54
21	ME:UACC_257	6.85	0.51
22	BR:BT_549	7.61	0.45
23	RE:RXF_393	7.72	0.39
24	ME:SK_MEL_28	8.35	0.34
25	RE:TK_10	8.95	0.33
26	BR:HS578T	8.58	0.28
27	PR:DU_145	8.6	0.14
28	PR:PC_3	8.42	0.07
29	CNS:U251	8.05	-0.02
30	CNS:SNB_19	7.5	-0.07
31	LE:RPMI_8226	8.12	-0.12
32	BR:T47D	8.46	-0.15
33	RE:ACHN	8.17	-0.2
34	RE:A498	8.16	-0.21
35	LC:EKVX	7.57	-0.23
36	OV:OVCAR_8	7.65	-0.25
37	LE:MOLT_4	8.4	-0.26
38	LE:CCRF_CEM	7.36	-0.28
39	CO:HCT_15	8.55	-0.29
40	LC:A549	8.55	-0.31
41	RE:SN12C	8.2	-0.32
42	CO:COLO205	7.21	-0.39
43	ME:SK_MEL_5	7.32	-0.5
44	BR:MDA_MB_231	6.63	-0.53
45	CO:HCC_2998	7.41	-0.55
46	CO:HT29	6.44	-0.58
47	OV:OVCAR_3	6.96	-0.63
48	LC:HOP_92	7.66	-0.7
49	LC:NCI_H522	6.65	-0.81
50	BR:MCF7	7.16	-0.84
51	CO:HCT_116	7.85	-0.86
52	LE:HL_60	7.87	-0.91
53	LC:NCI_H460	8.2	-0.99
54	OV:NCI_ADR_RES	7.66	-0.99
55	CO:KM12	7.68	-1.2
56	OV:OVCAR_5	8.62	-1.34
57	LE:K_562	8.05	-1.55
58	OV:IGROV1	7.63	-1.85
59	OV:SK_OV_3	8.02	-1.88
60	OV:OVCAR_4	8.35	-2.21
	Range	2.75	4.01
	Average	7.81	0.018666667
	Standard Deviation	0.63	0.918352369

B



C

	RECQ1	WRN	BLM	RECQL4	RECQL5
RECQ1	1	0.078	-0.004	-0.275	0.001
WRN	0.078	1	0.198	0.237	-0.127
BLM	-0.004	0.198	1	0.148	0.08
RECQL4	**-0.275**	0.237	0.148	1	0.066
RECQL5	0.001	-0.127	0.08	0.066	1

FIGURE 1 | RECQ1 transcript levels in the NCI-60 human tumor cell lines. (A) Table of average intensity (determined from log 2 intensity values from Affymetrix microarrays) and their combined *z*-score means presented in descending order. **(B)** Scatter plot depicting the distribution of RECQ1 transcript levels across the NCI-60. The numerical series (1-to-60) for the cell lines from panel **(A)** correspond to the *x*-axis. Cell lines from the central nervous system (shown in yellow) and melanoma (shown in pink) displayed higher transcript levels of RECQ1, while ovarian cancer cell lines (shown in blue) displayed the least. **(C)** Cross-correlation among RecQ family. Pearson's correlation coefficients between transcripts of the known five RecQ homologs in the NCI-60. Only significant correlation of RECQ1 in the NCI-60 is with the RECQL4 transcripts. Processing and normalization of transcript expression data from the NCI-60 has been described previously (Reinhold et al., 2012). Normalization of Affymetrix HG-U95, HG-U133, HG-U133 plus 2.0 and Affy HuEx 1.0 is done by GC robust multi-*array* average (GCRMA). Data is accessible at http://discover.nci.nih.gov by using RECQL (RECQ1) as input in Cellminer.

however, RECQ1 expression displayed significant inverse correlation with RECQL4 (**Figure 1C**).

Recent studies using the NCI-60 panel have shown that the genes whose expression at the mRNA level is correlated over diverse cell lines are likely to function together in a molecular network regulating these processes (Kohn et al., 2012, 2014). We postulated that the ability of RECQ1 to promote tumor cell migration and invasion involves changes in expression of specific genes that are critical to these processes. Using the CellMiner NCI-60 analysis tools, we searched for genes that were significantly correlated with RECQ1-expression across the NCI-60 panel and had established functions in cell migration and invasion (**Figure 2A**). Notably, RECQ1 expression displayed significant positive correlation with mesenchymal differentiation markers vimentin, N-cadherin, and fibronectin (VIM, FN1, and CDH2), and transcription factors that promote these processes (TWIST, SNAI2, and ZEB2). Furthermore, RECQ1 expression correlated significantly and negatively with genes maintaining cellular structural integrity (KRT8) and epithelial marker E-cadherin (CDH1). Correlation of the mRNA transcript levels of RECQ1 with VIM and CDH1 across the NCI-60 tumor cell lines is depicted in **Figure 2B**. The NCI-60 gene expression profile for CDH1 is inversely correlated to that of the gene VIM which is considered as a hallmark of mesenchymal-like conversion of epithelial cells in carcinomas (Zeisberg and Neilson, 2009; Kohn et al., 2014).

PREDICTIVE AND PROGNOSTIC POTENTIAL OF RECQ1 EXPRESSION IN CANCER

Predictions from *in silico* analysis across the NCI-60 panel support previously reported overexpression of RECQ1 in various cancers and indicate RECQ1 expression to be especially significant in tumors of central nervous system origin and melanoma. Evidently, RECQ1 is highly expressed in human brain glioblastoma relative to control brain tissues and its depletion affects proliferation of glioblastoma cells and causes an increased level of DNA damages (Mendoza-Maldonado et al., 2011). Examining RECQ1 gene expression using the ONCOMINE mRNA microarray database[4] revealed that RECQ1 is significantly upregulated in brain and central nervous system tumors, including glioblastoma, when compared to normal tissue. Overexpression of DNA repair genes is associated with metastasis in melanomas (Kauffmann et al., 2008), and the expression levels of RECQ1 significantly correlated with DNA repair genes displaying functional network which are commonly overexpressed in tumors with poorer prognosis in melanoma (Jewell et al., 2010). Given the demonstrated roles of RECQ1 in repairing DNA damage caused by chemotherapeutic agents such as ionizing radiation, camptothecin, and temozolomide, overexpression of RECQ1 may provide a survival advantage to melanoma cells by promoting the ability of cancer cells to tolerate genotoxic stress. In contrast, WRN and RECQL4 may have a tumor suppressor function in melanoma since melanoma has been reported in patients with loss of function mutations in these RecQ proteins (Howell and Bray, 2008; Monnat, 2010). Notably, within the RecQ family, RECQ1 expression correlated significantly and negatively with RECQL4 across the NCI-60 panel. Lao et al. (2013) have recently shown that the expression of BLM and RECQL4 is increased in primary colorectal cancer whereas expression levels of RECQ1 and RECQL5 are decreased. Consistent with the observation in primary colorectal cancer patient samples, low expression of RECQ1 and significantly high expression of RECQL4 were observed in colorectal cancer cell lines as compared to normal colonic mucosa indicating the feasibility of using cell lines to study the functional consequences of alterations in the expression levels of the RecQ helicases (Lao et al., 2013).

RecQ helicases contribute multiple biochemical activities to various DNA repair processes and loss of their functions leads to increased DNA damage, genomic instability, and enhanced sensitivity to certain chemotherapeutic agents (Brosh, 2013). Therefore, altered expression of RecQ helicases may be useful in predicting patient's response to these DNA damaging drugs used for treatment of cancer. Indeed RECQ1 expression correlated with cisplatin resistance in oral squamous cell carcinoma (Zhang et al., 2006) and the depletion of RECQ1 significantly augmented the *in vivo* anticancer effects of the drug cis-platinum (II) diammine dichloride (Arai et al., 2011). This is consistent with the fact that cisplatin and related platinum drugs induce inter-strand cross links in DNA that impair progression of replication forks and RECQ1 helicase functions are important to restore productive DNA replication (Sami and Sharma, 2013). However, RECQ1 expression was found to be elevated in ovarian cancer cells that were sensitive to carboplatin as compared to those which were carboplatin-resistant (Peters et al., 2005) indicating complex mechanisms of platinum resistance (Stewart, 2007). Thus, a better correlation of RECQ1 expression with the molecular characteristics and heterogeneity of cancer needs to be established in order to test the use of RECQ1 as a potential biomarker.

In addition to unlimited proliferative potential, a tumor cell must acquire the ability to migrate and invade normal tissues to become fully malignant (Hanahan and Weinberg, 2011). Invasion of tumor cells and metastatic spread to distant organs relies on complex molecular interactions including diminished epithelial characteristics and enhanced mesenchymal attributes (Kalluri and Weinberg, 2009). RECQ1 expression across the NCI-60 panel significantly correlated with a loss of the epithelial marker CDH1 and acquisition of expression of the mesenchymal markers including VIM. Correlation with VIM in the NCI-60 panel raises the question whether RECQ1 expression is associated with the epithelial to mesenchymal transition (EMT) which is among the central mechanisms to induce invasiveness and metastasis of tumors. Indeed, RECQ1-depletion decreased cell migration and invasion in cervical adenocarcinoma HeLa and breast cancer MDA-MB-231 cell lines (Li et al., 2014). Similarly, RECQ1 silencing in oral squamous cell carcinoma SCC-9 cells downregulated the expression level of immunosuppressive factors that are necessary for regulating the migration of tumor cells (Tao et al., 2014). Collectively these results propose that RECQ1 may contribute to tumor progression by regulating key genes that promote cancer cell migration, invasion, and metastasis. It is plausible that similar to what has been shown for a few other proteins involved in DNA damage response and repair, RECQ1 participates in transcription regulation either by binding directly

[4]www.oncomine.org

A

Gene	Correlation (P<0.05	Function
RECQ1 (RECQL)	1	DNA repair, transcription
VIM	0.465	Mesenchymal marker
FN1	0.386	Mesenchymal marker
CDH2	0.341	Mesenchymal marker
SNAI2	0.45	EMT-promoting transcription repressor
ZEB2	0.396	EMT-promoting transcription repressor
TWIST1	0.504	EMT-promoting transcription repressor
CDH1	-0.225	Epithelial marker
KRT8	-0.367	Cytoskeleton organization

B

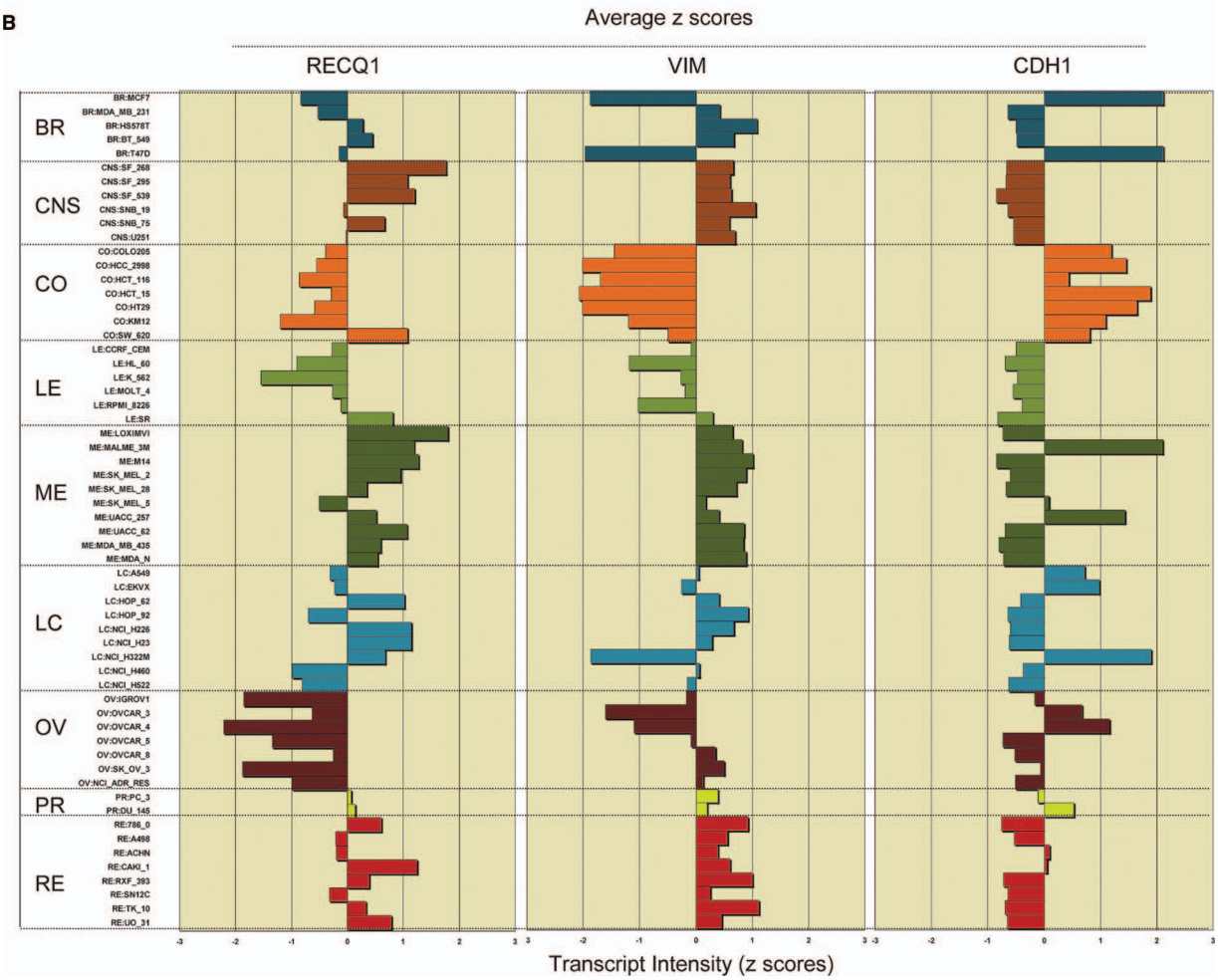


FIGURE 2 | Continued

FIGURE 2 | RECQ1 expression in the NCI-60 panel correlates with markers of tumor progression. (A) Significant correlations of RECQ1 with select genes involved the cell invasion, migration, and metastasis. The numbers in the table are expression profile correlations for expression of gene pairs in the NCI-60 panel. EMT, epithelial to mesenchymal transition; **(B)** RECQ1 expression is highly correlated with the mesenchymal marker vimentin. Comparison of expression profiles for RECQ1, VIM (vimentin), and CDH1 (E-cadherin) across the NCI-60 cell lines. Mean-centered transcript *z*-cores are plotted on the *x*-axis; bars to the right show increased expression, bars to the left show decreased expression relative to the expression mean. The cell lines on the *y*-axis are grouped by tissue of origin. BR, breast; CNS, central nervous system; CO, colon; LE, leukemia; ME, melanoma; LC, lung cancer; OV, ovarian; PR, prostrate; RE, renal. Data was generated querying RECQL (RECQ1) as input in Cellminer (http://discover.nci.nih.gov/cellminer/).

to DNA or through interaction with specific transcription factors (Featherstone and Jackson, 1999; Mullan et al., 2006; Kraus, 2008; Jaehnig et al., 2013; Broustas and Lieberman, 2014). A systematic investigation of RECQ1-regulated transcriptome may uncover the gene networks regulated by RECQ1 in the context of cancer progression.

Increased expression of several DNA repair proteins has been correlated with cellular invasiveness in cancer (Mitra et al., 2009; Barbano et al., 2011; Martinez-Marignac et al., 2011; Yuan et al., 2012); however, little effort has been made thus far to determine any role of RecQ helicase homologs in tumor progression and metastasis. Understanding the role of RECQ1 in conferring proliferative and invasive phenotype to cancer cells could be useful in developing therapeutic strategies to block primary tumor progression and metastases. Functional evidence from RECQ1 silencing, and the projected correlation with VIM expression in the NCI-60 panel implies an association of RECQ1 with more aggressive disease (Zeisberg and Neilson, 2009). Future studies should examine whether RECQ1 mRNA and/or protein levels can predict invasion and metastatic potential of a tumor. Predictions from large human cancer cell line panels that capture tumor heterogeneity, such as NCI-60, and the availability of clinically and molecularly annotated patient tumor datasets for multiple tumor types, such as TCGA, should provide additional opportunities to test the significance of RECQ1 in developing new *in vitro* preclinical models for cancer detection and treatment.

ACKNOWLEDGMENTS

This research was supported by the NIGMS/NIH under award number SC1GM093999 and NSF under HU-ADVANCE-IT mini-grant. The content is solely the responsibility of the author and does not necessarily represent the official views of the funding agencies.

REFERENCES

Arai, A., Chano, T., Futami, K., Furuichi, Y., Ikebuchi, K., Inui, T., et al. (2011). RECQL1 and WRN proteins are potential therapeutic targets in head and neck squamous cell carcinoma. *Cancer Res.* 71, 4598–4607. doi: 10.1158/0008-5472.CAN-11-0320

Barbano, R., Copetti, M., Perrone, G., Pazienza, V., Muscarella, L. A., Balsamo, T., et al. (2011). High RAD51 mRNA expression characterize estrogen receptor-positive/progesteron receptor-negative breast cancer and is associated with patient's outcome. *Int. J. Cancer* 129, 536–545. doi: 10.1002/ijc.25736

Berti, M., Chaudhuri, A. R., Thangavel, S., Gomathinayagam, S., Kenig, S., Vujanovic, M., et al. (2013). Human RECQ1 promotes restart of replication forks reversed by DNA topoisomerase I inhibition. *Nat. Struct. Mol. Biol.* 20, 347–354. doi: 10.1038/nsmb.2501

Branzei, D., and Foiani, M. (2007). RecQ helicases queuing with Srs2 to disrupt Rad51 filaments and suppress recombination. *Genes Dev.* 21, 3019–3026. doi: 10.1101/gad.1624707

Brosh, R. M. Jr. (2013). DNA helicases involved in DNA repair and their roles in cancer. *Nat. Rev. Cancer* 13, 542–558. doi: 10.1038/nrc3560

Broustas, C. G., and Lieberman, H. B. (2014). DNA damage response genes and the development of cancer metastasis. *Radiat. Res.* 181, 111–130. doi: 10.1667/RR13515.1

Bugreev, D. V., Brosh, R. M. Jr., and Mazin, A. V. (2008). RECQ1 possesses DNA branch migration activity. *J. Biol. Chem.* 283, 20231–20242. doi: 10.1074/jbc.M801582200

Cogoni, C., and Macino, G. (1999). Posttranscriptional gene silencing in *Neurospora* by a RecQ DNA helicase. *Science* 286, 2342–2344. doi: 10.1126/science.286.5448.2342

Cotton, R. T., Li, D., Scherer, S. E., Muzny, D. M., Hodges, S. E., Catania, R. L., et al. (2009). Single nucleotide polymorphism in RECQL and survival in resectable pancreatic adenocarcinoma. *HPB* 11, 435–444. doi: 10.1111/j.1477-2574.2009.00089.x

Croteau, D. L., Popuri, V., Opresko, P. L., and Bohr, V. A. (2014). Human RecQ helicases in DNA repair, recombination, and replication. *Annu. Rev. Biochem.* 83, 519–552. doi: 10.1146/annurev-biochem-060713-035428

Featherstone, C., and Jackson, S. P. (1999). Ku, a DNA repair protein with multiple cellular functions? *Mutat. Res.* 434, 3–15. doi: 10.1016/S0921-8777(99)00006-3

Futami, K., Kumagai, E., Makino, H., Goto, H., Takagi, M., Shimamoto, A., et al. (2008a). Induction of mitotic cell death in cancer cells by small interference RNA suppressing the expression of RecQL1 helicase. *Cancer Sci.* 99, 71–80. doi: 10.1111/j.1349-7006.2007.00647.x

Futami, K., Kumagai, E., Makino, H., Sato, A., Takagi, M., Shimamoto, A., et al. (2008b). Anticancer activity of RecQL1 helicase siRNA in mouse xenograft models. *Cancer Sci.* 99, 1227–1236. doi: 10.1111/j.1349-7006.2008.00794.x

Futami, K., Ogasawara, S., Goto, H., Yano, H., and Furuichi, Y. (2010). RecQL1 DNA repair helicase: a potential tumor marker and therapeutic target against hepatocellular carcinoma. *Int. J. Mol. Med.* 25, 537–545. doi: 10.3892/ijmm_00000375

Gray, L. T., Vallur, A. C., Eddy, J., and Maizels, N. (2014). G quadruplexes are genomewide targets of transcriptional helicases XPB and XPD. *Nat. Chem. Biol.* 10, 313–318. doi: 10.1038/nchembio.1475

Gyorffy, B., Lanczky, A., Eklund, A. C., Denkert, C., Budczies, J., Li, Q., et al. (2010). An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res. Treat.* 123, 725–731. doi: 10.1007/s10549-009-0674-9

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Helleday, T. (2003). Pathways for mitotic homologous recombination in mammalian cells. *Mutat. Res.* 532, 103–115. doi: 10.1016/j.mrfmmm.2003.08.013

Hickson, I. D. (2003). RecQ helicases: caretakers of the genome. *Nat. Rev. Cancer* 3, 169–178. doi: 10.1038/nrc1012

Howell, S. M., and Bray, D. W. (2008). Amelanotic melanoma in a patient with Rothmund–Thomson syndrome. *Arch. Dermatol.* 144, 416–417. doi: 10.1001/archderm.144.3.416

Jaehnig, E. J., Kuo, D., Hombauer, H., Ideker, T. G., and Kolodner, R. D. (2013). Checkpoint kinases regulate a global network of transcription factors in response to DNA damage. *Cell Rep.* 4, 174–188. doi: 10.1016/j.celrep.2013.05.041

Jewell, R., Conway, C., Mitra, A., Randerson-Moor, J., Lobo, S., Nsengimana, J., et al. (2010). Patterns of expression of DNA repair genes and relapse from melanoma. *Clin. Cancer Res.* 16, 5211–5221. doi: 10.1158/1078-0432.ccr-10-1521

Kalluri, R., and Weinberg, R. A. (2009). The basics of epithelial-mesenchymal transition. *J. Clin. Invest.* 119, 1420–1428. doi: 10.1172/jci39104

Kauffmann, A., Rosselli, F., Lazar, V., Winnepenninckx, V., Mansuet-Lupo, A., Dessen, P., et al. (2008). High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene* 27, 565–573. doi: 10.1038/sj.onc.1210700

Kawabe, T., Tsuyama, N., Kitao, S., Nishikawa, K., Shimamoto, A., Shiratori, M., et al. (2000). Differential regulation of human RecQ family helicases in cell transformation and cell cycle. *Oncogene* 19, 4764–4772. doi: 10.1038/sj.onc.1203841

Kohn, K. W., Zeeberg, B. M., Reinhold, W. C., and Pommier, Y. (2014). Gene expression correlations in human cancer cell lines define molecular interaction networks for epithelial phenotype. *PLoS ONE* 9:e99269. doi: 10.1371/journal.pone.0099269

Kohn, K. W., Zeeberg, B. R., Reinhold, W. C., Sunshine, M., Luna, A., and Pommier, Y. (2012). Gene expression profiles of the NCI-60 human tumor cell lines define molecular interaction networks governing cell migration processes. *PLoS ONE* 7:e35716. doi: 10.1371/journal.pone.0035716

Kraus, W. L. (2008). Transcriptional control by PARP-1: chromatin modulation, enhancer-binding, coregulation, and insulation. *Curr. Opin. Cell Biol.* 20, 294–302. doi: 10.1016/j.ceb.2008.03.006

Lao, V. V., Welcsh, P., Luo, Y., Carter, K. T., Dzieciatkowski, S., Dintzis, S., et al. (2013). Altered RECQ helicase expression in sporadic primary colorectal cancers. *Transl. Oncol.* 6, 458–469. doi: 10.1593/tlo.13238

Lau, N. C., Seto, A. G., Kim, J., Kuramochi-Miyagawa, S., Nakano, T., Bartel, D. P., et al. (2006). Characterization of the piRNA complex from rat testes. *Science* 313, 363–367. doi: 10.1126/science.1130164

Li, D., Frazier, M., Evans, D. B., Hess, K. R., Crane, C. H., Jiao, L., et al. (2006). Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. *J. Clin. Oncol.* 24, 1720–1728. doi: 10.1200/JCO.2005.04.4206

Li, X. L., Lu, X., Parvathaneni, S., Bilke, S., Zhang, H., Thangavel, S., et al. (2014). Identification of RECQ1-regulated transcriptome uncovers a role of RECQ1 in regulation of cancer cell migration and invasion. *Cell Cycle* 13. [Epub ahead of print].

Liu, H., D'Andrade, P., Fulmer-Smentek, S., Lorenzi, P., Kohn, K. W., Weinstein, J. N., et al. (2010). mRNA and microRNA expression profiles of the NCI-60 integrated with drug activities. *Mol. Cancer Ther.* 9, 1080–1091. doi: 10.1158/1535-7163.MCT-09-0965

Lu, X., Parvathaneni, S., Hara, T., Lal, A., and Sharma, S. (2013). Replication stress induces specific enrichment of RECQ1 at common fragile sites FRA3B and FRA16D. *Mol. Cancer* 12, 29. doi: 10.1186/1476-4598-12-29

Maizels, N., and Gray, L. T. (2013). The G4 genome. *PLoS Genet.* 9:e1003468. doi: 10.1371/journal.pgen.1003468

Martinez-Marignac, V. L., Rodrigue, A., Davidson, D., Couillard, M., Al-Moustafa, A. E., Abramovitz, M., et al. (2011). The effect of a DNA repair gene on cellular invasiveness: XRCC3 over-expression in breast cancer cells. *PLoS ONE* 6:e16394. doi: 10.1371/journal.pone.0016394

Mendoza-Maldonado, R., Faoro, V., Bajpai, S., Berti, M., Odreman, F., Vindigni, M., et al. (2011). The human RECQ1 helicase is highly expressed in glioblastoma and plays an important role in tumor cell proliferation. *Mol. Cancer* 10, 83. doi: 10.1186/1476-4598-10-83

Mitra, A., Jameson, C., Barbachano, Y., Sanchez, L., Kote-Jarai, Z., Peock, S., et al. (2009). Overexpression of RAD51 occurs in aggressive prostatic cancer. *Histopathology* 55, 696–704. doi: 10.1111/j.1365-2559.2009.03448.x

Monnat, R. J. Jr. (2010). Human RECQ helicases: roles in DNA metabolism, mutagenesis and cancer biology. *Semin. Cancer Biol.* 20, 329–339. doi: 10.1016/j.semcancer.2010.10.002

Mullan, P. B., Quinn, J. E., and Harkin, D. P. (2006). The role of BRCA1 in transcriptional regulation and cell cycle control. *Oncogene* 25, 5854–5863. doi: 10.1038/sj.onc.1209872

Nguyen, G. H., Tang, W., Robles, A. I., Beyer, R. P., Gray, L. T., Welsh, J. A., et al. (2014). Regulation of gene expression by the BLM helicase correlates with the presence of G-quadruplex DNA motifs. *Proc. Natl. Acad. Sci. U.S.A.* 111, 9905–9910. doi: 10.1073/pnas.1404807111

Peters, D., Freund, J., and Ochs, R. L. (2005). Genome-wide transcriptional analysis of carboplatin response in chemosensitive and chemoresistant ovarian cancer cells. *Mol. Cancer Ther.* 4, 1605–1616. doi: 10.1158/1535-7163.mct-04-0311

Popuri, V., Bachrati, C. Z., Muzzolini, L., Mosedale, G., Costantini, S., Giacomini, E., et al. (2008). The Human RecQ helicases, BLM and RECQ1, display distinct DNA substrate specificities. *J. Biol. Chem.* 283, 17766–17776. doi: 10.1074/jbc.M709749200

Popuri, V., Croteau, D. L., Brosh, R. M. Jr., and Bohr, V. A. (2012). RECQ1 is required for cellular resistance to replication stress and catalyzes strand exchange on stalled replication fork structures. *Cell Cycle* 11, 4252–4265. doi: 10.4161/cc.22581

Popuri, V., Hsu, J., Khadka, P., Horvath, K., Liu, Y., Croteau, D. L., et al. (2014). Human RECQL1 participates in telomere maintenance. *Nucleic Acids Res.* 42, 5671–5688. doi: 10.1093/nar/gku200

Reinhold, W. C., Sunshine, M., Liu, H., Varma, S., Kohn, K. W., Morris, J., et al. (2012). CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set. *Cancer Res.* 72, 3499–3511. doi: 10.1158/0008-5472.CAN-12-1370

Sami, F., and Sharma, S. (2013). Probing genome maintenance functions of human RECQ1. *Comput. Struct. Biotechnol. J.* 6, e201303014. doi: 10.5936/csbj.201303014

Sanada, S., Futami, K., Terada, A., Yonemoto, K., Ogasawara, S., Akiba, J., et al. (2013). RECQL1 DNA repair helicase: a potential therapeutic target and a proliferative marker against ovarian cancer. *PLoS ONE* 8:e72820. doi: 10.1371/journal.pone.0072820

Sharma, S. (2011). Non-B DNA secondary structures and their resolution by RecQ helicases. *J. Nucleic Acids* 2011, 724215. doi: 10.4061/2011/724215

Sharma, S., and Brosh, R. M. Jr. (2007). Human RECQ1 is a DNA damage responsive protein required for genotoxic stress resistance and suppression of sister chromatid exchanges. *PLoS ONE* 2:e1297. doi: 10.1371/journal.pone.0001297

Sharma, S., and Brosh, R. M. Jr. (2008). Unique and important consequences of RECQ1 deficiency in mammalian cells. *Cell Cycle* 7, 989–1000. doi: 10.4161/cc.7.8.5707

Sharma, S., Phatak, P., Stortchevoi, A., Jasin, M., and Larocque, J. R. (2012). RECQ1 plays a distinct role in cellular response to oxidative DNA damage. *DNA Repair (Amst.)* 11, 537–549. doi: 10.1016/j.dnarep.2012.04.003

Sharma, S., Sommers, J. A., Choudhary, S., Faulkner, J. K., Cui, S., Andreoli, L., et al. (2005). Biochemical analysis of the DNA unwinding and strand annealing activities catalyzed by human RECQ1. *J. Biol. Chem.* 280, 28072–28084. doi: 10.1074/jbc.M500264200

Sharma, S., Stumpo, D. J., Balajee, A. S., Bock, C. B., Lansdorp, P. M., Brosh, R. M., et al. (2007). RECQL, a member of the RecQ family of DNA helicases, suppresses chromosomal instability. *Mol. Cell. Biol.* 27, 1784–1794. doi: 10.1128/MCB.01620-06

Shoemaker, R. H. (2006). The NCI60 human tumour cell line anticancer drug screen. *Nat. Rev. Cancer* 6, 813–823. doi: 10.1038/nrc1951

Stewart, D. J. (2007). Mechanisms of resistance to cisplatin and carboplatin. *Crit. Rev. Oncol. Hematol.* 63, 12–31. doi: 10.1016/j.critrevonc.2007.02.001

Tao, J., Tao, S., Han, J., Zhou, Z., Zhang, X., Wang, H., et al. (2014). RECQL1 plays an important role in the development of tongue squamous cell carcinoma. *Cell Physiol. Biochem.* 33, 1579–1590. doi: 10.1159/000358721

Thangavel, S., Mendoza-Maldonado, R., Tissino, E., Sidorova, J. M., Yin, J., Wang, W., et al. (2010). Human RECQ1 and RECQ4 helicases play distinct roles in DNA replication initiation. *Mol. Cell. Biol.* 30, 1382–1396. doi: 10.1128/MCB.01290-09

Weinstein, J. N. (2006). Spotlight on molecular profiling: "integromic" analysis of the NCI-60 cancer cell lines. *Mol. Cancer Ther.* 5, 2601–2605. doi: 10.1158/1535-7163.MCT-06-0640

Xu, L., Geman, D., and Winslow, R. L. (2007). Large-scale integration of cancer microarray data identifies a robust common cancer signature. *BMC Bioinformatics* 8:275. doi: 10.1186/1471-2105-8-275

Yuan, S. S., Hou, M. F., Hsieh, Y. C., Huang, C. Y., Lee, Y. C., Chen, Y. J., et al. (2012). Role of MRE11 in cell proliferation, tumor invasion, and DNA repair in breast cancer. *J. Natl. Cancer Inst.* 104, 1485–1502. doi: 10.1093/jnci/djs355

Zeisberg, M., and Neilson, E. G. (2009). Biomarkers for epithelial-mesenchymal transitions. *J. Clin. Invest.* 119, 1429–1437. doi: 10.1172/JCI36183

Zhang, P., Zhang, Z., Zhou, X., Qiu, W., Chen, F., and Chen, W. (2006). Identification of genes associated with cisplatin resistance in human oral squamous cell carcinoma cell line. *BMC Cancer* 6:224. doi: 10.1186/1471-2407-6-224

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 September 2014; accepted: 19 November 2014; published online: 05 December 2014.
*Citation: Sharma S (2014) An appraisal of RECQ1 expression in cancer progression. Front. Genet. **5**:426. doi: 10.3389/fgene.2014.00426*
This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.

frontiers in
GENETICS

MINI REVIEW ARTICLE
published: 25 November 2014
doi: 10.3389/fgene.2014.00418

Dysregulation of antiviral helicase pathways in systemic lupus erythematosus

Luciana Oliveira[1], Nailú A. Sinicato[1], Mariana Postal[1], Simone Appenzeller[1] and Timothy B. Niewold[2]*

[1] *Rheumatology Unit, Department of Medicine, Faculty of Medical Science, State University of Campinas, Campinas, Brazil*
[2] *Mayo Clinic, Division of Rheumatology, Department of Immunology, Rochester, MN, USA*

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

Reviewed by:
Miriam Wittmann, University of Leeds, UK
Anca Macovei, International Rice Research Institute, Philippines

***Correspondence:**
Timothy B. Niewold, Mayo Clinic, Division of Rheumatology, Department of Immunology, 200 1st Street South West, Guggenheim Building 3-42, Rochester, MN 55905, USA
e-mail: niewold.timothy@mayo.edu

In the autoimmune disease systemic lupus erythematosus (SLE), our normal antiviral defenses are inappropriately activated, resulting in over-activity of the type I interferon (IFN) pathway. This increased activity of the type I IFN pathway is an important primary pathogenic factor in the disease. Emerging evidence has implicated the antiviral helicases in this process. The antiviral helicases normally function as nucleic acid receptors in viral immunity. Genetic variations in antiviral helicase genes have been associated with SLE, supporting the idea that helicase pathways are involved in the primary pathogenesis of SLE. Studies have documented functional consequences of these genetic variations within the type I IFN pathway in human cell lines and SLE patients. In this review, we summarize the function of helicases in the anti-viral immune response, and how this response is dysregulated in SLE patients. In particular, we will focus on known functional genetic polymorphisms in the IFIH1 (MDA5) and mitochondrial antiviral signaling protein genes which have been implicated in human SLE. These data provide fascinating evidence for dysregulation of helicase-mediated innate immunity in SLE, and may support novel therapeutic strategies in the disease.

Keywords: antiviral helicase, systemic lupus erythematosus, interferon

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multisystem inflammation commonly including the skin, kidneys, and joints, and other systems. While the pathogenesis of SLE is not completely understood, it seems likely that both genetic and environmental factors contribute to the disease. A number of genetic factors have been associated with SLE in recent years (Harley et al., 2008; Ghodke-Puranik and Niewold, 2013), providing a window into human disease pathogenesis. Interestingly, many of these genetic variations associated with risk of SLE have function within the type I interferon (IFN) pathway (Ghodke-Puranik and Niewold, 2013). Type I IFN is a classical anti-viral molecule which causes activation of antigen presenting cells within the innate immune system and increased expression of MHC and co-stimulatory molecules (Pestka et al., 2004).

Many lines of evidence support the idea that type I IFN plays a primary role in SLE pathogenesis (Niewold, 2011). Circulating type I IFN levels are elevated in many SLE patients (Weckerle et al., 2011), and this elevation is also observed in unaffected members of SLE families, suggesting that high IFN levels are a heritable risk factor for SLE (Niewold et al., 2007). Familial aggregation has been observed with other cytokines in SLE, such as tumor necrosis factor alpha and IL-10 (Grondal et al., 1999; Mangale et al., 2013), but in these cases unrelated family members such as spouses shared the trait as well, suggesting a contribution from environmental factors. Subsequent study of SLE-associated genetic factors has confirmed that SLE-risk genes contribute to the high IFN levels observed in SLE (Kariuki et al., 2008; Kariuki and Niewold, 2010; Agik et al., 2012; Niewold et al., 2012), and it seems that the high IFN trait is significantly polygenic (Harley et al., 2010; Kariuki et al., 2010; Koldobskaya et al., 2012; Jensen et al., 2013). The genetic data all support the concept that common gain-of-function variations in the type I IFN pathway are associated with SLE pathogenesis. Additionally, rare variants in the TREX1 gene have been described that are strongly associated with a SLE and Aicardi-Goutieres syndrome, a rare condition characterized by alterations in type I IFN and neurologic symptoms (Lee-Kirsch et al., 2007; Namjou et al., 2011). Recombinant human type I IFN has been administered as a therapeutic to treat some malignancies and chronic viral infection, and in some cases *de novo* SLE has developed (Ronnblom et al., 1990), which typically resolves when the type I IFN is stopped (Niewold and Swedler, 2005). These data taken together support the idea that type I IFN is a primary pathogenic factor in human SLE. While there are significant differences in SLE incidence between men and women (9:1 female), type I IFN pathway activation seems to be equal between men and women with SLE (Niewold et al., 2008a; Weckerle and Niewold, 2011). There is an increased incidence of SLE in African-Americans as compared to European-American populations (4:1), and in this case is seems that there are some differences in the way the pathway is activated, but high IFN is clearly seen in both populations (Ko et al., 2012, 2013). Thus type I IFN is a common pathway to SLE susceptibility, and it follows that molecules operating upstream of type I IFN production would play a role in disease. A large body of work has supports the relevance of the endosomal Toll-like receptors in SLE pathogenesis (Lovgren et al., 2004; Salloum and Niewold, 2011).

In this review, we will focus on emerging data which implicates RNA helicases in type I IFN pathway dysregulation in human SLE. These data may also be relevant to other autoimmune diseases, as a number of conditions have been associated with increased type I IFN, including dermatomyositis, Sjogren's syndrome, neuromyelitis optica, and others (Niewold et al., 2008b, 2011; Sweiss et al., 2011; Feng et al., 2012; Mavragani et al., 2013). In particular, we will focus on known functional genetic polymorphisms in the IFIH1 (MDA5) and mitochondrial antiviral signaling protein (MAVS) genes which function in helicase pathways, and have been implicated in human SLE.

PATTERN RECOGNITION RECEPTORS INVOLVED IN ANTI-VIRAL RESPONSES AND SLE

Several families of receptors that recognize pathogen-associated molecular patterns (PAMPs) have been described, such as the Toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I)-like receptor (RLRs). TLRs are transmembrane receptors expressed in specific immune cells, such as dendritic cells and macrophages. TLR7, 8, and 9 are expressed in the endosomal membrane, and can recognize viral nucleic acid. In anti-viral immunity, viral immune complexes are taken up via Fc receptors, and then delivered to the endosome resulting in TLR activation. RLRs, on the other hand, are cytosolic proteins that can recognize viral nucleic acid in the cytosol. Activation of either TLRs or RLRs results in IFN production and an anti-viral response (**Figure 1**).

The endosomal TLRs have been implicated in SLE pathogenesis in a number of previous studies. Genetic variations in TLR7 are associated with SLE susceptibility in humans (Deng et al., 2013). Mice which carry a duplication of the endosomal TLR region of the X-chromosome have enhanced susceptibility to an SLE-like disease (Subramanian et al., 2006). Interestingly, the autoantibodies which are characteristically produced in SLE target components of the cell nucleus, for example antibodies against double-stranded DNA and nuclear RNA-binding proteins. These SLE-associated autoantibodies form immune complexes that contain RNA and DNA, and the immune complexes can result in activation of the TLR system with subsequent type I IFN production (Lovgren et al., 2004, 2006). Thus, the anti-nuclear immune response that characterizes SLE produces immune complexes that are viral mimics, subverting normal viral immunity. These autoantibodies are frequently high titer and continuously present in SLE, and the

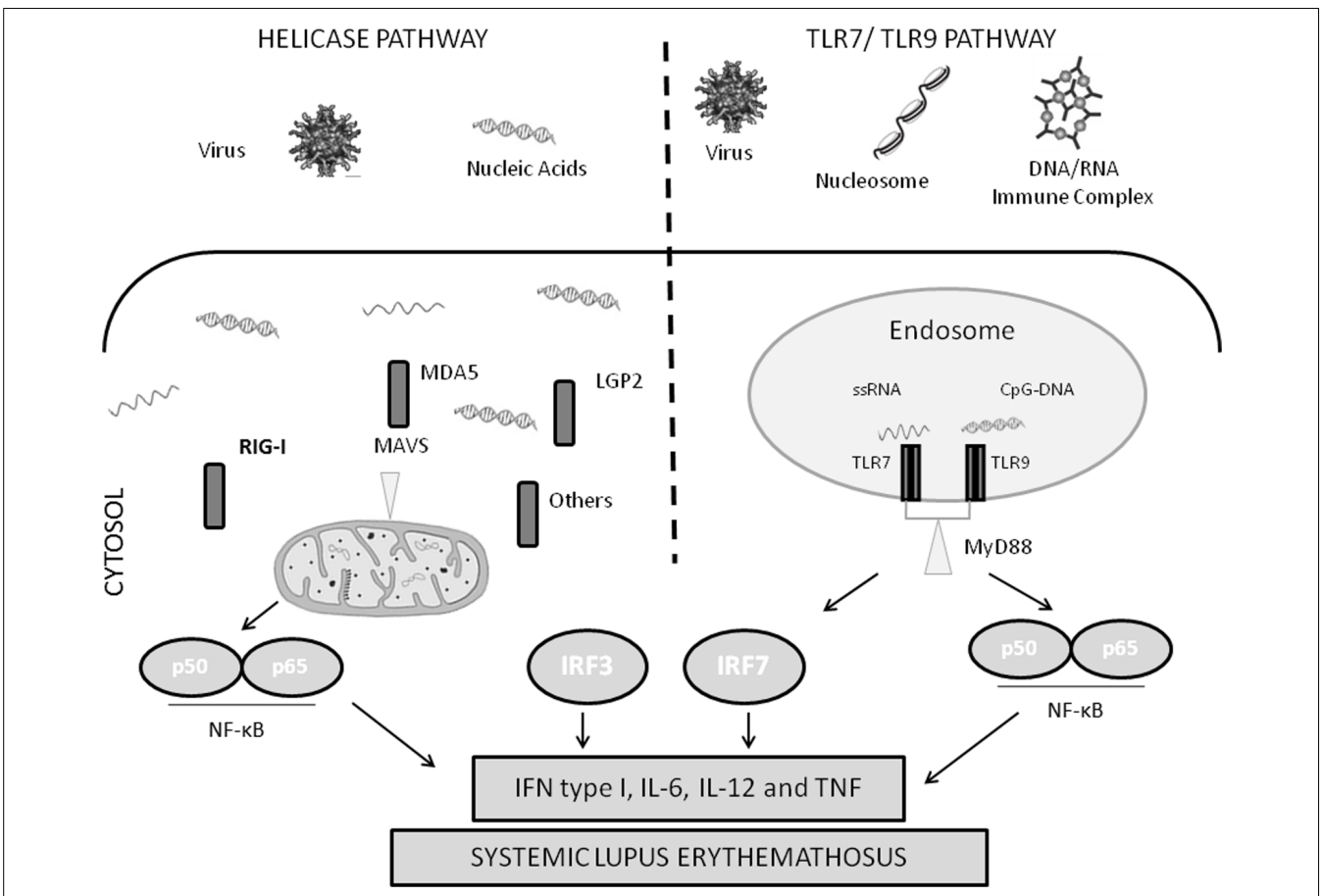


FIGURE 1 | Diagram of nucleic acid sensing antiviral immune response pathways. IFN, type I interferon; IL, interleukin; IRF, interferon regulatory factor; LGP2, laboratory of genetics and physiology 2; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma differentiation-associated gene 5; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor-κB; RIG-I, retinoic acid-inducible gene I; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRIF, Toll/IL-1R-domain-containing adaptor inducing interferon β.

antigens are ubiquitous, resulting in consistent inappropriate activation of the type I IFN pathway in SLE (Salloum and Niewold, 2011). Supporting this idea, one of the accepted and effective treatments for SLE, hydroxychloroquine, seems to interrupt endosomal TLR signaling (Kuznik et al., 2011; Sacre et al., 2012).

RNA HELICASES

Ribonucleic acid helicases are involved in almost all cellular processes involving RNA (Steimer and Klostermeier, 2012). These enzymes use ATP to bind or remodel RNA and RNA–protein complexes (Linder and Jankowsky, 2011). Based on their shared conserved motifs and three dimensional core structures, RNA helicases are grouped into families and superfamilies (Linder and Jankowsky, 2011; Steimer and Klostermeier, 2012). The majority of RNA helicases belong to superfamily 2 (SF2; Pyle, 2008; Steimer and Klostermeier, 2012). DEAD box proteins are the largest family of helicases in SF2, and in humans these helicases have essential physiological roles in cellular RNA metabolism (Linder and Jankowsky, 2011). The DEAD box helicases work by destabilizing short RNA duplexes close to the binding site of the helicase. In contrast, the DExH group of helicases work in a progressive fashion, unwinding longer stretches of RNA (Pyle, 2008). DEAD box helicases frequently play a role in viral immunity by acting as sensors cytosolic viral nucleic acids. Besides the RLR DEAD box helicases which include RIG-I, MDA5, and LGP2, other DEAD box helicases likely perform this role as well, including DDX1, DDX3, DDX36, DDX41, DDX60, and others (Fullam and Schroder, 2013). In addition to sensing nucleic acid, some of these functions may be helicases further downstream in the pattern recognition receptor signaling pathways, potentially playing roles in transcriptional regulation (Fullam and Schroder, 2013). Interestingly, it appears that some RNA helicases are important for viral replication, suggesting that viruses have adopted this cellular mechanism to their own advantage in some cases (Fullam and Schroder, 2013).

Retinoic acid-inducible gene-I and MDA5 are proteins encoded by the DDX58 and IFIH1 genes, respectively. These RLRs are induced by type I IFN, and each recognize specific types of viruses (Yoneyama and Fujita, 2008). RIG-I and MDA5 recognize distinct viral RNA structures containing 5′ triphosphate in single and double-stranded RNA (Shrivastav and Niewold, 2013). These two RLRs demonstrate some specificity in the types of nucleic acids they recognize: while MDA5 senses picornavirus-derived nucleic acid, RIG-I senses other viral nucleic acids, such as those derived from influenza A (Yoneyama and Fujita, 2008). This differential recognition is based on the distinct RNA patterns generated by different viruses (Yoneyama and Fujita, 2008).

Activation of RIG-I and MDA5 by nucleic acid leads to binding of the mitochondrial adaptor IFN β promoter stimulater 1 (IPS-1) also known as MAVS (Reikine et al., 2014). As the name suggests, MAVS is found in the mitochondrial membrane, and is critical to signal transduction via MDA5 and RIG-I. MAVS forms large multimeric polymers on the mitochondrial membrane in combination with RIG-I and MDA5 bound to target nucleic acids, forming an active signaling complex (Reikine et al., 2014). This leads to activation of NF-κB, IRF3, and IRF7 (Reikine et al., 2014). These transcription factors are involved in IFN and interferon-stimulated gene expression, and the production of type-1 IFN and pro-inflammatory cytokines (Shrivastav and Niewold, 2013; **Figure 1**).

MDA5/IFIH1

IFIH1 is the gene that encodes MDA5, and a common coding-change polymorphism in the IFIH1 gene has been associated with risk of SLE and other autoimmune diseases in humans (Smyth et al., 2006; Sutherland et al., 2007; Harley et al., 2008; Gateva et al., 2009; Strange et al., 2010; Molineros et al., 2013). This A946T polymorphism in IFIH1 was identified in case-control genetic studies of SLE (Harley et al., 2008; Gateva et al., 2009), and interestingly this polymorphism was the major finding in a recent admixture-mapping genetic screen to identify genes associated with SLE in African-Americans (Molineros et al., 2013), supporting relevance of this polymorphism across multiple ancestral backgrounds. This polymorphism appears to be gain-of-function in nature, being associated with increased IFIH1 mRNA expression (Downes et al., 2010), increased sensitivity to type I IFN and increased IFN-induced gene expression in circulating blood cells from SLE patients (Robinson et al., 2011), and modulation of inflammation- and apoptosis-related gene expression (Molineros et al., 2013). These studies support the general idea that over-activity of the anti-viral helicases would result in greater type I IFN signaling and risk of SLE. Rare loss-of-function variations in IFIH1 have been discovered in the IFIH1 gene, and interestingly these loss-of-function variants appear to be protective against autoimmune disease (Nejentsev et al., 2009), further supporting the idea that increased function of IFIH1/MDA5 is associated with risk of autoimmune disease.

Studies in mice have also supported this hypothesis. A recent study demonstrated that a single coding-change mutation in IFIH1 (Gly821Ser) generated by *N*-ethyl-*N*-nitrosourea (ENU) resulted in constitutive activation of MDA5 (Funabiki et al., 2014). Mice with this mutation developed a systemic autoimmune disease similar to lupus, with nephritis characterized by lymphocyte infiltration as well as deposition of immunoglobulin and complement, systemic inflammation in the heart and lung, and increased tumor necrosis factor alpha, IL-6 and type I IFN (Funabiki et al., 2014). This gain-of-function mutation in the mouse line has not been observed in humans, but it supports the general concept that gain of function in IFIH1 is associated with autoimmunity.

MAVS

Genetic studies in human SLE have also identified a functional coding-change polymorphism in MAVS, a key adapter of both the RIG-I and MDA5 helicases. The C97F polymorphism in MAVS substantially reduced the expression of type I IFN and other proinflammatory mediators in human cell lines (Pothlichet et al., 2011). Interestingly, this variation was almost exclusively found in the African-American population, with a frequency of 10.2% in controls (Pothlichet et al., 2011). In African–American patients with SLE, the C79F allele was associated with low type I IFN and was more than twice as common (22.4% frequency) in SLE patients who lacked autoantibodies to RNA-binding proteins. This study demonstrated that a coding-change genetic variation in the gene

encoding MAVS has a functional impact on the antiviral IFN pathway in humans, and is associated with a serologic subgroup of SLE patients (Pothlichet et al., 2011). These studies in both MAVS and IFIH1 demonstrate the importance of variations in these genes upon immune function and autoimmune disease risk in human populations.

CONCLUSION

Dysregulation of anti-viral helicase immune responses represent a primary pathogenic factor in human SLE. This is demonstrated by the presence of coding-change polymorphisms in both the IFIH1 and MAVS genes which modulate function of the type I IFN pathway and risk of SLE in humans. While immune complexes formed by SLE autoantibodies and nuclear material seem to be the likely trigger for endosomal TLR stimulation in SLE, the exact triggers of the cytosolic anti-viral helicases in human SLE are somewhat less clear. Viruses may stimulate some of the early events in SLE patients, as strong epidemiological data implicates Epstein–Barr virus infection in the initial pathogenesis of SLE (James et al., 1997; Poole et al., 2006). It is possible that this represents a gene – environment interaction in human SLE – a hypersensitive or over-active anti-viral helicase system coupled with a viral trigger, such as Epstein–Barr virus infection, which then results in an exaggerated type I IFN response and subsequent misdirection of the adaptive immune response against self-antigens. It is also possible that viral-like elements, such as LINE elements, may also play a role in the chronic stimulation of these cytosolic nucleic acid receptors (Crow, 2010). Both Epstein-Barr virus and LINE-1 RNAs could potentially be seen as "foreign" by the cytosolic helicases. The fact that both the TLR and cytosolic pathways of viral recognition are involved in human SLE and the convergence of these pathways upon type I IFN and anti-viral responses is striking, suggesting that over-active anti-viral immunity represents a major common pathogenic pathway in human SLE. A number of therapeutics have been developed to target type I IFN in SLE, including monoclonal antibodies against IFN-α (Merrill et al., 2011; Kalunian et al., 2012; McBride et al., 2012), as well as a vaccination strategy aimed at inducing antibodies against IFN-α (Lauwerys et al., 2013). These studies are in early stages, phase I to phase II, and thus far the data generally support relative safety and proof-of-mechanism. It is too early to make conclusions about efficacy, but some of the larger phase II studies have reported potential subset effects within the overall SLE patient groups (Kalunian et al., 2012). This would suggest that anti-IFN therapies may not work for every patient, but the genetics data we discuss in this review may also suggest this outcome. It seems that the IFN pathway is impacted by a number of genetic factors, and these factors will not be shared by all patients. Therapeutics targeting the RNA helicases could be potentially interesting in SLE, and further understanding of the specific dysregulation of the helicase pathways in human SLE such as the work summarized in this review could help to determine optimal points of intervention in the pathway and in which group of patients.

ACKNOWLEDGMENTS

Simone Appenzeller – Conselho Nacional de Desenvolvimento Cientifico e Tecnologico de Brasil, Visiting Researcher Project Grant, Program Ciencia Sem Frontieras; Timothy B. Niewold – Research grants from the NIH (AR060861, AI083790, AI071651), Rheumatology Research Foundation, the Mayo Clinic Foundation, the Lupus Foundation of Minnesota, and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico de Brasil, Visiting Researcher Project Grant, Program Ciencia Sem Frontieras.

REFERENCES

Agik, S., Franek, B. S., Kumar, A. A., Kumabe, M., Utset, T. O., Mikolaitis, R. A., et al. (2012). The autoimmune disease risk allele of UBE2L3 in African American patients with systemic lupus erythematosus: a recessive effect upon subphenotypes. *J. Rheumatol.* 39, 73–78. doi: 10.3899/jrheum.110590

Crow, M. K. (2010). Long interspersed nuclear elements (LINE-1): potential triggers of systemic autoimmune disease. *Autoimmunity* 43, 7–16. doi: 10.3109/08916930903374865

Deng, Y., Zhao, J., Sakurai, D., Kaufman, K. M., Edberg, J. C., Kimberly, R. P., et al. (2013). MicroRNA-3148 modulates allelic expression of toll-like receptor 7 variant associated with systemic lupus erythematosus. *PLoS Genet.* 9:e1003336. doi: 10.1371/journal.pgen.1003336

Downes, K., Pekalski, M., Angus, K. L., Hardy, M., Nutland, S., Smyth, D. J., et al. (2010). Reduced expression of IFIH1 is protective for type 1 diabetes. *PLoS ONE* 55:e12646. doi: 10.1371/journal.pone.0012646

Feng, X., Reder, N. P., Yanamandala, M., Hill, A., Franek, B. S., Niewold, T. B., et al. (2012). Type I interferon signature is high in lupus and neuromyelitis optica but low in multiple sclerosis. *J. Neurol. Sci.* 313, 48–53. doi: 10.1016/j.jns.2011.09.032

Fullam, A., and Schroder, M. (2013). DExD/H-box RNA helicases as mediators of anti-viral innate immunity and essential host factors for viral replication. *Biochim. Biophys. Acta* 1829, 854–865. doi: 10.1016/j.bbagrm.2013.03.012

Funabiki, M., Kato, H., Miyachi, Y., Toki, H., Motegi, H., Inoue, M., et al. (2014). Autoimmune disorders associated with gain of function of the intracellular sensor MDA5. *Immunity* 40, 199–212. doi: 10.1016/j.immuni.2013.12.014

Gateva, V., Sandling, J. K., Hom, G., Taylor, K. E., Chung, S. A., Sun, X., et al. (2009). A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat. Genet.* 41, 1228–1233. doi: 10.1038/ng.468

Ghodke-Puranik, Y., and Niewold, T. B. (2013). Genetics of the type I interferon pathway in systemic lupus erythematosus. *Int. J. Clin. Rheumtol.* 8, 657–669. doi: 10.2217/ijr.13.58

Grondal, G., Kristjansdottir, H., Gunnlaugsdottir, B., Arnason, A., Lundberg, I., Klareskog, L., et al. (1999). Increased number of interleukin-10-producing cells in systemic lupus erythematosus patients and their first-degree relatives and spouses in Icelandic multicase families. *Arthritis Rheum.* 42, 1649–1654. doi: 10.1002/1529-0131(199908)42:8<1649::AID-ANR13>3.0.CO;2-D

Harley, J. B., Alarcon-Riquelme, M. E., Criswell, L. A., Jacob, C. O., Kimberly, R. P., Moser, K. L., et al. (2008). Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat. Genet.* 40, 204–210. doi: 10.1038/ng.81

Harley, T. W. I., Niewold, T. B., Stormont, R. M., Kaufman, K. M., Glenn, S. B., Franek, B. S., et al. (2010). The role of genetic variation near interferon-kappa in systemic lupus erythematosus. *J. Biomed. Biotechnol.* 2010:706825. doi: 10.1155/2010/706825

James, J. A., Kaufman, K. M., Farris, A. D., Taylor-Albert, E., Lehman, T. J., and Harley, J. B. (1997). An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J. Clin. Invest.* 100, 3019–3026. doi: 10.1172/JCI119856

Jensen, M. A., Patterson, K. C., Kumar, A. A., Kumabe, M., Franek, B. S., and Niewold, T. B. (2013). Functional genetic polymorphisms in ILT3 are associated with decreased surface expression on dendritic cells and increased serum cytokines in lupus patients. *Ann. Rheum. Dis.* 72, 596–601. doi: 10.1136/annrheumdis-2012-202024

Kalunian, K., Merrill, J. T., Maciuca, R., Ouyang, W., McBride, J. M., Townsend, M. J., et al. (2012). Efficacy and safety of rontalizumab (Anti-Interferon Alpha) in SLE subjects with restricted immunosuppressant use: results of a randomized, double-blind, placebo-controlled phase 2 study. *Arthritis Rheum.* 64:2622. doi: 10.1002/art.40353

Kariuki, S. N., Crow, M. K., and Niewold, T. B. (2008). The PTPN22 C1858T polymorphism is associated with skewing of cytokine profiles toward high

interferon-alpha activity and low tumor necrosis factor alpha levels in patients with lupus. *Arthritis Rheum.* 58, 2818–2823. doi: 10.1002/art.23728

Kariuki, S. N., Franek, B. S., Kumar, A. A., Arrington, J., Mikolaitis, R. A., Utset, T. O., et al. (2010). Trait-stratified genome-wide association study identifies novel and diverse genetic associations with serologic and cytokine phenotypes in systemic lupus erythematosus. *Arthritis Res. Ther.* 12:R151. doi: 10.1186/ar3101

Kariuki, S. N., and Niewold, T. B. (2010). Genetic regulation of serum cytokines in systemic lupus erythematosus. *Transl. Res.* 155, 109–117. doi: 10.1016/j.trsl.2009.08.012

Ko, K., Franek, B. S., Marion, M., Kaufman, K. M., Langefeld, C. D., Harley, J. B., et al. (2012). Genetic ancestry, serum interferon-alpha activity, and autoantibodies in systemic lupus erythematosus. *J. Rheumatol.* 39, 1238–1240. doi: 10.3899/jrheum.111467

Ko, K., Koldobskaya, Y., Rosenzweig, E., and Niewold, T. B. (2013). Activation of the interferon pathway is dependent upon autoantibodies in African-American SLE patients, but Not in European-American SLE patients. *Front. Immunol.* 4:309. doi: 10.3389/fimmu.2013.00309

Koldobskaya, Y., Ko, K., Kumar, A. A., Agik, S., Arrington, J., Kariuki, S. N., et al. (2012). Gene-expression-guided selection of candidate loci and molecular phenotype analyses enhance genetic discovery in systemic lupus erythematosus. *Clin. Dev. Immunol.* 2012:682018. doi: 10.1155/2012/682018

Kuznik, A., Bencina, M., Svajger, U., Jeras, M., Rozman, B., and Jerala, R. (2011). Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. *J. Immunol.* 186, 4794–4804. doi: 10.4049/jimmunol.1000702

Lauwerys, B. R., Hachulla, E., Spertini, F., Lazaro, E., Jorgensen, C., Mariette, X., et al. (2013). Down-regulation of interferon signature in systemic lupus erythematosus patients by active immunization with interferon alpha-kinoid. *Arthritis Rheum.* 65, 447–456. doi: 10.1002/art.37785

Lee-Kirsch, M. A., Gong, M., Chowdhury, D., Senenko, L., Engel, K., Lee, Y. A., et al. (2007). Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat. Genet.* 39, 1065–1067. doi: 10.1038/ng2091

Linder, P., and Jankowsky, E. (2011). From unwinding to clamping – the DEAD box RNA helicase family. *Nat. Rev. Mol. Cell Biol.* 12, 505–516. doi: 10.1038/nrm3154

Lovgren, T., Eloranta, M. L., Bave, U., Alm, G. V., and Ronnblom, L. (2004). Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis Rheum.* 50, 1861–1872. doi: 10.1002/art.20254

Lovgren, T., Eloranta, M. L., Kastner, B., Wahren-Herlenius, M., Alm, G. V., and Ronnblom, L. (2006). Induction of interferon-alpha by immune complexes or liposomes containing systemic lupus erythematosus autoantigen- and Sjogren's syndrome autoantigen-associated RNA. *Arthritis Rheum.* 54, 1917–1927. doi: 10.1002/art.21893

Mangale, D., Kariuki, S. N., Chrabot, B. S., Kumabe, M., Kelly, J. A., Harley, J. B., et al. (2013). Familial aggregation of high tumor necrosis factor alpha levels in systemic lupus erythematosus. *Clin. Dev. Immunol.* 2013:267430. doi: 10.1155/2013/267430

Mavragani, C. P., Niewold, T. B., Chatzigeorgiou, A., Danielides, S., Thomas, D., Kirou, K. A., et al. (2013). Increased serum type I interferon activity in organ-specific autoimmune disorders: clinical, imaging, and serological associations. *Front. Immunol.* 4:238. doi: 10.3389/fimmu.2013.00238

McBride, J. M., Jiang, J., Abbas, A. R., Morimoto, A., Li, J., Maciuca, R., et al. (2012). Safety and pharmacodynamics of rontalizumab in patients with systemic lupus erythematosus: results of a phase I, placebo-controlled, double-blind, dose-escalation study. *Arthritis Rheum.* 64, 3666–3676. doi: 10.1002/art.34632

Merrill, J. T., Wallace, D. J., Petri, M., Kirou, K. A., Yao, Y., White, W. I., et al. (2011). Lupus interferon skin activity study, safety profile and clinical activity of sifalimumab, a fully human anti-interferon alpha monoclonal antibody, in systemic lupus erythematosus: a phase I, multicentre, double-blind randomised study. *Ann. Rheum. Dis.* 70, 1905–1913. doi: 10.1136/ard.2010.144485

Molineros, J. E., Maiti, A. K., Sun, C., Looger, L. L., Han, S., Kim-Howard, X., et al. (2013). Admixture mapping in lupus identifies multiple functional variants within IFIH1 associated with apoptosis, inflammation, and autoantibody production. *PLoS Genet.* 9:e1003222. doi: 10.1371/journal.pgen.1003222

Namjou, B., Kothari, P. H., Kelly, J. A., Glenn, S. B., Ojwang, J. O., Adler, A., et al. (2011). Evaluation of the TREX1 gene in a large multi-ancestral lupus cohort. *Genes Immun.* 12, 270–279. doi: 10.1038/gene.2010.73

Nejentsev, S., Walker, N., Riches, D., Egholm, M., and Todd, J. A. (2009). Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* 324, 387–389. doi: 10.1126/science.1167728

Niewold, T. B. (2011). Interferon alpha as a primary pathogenic factor in human lupus. *J. Interferon Cytokine Res.* 31, 887–892. doi: 10.1089/jir.2011.0071

Niewold, T. B., Adler, J. E., Glenn, S. B., Lehman, T. J., Harley, J. B., and Crow, M. K. (2008a). Age- and sex-related patterns of serum interfeon-alpha activity in lupus families. *Arthritis Rheum.* 58, 2113–2119. doi: 10.1002/art.23619

Niewold, T. B., Rivera, T. L., Buyon, J. P., and Crow, M. K. (2008b). Serum type I interferon activity is dependent on maternal diagnosis in anti-SSA/Ro-positive mothers of children with neonatal lupus. *Arthritis Rheum.* 58, 541–546. doi: 10.1002/art.23191

Niewold, T. B., Hua, J., Lehman, T. J., Harley, J. B., and Crow, M. K. (2007). High serum IFN-alpha activity is a heritable risk factor for systemic lupus erythematosus. *Genes Immun.* 8, 492–502. doi: 10.1038/sj.gene.6364408

Niewold, T. B., Kelly, J. A., Kariuki, S. N., Franek, B. S., Kumar, A. A., Kaufman, K. M., et al. (2012). IRF5 haplotypes demonstrate diverse serological associations which predict serum interferon alpha activity and explain the majority of the genetic association with systemic lupus erythematosus. *Ann. Rheum. Dis.* 71, 463–468. doi: 10.1136/annrheumdis-2011-200463

Niewold, T. B., and Swedler, W. I. (2005). Systemic lupus erythematosus arising during interferon-alpha therapy for cryoglobulinemic vasculitis associated with hepatitis C. *Clin. Rheumatol.* 24, 178–181. doi: 10.1007/s10067-004-1024-2

Niewold, T. B., Wu, S. C., Smith, M., Morgan, G. A., and Pachman, L. M. (2011). Familial aggregation of autoimmune disease in juvenile dermatomyositis. *Pediatrics* 127, e1239–e1246. doi: 10.1542/peds.2010-3022

Pestka, S., Krause, C. D., and Walter, M. R. (2004). Interferons, interferon-like cytokines, and their receptors. *Immunol. Rev.* 202, 8–32. doi: 10.1111/j.0105-2896.2004.00204.x

Poole, B. D., Scofield, R. H., Harley, J. B., and James, J. A. (2006). Epstein-Barr virus and molecular mimicry in systemic lupus erythematosus. *Autoimmunity* 39, 63–70. doi: 10.1080/08916930500484849

Pothlichet, J., Niewold, T. B., Vitour, D., Solhonne, B., Crow, M. K., and Si-Tahar, M. (2011). A loss-of-function variant of the antiviral molecule MAVS is associated with a subset of systemic lupus patients. *EMBO Mol. Med.* 3, 142–152. doi: 10.1002/emmm.201000120

Pyle, A. M. (2008). Translocation and unwinding mechanisms of RNA and DNA helicases. *Annu. Rev. Biophys.* 37, 317–336. doi: 10.1146/annurev.biophys.37.032807.125908

Reikine, S., Nguyen, J. B., and Modis, Y. (2014). Pattern recognition and signaling mechanisms of RIG-I and MDA5. *Front. Immunol.* 5:342. doi: 10.3389/fimmu.2014.00342

Robinson, T., Kariuki, S. N., Franek, B. S., Kumabe, M., Kumar, A. A., Badaracco, M., et al. (2011). Autoimmune disease risk variant of IFIH1 is associated with increased sensitivity to IFN-alpha and serologic autoimmunity in lupus patients. *J. Immunol.* 187, 1298–1303. doi: 10.4049/jimmunol.1100857

Ronnblom, L. E., Alm, G. V., and Oberg, K. E. (1990). Possible induction of systemic lupus erythematosus by interferon-alpha treatment in a patient with a malignant carcinoid tumour. *J. Intern. Med.* 227, 207–210. doi: 10.1111/j.1365-2796.1990.tb00144.x

Sacre, K., Criswell, L. A., and McCune, J. M. (2012). Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus . *Arthritis Res. Ther.* 14:R155. doi: 10.1186/ar3895

Salloum, R., and Niewold, T. B. (2011). Interferon regulatory factors in human lupus pathogenesis. *Transl. Res.* 157, 326–331. doi: 10.1016/j.trsl.2011.01.006

Shrivastav, M., and Niewold, T. B. (2013). Nucleic acid sensors and type I interferon production in systemic lupus erythematosus. *Front. Immunol.* 4:319. doi: 10.3389/fimmu.2013.00319

Smyth, D. J., Cooper, J. D., Bailey, R., Field, S., Burren, O., Smink, L. J., et al. (2006). A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. *Nat. Genet.* 38, 617–619. doi: 10.1038/ng1800

Steimer, L., and Klostermeier, D. (2012). RNA helicases in infection and disease. *RNA Biol.* 9, 751–771. doi: 10.4161/rna.20090

Strange, A., Capon, F., Spencer, C. C., Knight, J., Weale, M. E., Allen, M. H., et al. (2010). A genome-wide association study identifies new psoriasis susceptibility

loci and an interaction between HLA-C and ERAP1. *Nat. Genet.* 42, 985–990. doi: 10.1038/ng.694

Subramanian, S., Tus, K., Li, Q. Z., Wang, A., Tian, X. H., Zhou, J., et al. (2006). A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9970–9975. doi: 10.1073/pnas.0603912103

Sutherland, A., Davies, J., Owen, C. J., Vaikkakara, S., Walker, C., Cheetham, T. D., et al. (2007). Genomic polymorphism at the interferon-induced helicase (IFIH1) locus contributes to Graves' disease susceptibility. *J. Clin. Endocrinol. Metab.* 92, 3338–3341. doi: 10.1210/jc.2007-0173

Sweiss, N. J., Zhang, W., Franek, B. S., Kariuki, S. N., Moller, D. R., Patterson, K. C., et al. (2011). Linkage of type I interferon activity and TNF-alpha levels in serum with sarcoidosis manifestations and ancestry. *PLoS ONE* 6:e29126. doi: 10.1371/journal.pone.0029126.t001

Weckerle, C. E., Franek, B. S., Kelly, J. A., Kumabe, M., Mikolaitis, R. A., Green, S. L., et al. (2011). Network analysis of associations between serum interferon-alpha activity, autoantibodies, and clinical features in systemic lupus erythematosus. *Arthritis Rheum.* 63, 1044–1053. doi: 10.1002/art.30187

Weckerle, C. E., and Niewold, T. B. (2011). The unexplained female predominance of systemic lupus erythematosus: clues from genetic and cytokine studies. *Clin. Rev. Allergy. Immunol.* 40, 42–49. doi: 10.1007/s12016-009-8192-4

Yoneyama, M., and Fujita, T. (2008). Structural mechanism of RNA recognition by the RIG-I-like receptors. *Immunity* 29, 178–181. doi: 10.1016/j.immuni.2008.07.009

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 08 September 2014; accepted: 10 November 2014; published online: 25 November 2014.

*Citation: Oliveira L, Sinicato NA, Postal M, Appenzeller S and Niewold TB (2014) Dysregulation of antiviral helicase pathways in systemic lupus erythematosus. Front. Genet. **5**:418. doi: 10.3389/fgene.2014.00418*

This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.

frontiers in
GENETICS

REVIEW ARTICLE
published: 29 October 2014
doi: 10.3389/fgene.2014.00366

Structural mechanisms of human RecQ helicases WRN and BLM

Ken Kitano*

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Japan

Edited by:
Masayuki Seki, Tohoku Pharmaceutical University, Japan

Reviewed by:
Arthur J. Lustig, Tulane University, USA
George A. Garinis, University of Crete, Greece

***Correspondence:**
Ken Kitano, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan
e-mail: kkitano@is.naist.jp

The RecQ family DNA helicases Werner syndrome protein (WRN) and Bloom syndrome protein (BLM) play a key role in protecting the genome against deleterious changes. In humans, mutations in these proteins lead to rare genetic diseases associated with cancer predisposition and accelerated aging. WRN and BLM are distinguished from other helicases by possessing signature tandem domains toward the C terminus, referred to as the RecQ C-terminal (RQC) and helicase-and-ribonuclease D-C-terminal (HRDC) domains. Although the precise function of the HRDC domain remains unclear, the previous crystal structure of a WRN RQC-DNA complex visualized a central role for the RQC domain in recognizing, binding and unwinding DNA at branch points. In particular, a prominent hairpin structure (the β-wing) within the RQC winged-helix motif acts as a scalpel to induce the unpairing of a Watson–Crick base pair at the DNA duplex terminus. A similar RQC-DNA interaction was also observed in the recent crystal structure of a BLM-DNA complex. I review the latest structures of WRN and BLM, and then provide a docking simulation of BLM with a Holliday junction. The model offers an explanation for the efficient branch migration activity of the RecQ family toward recombination and repair intermediates.

Keywords: Werner syndrome, Bloom syndrome, WRN, BLM, DNA helicase, Holliday junction, structural biology

INTRODUCTION

RecQ helicases, a family of DNA unwinding enzymes that belong to the SF2 superfamily helicases, play crucial roles at multiple steps in DNA recombination, replication and repair. Whereas the genomes of bacteria typically encode a single recQ gene, the human genome contains five recQ genes that encode Werner syndrome protein (WRN), Bloom syndrome protein (BLM), RECQ1, RECQ4, and RECQ5. Mutations in WRN and BLM are associated with the rare genetic diseases Werner and Bloom syndromes, respectively. These two diseases are characterized by a high frequency of cancer predisposition, illustrating the primary importance of WRN and BLM in preventing tumorigenesis. Indeed, cells derived from afflicted patients show pronounced genomic instabilities such as sister chromatid exchange and telomere shortening.

The Werner and Bloom syndromes, however, are also characterized by many distinct clinical symptoms: Werner patients display features of accelerated aging including the early onset of osteoporosis, atherosclerosis, arteriosclerosis, type II diabetes and cataracts (Goto, 2000; Goto et al., 2013; Oshima et al., 2013), while Bloom patients display severe growth retardation with short stature, immunodeficiency, sunlight sensitivity and a predisposition to a wide spectrum of cancers (Manthei and Keck, 2013). The different clinical features of the disorders, and the fact that the functional loss of either WRN or BLM cannot be compensated for by the presence of the other protein (or of other RecQ members), support the notion that WRN and BLM have distinct functions in cells.

To date, a number of reviews on the biological functions of WRN and BLM have been published, including the latest ones that discuss the diverse genome-maintenance mechanisms of the RecQ family (Larsen and Hickson, 2013; Croteau et al., 2014). In this review, I will focus on structural aspects of WRN and BLM, which are an exciting area of current RecQ research. In particular, structures and functions of the RecQ C-terminal (RQC) and helicase-and-ribonuclease D-C-terminal (HRDC) domains of WRN and BLM are discussed. These two domains are conserved in tandem on the C-terminal side of each protein's ATPase domain, but also display several divergent features; the sequence identity within the ATPase domain of WRN and BLM is ∼30%, while the identities within the RQC and HRDC domains are ∼10 and ∼20%, respectively. An understanding of the structural and functional differences between these domains may yield insights into the onset of the two distinct diseases.

Furthermore, I present a novel docking simulation of BLM with a Holliday junction (HJ), using the recently determined crystal structure of a BLM-DNA complex (Swan et al., 2014). The model offers explanations for the efficient branch migration activities of BLM and also of WRN.

DOMAINS OF WRN AND BLM

Domain diagrams of WRN and BLM are shown in **Figure 1**. WRN and BLM are multi-domain helicases composed of 1,432 and 1,417 amino acids (a.a.), respectively. The two proteins share the structured ATPase, RQC, and HRDC domains, while an exonuclease domain is present only at the N terminus of WRN. Previous crystal structures of the exonuclease domain from human (Perry et al., 2006) and mouse (Choi et al., 2007) WRNs in the absence of DNA suggested a nuclease mechanism mediated by two metal ions, although the *in vivo* role of this domain is still unknown.

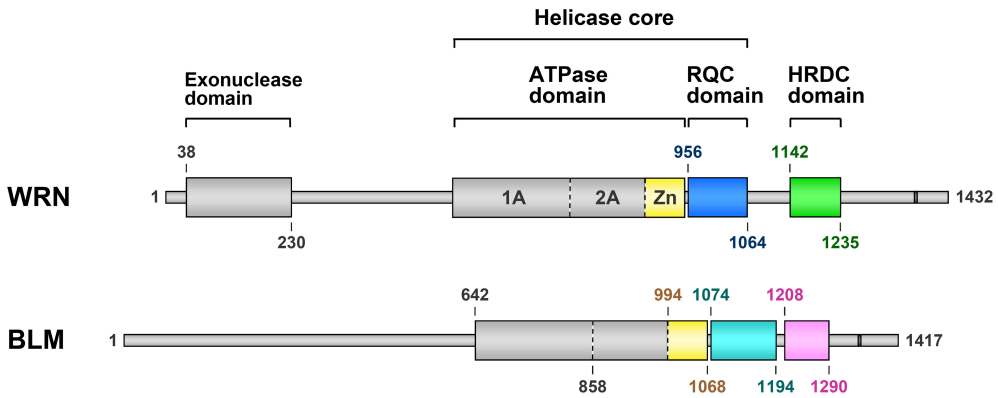


FIGURE 1 | Domain diagrams of human WRN and BLM. Werner syndrome protein and Bloom syndrome protein share three structurally folded domains comprising an ATPase domain, an RQC domain (colored blue in WRN and cyan in BLM) and an HRDC domain (green and pink, respectively). The Zn subdomain (yellow) is located at the C-terminal end of the ATPase domain. Nuclear localization signals of WRN (Matsumoto et al., 1997) and BLM (Kaneko et al., 1997) are depicted as dark gray bars. The domain boundaries (a.a. numbers) were determined from the available 3D structures of WRN (Hu et al., 2005; Perry et al., 2006; Kitano et al., 2007, 2010) and BLM (Kim and Choi, 2010; Sato et al., 2010; Kim et al., 2013; Swan et al., 2014).

RQC DOMAIN

WRN RQC

The RQC domain, which is tethered to the zinc-binding subdomain (Zn) of the ATPase domain with a short linker, is unique to the RecQ family of proteins. This region folds into a winged-helix motif, a subset of the helix-turn-helix superfamily (Hu et al., 2005; Kitano et al., 2010; Kim et al., 2013; Swan et al., 2014). Helix-turn-helix motifs including the winged helix are known as major double-stranded (ds) DNA-binding domains and are found in many nuclear proteins (Gajiwala and Burley, 2000; Harami et al., 2013).

Figure 2 shows the co-crystal structure of the WRN RQC domain bound to a DNA duplex (Kitano et al., 2010), whose determination in 2010 represented the first example of a RecQ-DNA complex. The structure revealed two unexpected features of the RQC domain. First, the RQC domain binds duplex DNA in a novel DNA-interaction mode that differs from all known examples of winged-helix and other helix-turn-helix proteins. The recognition helix, a principal component of helix-turn-helix motifs that are usually embedded within DNA grooves, was unprecedentedly excluded from the interaction. Second, the structure successfully captured a DNA-unwinding event by the RQC domain. The RQC

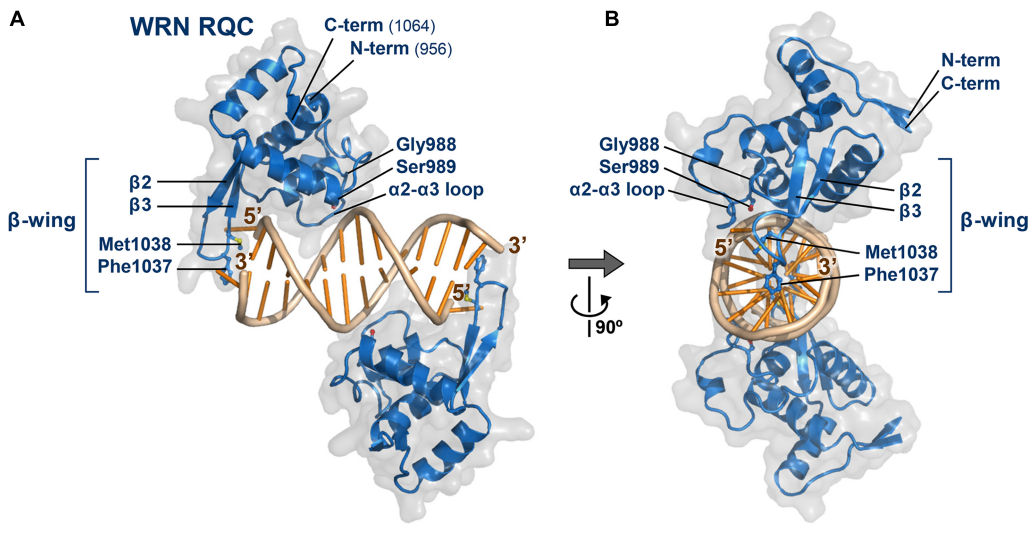


FIGURE 2 | Structure of the WRN RQC domain bound to dsDNA. **(A)** Crystal structure of WRN RQC bound to the 14-base-pair duplex (PDB ID: 3AAF; Kitano et al., 2010). The two RQC monomers (blue) bind to each DNA blunt terminus and unpair the terminal base pairs. The molecular surfaces of each domain are shown in transparent gray. Secondary-structure elements are labeled, and side chains of the key interacting amino acids are shown as stick models. The unpaired 5′-nucleotide is held tightly by RQC to prevent its reannealing, whereas the 3′-nucleotide is mostly disordered. All figures displaying 3D structures within this paper were prepared using PyMOL (DeLano Scientific). **(B)** View following 90° rotation along the y-axis.

domain specifically interacted with a blunt end of the DNA duplex and, in the absence of any other domain, unpaired a Watson–Crick base pair using the prominent hairpin structure β2–β3, which corresponds to the so-called β-wing of the winged-helix fold.

BLM RQC

Last year, the crystal structure of the BLM RQC domain bound to a phosphate ion (**Figure 3A**) was determined (Kim et al., 2013), and, subsequently, the co-crystal structure of a BLM large fragment (a.a. 640–1291) in complex with a 3′-overhang DNA duplex (**Figures 3B,C**) was determined (Swan et al., 2014). The latter structure includes all of the ATPase, RQC, and HRDC domains, but interactions with the duplex region of the DNA were concentrated on the RQC domain surface; the BLM RQC domain binds to the dsDNA terminus in the same binding mode as had been observed with the truncated WRN RQC domain (Kitano et al., 2010).

The structure of the BLM RQC domain, however, includes three distinct features (**Figure 3A**). First, aromatic and non-polar residues at the tip of the β-wing, key elements that WRN uses for DNA strand separation, are each replaced by polar and acidic residues in BLM. A detailed discussion of this feature is given below. Second, a BLM-specific 14-a.a. insertion (referred to as the BLM insertion) between the N-terminal helices exhibits a looping-out structure that extends at right angles to the β-wing. Third, the C-terminal residues of BLM RQC adopt a novel extended structure (referred to as the C-term extended loop) by being tightly packed against the domain core. These unique structures in BLM RQC may be associated with the preferential activity of BLM toward HJs (Kim et al., 2013).

RQC IS AN UNCONVENTIONAL WINGED-HELIX DOMAIN

Figure 4 shows a comparison of the DNA-binding modes of the WRN (A) and BLM (B) RQC domains with those of the conventional winged-helix domains of the transcription factors ETS (Kodandapani et al., 1996; C) and RFX1 (Gajiwala et al., 2000; D). The conventional winged-helix domains all bind to DNA via principal contacts of a recognition helix (colored green in C,

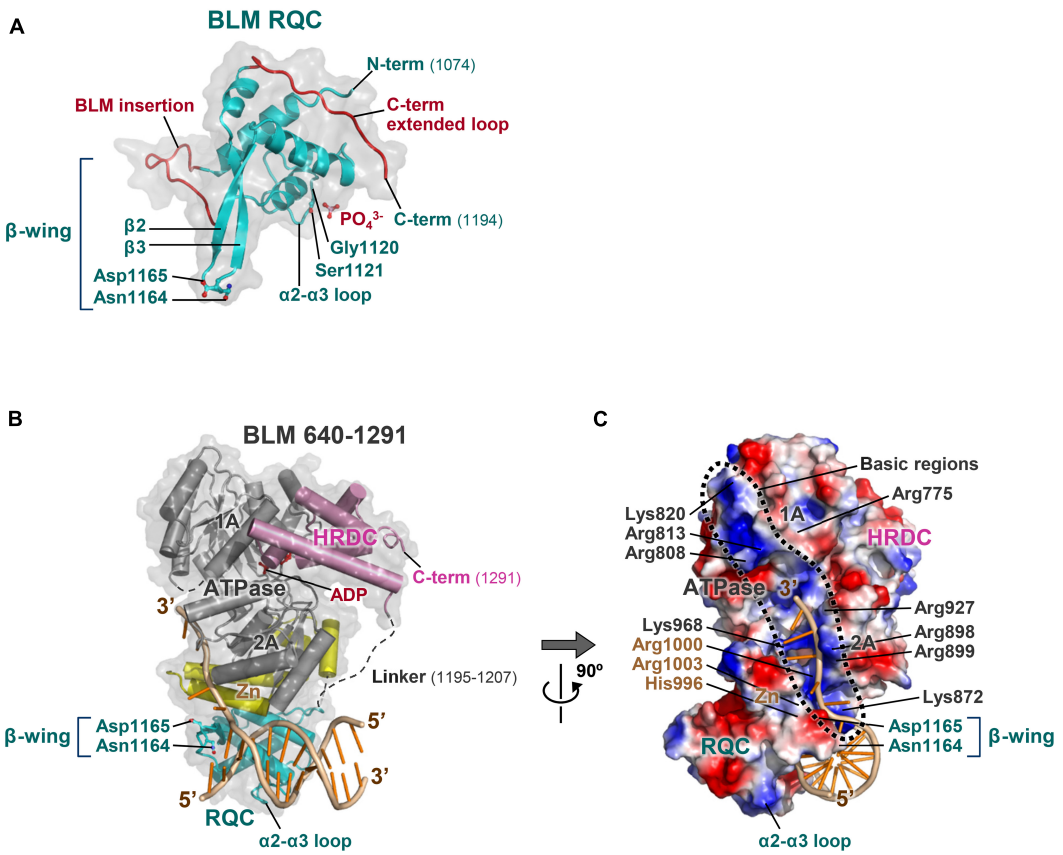


FIGURE 3 | Structures of the BLM RQC domain and of BLM 640–1291 bound to DNA. (A) Crystal structure of BLM RQC bound to a phosphate ion (PDB ID: 3WE2; Kim et al., 2013). Two BLM-specific loop regions, BLM-insertion (a.a. 1093–1106) and C-term extended loop (a.a. 1183–1194), are colored red. The phosphate ion forms a hydrogen bond with Ser1121, mimicking one of the phosphate groups in DNA substrates. **(B)** Crystal structure of BLM 640–1291 bound to a 3′-overhang duplex (PDB ID: 4O3M; Swan et al., 2014). The RQC and HRDC domains are in cyan and pink, respectively, while the Zn subdomain within the ATPase domain is in yellow. ADP (red) is bound to the inter-subdomain cleft between the ATPase subdomains 1A and 2A (gray). **(C)** Surface potential representation of BLM 640–1291 in a view following 90° rotation along the y-axis. The basic regions along the ATPase domain are encircled by a dashed line, and include a number of basic residues: Arg775, Arg808, Arg813, and Lys820 within subdomain 1A; Lys872, Arg898, Arg899, Arg927, and Lys968 within subdomain 2A; and His996, Arg1000, and Arg1003 within the Zn subdomain.

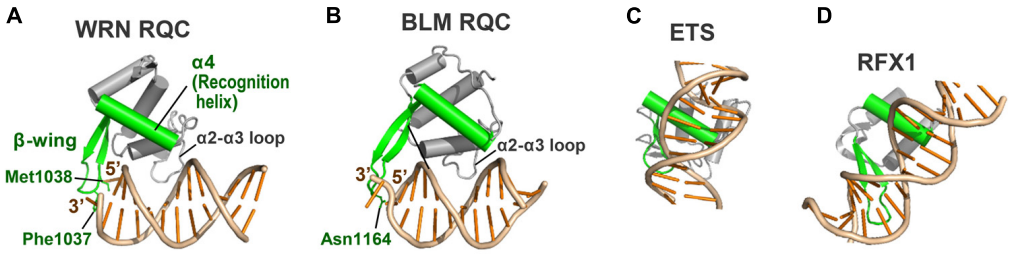


FIGURE 4 | Comparison of the RQC domains with conventional winged-helix domains. (A) WRN RQC domain (PDB ID: 3AAF; Kitano et al., 2010). The RQC domain binds the DNA duplex terminus via the α2–α3 loop and the β-wing (colored green), while the helix α4 (recognition helix; also green) is located apart from the DNA. **(B)** BLM RQC domain (PDB ID: 4O3M; Swan et al., 2014). The orientation and colors of the domain are comparable to those in **(A)**. **(C)** PU.1 ETS winged-helix domain (PDB ID: 1PUE; Kodandapani et al., 1996). The recognition helix and β-wing bind the major and minor grooves, respectively. **(D)** RFX1 winged-helix domain (PDB ID: 1DP7; Gajiwala et al., 2000). The recognition helix and β-wing bind the minor and major grooves, respectively.

D) deep in the major or minor groove of DNA. This arrangement facilitates sequence-specific DNA binding that can induce a bend in the DNA (Gajiwala and Burley, 2000; Harami et al., 2013).

In contrast, the recognition helix (α4) of WRN RQC (A) and BLM RQC (B) is located more than 4 Å away from the bound DNA and is not involved in the direct interaction with DNA. Instead, the positively charged loop between helices α2 and α3 (the α2–α3 loop) serves as the prominent DNA binding site by interfacing with the major groove of the DNA, and the β-wing (also green) exhibits a unique interaction with the terminus of the duplex. Considering that the fundamental role of the recognition helix is to promote sequence-specific DNA recognition, its exceptional lack of use in RQC seems essential for realizing sequence-independent helicase reactions (Kitano et al., 2010). The RQC domain, unlike the conventional winged-helix domains, does not form a hydrogen bond with the bases or induce a bend in the duplex.

The protruding β-wing within the RQC domain is also essential for WRN and BLM to prevent non-specific binding to DNA, since the β-wing exhibits steric hindrance with linear paired bases (Kitano et al., 2010). Due to this conflict effect, the proteins can bind only to branched sites that contain a terminus of the duplex, a structural explanation for the DNA structure-specific activities of the RecQ family. Electron microscopic analyses of full-length WRN (Compton et al., 2008) and BLM (Huber et al., 2006) also showed that the two proteins do not bind DNA in the interior of the linear B-form conformation.

The α2–α3 loop of the RQC domains plays a major role in the interaction with DNA. On this loop, a conserved serine of WRN (Ser989 in **Figure 2**) and BLM (Ser1121 in **Figure 3A**; the phosphate ion mimics one of the DNA phosphates) forms a hydrogen bond with a backbone phosphate of the DNAs (Kitano et al., 2010; Kim et al., 2013; Swan et al., 2014). Single mutation of these serines disturbs the DNA-binding ability of WRN RQC (Kitano et al., 2010) and BLM RQC (Kim et al., 2013), showing their common importance for DNA interaction. Another key residue on this loop is a glycine (WRN Gly988 and BLM Gly1120), which is adjacent to the serine and important to provide the α2–α3 loop with the flexibility required for DNA interaction (Kitano et al., 2010). Single mutation of Gly1120 in BLM also causes partial loss-of-function of the full-length protein (Mirzaei and Schmidt, 2012).

THE β-WING, A HAIRPIN SCALPEL FOR DNA STRAND SEPARATION

The β-wing of the RQC domain extends from the edge of the domain surface and, during the helicase catalytic reactions, acts as a scalpel for splitting a DNA duplex. **Figure 5A** shows a schematic depiction of the WRN β-wing interaction with the last paired bases of the partially unwound DNA duplex (Kitano et al., 2010). The aromatic (Phe1037) and non-polar (Met1038) residues at the hairpin tip cut into the stacked bases from the duplex terminus, resulting in a loss of base–base stacking and the separation of both strands.

On the other hand, the β-wing of BLM (**Figure 5B**) is capped by polar (Asn1164) and acidic (Asp1165) residues (Kim et al., 2013; Swan et al., 2014). Asn1164 (the counterpart of WRN Phe1037) also functions to wedge apart the DNA strands by interacting with the last paired base at the 3′ terminus. The importance of both WRN Phe1037 and BLM Asn1164 in DNA-unwinding reactions was confirmed by mutagenesis helicase assays (Tadokoro et al.,

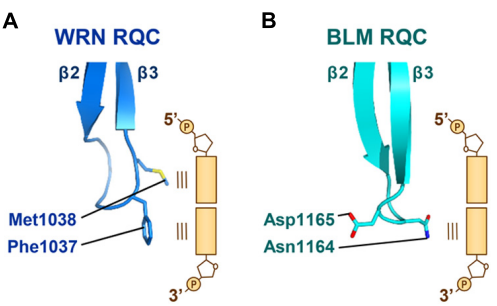


FIGURE 5 | Interactions of the WRN and BLM β-wings with DNA. (A) The β-wing of WRN RQC (PDB ID: 3AAF; Kitano et al., 2010) is capped by aromatic (Phe1037) and non-polar (Met1038) residues, which stack onto the last paired bases at the 3′ and 5′ termini, respectively. **(B)** The β-wing of BLM RQC (PDB ID: 4O3M; Swan et al., 2014) is capped by polar (Asn1164) and acidic (Asp1165) residues. Asn1164 interacts with the last paired base at the 3′ terminus, while Asp1165 faces in the other direction.

2012; Swan et al., 2014). In contrast, the acidic side chain of BLM Asp1165 (the counterpart of WRN Met1038) does not interact with the duplex but faces in the other direction. When binding to multi-stranded DNAs like a HJ, such electronegativity at the tip of the β-wing may result in an electrostatic repulsion against the neighboring DNA strands. In agreement with this idea, the DNA-binding activity of purified BLM is weaker than that of WRN (Kamath-Loeb et al., 2012; Kim et al., 2013). The electrostatic repulsive power between the acidic BLM β-wing and DNA strands, as discussed below, may be adapted for use in catalyzing the branch migration of HJs.

HRDC DOMAIN

WRN HRDC AND BLM HRDC

In contrast to the accumulated knowledge pertaining to the unique functions of the RQC domain, the function of the C-terminal HRDC domain remains unclear. In human RecQs, only WRN and BLM possess the HRDC domain, whereas the other three members (RECQ1, RECQ4, and RECQ5) completely lack HRDC sequences (Larsen and Hickson, 2013; Croteau et al., 2014). The existing data concerning WRN (Lee et al., 2005) and BLM (Wu et al., 2005) suggest that the HRDC domain is not essential for conventional helicase activity on forked duplexes.

Figure 6 shows the crystal structure of the WRN HRDC domain (A, B; Kitano et al., 2007) and the NMR structure of the BLM HRDC domain (C, D; Kim and Choi, 2010; Sato et al., 2010). The two HRDC domains fold into a common globular bundle of five α-helices and one 3_{10}-helix connected by short loop regions. However, the amino acids located on the domain surfaces are poorly conserved, yielding distinct surface properties for each protein. For example, the WRN HRDC domain surface retains both acidic and basic regions (B), whereas the BLM HRDC surface is largely electronegative with many acidic residues exposed to the solvent (D). The isoelectric point (pI) of BLM HRDC (5.1) is also much lower than that of WRN HRDC (8.1; Sato et al., 2010).

The distinct charge distributions of the two HRDC domains suggest different roles for the domain in each protein. As its name implies, the HRDC domain was originally found in several bacterial DNA helicases such as PcrA (Subramanya et al., 1996; Velankar et al., 1999) and Rep (Korolev et al., 1997), in addition to the RNase D family of nucleases (Zuo et al., 2005). Consequently, interest in the HRDC domains has focused on their DNA-binding ability (Morozov et al., 1997). The isolated HRDC domain of Sgs1 (the yeast ortholog of BLM) was shown to bind DNA weakly using the electropositive surface area of the domain (Liu et al.,

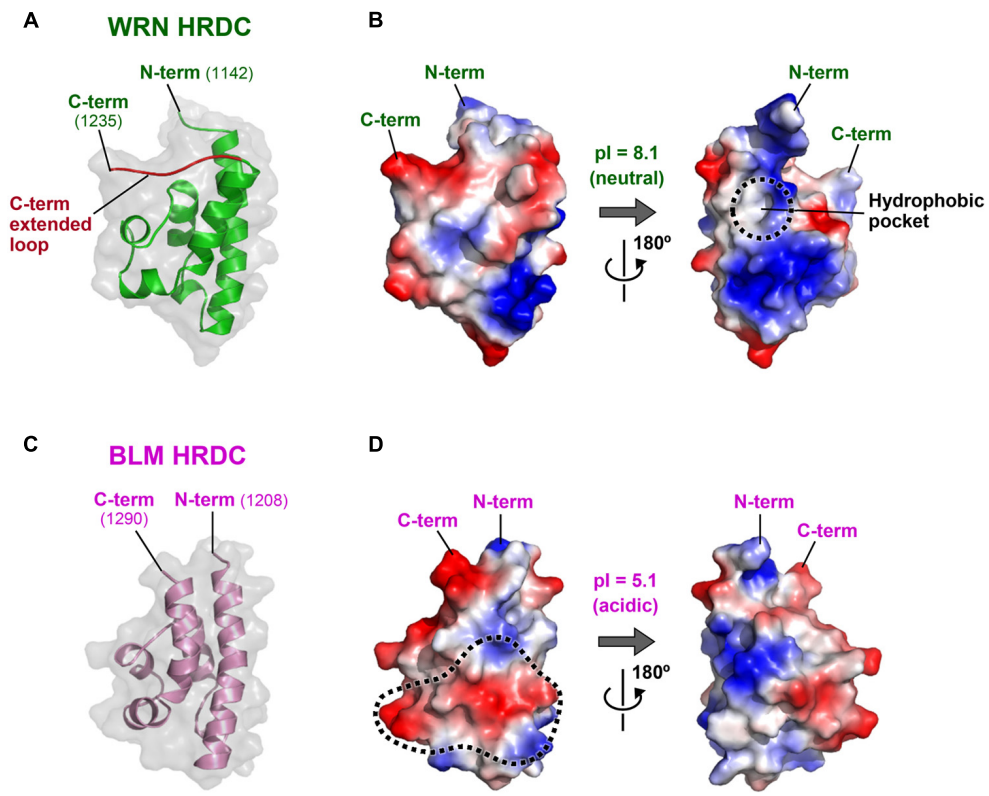


FIGURE 6 | Structures of the WRN and BLM HRDC domains. (A) Crystal structure of WRN HRDC (PDB ID: 2E1E; Kitano et al., 2007). The WRN-specific C-term extended loop (a.a. 1227–1235) is colored red. **(B)** Surface potential of WRN HRDC. Front (left) and back (right) views. A WRN-specific hydrophobic pocket at the back surface is encircled by a dashed line. **(C)** NMR structure of BLM HRDC (PDB ID: 2RRD; Sato et al., 2010). The molecule is viewed in the same orientation as in **(A)**. **(D)** Surface potential of BLM HRDC. A front (left) surface area corresponding to the proposed DNA interaction area of Sgs1 (Liu et al., 1999) is encircled by a dashed line.

1999). However, both the WRN and BLM HRDC structures show that the proposed DNA-binding surface area of Sgs1 is not conserved (Kitano et al., 2007; Sato et al., 2010). The corresponding region of BLM HRDC (encircled by a dashed line in **Figure 6D**) is highly electronegative, and is therefore unlikely to be involved in direct DNA interaction. Consistent with these observations, neither of the purified WRN (Kitano et al., 2007) nor BLM HRDCs (Sato et al., 2010; Kim et al., 2013) exhibit detectable DNA-binding ability *in vitro*.

POSSIBLE FUNCTION OF WRN HRDC

As depicted in **Figure 1**, the HRDC domains of WRN and BLM are connected to their adjacent RQC domains by different lengths of linker. The linker of WRN comprises 77 residues (a.a. 1065–1141), which is six times longer than that of BLM (13 residues; a.a. 1195–1207). The long linker region of WRN is probably unstructured (Kitano et al., 2007), which may contribute to the spatial separation of the HRDC domain from the RQC domain. Such isolation may be advantageous for interactions with other proteins. For example, the WRN HRDC domain may interact with other protein partner(s) that recognize DNA double-strand breaks (DSBs), thus facilitating indirect recruitment of WRN to the site for repair (Lan et al., 2005; Kitano et al., 2007).

The WRN HRDC domain also possesses a C-term extended loop (colored red in **Figure 6A**) that is tightly packed against the upper surface of the folding core. This extra structure increases the surface area of WRN HRDC, and assists in the formation of WRN-specific structures such as a hydrophobic pocket at the back of the domain (encircled by a dashed line in **Figure 6B**). This hydrophobic pocket is a candidate for interaction with other proteins (Kitano et al., 2007).

ATPase DOMAIN

The ATPase domain is the largest and most highly conserved component of the RecQ family (**Figure 1**). The domain is constituted of two RecA-like subdomains (1A and 2A) that are characteristic of a wide variety of DNA and RNA helicases (Singleton et al., 2007). The ATPase domain is responsible for binding and hydrolysis of ATP (Vindigni et al., 2010; Swan et al., 2014). The C-terminal portion of the ATPase domain also includes the Zn subdomain (colored yellow), which is unique to the RecQ family and is often combined with the RQC domain sequence. Crystal structures of bacterial (Bernstein et al., 2003) and human (Pike et al., 2009; Swan et al., 2014) RecQs showed that the Zn subdomain is tightly packed against subdomain 2A of the ATPase domain, and is thus structurally a part of the ATPase domain.

By analogy with other helicases (Singleton et al., 2007), the ATPase domain of the RecQ family was originally referred to as a "helicase domain." However, this designation often caused misunderstanding, since the domain itself does not possess helicase activities; the isolated ATPase domains of WRN (Von Kobbe et al., 2003; Lee et al., 2005) and BLM (Janscak et al., 2003) do not exhibit efficient DNA-binding or unwinding activity. As described above, the RQC domain, not the ATPase domain, constitutes the primary DNA-binding site in members of the RecQ family and also catalyzes the direct unpairing of DNA duplexes (Kitano et al., 2010; Swan et al., 2014).

The ATPase domains of WRN and BLM, therefore, function simply as an ATP-dependent DNA translocation module that supplies a driving force for the helicase reactions. The ATPase and RQC domains are both required for the processive helicase reactions, combining to form a "helicase core" in the RecQ family.

BLM-HJ BINDING MODEL

DISSOLUTION OF DOUBLE HJ BY BLM AND TOPOISOMERASE IIIα

Unlike most other helicases, WRN and BLM (and some other RecQ members) preferentially act on DNA structures that resemble recombination and repair intermediates (Larsen and Hickson, 2013; Croteau et al., 2014). Such structure-specific activities of RecQs account for many of their key functions in DNA metabolic pathways. For example, BLM acts in concert with topoisomerase IIIα to resolve the DSB repair intermediate double HJ, producing exclusively non-crossover products (Wu and Hickson, 2003; Wu et al., 2005; Plank et al., 2006; Seki et al., 2006). This process, referred to as double HJ dissolution, is crucial for suppressing the sister-chromatid exchanges that cause early neoplastic transformation of cells (Larsen and Hickson, 2013; Manthei and Keck, 2013). In double HJ dissolution, a branch migration activity of BLM (Karow et al., 2000) is used to efficiently bring two HJs toward each other, until they form a hemicatenane intermediate that can be decatenated by topoisomerase IIIα.

In **Figures 7A,B,** I present a new binding model of a BLM dimer with a HJ. This model has been constructed in silico by superimposing the BLM 640–1291 structure (Swan et al., 2014) onto our previous docking simulation of the WRN RQC-HJ complex (Kitano et al., 2010). The BLM 640–1291 structure was used without modification, while the north and south arms of the HJ (the vertical duplexes in **Figure 7A**) were manually tilted by 21° rotation from their ideal vertical positions, to avoid steric conflicts with the two ATPase domains. The obtained model shows that the two molecules of BLM can simultaneously bind to the single HJ with no significant steric hindrance to each other. Each RQC domain binds the east or west (the horizontal) arm of the HJ with twofold symmetry, while the ATPase domains interact with the north and south arms. Binding of a BLM dimer to a single HJ is also suggested by a recent single-molecule visualization study (Gyimesi et al., 2013).

In the electrostatic surface potentials of BLM 640–1291 (**Figure 3C**), a line of basic regions (encircled by a dashed line) that traverses subdomains 1A, 2A, and Zn of the ATPase domain is observed. The lower part of these regions binds to the 3′-overhang ssDNA, suggesting a role for these regions as a DNA translocation route for the helicase reactions. In the present BLM-HJ model (**Figure 7A**), the basic regions in each ATPase domain run precisely in parallel with the north or south duplexes, representing the best operational arrangement for the translocations of the two arms.

PROPOSED BRANCH MIGRATION MECHANISM

The BLM-HJ model (**Figures 7A,B**) offers several plausible explanations for the branch migration reactions undertaken by BLM.

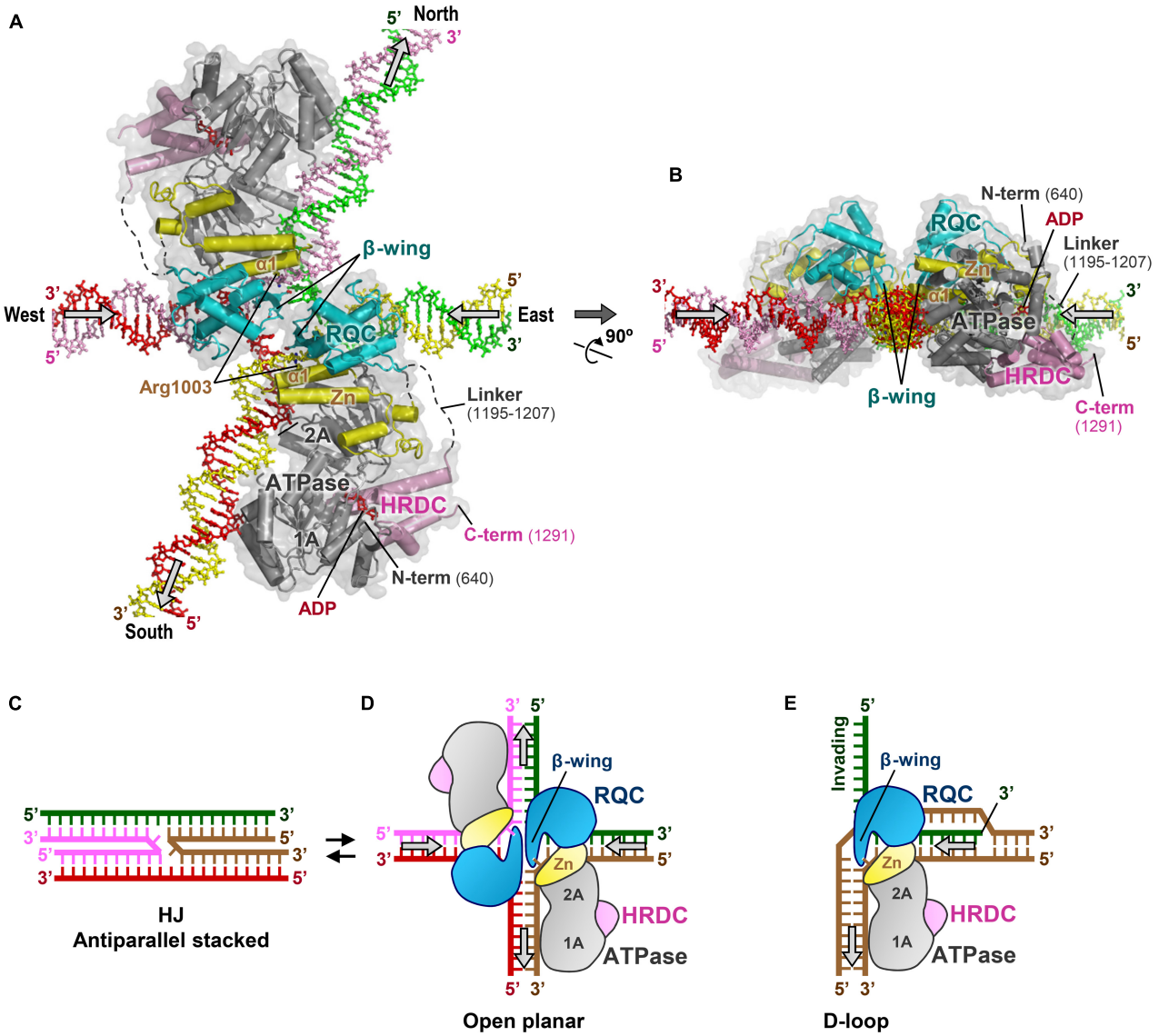


FIGURE 7 | Binding model of BLMs to HJ. (A) 3D docking simulation of a BLM dimer to a HJ. BLMs are in the same colors as in **Figure 3B**. The arrows on the HJ arms represent the directions of DNA translocations required for branch migration. This model was constructed in silico by the following procedures: first, the structure of BLM 640–1291 (PDB ID: 4O3M; Swan et al., 2014) was superimposed onto our previous binding model of the WRN RQC-HJ complex (Kitano et al., 2010) using the RQC domain and DNA duplex region as references. The north–south arms of the HJ were then manually tilted by 21° on the same plane using Coot (Emsley et al., 2010), so that the ATPase subdomains 1A and 2A can simultaneously interact with the duplexes without steric conflicts. The present interactions between the RQC domains and the HJ are consistent with a previous mutagenesis binding assay of BLM RQC with HJ (Kim et al., 2013). **(B)** View after 90° rotation along the x-axis. **(C)** Schematic representation of a HJ in the antiparallel stacked conformation. **(D)** Schematic representation of **(A)**, with the HJ in the ideal open-planar conformation. **(E)** Schematic representation depicting release of the 3′-invading strand (green) by WRN or BLM (Kitano et al., 2010).

First, co-insertion of the two acidic β-wings into a small hole of the HJ enables the BLMs to catalyze simultaneous unpairing of the east and west arms of the HJ. This is reminiscent of the acidic hairpin of bacterial RuvA, a protein that also catalyzes branch migration of HJs (Ingleston et al., 2000; Yamada et al., 2004). By analogy with the proposed mechanism for RuvA, negative charges on the BLM β-wings may serve to repel the DNA backbones from the junction center and/or to repel the other β-wing within the same hole by electrostatic repulsion. The resultant mechanical force to enlarge the hole could result in the disruption of base pairs near the crossover point, thereby enhancing strand-exchange reactions.

Second, nucleotides that are newly unpaired by the RQC domains may be translocated under guidance of the Zn subdomain, which abuts the ATPase subdomain 2A. In the present model, the first helix, α1 (a.a. 995–1007), of each Zn subdomain is extended in parallel with the yellow or pink strand at the intersection, exposing the basic residue Arg1003 toward the unpaired

3′-nucleotides. This residue probably forms a salt bridge with the DNA backbone phosphate. Therefore, the two Zn subdomains located around the central hole seem likely to act as a guiderail for the unpaired 3′-nucleotides to move smoothly into the north-south duplexes, helping them to rapidly anneal with new partners. At the same time, the Zn subdomain would also act as a joint to adjust the relative orientation of the ATPase domain against the RQC domain.

Third, the two ATPase domains would pull the north and south duplexes in opposite directions, by the conventional inch-worm mechanism (Singleton et al., 2007). Hydrolysis of an ATP at the interface of the ATPase subdomains 1A-2A would result in a rigid-body movement of subdomain 1A, a conformational change of the ATPase domain as a motor. The resultant translocations of the north–south duplexes by the ATPase domains would in turn allow each RQC domain to melt the next base pairs of the east–west duplexes, thereby driving the branch migration reactions.

In summary, although a genuine BLM-HJ co-crystal structure is still lacking, the proposed binding model yields a possible HJ branch migration mechanism for BLM, in which DNA unwinding by the RQC domains and DNA annealing/translocation by the ATPase domains are effectively coordinated.

SEPARATION OF UNPAIRING MODULE FROM TRANSLOCATION MODULE

Many other helicases such as bacterial UvrD (Lee and Yang, 2006; **Figure 8B**) and archaeal Hel308 (Buttner et al., 2007; **Figure 8C**) also possess a conserved β-hairpin to act as an unwinding element. However, these hairpins are located directly within the ATPase domains (i.e., helicase domains) and cannot be inserted into the narrow hole of the HJ, since severe steric conflicts would occur between their ATPase domains and DNA strands (Kitano et al., 2010). This is the reason why PcrA (a homolog of UvrD) cannot efficiently promote migration of HJs (Constantinou et al., 2000; Karow et al., 2000).

In contrast, WRN and BLM prepare the unwinding element in the compact RQC domain (**Figure 8A**). For the RecQ family, separation of the unwinding module (the RQC domain) from the translocation module (the ATPase domain) is likely to be crucial to process multi-stranded DNAs such as the HJs.

POSSIBLE FUNCTION OF BLM HRDC

The co-crystal structure of the BLM-DNA complex (**Figure 3B**) also showed that the BLM HRDC domain is situated ∼28 Å apart from the DNA substrate and binds to the ATPase domain within the same molecule (Swan et al., 2014). The domain-domain interface includes an ATP-binding cleft formed between the ATPase subdomains 1A and 2A. Therefore, the BLM HRDC domain may somehow be associated with the hydrolysis of ATP within the ATPase domain, although the detailed mechanism of action is not known.

On the other hand, the HJ is known to adopt a dynamic structure between antiparallel stacked (**Figure 7C**) and open planar conformations (**Figure 7D**; Liu and West, 2004). The former is favored in the presence of divalent cations such as Mg^{2+}, but is inhibitory to branch migration reactions due to its closed structure, stabilized by strong van der Waals contacts and hydrogen bonds (Ortiz-Lombardia et al., 1999; Eichman et al., 2000). Therefore, proteins that promote branch migration must first open the four arms, as does RuvA, which binds exclusively to the open planar conformation of the HJ (Ingleston et al., 2000; Yamada et al., 2004).

In the current BLM-HJ model (**Figures 7A,B**), the two HRDC domains are located in the empty spaces between the north–west and east–south arms of the HJ. Considering the electronegativity of the BLM HRDC domain surface, the HRDC domain may

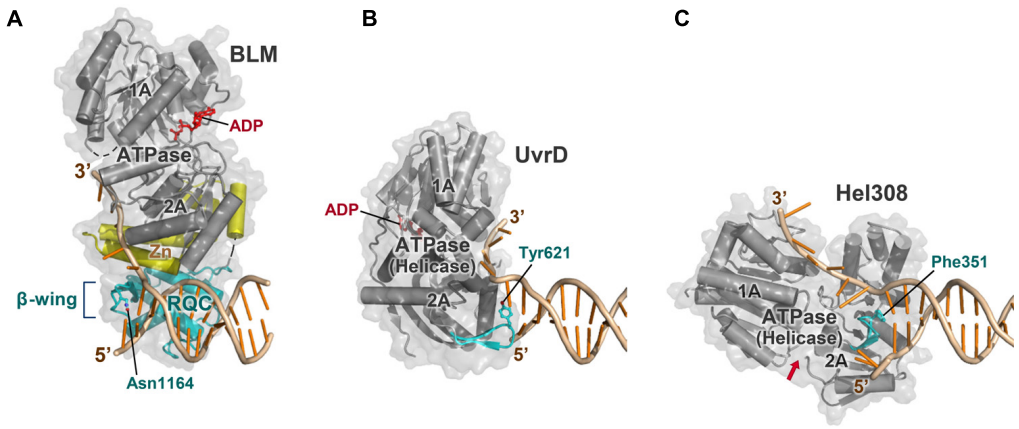


FIGURE 8 | Comparison of BLM with other helicases. (A) BLM 640–1291 bound to a 3′-overhang duplex (PDB ID: 4O3M; Swan et al., 2014). The strand-separating element (β-wing) is within the RQC domain (colored cyan), which is bridged to the ATPase domain (gray) by the Zn subdomain (yellow). The complex is viewed in the same orientation as in **Figure 3B** except for the omission of the HRDC structure; in **(A–C)**, other portions of the enzyme outside the helicase core are omitted for clarity. **(B)** ATPase (helicase) domain of *E. coli* UvrD bound to a 3′-overhang duplex (PDB ID: 2IS6; Lee and Yang, 2006). The strand-separating element (β-hairpin; cyan) is included within the ATPase subdomain 2A. The structure is comparable to that of PcrA (Velankar et al., 1999). **(C)** ATPase (helicase) domain of archaeal Hel308 bound to a 3′-overhang duplex (PDB ID: 2P6R; Buttner et al., 2007). The strand-separating element (β-hairpin) is also within the ATPase subdomain 2A. The ATP-binding cleft is indicated by a red arrow. In **(A–C)**, Asn1164 (BLM), Tyr621 (UvrD), and Phe351 (Hel308), respectively, stack onto the last paired base at the 3′ terminus.

act as a wedge to open the HJ arms by electrostatic repulsion (Kim et al., 2013).

OTHER DNA SUBSTRATES

In addition to the established importance of BLM in double HJ dissolution, BLM may also be required for other genomic events such as segregation of sister chromatids in mitosis (Chan et al., 2007; Larsen and Hickson, 2013; Manthei and Keck, 2013) as well as the recombination process in meiosis (De Muyt et al., 2012; Zakharyevich et al., 2012). Although the precise *in vivo* substrates of BLM in these pathways remain to be elucidated, a mechanism similar to the branch migration function of BLM in resolving multi-stranded DNAs may be utilized.

WRN can also catalyze branch migration of HJs *in vitro* (Constantinou et al., 2000), but fails to substitute for BLM in double HJ dissolution reactions (Wu et al., 2005). Alternatively, WRN may be involved in another DSB repair pathway such as non-homologous end-joining (Lan et al., 2005; Yan et al., 2005) by interacting with its key protein Ku70/80 (Croteau et al., 2014). Furthermore, WRN also plays a role in protecting chromosome ends by interacting with telomere maintenance proteins like TRF2 and POT1 (Croteau et al., 2014). **Figure 7E** shows a schematic view of the displacement loop (D-loop) bound by WRN (or BLM). Since the structure of the D-loop is comparable to that of the right half of the HJ (Kitano et al., 2010), it is tempting to speculate that WRN catalyzes the dissociation of telomeric D-loops in the replication and recombination processes (Opresko et al., 2004; Brosh, 2013).

WRN and BLM are additionally capable of unwinding non-Watson-Crick/Hoogsteen base pairs such as G-quadruplex (G4) DNA (Larsen and Hickson, 2013; Croteau et al., 2014). The G4-unwinding activity of WRN and BLM may be important for the efficient replication of telomeres (Brosh, 2013) as well as for the regulation of gene expression (Johnson et al., 2010; Nguyen et al., 2014). The purified RQC domain of BLM binds to G4 DNA with high affinity (Huber et al., 2006). Future structural studies of complexes with G4 DNA should reveal the mechanism by which WRN and BLM unwind such abnormal DNA structures.

CONCLUDING REMARKS

Recent advances in the structural studies of WRN and BLM, in particular the discovery of the "DNA zip-slider" function of the RQC domain to catalyze strand separation, have greatly improved our understanding of WRN and BLM in terms of their preferential activities toward recombination and repair intermediates. In this paper, I have focused on the structures of WRN and BLM, but it should be mentioned that other important RecQ structures that could not be discussed here are also available, including those of *Escherichia coli* RecQ (Bernstein et al., 2003; Bernstein and Keck, 2005), *Deinococcus radiodurans* RecQ (Killoran and Keck, 2008; Liu et al., 2013) and human RECQ1 (a protein that is not associated with genetic disease; Pike et al., 2009). The structure of *E. coli* RecQ without DNA (Bernstein et al., 2003) gave us the first structural image of the RecQ-family helicase core, although recent data imply that the β-wing of bacterial RecQs is not involved in DNA unwinding (Pike et al., 2009; Hoadley and Keck, 2010). The β-wing of RECQ1 (Pike et al., 2009), in contrast, probably functions in a manner similar to that of WRN. Reviews on these structures are available elsewhere (Killoran and Keck, 2006; Vindigni et al., 2010).

Besides their biological importance in the prevention of tumorigenesis and accelerated aging, WRN and BLM are also new targets for cancer chemotherapy (Futami et al., 2007; Opresko et al., 2007; Arai et al., 2011; Moser et al., 2012; Brosh, 2013). Recent high-throughput screens of chemical compound libraries identified two compounds, NSC19630 (Aggarwal et al., 2011) and ML216 (Nguyen et al., 2013), as specific inhibitors of WRN and BLM, respectively. Although the mechanism by which NSC19630 interferes with WRN function is unknown, ML216 was shown to inhibit the helicase activity of BLM 636–1298, a fragment similar to that used in the structure determination (Swan et al., 2014), by competing with its DNA-binding activity (Nguyen et al., 2013). Future co-crystallizations of WRN and BLM with these inhibitors may lead us to novel drug design strategies targeting the RecQ family of proteins.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid to Ken Kitano from the Inamori Foundation and the JSPS KAKENHI grant number 25440024. I am grateful to I. Smith for his suggestions with manuscript preparation.

REFERENCES

Aggarwal, M., Sommers, J. A., Shoemaker, R. H., and Brosh, R. M. Jr. (2011). Inhibition of helicase activity by a small molecule impairs Werner syndrome helicase (WRN) function in the cellular response to DNA damage or replication stress. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1525–1530. doi: 10.1073/pnas.1006423108

Arai, A., Chano, T., Futami, K., Furuichi, Y., Ikebuchi, K., Inui, T., et al. (2011). RECQL1 and WRN proteins are potential therapeutic targets in head and neck squamous cell carcinoma. *Cancer Res.* 71, 4598–4607. doi: 10.1158/0008-5472.CAN-11-0320

Bernstein, D. A., and Keck, J. L. (2005). Conferring substrate specificity to DNA helicases: role of the RecQ HRDC domain. *Structure* 13, 1173–1182. doi: 10.1016/j.str.2005.04.018

Bernstein, D. A., Zittel, M. C., and Keck, J. L. (2003). High-resolution structure of the *E. coli* RecQ helicase catalytic core. *EMBO J.* 22, 4910–4921. doi: 10.1093/emboj/cdg500

Brosh, R. M. Jr. (2013). DNA helicases involved in DNA repair and their roles in cancer. *Nat. Rev. Cancer* 13, 542–558. doi: 10.1038/nrc3560

Buttner, K., Nehring, S., and Hopfner, K. P. (2007). Structural basis for DNA duplex separation by a superfamily-2 helicase. *Nat. Struct. Mol. Biol.* 14, 647–652. doi: 10.1038/nsmb1246

Chan, K. L., North, P. S., and Hickson, I. D. (2007). BLM is required for faithful chromosome segregation and its localization defines a class of ultrafine anaphase bridges. *EMBO J.* 26, 3397–3409. doi: 10.1038/sj.emboj.7601777

Choi, J. M., Kang, S. Y., Bae, W. J., Jin, K. S., Ree, M., and Cho, Y. (2007). Probing the roles of active site residues in the 3′-5′ exonuclease of the Werner syndrome protein. *J. Biol. Chem.* 282, 9941–9951. doi: 10.1074/jbc.M609657200

Compton, S. A., Tolun, G., Kamath-Loeb, A. S., Loeb, L. A., and Griffith, J. D. (2008). The Werner syndrome protein binds replication fork and Holliday junction DNAs as an oligomer. *J. Biol. Chem.* 283, 24478–24483. doi: 10.1074/jbc.M803370200

Constantinou, A., Tarsounas, M., Karow, J. K., Brosh, R. M., Bohr, V. A., Hickson, I. D., et al. (2000). Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. *EMBO Rep.* 1, 80–84. doi: 10.1093/embo-reports/kvd004

Croteau, D. L., Popuri, V., Opresko, P. L., and Bohr, V. A. (2014). Human RecQ helicases in DNA repair, recombination, and replication. *Annu. Rev. Biochem.* 83, 519–552. doi: 10.1146/annurev-biochem-060713-035428

De Muyt, A., Jessop, L., Kolar, E., Sourirajan, A., Chen, J., Dayani, Y., et al. (2012). BLM helicase ortholog Sgs1 is a central regulator of meiotic recombination intermediate metabolism. *Mol. Cell* 46, 43–53. doi: 10.1016/j.molcel.2012.02.020

Eichman, B. F., Vargason, J. M., Mooers, B. H., and Ho, P. S. (2000). The Holliday junction in an inverted repeat DNA sequence: sequence effects on the structure of four-way junctions. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3971–3976. doi: 10.1073/pnas.97.8.3971

Emsley, P., Lohkamp, B., Scott, W. G., and Cowtan, K. (2010). Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* 66, 486–501. doi: 10.1107/S0907444910007493

Futami, K., Takagi, M., Shimamoto, A., Sugimoto, M., and Furuichi, Y. (2007). Increased chemotherapeutic activity of camptothecin in cancer cells by siRNA-induced silencing of WRN helicase. *Biol. Pharm. Bull.* 30, 1958–1961. doi: 10.1248/bpb.30.1958

Gajiwala, K. S., and Burley, S. K. (2000). Winged helix proteins. *Curr. Opin. Struct. Biol.* 10, 110–116. doi: 10.1016/S0959-440X(99)00057-3

Gajiwala, K. S., Chen, H., Cornille, F., Roques, B. P., Reith, W., Mach, B., et al. (2000). Structure of the winged-helix protein hRFX1 reveals a new mode of DNA binding. *Nature* 403, 916–921. doi: 10.1038/35002634

Goto, M. (2000). Werner's syndrome: from clinics to genetics. *Clin. Exp. Rheumatol.* 18, 760–766.

Goto, M., Ishikawa, Y., Sugimoto, M., and Furuichi, Y. (2013). Werner syndrome: a changing pattern of clinical manifestations in Japan (1917∼2008). *Biosci. Trends* 7, 13–22. doi: 10.5582/bst.2013.v7.1.13

Gyimesi, M., Pires, R. H., Billington, N., Sarlos, K., Kocsis, Z. S., Modos, K., et al. (2013). Visualization of human Bloom's syndrome helicase molecules bound to homologous recombination intermediates. *FASEB J.* 27, 4954–4964. doi: 10.1096/fj.13-234088

Harami, G. M., Gyimesi, M., and Kovacs, M. (2013). From keys to bulldozers: expanding roles for winged helix domains in nucleic-acid-binding proteins. *Trends Biochem. Sci.* 38, 364–371. doi: 10.1016/j.tibs.2013.04.006

Hoadley, K. A., and Keck, J. L. (2010). Werner helicase wings DNA binding. *Structure* 18, 149–151. doi: 10.1016/j.str.2010.01.007

Hu, J. S., Feng, H., Zeng, W., Lin, G. X., and Xi, X. G. (2005). Solution structure of a multifunctional DNA- and protein-binding motif of human Werner syndrome protein. *Proc. Natl. Acad. Sci. U.S.A.* 102, 18379–18384. doi: 10.1073/pnas.0509380102

Huber, M. D., Duquette, M. L., Shiels, J. C., and Maizels, N. (2006). A conserved G4 DNA binding domain in RecQ family helicases. *J. Mol. Biol.* 358, 1071–1080. doi: 10.1016/j.jmb.2006.01.077

Ingleston, S. M., Sharples, G. J., and Lloyd, R. G. (2000). The acidic pin of RuvA modulates Holliday junction binding and processing by the RuvABC resolvasome. *EMBO J.* 19, 6266–6274. doi: 10.1093/emboj/19.22.6266

Janscak, P., Garcia, P. L., Hamburger, F., Makuta, Y., Shiraishi, K., Imai, Y., et al. (2003). Characterization and mutational analysis of the RecQ core of the Bloom syndrome protein. *J. Mol. Biol.* 330, 29–42. doi: 10.1016/S0022-2836(03)00534-5

Johnson, J. E., Cao, K., Ryvkin, P., Wang, L. S., and Johnson, F. B. (2010). Altered gene expression in the Werner and Bloom syndromes is associated with sequences having G-quadruplex forming potential. *Nucleic Acids Res.* 38, 1114–1122. doi: 10.1093/nar/gkp1103

Kamath-Loeb, A., Loeb, L. A., and Fry, M. (2012). The Werner syndrome protein is distinguished from the Bloom syndrome protein by its capacity to tightly bind diverse DNA structures. *PLoS ONE* 7:e30189. doi: 10.1371/journal.pone.0030189

Kaneko, H., Orii, K. O., Matsui, E., Shimozawa, N., Fukao, T., Matsumoto, T., et al. (1997). BLM (the causative gene of Bloom syndrome) protein translocation into the nucleus by a nuclear localization signal. *Biochem. Biophys. Res. Commun.* 240, 348–353. doi: 10.1006/bbrc.1997.7648

Karow, J. K., Constantinou, A., Li, J. L., West, S. C., and Hickson, I. D. (2000). The Bloom's syndrome gene product promotes branch migration of Holliday junctions. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6504–6508. doi: 10.1073/pnas.100448097

Killoran, M. P., and Keck, J. L. (2006). Sit down, relax and unwind: structural insights into RecQ helicase mechanisms. *Nucleic Acids Res.* 34, 4098–4105. doi: 10.1093/nar/gkl538

Killoran, M. P., and Keck, J. L. (2008). Structure and function of the regulatory C-terminal HRDC domain from *Deinococcus radiodurans* RecQ. *Nucleic Acids Res.* 36, 3139–3149. doi: 10.1093/nar/gkn143

Kim, S. Y., Hakoshima, T., and Kitano, K. (2013). Structure of the RecQ C-terminal domain of human Bloom syndrome protein. *Sci. Rep.* 3, 3294. doi: 10.1038/srep03294

Kim, Y. M., and Choi, B. S. (2010). Structure and function of the regulatory HRDC domain from human Bloom syndrome protein. *Nucleic Acids Res.* 38, 7764–7777. doi: 10.1093/nar/gkq586

Kitano, K., Kim, S. Y., and Hakoshima, T. (2010). Structural basis for DNA strand separation by the unconventional winged-helix domain of RecQ helicase WRN. *Structure* 18, 177–187. doi: 10.1016/j.str.2009.12.011

Kitano, K., Yoshihara, N., and Hakoshima, T. (2007). Crystal structure of the HRDC domain of human Werner syndrome protein, WRN. *J. Biol. Chem.* 282, 2717–2728. doi: 10.1074/jbc.M610142200

Kodandapani, R., Pio, F., Ni, C. Z., Piccialli, G., Klemsz, M., Mckercher, S., et al. (1996). A new pattern for helix-turn-helix recognition revealed by the PU.1 ETS-domain-DNA complex. *Nature* 380, 456–460. doi: 10.1038/380456a0

Korolev, S., Hsieh, J., Gauss, G. H., Lohman, T. M., and Waksman, G. (1997). Major domain swiveling revealed by the crystal structures of complexes of *E. coli* Rep helicase bound to single-stranded DNA and ADP. *Cell* 90, 635–647. doi: 10.1016/S0092-8674(00)80525-5

Lan, L., Nakajima, S., Komatsu, K., Nussenzweig, A., Shimamoto, A., Oshima, J., et al. (2005). Accumulation of Werner protein at DNA double-strand breaks in human cells. *J. Cell Sci.* 118, 4153–4162. doi: 10.1242/jcs.02544

Larsen, N. B., and Hickson, I. D. (2013). RecQ helicases: conserved guardians of genomic integrity. *Adv. Exp. Med. Biol.* 767, 161–184. doi: 10.1007/978-1-4614-5037-5_8

Lee, J. W., Kusumoto, R., Doherty, K. M., Lin, G. X., Zeng, W., Cheng, W. H., et al. (2005). Modulation of Werner syndrome protein function by a single mutation in the conserved RecQ domain. *J. Biol. Chem.* 280, 39627–39636. doi: 10.1074/jbc.M506112200

Lee, J. Y., and Yang, W. (2006). UvrD helicase unwinds DNA one base pair at a time by a two-part power stroke. *Cell* 127, 1349–1360. doi: 10.1016/j.cell.2006.10.049

Liu, S., Zhang, W., Gao, Z., Ming, Q., Hou, H., Lan, W., et al. (2013). NMR structure of the *N*-terminal-most HRDC1 domain of RecQ helicase from *Deinococcus radiodurans*. *FEBS Lett.* 587, 2635–2642. doi: 10.1016/j.febslet.2013.06.048

Liu, Y., and West, S. C. (2004). Happy Hollidays: 40th anniversary of the Holliday junction. *Nat. Rev. Mol. Cell Biol.* 5, 937–944. doi: 10.1038/nrm1502

Liu, Z., Macias, M. J., Bottomley, M. J., Stier, G., Linge, J. P., Nilges, M., et al. (1999). The three-dimensional structure of the HRDC domain and implications for the Werner and Bloom syndrome proteins. *Structure* 7, 1557–1566. doi: 10.1016/S0969-2126(00)88346-X

Manthei, K. A., and Keck, J. L. (2013). The BLM dissolvasome in DNA replication and repair. *Cell. Mol. Life Sci.* 70, 4067–4084. doi: 10.1007/s00018-013-1325-1

Matsumoto, T., Shimamoto, A., Goto, M., and Furuichi, Y. (1997). Impaired nuclear localization of defective DNA helicases in Werner's syndrome. *Nat. Genet.* 16, 335–336. doi: 10.1038/ng0897-335

Mirzaei, H., and Schmidt, K. H. (2012). Non-Bloom syndrome-associated partial and total loss-of-function variants of BLM helicase. *Proc. Natl. Acad. Sci. U.S.A.* 109, 19357–19362. doi: 10.1073/pnas.1210304109

Morozov, V., Mushegian, A. R., Koonin, E. V., and Bork, P. (1997). A putative nucleic acid-binding domain in Bloom's and Werner's syndrome helicases. *Trends Biochem. Sci.* 22, 417–418. doi: 10.1016/S0968-0004(97)01128-6

Moser, R., Toyoshima, M., Robinson, K., Gurley, K. E., Howie, H. L., Davison, J., et al. (2012). MYC-driven tumorigenesis is inhibited by WRN syndrome gene deficiency. *Mol. Cancer Res.* 10, 535–545. doi: 10.1158/1541-7786.MCR-11-0508

Nguyen, G. H., Dexheimer, T. S., Rosenthal, A. S., Chu, W. K., Singh, D. K., Mosedale, G., et al. (2013). A small molecule inhibitor of the BLM helicase modulates chromosome stability in human cells. *Chem. Biol.* 20, 55–62. doi: 10.1016/j.chembiol.2012.10.016

Nguyen, G. H., Tang, W., Robles, A. I., Beyer, R. P., Gray, L. T., Welsh, J. A., et al. (2014). Regulation of gene expression by the BLM helicase correlates with the presence of G-quadruplex DNA motifs. *Proc. Natl. Acad. Sci. U.S.A.* 111, 9905–9910. doi: 10.1073/pnas.1404807111

Opresko, P. L., Calvo, J. P., and Von Kobbe, C. (2007). Role for the Werner syndrome protein in the promotion of tumor cell growth. *Mech. Ageing Dev.* 128, 423–436. doi: 10.1016/j.mad.2007.05.009

Opresko, P. L., 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, 763–774. doi: 10.1016/j.molcel.2004.05.023

Ortiz-Lombardia, M., Gonzalez, A., Eritja, R., Aymami, J., Azorin, F., and Coll, M. (1999). Crystal structure of a DNA Holliday junction. *Nat. Struct. Biol.* 6, 913–917. doi: 10.1038/13277

Oshima, J., Martin, G. M., and Hisama, F. M. (2013). "Chapter 18 – the biological basis of aging: implications for medical genetics," in *Emery and Rimoin's Principles and Practice of Medical Genetics*, eds D. L. Rimoin, R. E. Pyeritz, and B. Korf (Philadelphia, PA: Elsevier), 1–19. doi: 10.1016/B978-0-12-383834-6.00022-7

Perry, J. J., Yannone, S. M., Holden, L. G., Hitomi, C., Asaithamby, A., Han, S., et al. (2006). WRN exonuclease structure and molecular mechanism imply an editing role in DNA end processing. *Nat. Struct. Mol. Biol.* 13, 414–422. doi: 10.1038/nsmb1088

Pike, A. C., Shrestha, B., Popuri, V., Burgess-Brown, N., Muzzolini, L., Costantini, S., et al. (2009). Structure of the human RECQ1 helicase reveals a putative strand-separation pin. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1039–1044. doi: 10.1073/pnas.0806908106

Plank, J. L., Wu, J., and Hsieh, T. S. (2006). Topoisomerase IIIα and Bloom's helicase can resolve a mobile double Holliday junction substrate through convergent branch migration. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11118–11123. doi: 10.1073/pnas.0604873103

Sato, A., Mishima, M., Nagai, A., Kim, S. Y., Ito, Y., Hakoshima, T., et al. (2010). Solution structure of the HRDC domain of human Bloom syndrome protein BLM. *J. Biochem.* 148, 517–525. doi: 10.1093/jb/mvq097

Seki, M., Nakagawa, T., Seki, T., Kato, G., Tada, S., Takahashi, Y., et al. (2006). Bloom helicase and DNA topoisomerase IIIα are involved in the dissolution of sister chromatids. *Mol. Cell. Biol.* 26, 6299–6307. doi: 10.1128/MCB.00702-06

Singleton, M. R., Dillingham, M. S., and Wigley, D. B. (2007). Structure and mechanism of helicases and nucleic acid translocases. *Annu. Rev. Biochem.* 76, 23–50. doi: 10.1146/annurev.biochem.76.052305.115300

Subramanya, H. S., Bird, L. E., Brannigan, J. A., and Wigley, D. B. (1996). Crystal structure of a DExx box DNA helicase. *Nature* 384, 379–383. doi: 10.1038/384379a0

Swan, M. K., Legris, V., Tanner, A., Reaper, P. M., Vial, S., Bordas, R., et al. (2014). Structure of human Bloom's syndrome helicase in complex with ADP and duplex DNA. *Acta Crystallogr. D Biol. Crystallogr.* 70, 1465–1475. doi: 10.1107/S139900471400501X

Tadokoro, T., Kulikowicz, T., Dawut, L., Croteau, D. L., and Bohr, V. A. (2012). DNA binding residues in the RQC domain of Werner protein are critical for its catalytic activities. *Aging (Albany, N.Y.)* 4, 417–430.

Velankar, S. S., Soultanas, P., Dillingham, M. S., Subramanya, H. S., and Wigley, D. B. (1999). Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. *Cell* 97, 75–84. doi: 10.1016/S0092-8674(00)80716-3

Vindigni, A., Marino, F., and Gileadi, O. (2010). Probing the structural basis of RecQ helicase function. *Biophys. Chem.* 149, 67–77. doi: 10.1016/j.bpc.2010.03.012

Von Kobbe, C., Thoma, N. H., Czyzewski, B. K., Pavletich, N. P., and Bohr, V. A. (2003). Werner syndrome protein contains three structure-specific DNA binding domains. *J. Biol. Chem.* 278, 52997–53006. doi: 10.1074/jbc.M308338200

Wu, L., Chan, K. L., Ralf, C., Bernstein, D. A., Garcia, P. L., Bohr, V. A., et al. (2005). The HRDC domain of BLM is required for the dissolution of double Holliday junctions. *EMBO J.* 24, 2679–2687. doi: 10.1038/sj.emboj.7600740

Wu, L., and Hickson, I. D. (2003). The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426, 870–874. doi: 10.1038/nature02253

Yamada, K., Ariyoshi, M., and Morikawa, K. (2004). Three-dimensional structural views of branch migration and resolution in DNA homologous recombination. *Curr. Opin. Struct. Biol.* 14, 130–137. doi: 10.1016/j.sbi.2004.03.005

Yan, H., Mccane, J., Toczylowski, T., and Chen, C. (2005). Analysis of the Xenopus Werner syndrome protein in DNA double-strand break repair. *J. Cell Biol.* 171, 217–227. doi: 10.1083/jcb.200502077

Zakharyevich, K., Tang, S., Ma, Y., and Hunter, N. (2012). Delineation of joint molecule resolution pathways in meiosis identifies a crossover-specific resolvase. *Cell* 149, 334–347. doi: 10.1016/j.cell.2012.03.023

Zuo, Y., Wang, Y., and Malhotra, A. (2005). Crystal structure of *Escherichia coli* RNase D, an exoribonuclease involved in structured RNA processing. *Structure* 13, 973–984. doi: 10.1016/j.str.2005.04.015

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 August 2014; paper pending published: 22 September 2014; accepted: 30 September 2014; published online: 29 October 2014.

*Citation: Kitano K (2014) Structural mechanisms of human RecQ helicases WRN and BLM. Front. Genet. **5**:366. doi: 10.3389/fgene.2014.00366*

This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.

frontiers in
GENETICS

REVIEW ARTICLE
published: 21 October 2014
doi: 10.3389/fgene.2014.00372

Molecular and cellular functions of the FANCJ DNA helicase defective in cancer and in Fanconi anemia

***Robert M. Brosh Jr.*[1]* and Sharon B. Cantor*[2]**

[1] *Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA*
[2] *Department of Cancer Biology, University of Massachusetts Medical School – UMASS Memorial Cancer Center, Worcester, MA, USA*

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

Reviewed by:
Kaoru Tominaga, Jichi Medical University, Japan
George A. Garinis, Institute of Molecular Biology and Biotechnology-FORTH and University of Crete, Greece

***Correspondence:**
Robert M. Brosh Jr., Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, NIH Biomedical Research Center, 251 Bayview Boulevard, Baltimore, MD 21224, USA
e-mail: broshr@mail.nih.gov

The FANCJ DNA helicase is mutated in hereditary breast and ovarian cancer as well as the progressive bone marrow failure disorder Fanconi anemia (FA). FANCJ is linked to cancer suppression and DNA double strand break repair through its direct interaction with the hereditary breast cancer associated gene product, BRCA1. FANCJ also operates in the FA pathway of interstrand cross-link repair and contributes to homologous recombination. FANCJ collaborates with a number of DNA metabolizing proteins implicated in DNA damage detection and repair, and plays an important role in cell cycle checkpoint control. In addition to its role in the classical FA pathway, FANCJ is believed to have other functions that are centered on alleviating replication stress. FANCJ resolves G-quadruplex (G4) DNA structures that are known to affect cellular replication and transcription, and potentially play a role in the preservation and functionality of chromosomal structures such as telomeres. Recent studies suggest that FANCJ helps to maintain chromatin structure and preserve epigenetic stability by facilitating smooth progression of the replication fork when it encounters DNA damage or an alternate DNA structure such as a G4. Ongoing studies suggest a prominent but still not well-understood role of FANCJ in transcriptional regulation, chromosomal structure and function, and DNA damage repair to maintain genomic stability. This review will synthesize our current understanding of the molecular and cellular functions of FANCJ that are critical for chromosomal integrity.

Keywords: FANCJ, helicase, DNA repair, replication, Fanconi anemia, cancer, genomic stability, G-quadruplex

DISCOVERY OF BACH1/BRIP1/FANCJ AND ITS EMERGENCE AS A PROMINENT PLAYER IN HUMAN GENETIC DISEASE AND TUMOR SUPPRESSION

FANCJ (originally named BRCA1 interacting C-terminal helicase (BACH1) or BRCA1 interacting helicase (BRIP1) was first discovered by its physical interaction with BRCA1, a known tumor suppressor and mediator of double strand break (DSB) repair (Cantor et al., 2001). Consistent with FANCJ's association with BRCA1, FANCJ-deficient cells are sensitive to DNA cross-linking agents (Bridge et al., 2005; Levitus et al., 2005; Litman et al., 2005) and mildly sensitive to ionizing radiation (IR; Peng et al., 2006), and display a defect in homologous recombination (HR) repair of DSBs (Litman et al., 2005). The first clinical evidence for the importance of FANCJ was the identification of germ line sequence changes in FANCJ that were associated with early breast cancer in two individuals that displayed normal genotypes for BRCA1 and BRCA2 (Cantor et al., 2001). Subsequent studies solidified the causal relationship of FANCJ mutations as low penetrance breast and ovarian cancer alleles (Seal et al., 2006; Rafnar et al., 2011; for review, see Cantor and Guillemette, 2011).

In accord with its role as a tumor suppressor, FANCJ was identified as the gene mutated in the J complementation group of Fanconi anemia (FA), a rare disorder characterized by progressive bone marrow failure, skeletal abnormalities, and cancer (Levitus et al., 2005; Levran et al., 2005; Litman et al., 2005). Currently, there are 16 FA complementation groups. The corresponding genes encode proteins implicated in a complex pathway of interstrand cross-link (ICL) repair that corrects damage when the two complementary strands of the DNA double helix become covalently linked, a type of lesion that blocks cellular DNA replication and transcription (**Figure 1**). The reader is referred to several recently published excellent reviews on the functions of the FA gene products and the overall workings of the FA pathway and its importance in chromosomal stability (Kee and D'Andrea, 2012; Kottemann and Smogorzewska, 2013; Walden and Deans, 2014). One notable finding is that FANCJ is not required for DNA damage induced FANCD2 monoubiquitination, suggesting that the helicase functions downstream of this key activation step of the FA pathway (Litman et al., 2005). FANCJ likely operates with other downstream BRCA-FA proteins, such as BRCA1, and related factors also classified as tumor suppressors to facilitate recombinational repair (potentially following unhooking of the processed cross-link; **Figure 1**). In addition, it is believed that FANCJ functions in a broader role to suppress replication stress (see subsequent sections).

The FANCJ gene encodes a protein of 1,249 amino acids with a conserved ATPase helicase core domain comprised of eight motifs [0 (Q), I, Ia, II, III, IV, V, VI] found in DEAH superfamily two helicases (**Figure 2**). A signature motif in this FANCJ family of DNA helicases is an Iron–Sulfur (Fe–S) cluster, characterized by four conserved cysteine residues, residing within

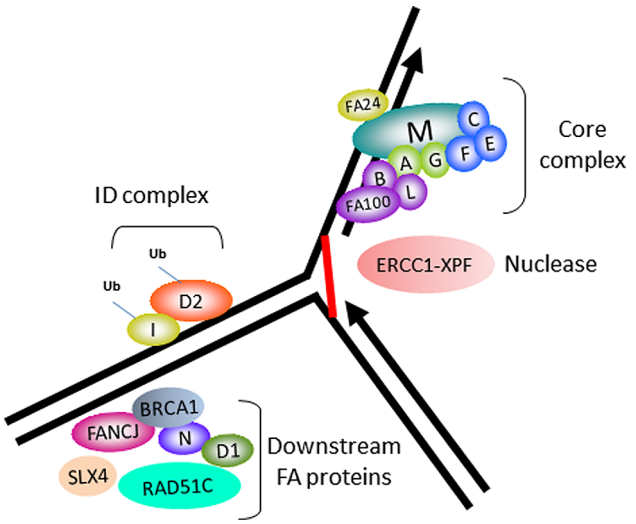


FIGURE 1 | Fanconi anemia (FA) proteins implicated in interstrand cross-link (ICL) repair. Cartoon schematic of the FA proteins implicated in the ICL repair pathway. The integrity of the core complex is required for FANCD2/FANCI monoubiquitination, a key activation step of the FA pathway. ERCC1-XPF is the nuclease implicated in ICL processing. FANCJ and certain other FA proteins are not required for FANCD2/FANCI monoubiquitination, and are therefore implicated in downstream events of the FA pathway, such as homologous recombination (HR) repair of the processed ICL.

the helicase core domain (Cantor et al., 2004; Rudolf et al., 2006). Members of the Fe–S helicase cluster family function in preserving the genome, such as XPD and RTEL helicases (Wu et al., 2009). Recently solved crystal structures and biochemical studies of thermophilic species of XPD suggest that the Fe–S cluster functions with an Arch domain that is proposed to be a wedge propelling strand separation. Moreover, two conserved RecA-like domains mediate ATP binding, protein–DNA interactions, and helicase translocation mediated by ATP-induced conformational changes (Rudolf et al., 2006; Fan et al., 2008; Wolski et al., 2008; Kuper et al., 2011). Studies on Fe–S cluster helicases XPD (Mui et al., 2011; Sontz et al., 2012) and DinG (Ren et al., 2009), as well as other DNA processing enzymes (Grodick et al., 2014), have implicated the Fe–S cluster as a redox sensor that facilitates DNA damage detection; however, a formal role of the FANCJ Fe–S cluster in this capacity has not been determined.

CATALYTIC ACTIVITIES AND DNA SUBSTRATE SPECIFICITY OF FANCJ HELICASE

Biochemical characterization of the purified recombinant FANCJ protein using a classic DNA directionality substrate (Cantor et al., 2004) or oligonucleotide-based partial duplex substrates with single-stranded DNA overhangs of defined polarity (Gupta et al., 2005) determined the 5′–3′ directionality of FANCJ as an ATP-dependent DNA helicase. Biophysical analyses of FANCJ assembly state suggest that the helicase exists in an equilibrium, as a monomer, and dimer in solution. Furthermore, biochemical studies showed that the dimeric form of FANCJ displays maximal catalytic activity as an ATPase and DNA helicase on relatively short forked duplex substrates of 20 base pairs (bp; Wu et al., 2012). FANCJ is limited in its processivity; therefore, it poorly unwinds substrates with duplexes of ∼50 bp or greater. However, on both shorter and longer duplexes its helicase activity is markedly stimulated by the single-stranded DNA binding protein replication protein A (RPA; Gupta et al., 2007).

The DNA substrate preference of FANCJ has been studied in some detail (**Figure 3**). A 5′ tail of 15 nucleotides (nt) is required and 35 nt is optimal for FANCJ to catalyze appreciable unwinding of the simple 5′ tailed duplex (Gupta et al., 2005). FANCJ preferentially binds and unwinds forked duplex DNA substrates (Gupta et al., 2005), and is also active on a 5′ flap (but not 3′ flap) substrate (**Figure 3**), consistent with its translocation directionality. Backbone continuity in the pre-existing 5′ single-stranded DNA tail, but not the 3′ single-stranded tail, of the forked duplex substrate within six nt of the single-stranded DNA-double stranded DNA junction is required for FANCJ to initiate unwinding of the adjacent duplex. However, disruption of the sugar phosphate backbone by a polyglycol modification in *either* the translocating or non-translocating strand within the duplex region inhibits FANCJ helicase activity (Gupta et al., 2006). This finding demonstrates that FANCJ senses both strands during the elongation phase of the unwinding reaction. Inhibition of FANCJ helicase by the polyglycol modification in either strand of the duplex substrate can be overcome by increasing the 5′ single-stranded DNA loading tail of the substrate (Gupta et al., 2006), suggesting that loading of multiple FANCJ molecules under multi-turnover conditions drives forward the DNA unwinding reaction even when the helicase encounters a formidable obstacle to progression. Interestingly, FANCJ helicase activity is not inhibited by the presence of abasic sites in either the translocating or non-translocating strands within the duplex region of the forked DNA substrate (Gupta et al., 2006), suggesting that FANCJ's electrostatic interactions with the sugar phosphate backbone dominate over base-stacking interactions. FANCJ helicase activity is also inhibited in a translocating strand specific manner by an alkyl phosphotriester lesion that introduces a hydrophobic group into the nucleic acid backbone and neutralizes the negatively charged phosphodiester moiety (Suhasini et al., 2012). Presumably, the physical attributes of the alkyltriester damage or its effect on double helical rigidity differentially affect FANCJ unwinding compared to the polyglycol linkage, which inhibited irrespective of the strand. A number of chemical genotoxins cause the formation of phosphotriester adducts which can persist for a long time in genomic DNA (Jones et al., 2010). These lesions and other DNA adducts may exert their mutagenic and carcinogenic effects by inhibiting DNA metabolizing enzymes, including helicases such as FANCJ (Suhasini and Brosh, 2010).

To assess the possibility that FANCJ has a catalytic role in processing an ICL intermediate in a DSB repair pathway, we assessed its activity on a three-stranded displacement loop (D-loop) DNA substrate that represents a key early intermediate of HR repair (Gupta et al., 2005). Following unhooking of an ICL, a D-loop arises after RAD51-mediated strand invasion and base-pairing of a single-stranded DNA overhang formed at a resected DSB. Notably, FANCJ unwinds a D-loop without a 5′ single-stranded

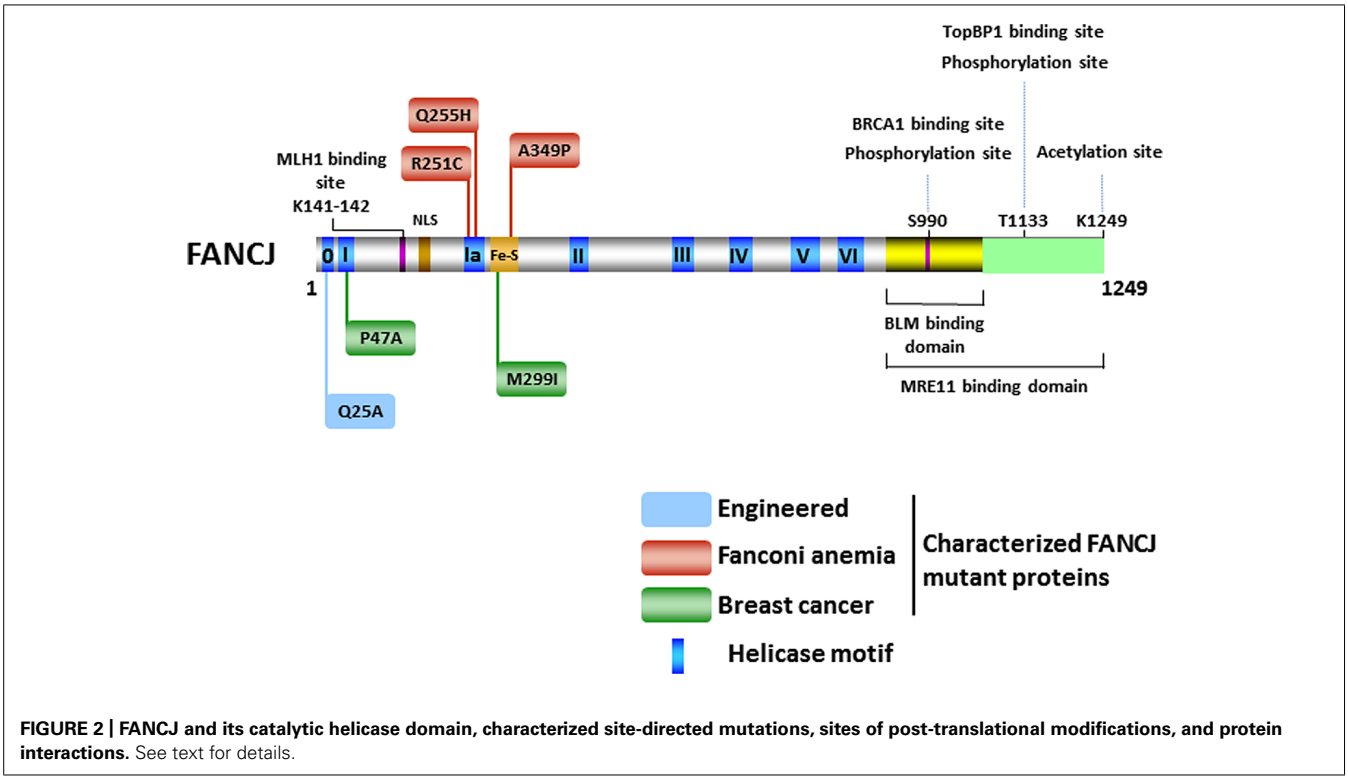


FIGURE 2 | FANCJ and its catalytic helicase domain, characterized site-directed mutations, sites of post-translational modifications, and protein interactions. See text for details.

DNA tail suggesting that the DNA junctions in the D-loop substrate enable FANCJ to overcome its usually strict requirement for loading on a 5′ single-stranded DNA tail to initiate unwinding (**Figure 3**). However, FANCJ fails to unwind a four-stranded Holliday Junction structure, another key intermediate of HR repair that can lead to cross-over or non-crossover recombinant products (Gupta et al., 2005). Thus, FANCJ may be exquisitely tailored to act upon D-loop intermediates to suppress HR or homeologous recombination. Alternatively, FANCJ may act upon D-loops to enable synthesis-dependent strand annealing, a pathway of DSB repair distinct from the classic Holliday Junction resolution pathway.

In addition to unwinding conventional duplex DNA substrates, FANCJ resolves alternate DNA structures including DNA triplexes (Sommers et al., 2009) or G4 (London et al., 2008; Wu et al., 2008) that form by Hoogsteen hydrogen bonding (**Figure 3**). For both triplexes and G4s, FANCJ requires a 5′ single-stranded DNA tail, consistent with its 5′–3′ directionality of translocation. For unwinding triplexes this 5′ tail must reside on the pyrimidine motif third strand that invades the major groove of the underlying DNA double helix. FANCJ has the capacity to resolve intermolecular (two-stranded or four-stranded) as well as unimolecular G4 substrates (Bharti et al., 2013), which is likely important to suppress replication-associated G4 substrates and in turn DSB formations (discussed below).

Aside from unwinding DNA, FANCJ has the ability to harness the energy from ATP hydrolysis to disrupt protein–DNA interactions. Attesting to its robust capacity, FANCJ was shown to disrupt the high affinity interaction of biotin bound to a biotinylated oligonucleotide in an ATP-dependent manner (Sommers et al., 2009). Of greater biological relevance, FANCJ can destabilize a RAD51-single-stranded DNA filament (**Figure 4A**), and therefore inhibit DNA strand exchange activity of RAD51 (Sommers et al., 2009). Thus, FANCJ may limit promiscuous recombination. Alternatively, by removing RAD51 from the 3′ invading strand of the nucleoprotein filament, FANCJ could enable loading of the DNA polymerase and promotes DNA synthesis.

FANCJ helicase and translocase activities are also modulated by protein interactions. In particular, FANCJ is blocked from unwinding partial duplex DNA substrates bound by double stranded DNA-interacting proteins (e.g., catalytically inactive restriction endonuclease, or the telomere binding proteins TRF1, TRF2). However, this inhibition is overcome by the presence of RPA in the FANCJ reaction mixtures under conditions that RPA alone had little effect (Sommers et al., 2014; **Figure 4B**). The ability of RPA to stimulate FANCJ displacement of TRF1 or TRF2 from forked duplex substrates harboring telomeric repeats may be important for remodeling chromosome ends during DNA replication or repair. Indeed, FANCJ was localized to telomeres of living cells that operate according to the alternative lengthening of telomere (ALT) pathway (Dejardin and Kingston, 2009). Since RPA also increases the ability of FANCJ to unwind duplex and G4s (Wu et al., 2008), RPA–FANCJ interactions may both clear protein obstacles and resolve alternate DNA structures during cellular replication to preserve the genome (see below).

Our biochemical studies further suggest that RPA is important for enabling FANCJ to bypass bulky adducts or helix-distorting lesions such as thymine glycol, an oxidative base damage that can be mutagenic or lethal (Wallace, 2002). FANCJ helicase activity is strongly inhibited by a single thymine glycol in either

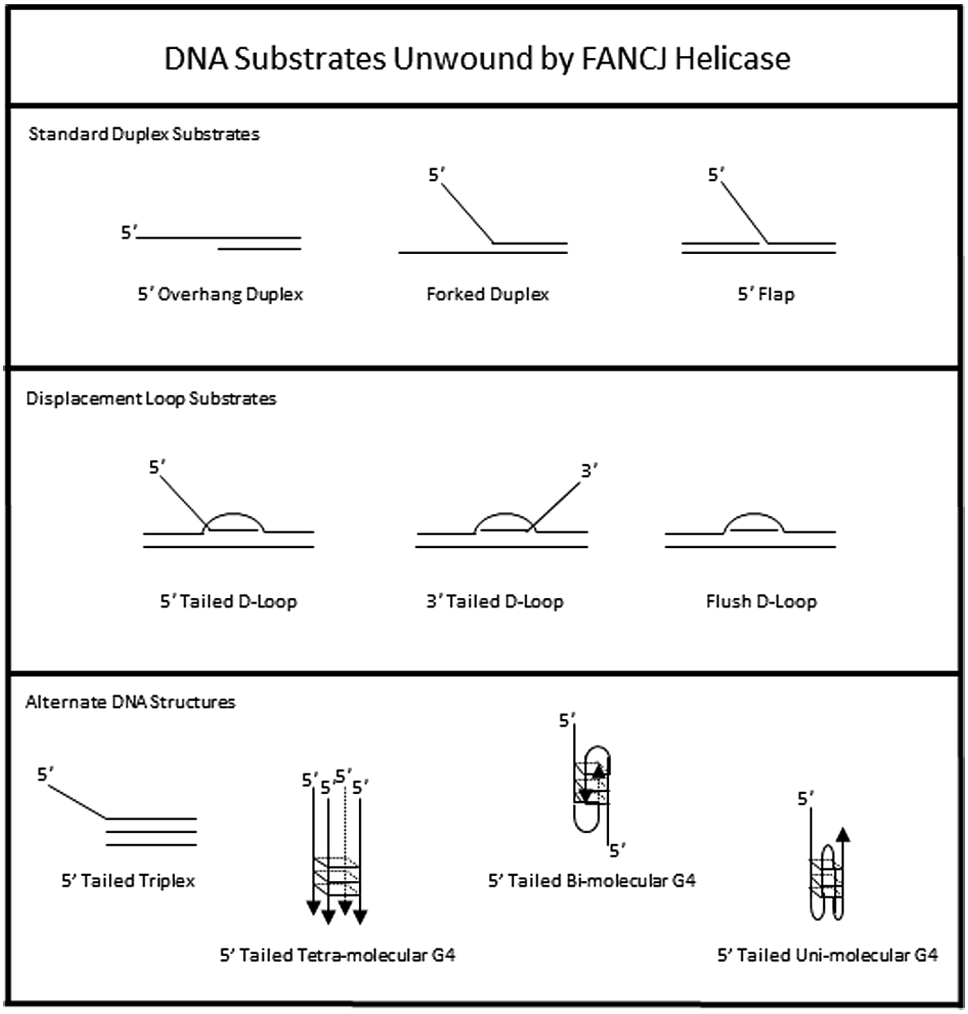


FIGURE 3 | DNA substrate specificity of FANCJ helicase. See text for details.

the translocating or non-translocating strand of a DNA duplex (Suhasini et al., 2009). However, RPA stimulates FANCJ to efficiently unwind the substrate harboring thymine glycol in the non-translocating strand, but fails to do so when the thymine glycol resides in the translocating strand. The demonstrated high affinity interaction of RPA with single-stranded DNA harboring a single thymine glycol (Suhasini et al., 2009), together with the strand-specific RPA stimulation of FANCJ helicase activity on the DNA substrate harboring the thymine glycol, suggest a model in which RPA promotes strand displacement. Specifically, the exposed thymine glycol in the non-translocating strand of the partially unwound DNA substrate is readily bound by RPA, resulting in RPA coating of the strand displaced by FANCJ, stabilized duplex separation, and further FANCJ helicase progression past the thymine glycol leading to complete separation of the complementary strands. Such a mechanism may be important for the role of FANCJ to insure timely progression through S phase (Kumaraswamy and Shiekhattar, 2007), or in an environment of heightened oxidative stress. Given the emerging evidence that the FA pathway suppresses DNA damage induced by products of normal cellular metabolism such as aldehydes (Nalepa and Clapp, 2014), it will be of interest to assess how such aldehyde-induced lesions affect FANCJ and its role in DNA repair.

POST-TRANSLATIONAL MODIFICATIONS OF FANCJ

There has been considerable interest in the effect of post-translational modifications on the functions of DNA repair proteins and checkpoint signaling, both important components of the DNA damage response. Post-translational modifications of DNA damage response proteins can affect their subcellular localization, chromatin association, DNA and protein interactions, stability, and catalytic activity. Phosphorylation of FANCJ mediates interactions promoting repair and checkpoint responses. The first identified was the phosphorylation of Ser-990, which is essential for FANCJ binding to the tandem C-terminal BRCT motifs of BRCA1 (Yu et al., 2003; **Figure 2**). The interface between FANCJ and the interacting BRCA1 BRCT repeats was further

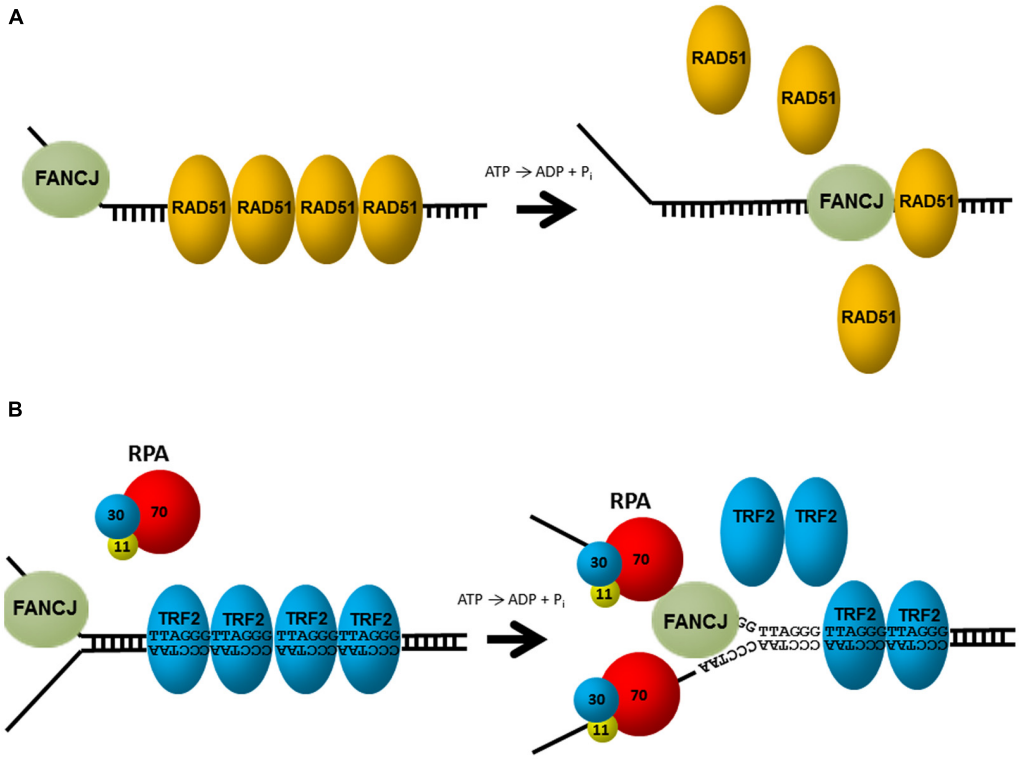


FIGURE 4 | FANCJ uses its motor ATPase to displace proteins bound to DNA. (A) FANCJ catalytically displaces the major DNA recombinase protein RAD51. See reference Sommers et al. (2009) and text for details. **(B)** Replication protein A (RPA) stimulates FANCJ to eject the shelterin protein TRF1 (or TRF2) bound to telomeric repeat duplex DNA. See reference Sommers et al. (2014) and text for details.

defined in structural studies (Yu et al., 2003; Botuyan et al., 2004; Shiozaki et al., 2004). Loss of this Ser-990 phosphorylation limits HR, but also enhances polymerase η dependent bypass suggesting that BRCA1 binding to FANCJ is important for directing the mechanism of DNA damage repair (Xie et al., 2010). Interestingly, the region of FANCJ that binds to the Bloom's syndrome protein (BLM) overlaps with the FANCJ Ser-990 phosphorylation site (Suhasini et al., 2011; **Figure 2**), raising the possibility that the protein interaction of FANCJ with BLM is affected by phosphoSer-990. More recently, a second FANCJ phosphorylation dependent interaction was identified at Thr-1133 (**Figure 2**). Phosphorylated FANCJ Thr-1133 interacts with the BRCT repeats of Topoisomerase IIb binding protein 1 (TopBP1) to promote an ATR-dependent checkpoint in response to replication stress (Gong et al., 2010).

Acetylation of FANCJ at lysine 1249, the last C-terminal amino acid (**Figure 2**), affects the DNA damage response similar to Ser-990 phosphorylation. When acetylation is prevented, cellular ICL resistance is achieved by a reduced need for Rad54-mediated HR repair and enhanced dependence on the translesion polymerase η. This modulation of repair pathway mechanism could stem from the role of FANCJ and its acetylation upregulating DNA end resection required for HR. Dynamic acetylation of FANCJ was also found to maintain checkpoint signaling following DNA damage (Xie et al., 2012). Continued studies of post-translational modifications on FANCJ and its partners are warranted. For example, it will be important to determine if a post-translational modification of FANCJ or the Bloom's syndrome helicase (BLM) influences the interaction between the two DNA helicases (**Figure 2**), especially because FANCJ status dramatically influences BLM protein stability by a proteasomal degradation pathway (Suhasini et al., 2011). It remains to be seen if the phosphorylation or acetylation state of FANCJ affects its catalytic activity on a specific DNA substrate. On a forked duplex DNA substrate, acetylation at 1249 did not influence its activity (Xie et al., 2012). Alternatively, post-translational modification of FANCJ may influence its subcellular localization. Recently it was reported that ICL induces localization of FANCJ to the centrosome and FANCJ is involved in regulation of centrosome biogenesis (Zou et al., 2013).

NOVEL INSIGHTS TO FANCJ STRUCTURE-FUNCTION RELATIONSHIPS BY CHARACTERIZATION OF SITE-DIRECTED MUTANTS

Characterization of helicase missense mutants may be informative for dissecting the molecular basis of disease, or potential dominant negative effects of debilitating missense mutations (Suhasini and Brosh, 2013). For FANCJ, the clinical spectrum of mutations includes missense mutations genetically linked to FA and/or cancer. This is not the general case for a number of disease-causing helicase mutations. For example, only recently were several WRN

missense mutations genetically linked to the premature aging disorder Werner syndrome identified and found to be in conserved catalytic domains of the WRN protein (Friedrich et al., 2010). The vast majority of WRN mutations are limited to frameshift or nonsense codons resulting in truncated proteins.

The first FANCJ mutants to be studied were missense variants (P47A, M299I; **Figure 2**) identified in individuals with early breast cancer and normal genotypes for BRCA1 or BRCA2 (Cantor et al., 2001). Tumors of the individuals who carried these two germline FANCJ mutations also carried a copy of the wild-type (WT) allele, suggesting that loss of function may have contributed to the penetrance of the mutant allele by a dominant negative mechanism. Biochemical analysis of the corresponding purified recombinant FANCJ proteins demonstrated that both missense mutations (P47A, M299I; **Figure 2**) affected catalytic activity in a distinct manner. The P47A substitution in the highly conserved Walker A box (motif I) inactivated the ATPase and helicase functions of FANCJ, whereas the M299I mutation located in the Fe-S cluster upregulated its ATPase activity (Cantor et al., 2004). A subsequent study demonstrated that the FANCJ-M299I protein could harness its elevated ATP hydrolysis to unwind a DNA substrate with damage in its sugar phosphate backbone in a more proficient manner (Gupta et al., 2006). Based on these studies, it was proposed that perturbation of FANCJ catalytic activity interferes with the helicase's normal role in the DNA damage response leading to tumorigenesis; however, a better understanding of FANCJ's precise role in cellular transformation is required. As discussed below, expression of certain FANCJ mutant proteins in a normal FANCJ background confer sensitivity to DNA damaging agents, suggesting that a single mutant FANCJ allele could be pathogenic. Further characterization of FANCJ variants using cell- and animal-based models may be helpful.

Analysis of the FA-associated FANCJ-A349P mutant provided insight to the pathogenesis of FA and the role of the Fe-S domain (Wu et al., 2010; **Figure 2**). The alanine to proline substitution is adjacent to one of the highly conserved cysteine residues important for chelation of Fe atoms. Inheritance of the A349P mutation and a second mutation encoding a prematurely truncated FANCJ protein resulted in intrauterine growth failure and death as a stillborn fetus with a gestational age of 22 weeks (Levran et al., 2005). Our genetic analysis demonstrated that expression of the FANCJ-A349P mutant allele in FANCJ-null FA-J patient cells failed to rescue sensitivity to the DNA cross-linking agent MMC; similarly, expression of FANCJ-A349P in fancj null chicken DT40 cells failed to rescue cisplatin sensitivity (Wu et al., 2010). Moreover, expression of the FANCJ-A349P mutant in FA-J cells or fancj null chicken cells failed to restore resistance to the G4 ligand telomestatin (TMS). These studies suggested that the FANCJ-A349P mutant is defective for ICL processing and G4 unwinding *in vivo*. Moreover, expression of FANCJ-A349P in cells expressing the normal FANCJ protein exerted a dominant negative effect, presenting the possibility that a single mutant FANCJ allele could be pathogenic. *In vitro* analysis revealed that the A349P substitution interfered with the functionality of the FANCJ Fe–S cluster and uncoupled ATP-dependent DNA translocation from helicase activity on duplex or G4 DNA substrates (Wu et al., 2010). Thus, an intact Fe–S domain is critical for FANCJ DNA unwinding and this activity is fundamentally important for FANCJ ICL repair and G4 DNA metabolism (Wu et al., 2010). It remains to be determined if the functions of FANCJ in ICL repair and G4 unwinding are linked or if separation-of-function mutants exist. Ongoing efforts in this area could elucidate the importance of FANCJ in the FA pathway of cross-link repair versus functions outside the FA pathway that may also be significant for genome stability.

FANCJ also has a conserved Q motif (also called motif 0) found in both RNA and DNA helicases that is predicted to coordinate ATP binding and hydrolysis to catalytic DNA strand separation (Tanner et al., 2003; **Figure 2**). Biophysical analysis of the purified recombinant FANCJ-Q25A mutant protein disrupted in the Q motif revealed that its assembly state was dramatically altered compared to the recombinant WT FANCJ protein. FANCJ-Q25A protein was only a monomer, whereas FANCJ-WT protein was nearly equally monomer and dimer (Wu et al., 2012). Thus, the Q motif in FANCJ plays a critical role in multimerization. FANCJ-Q25A was defective for DNA binding, ATP hydrolysis, and helicase activity. Consistent with the biochemical results, expression of the FANCJ-Q25A mutant protein in fancj null cells failed to rescue their sensitivity to a DNA cross-linking agent or G4 ligand. Moreover, expression of the FANCJ-Q25A mutant allele in a WT FANCJ background resulted in dominant negative phenotypes for ICL or G4 ligand resistance. Co-immunoprecipitation experiments with nuclear extracts demonstrated that the FANCJ-Q25A mutant protein retained its ability to interact with known protein partners of FANCJ (TopBP1, BRCA1), suggesting that these retained interactions could contribute to the dominant negative nature of the mutant allele; however, further studies are required to ascertain the precise mechanism. From a clinical perspective, it is of significance that the FANCJ-Q25A mutant is similar to a patient-derived BLM missense mutation in the Q motif (Q672R) that impairs BLM foci formation after cellular exposure to agents that impose replication stress (Ellis et al., 1995; Wu et al., 2012). Therefore, dimer formation may be essential for FANCJ focal accumulation and function *in vivo*.

Two FA patient-derived missense mutations in motif Ia, R251C, and Q255H, were characterized by the Wu lab (Guo et al., 2014; **Figure 2**). Although expression of either R251C or Q255H mutant could not rescue the cisplatin sensitivity of a fancj null cell line, the two mutations exerted markedly different effects on the biochemical functions of FANCJ *in vitro*. Both FANCJ motif Ia mutants abolished DNA helicase activity. The R251C mutation strongly interfered with FANCJ DNA binding and consequently its DNA-dependent ATPase activity. Instead, the Q255H mutant displayed elevated FANCJ DNA binding, a normal ATPase function, and ability to translocate on single-stranded DNA. In this regard, the Q255H mutant behaved similarly to the FANCJ-A349P mutant which was also able to translocate on single-stranded DNA in an ATP-dependent manner but failed to unwind even short 20 bp duplex substrates (Wu et al., 2010). For either the Q255H or R251C mutant, the ability to translocate on single-stranded DNA did not translate into efficient disruption of protein-DNA complexes. Collectively, these studies suggest that FANCJ translocase activity without protein complex disruption

and/or DNA unwinding is insufficient for its *in vivo* function. Nevertheless, the dominant negative phenotype exerted by FANCJ mutant alleles (A349P, Q25A, R251C, and Q255H) that impair DNA helicase (but not necessarily ATPase activity) demonstrate that catalytic DNA unwinding is vital for FANCJ function and suggests that FANCJ heterozygosity may contribute to tumorigenicity or disease-associated phenotypes. For a further discussion of helicase-inactivating mutations as a basis for dominant negative phenotypes, see (Wu and Brosh, 2010b).

Evidence continues to build that FANCJ and other players in the FA pathway are *bona fide* tumor suppressor genes, even outside their roles in the FA pathway (Pickering et al., 2013; Park et al., 2014; Pauty et al., 2014). For example, whole genome-sequencing of Icelanders led researchers to discover frameshift mutations in the BRIP1 (FANCJ) gene that vastly elevate the risk of invasive ovarian cancer (Rafnar et al., 2011). Moreover, a BRIP1 frameshift mutation was associated with a 36% increased risk of cancer in general and a reduced lifespan of 3.6 years compared to non-carriers (Rafnar et al., 2011). This work and that of others, reviewed in (Cantor and Guillemette, 2011), emphasizes the prominent role of FANCJ as a tumor suppressor, which may be informative for future studies in personalized medicine that exploit the mutational status of FANCJ and other DNA repair helicases (see below). The spectrum of associated cancers could be broad. Indeed, we found a number of protein coding mutations in FANCJ in melanoma genomes (one allele), suggesting that FANCJ deficiency may be a risk factor for skin cancer and possibly associated tumors could be sensitive to ICL-inducing agents (Guillemette et al., 2014).

FANCJ AND ITS PROTEIN PARTNERS ARE RECRUITED TO DNA DAMAGE FOCI IN A REGULATED AND LESION-SPECIFIC MANNER

Given the direct interaction of FANCJ with BRCA1, the dependency of these proteins on each other for localization at lesions has been examined. Indeed, these proteins colocalize at sites of DNA damage foci following HU, IR, and at laser-induced stripes (Cantor et al., 2001; Greenberg et al., 2006). BRCA1 mutant cells display reduced immunofluorescent focal staining for FANCJ in untreated cells as well as cells exposed to DNA damaging agents (Cantor et al., 2001; Gupta et al., 2007). However, the contribution of FANCJ to BRCA1 localization to DNA damage sites may be time or context dependent. FANCJ status did not affect recruitment of BRCA1 to laser-induced DSBs or psoralen (Pso)-ICLs (Suhasini et al., 2013). However, the number and intensity of BRCA1 foci in FANCJ-deficient cells exposed to IR was reduced at time points as early as 1 h post irradiation (Peng et al., 2006). Thus, there may be separate pools of FANCJ and BRCA1 or a sub-fraction of FANCJ may contribute to BRCA1 recruitment or retention at foci after IR exposure. Controlling the localization of BRCA1 to sites of DNA damage would directly affect its DNA repair function, and have potential consequences for cellular homeostasis. For example, oncogenic RAS transformation down-regulates FANCJ expression, which causes BRCA1 dissociation from chromatin, resulting in an impaired DNA damage response leading to cellular senescence (Tu et al., 2011).

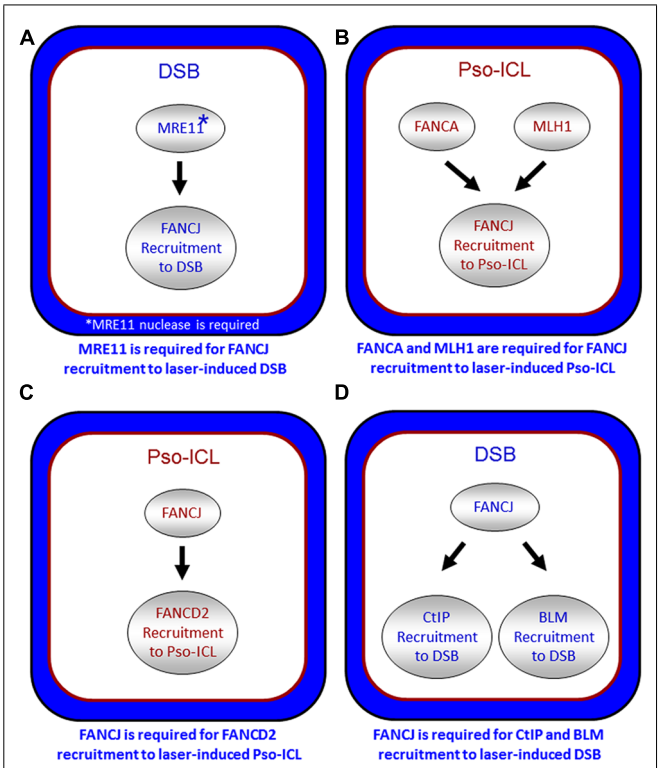


FIGURE 5 | Spatio-temporal recruitment of FANCJ and its protein partners to site of DNA damage. Confocal microscopy studies with living cells were used to elucidate the requirements of FANCJ or its interacting DNA repair factors to laser-induced psoralen (Pso)-interstrand cross-links **(A,C)** or laser-induced double strand breaks (DSB; **B,D**). See reference Suhasini et al. (2013) and text for details.

It is also important to note that other DNA repair proteins function with or in parallel with BRCA1 localize FANCJ to sites of DNA breaks. In particular, the DSB repair protein MRE11 and its associated nuclease activity is necessary for efficient FANCJ recruitment to laser-induced DSBs (Suhasini et al., 2013; **Figure 5A**). Consistent with this observation, CtIP is also delayed in its recruitment to DSBs in cells that are deficient in MRE11 exonuclease, that have reduced FANCJ recruitment to DSBs (Suhasini et al., 2013). Recent work from the Paull laboratory has implicated a catalytic role of CtIP in 5′ strand resection that is involved in the removal of DNA adducts at DNA breaks (Makharashvili et al., 2014). Perhaps FANCJ collaborates with CtIP to remove secondary structures therefore enabling CtIP to efficiently incise and process DNA ends, such as at common fragile sites (Wang et al., 2014). It is plausible that FANCJ's role in end resection may be independent of BRCA1, similar to what was determined for CtIP (Polato et al., 2014).

A critical protein partner that FANCJ collaborates with is RPA (Gupta et al., 2007). FANCJ and RPA robustly co-localize after DNA damage induced by IR or MMC or replication stress imposed by hydroxyurea (HU), which depletes nucleotide pools. RPA foci formation was not dependent on FANCJ mutational status after MMC treatment or IR exposure (Gupta et al., 2007), suggesting that FANCJ helicase activity is not a prerequisite for

creating single-stranded DNA at DNA breaks that RPA nucleates on. However, RPA foci are reduced in FANCJ-deficient cells exposed to HU for a short time period (20 min; Gong et al., 2010) or ultraviolet (UV) light (Guillemette et al., 2014), suggesting FANCJ helicase activity provides single-stranded DNA loading zones for RPA when the replication fork is stalled. RPA binds to FANCJ and stimulates its helicase (Gupta et al., 2007) and DNA-protein displacement activities (Sommers et al., 2014), as mentioned above. Based on these observations, we favor the hypothesis that FANCJ together with RPA binds a key DSB repair intermediate or stalled replication fork structure and RPA stimulates FANCJ's helicase and/or protein displacement activity to allow appropriate and efficient processing during the maturation of the DNA intermediate.

FANCJ also binds directly to the mismatch repair (MMR) protein MLH1 (Peng et al., 2007). While MLH1 binding did not demonstrate any notable changes in FANCJ helicase activity *in vitro*, MLH1 is critical for FANCJ localization to sites of DNA crosslinks. In particular, using a laser confocal microscopy approach with living cells, we examined the recruitment of FANCJ to laser-activated Pso-ICLs (Suhasini et al., 2013). This analysis demonstrated that FANCJ relies on MLH1 and a member of the FA core complex, FANCA, to recruit efficiently to the laser-activated Pso-ICL (**Figure 5B**). FANCJ localization to UV light induced DNA crosslinks also requires the MLH1 interaction as well as the upstream MMR protein MSH2 (Guillemette et al., 2014). This is logical given that the physical interaction between FANCJ and MLH1 is required for cells to properly respond to agents that induce ICL damage or UV damage (Peng et al., 2007; Guillemette et al., 2014). Localization of FANCJ by MMR proteins may in turn limit deleterious MMR functions at a stalled fork. This idea is based on the fact that defects in cells lacking the FANCJ-MLH1 interaction are suppressed by depletion of MSH2 (Peng et al., 2014). Coordination by FANCA and MMR proteins could ensure that FANCJ helicase function is set to unwind DNA or displace proteins to restore replication fork progression following ICLs or other replication blocking agents.

The relationship of FANCJ with FANCD2 is more complex. While FANCJ operates downstream of FANCD2 monoubiquitination (Litman et al., 2005; **Figure 1**), FANCD2 recruitment to Pso-ICLs, not but laser-induced DSBs, was dependent on FANCJ (Suhasini et al., 2013; **Figure 5C**). Consistent with these observations, FANCJ is recruited to Pso-ICLs much earlier than FANCD2. These findings correlate with those of the Andreassen lab that reported that FANCJ foci formed normally in FANCD2-deficient cells after exposure to the DNA cross-linker MMC (Zhang et al., 2010). In more recent studies, the Kupfer lab reported that FANCD2 is required for proper chromatin localization of FANCJ, suggesting a mechanism whereby FANCD2 helps to regulate FANCJ's role in downstream events of the FA pathway (Chen et al., 2014). In addition, they propose a model in which FANCJ sequesters non-monoubiquitinated FANCD2 from chromatin in the absence of DNA damage; therefore, a collaborative interaction between FANCJ and FANCD2 could exist that is necessary for the appropriate DNA damage-induced chromatin association of the two FA proteins.

FANCJ also binds and serves to localize the BLM helicase to DSBs (Suhasini et al., 2013; **Figure 5D**). The precise role of FANCJ in BLM recruitment to laser-induced DSBs or its function at DNA ends is being investigated, and a potential partnership between FANCJ and BLM in processive strand resection is a possibility (Suhasini and Brosh, 2012). Based on biochemical evidence that FANCJ and BLM helicases synergistically unwind damaged DNA (Suhasini et al., 2011), we proposed a model that the two helicases with opposite directionalities of translocation move together in a complex as part of the end resection machinery involving RPA and the 5′ structure-specific nucleases DNA2 or EXO-1 to catalytically resect single-stranded DNA to provide the 3′ single-stranded overhang for strand invasion step of HR repair (Suhasini and Brosh, 2012). Our recent work showing that FANCJ or a human RecQ helicase (RECQ1) can efficiently dislodge protein bound to duplex DNA in a RPA-dependent manner (Sommers et al., 2014) poses a scenario in which FANCJ and BLM with their interacting partner RPA displace proteins bound near double-stranded ends and resolve secondary structure or damaged DNA to enable processive and kinetically efficient end resection.

Clearly a concerted hierarchy exists for FANCJ and its interacting partners to be recruited to DNA damage sites and subsequently act. Further studies are needed because they may provide insights to DNA repair pathway cascades or the cross-talk between DNA damage response regimes. For example, the interaction of FANCJ with the MRN complex and BLM helicase suggests that FANCJ may have both early and late roles in DSB repair. FANCJ has the ability to inhibit MRE11 3′–5′ exonuclease activity (Suhasini et al., 2013), which may serve to harness initial end trimming by MRE11 to avoid excessive end resection that would generate 5′ single-stranded tailed duplex. Secondly FANCJ with RPA may facilitate processive strand resection by its interaction with the BLM-DNA2 or BLM-EXO-1 complexes to yield the 3′ single-stranded tailed duplex. Biochemical reconstitution experiments with purified proteins and defined DNA substrates, as well as carefully designed cell-based assays should address the efficacy of these models.

To ensure a robust DNA damage response and coordinate repair processing, it appears that more than one pathway contributes to the localization of FANCJ. As illustrated above, FANCJ recruitment to ICLs or DSBs is determined by proteins that either interact directly with FANCJ or operate in the same pathway. Indeed, both nucleotide excision repair (NER) and MMR proteins promote the localization of the FANCJ to sites of UV-induced lesions (Guillemette et al., 2014; **Figure 6**). MMR proteins initially recruit FANCJ. However, the further accumulation of FANCJ requires dual incision by the NER endonucleases XPF and XPG (Guillemette et al., 2014). Conceivably, the NER-dependent incision provides an ideal substrate for FANCJ at the lesion site. The combined MMR and NER localization of FANCJ ensures an S-phase checkpoint, lesion repair, and the suppression of UV-induced mutations. Supporting that multiple pathways contribute to high fidelity repair after UV irradiation, similar to skin tumors from XP patients (Dumaz et al., 1993), MMR-deficient (Borgdorff et al., 2006) and FANCJ-deficient (Guillemette et al., 2014) cells display an elevated frequency of UV-induced C > T point mutations. Moreover, along with NER genes, FANCJ and MMR genes

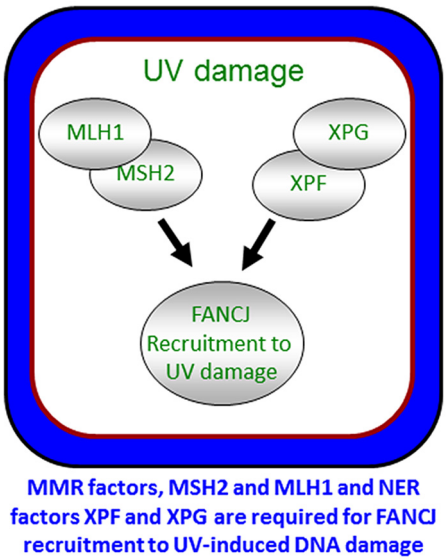


FIGURE 6 | Localization of FANCJ to UV-induced DNA damage. FANCJ recruitment to UV-induced DNA damage is dependent on the mismatch repair factors MLH1 and MSH2, and NER factors XPF and XPG. See reference Guillemette et al. (2014) and text for details.

are mutated in melanoma (Guillemette et al., 2014). The NER and FA-associated XPF protein promotes RPA phosphorylation in S-phase cells (Bomgarden et al., 2006). Given that XPF promotes FANCJ accumulation in S-phase cells, it follows that FANCJ also functions to promote RPA phosphorylation throughout S-phase. This function could be shared by FANCJ partners, such as BLM or the FA pathway, explaining its link to the UV response and checkpoints that limit genomic instability (Suhasini et al., 2011; Kelsall et al., 2012; Nalepa et al., 2013; Singh et al., 2013).

ROLE OF FANCJ AND THE FA PATHWAY IN THE BROADER CONTEXT BEYOND DNA REPAIR

Much emphasis has been placed on the impact of ICLs on actively dividing cells and role of the FA pathway in repairing such lesions encountered by the replication for. ICLs may arise naturally or be induced exogenously by chemicals used in chemotherapy. It is important to note that ICLs may have detrimental effects in non-dividing cells, given that they would interfere with other processes such as transcription. This has become a topic of increased interest, particularly because the processes of DNA repair and transcription are both highly important and inter-related. Human diseases with inherited defects in classic DNA repair genes (e.g., nucleotide excision repair) often display transcriptional deficiencies which are likely to culminate in pleiotropic symptoms including developmental abnormalities and features of premature aging [for review, see (Kamileri et al., 2012a)]. For example, the XPF-ERCC1 nuclease responsible for ICL unhooking in the FA pathway is recruited to active promoters and implicated in chromatin modifications that influence transcriptional activation (Le May et al., 2010; Kamileri et al., 2012b). Alterations to chromatin packaging due to mutations in DNA repair genes are suspected to underlie the phenotypic defects that contribute to developmental disorders that extend beyond DNA repair and maintenance of genomic stability. The connection of ICL accumulation to perturbed transcriptional regulation in an ERCC1- defective model of a human progeroid syndrome characterized by loss of fat tissue suggests how DNA damage can inflict detrimental effects in non-dividing cells (Karakasilioti et al., 2013). Defects in the FA pathway fall into this class of diseases as the clinical symptoms include not only cancer and hematologic abnormalities but often a range of congenital issues that can include skeletal defects and short stature, as well as renal dysfunction, abnormal pigmentation, and osteoporosis (Kee and D'Andrea, 2012). As mentioned above, FANCD2 is proposed to control FANCJ's localization to chromatin and its involvement in downstream events of the FA pathway (Chen et al., 2014). Therefore, it is of great importance to ascertain the probable dependent and independent roles of FANCJ and the FA pathway in transcriptional regulation. In terms of FANCJ, a leading hypothesis builds from its role as a G4 resolving enzyme that may target predicted G-quadruplexes (G4s) found near promoter elements believed to regulate transcription initiation (see below).

INVOLVEMENT OF FANCJ DURING REPLICATION STRESS

Replication stress can be a source of genomic instability. Cellular data have implicated FANCJ in helping to cope with replication stress induced exogenously by chemical exposure. Human cells mutated in FANCJ or acutely depleted of FANCJ by RNA interference are sensitive to DNA cross-linking agents that block fork progression (Bridge et al., 2005; Litman et al., 2005; Peng et al., 2007), or HU that causes fork stalling (Suhasini et al., 2011). Even in the absence of agents that exogenously induce replication, a role for FANCJ helicase activity to insure timely progression through S phase has been demonstrated (Kumaraswamy and Shiekhattar, 2007). FANCJ is implicated in intra-S phase checkpoint signaling through its interaction with TopBP1, which allows for activation of ataxia telangiectasia and Rad3-related (ATR), a requirement for checkpoint kinase 1 (CHK1; Gong et al., 2010). However, it is still unclear what the function(s) of FANCJ may be to influence events associated with fork stalling. When the replisome encounters a replication-blocking lesion or stalls due to replication stress induced by a small molecule that impedes the replicative helicase or depletes the nucleotide pool, the nascent leading and lagging strands can anneal to each other to form a Holliday Junction-like chicken foot DNA structure [for review, see (Atkinson and McGlynn, 2009)]. This process is known as fork regression or fork reversal, and provides a mechanism for repair machinery to gain access to the lesion as well as stabilize the fork. Whereas certain RecQ helicases (e.g., WRN, BLM; Machwe et al., 2006; Ralf et al., 2006) were shown to support fork regression, FANCJ is not directly implicated in this process. However, as a 5′–3′ DNA helicase, FANCJ may collaborate with a 3′–5′ helicase (e.g., WRN, BLM) to promote fork regression. Given that FANCJ has already been demonstrated to interact with BLM (Suhasini et al., 2011), the FANCJ–BLM partnership may contribute to concerted fork regression by opposite polarity helicases.

Aside from its proposed role in DNA end processing to initiate HR repair (previous section), FANCJ may help to process

DNA structures associated with stalled replication forks or regulate such processing events. The interaction between FANCJ and MLH1 appears to be critical for cells to recover from replication stress induced by ICLs or the DNA polymerase inhibitor, aphidicolin. As mentioned above, deletion of MSH2 suppresses replication restart defects in cells lacking the FANCJ–MLH1 interaction (Peng et al., 2014). Thus, to restart stalled replication forks, FANCJ through its MLH1 interaction could normally unwind and eliminate DNA structures bound by MSH2 or displace MSH2 from such structures. Mre11 has been implicated in the restart of stalled replication forks (Bryant et al., 2009; Hashimoto et al., 2010). As proposed for the tumor suppressor and HR factor BRCA2 as well as the DNA damage sensor poly (ADP-ribose) polymerase 1 (PARP1; Schlacher et al., 2011; Ying et al., 2012), FANCJ may help to prevent uncontrolled MRE11-dependent degradation of stalled replication forks by inhibiting its nuclease activity. Such an anti-nuclease role may aid in fork stabilization without excessive single-stranded DNA production to allow for regulated checkpoint signal transduction and effective repair of the replication-blocking lesion.

FANCJ RESOLVES G-QUADRUPLEX DNA TO ENABLE SMOOTH REPLICATION AND PRESERVE GENOMIC INTEGRITY

G-quadruplexes, composed of planar stacks of four guanine residues engaged in Hoogsteen hydrogen bonding, are now believed to form *in vivo* and exert biological effects on DNA replication and transcription, and play unique structural roles in telomere capping (Wu and Brosh, 2010a; Bochman et al., 2012; Maizels and Gray, 2013). FANCJ, and certain other DNA helicases (e.g., PIF1, WRN, BLM), resolve G4 DNA structures *in vitro*; however, the biological significance of G4 resolution by the various human DNA helicases *in vivo* is less clear. Human cells deficient in FANCJ, but not FANCA or FANCD2, were found to be sensitive to the G4 binding drug TMS, suggesting a role of the helicase to preserve genomic stability at G4-forming sequences that is independent of the classic FA DNA repair pathway (Wu et al., 2008); furthermore, a FA-J human patient cell line was found to accumulate deletions at predicted G4-forming sequences in the genome (London et al., 2008). Although a number of DNA helicases have been shown to unwind intermolecular G4 structures, FANCJ is distinct among the Fe-S cluster helicases tested in its ability to resolve entropically favored intramolecular G4 substrates (Bharti et al., 2013). Consistent with the biochemical results, a deficiency in FANCJ, but not the Fe–S helicases DDX11 or XPD, sensitized human cells to TMS as measured by induction of the DNA damage marker γ-H2AX (Bharti et al., 2013). These findings suggest that FANCJ has a specialized function among the Fe–S helicases to facilitate smooth replication of genomic regions prone to form G4s in order to prevent fork breakage (**Figure 7**). A recent study using Xenopus egg extract and single-stranded plasmid DNA template with a predicted G4-forming sequence validated the role of FANCJ to promote DNA synthesis through G4 structures (Castillo et al., 2014). It will be of interest to determine if a putative role of FANCJ at telomeres involves its ability to resolve the G-rich telomeric tail and influence telomere capping events.

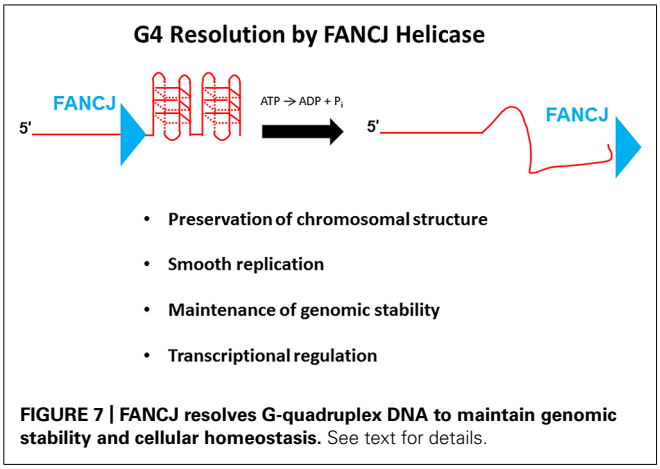


FIGURE 7 | FANCJ resolves G-quadruplex DNA to maintain genomic stability and cellular homeostasis. See text for details.

Recent advances using the chicken DT40 cell lines have improved our understanding of the role of FANCJ in DNA repair and G4 DNA metabolism. Human FANCJ can rescue the sensitivity of chicken brip1/fancj mutant cells to agents that induce DNA cross-links (Bridge et al., 2005) or stabilize G4 structures (Wu et al., 2008), indicating a conservation of function in the vertebrate species. In addition to its role in protecting brip1 cells from G4-associated genomic instability, the chicken ortholog of FANCJ serves a more general protective role in chromosomal maintenance that appears to operate outside the FA pathway (Kitao et al., 2011; **Figure 7**). In subsequent work, it was shown that FANCJ, together with the RecQ helicases WRN and BLM, preserve epigenetic stability by helping to efficiently couple histone recycling with replication fork progression through their ability to resolve G4s that impede smooth DNA synthesis (Sarkies et al., 2012). FANCJ seems to be a central player in the maintenance of epigenetic stability by collaborating as well with the REV1 translesion polymerase at G-rich sequences predicted to form quadruplexes. The precise molecular mechanisms of how these processes occur are still not well understood, but the model proposed by the Sale group predicts that FANCJ with its opposite polarity of directional movement may initiate its action on the opposite side of G4 structure(s) as that of the 3′–5′ RecQ heli or the TLS polymerase REV1 (Sarkies et al., 2012).

To examine the role of FANCJ in cellular DNA replication, the Niedzwiedz lab employed a DNA fiber analysis in isogenic fancj null and WT DT40 cells and determined that FANCJ helps to promote replication fork progression at regions prone to stalling, such as G4-forming sequences (Schwab et al., 2013). Furthermore, stabilization of G4 structures in FANCJ-deficient cells by the G4 binder TMS quenched fork progression, resulting in uncoupling of leading and lagging strand synthesis. The ability of FANCJ to enable smooth replication fork progression would help to maintain normal chromatin structure by preventing its condensation and reorganization.

FANCJ-deficient DT40 cells exposed to the G4 ligand TMS displayed stronger staining by a murine monoclonal antibody specific for binding G4 DNA compared to untreated fancj null cells, or normal cells exposed to TMS (Henderson et al., 2013). Enrichment of G4 DNA in FANCJ-deficient cells exposed to TMS

provided the first evidence that genetic and environmental conditions can synergize in the accumulation of G4 DNA *in vivo*. The emerging evidence for a role of FANCJ in replication of G4 motifs in avian cells supports the hypothesis for a conserved role of FANCJ in humans. Nonetheless, there are likely additional functions of FANCJ G4 resolving activity *in vivo*, including the control of gene expression (**Figure 7**); however, this hypothesis remains to be formally tested in human cells. It is well known that G-rich sequences predicted to form G4 are enriched in promoter regions especially at transcriptional start sites (Huppert and Balasubramanian, 2007), and a correlation exists between the presence of predicted G4 and promoter-proximal transcriptional pausing (Eddy et al., 2011). Although a definitive role of G4 resolution by FANCJ to control gene expression has not yet been elucidated, other DNA helicases including XPB, XPD (Gray et al., 2014), BLM (Nguyen et al., 2014), and RECQ1 (Li et al., 2014) bind G4 structures and regulate expression of genes characterized by the presence of G4 DNA motifs. It is unclear if differential roles in transcriptional regulation exist for G4 binding versus resolution by a DNA helicase, given that certain helicases [e.g., RECQ1 (Popuri et al., 2008; Wu et al., 2008) and XPB (Gray et al., 2014)] bind but poorly unwind G4 DNA *in vitro*; furthermore, it is yet unclear if human XPD resolves G4 DNA because different results were obtained from *in vitro* studies using XPD proteins from two distinct thermophilic species (Bharti et al., 2013; Gray et al., 2014). Given the evidence that FANCJ and its homologs play an important role in G4 metabolism, it seems likely that FANCJ unwinding of G4s will influence gene expression of proto-oncogenes where G4 motifs are prominent (Eddy and Maizels, 2006; Duquette et al., 2007; Brown et al., 2011). Further studies are needed to establish direct links and meaningful relationships between disease pathogenesis and regulated expression of messenger RNA and microRNA molecules by FANCJ and other DNA helicases.

FANCJ AS A POTENTIAL TARGET FOR CLINICAL AND PHARMACEUTICAL TREATMENT

Our group and others have been keenly interested in the prospect of helicase-based biomarkers and targeting DNA helicases like FANCJ to enhance existing or developing therapeutic strategies for treating cancers (Brosh, 2013). This prospect has been fueled by observations that the expression of many DNA damage response genes is up-regulated in rapidly proliferating cells and tumors, leading to their resistance to chemotherapy drugs or radiation used to combat cancer. In two recent studies, the influence of FANCJ expression on sensitivity of cancer cells or tissues to chemotherapy drugs was determined. In the first, Nakanishi et al. (2012) found that FANCJ expression in tumor tissues was elevated compared to normal epithelial tissue, which correlated with resistance to the chemotherapy drug 5-fluorouracil (5-FU) in tumors with normal MLH1 expression. Mori et al. (2013) determined that gastric cancer cells exposed to 5-FU down-regulated FANCJ expression, leading to their enhanced sensitivity to the ICL-inducing agent oxaliplatin. Such observations raise the exciting possibility that FANCJ is differentially expressed in tumors which may serve as a useful predictive biomarker for designing treatment strategies tailored to the cancer type.

From a related perspective, the development of small molecules that target FANCJ for helicase inhibition may provide a means to achieve synthetic lethality with chemotherapy drugs or in a defined genetic background to kill cancer cells, provided that a therapeutic window is achieved. Our work on the discovery of a WRN helicase inhibitor provided a proof-of-principle for the helicase-targeted approach to induce chemical or genetic synthetic lethality of cancer cells (Aggarwal et al., 2011, 2013a,b). The observation that a BLM helicase inhibitor can cause elevated sister chromatid exchange in cultured cells is provocative (Nguyen et al., 2013), and sets the stage to search for FANCJ inhibitors, given FANCJ's interaction with BLM and the finding that acute depletion of FANCJ causes elevated sister chromatid exchange (Suhasini et al., 2011). On the opposite side of the spectrum, it might also be useful to conduct small molecule screens to identify compounds that restore the function of a misfolded helicase protein caused by a disease-linked mutation. Given that a number of missense mutations in FANCJ are linked to FA or associated with cancer, FANCJ may be a good candidate protein to target for intervention.

Lastly, the function of FANCJ in G4 DNA metabolism suggests an avenue to explore. G4 structures which form at telomeres or the promoters of proto-oncogenes most likely play a prominent role in the ability of cancer cells to thrive or senesce. Indeed, telomerase inhibitors which prevent the elongation of the G-rich telomere tails at chromosome ends are in clinical trials (Buseman et al., 2012). If G4 structures in cancer cells are stabilized by G4 ligands directly or by blocking telomeric G4 unwinding with small molecules against G4 resolving helicases such as FANCJ or PIF1, then cancer cells may be caused to senesce and ultimately targeted for elimination. It seems probable that further studies will address the efficacy of targeting FANCJ and other DNA helicases as a possible strategy in cancer therapy.

SUMMARY

In this review, we have attempted to provide a comprehensive view of what now appears to be multi-faceted roles of FANCJ in cellular DNA metabolism. Discovered 13 years ago as a BRCA1-interacting protein, FANCJ helicase is now regarded as a *bona fide* tumor suppressor and genetically linked to the cancer-prone disorder FA characterized by progressive bone marrow failure; however, the precise function(s) of FANCJ in the FA pathway of ICL repair is still not well understood. In addition, cellular and biochemical studies have demonstrated an important role of FANCJ to resolve G4 DNA structures that potentially interfere with normal chromosome packaging, replication, and transcription. FANCJ interacts with a number of DNA damage response proteins and appears to be a key player in maintaining chromosomal stability through its involvement in DNA repair and checkpoint signaling. With FANCJ's emergence as a uniquely important genome caretaker, future studies may explore FANCJ as a potential target for clinical and therapeutic strategies. In spite of the tremendous progress, much still remains to be learned about FANCJ and avenues for biomedical advances.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute on Aging, and

DNA structures associated with stalled replication forks or regulate such processing events. The interaction between FANCJ and MLH1 appears to be critical for cells to recover from replication stress induced by ICLs or the DNA polymerase inhibitor, aphidicolin. As mentioned above, deletion of MSH2 suppresses replication restart defects in cells lacking the FANCJ–MLH1 interaction (Peng et al., 2014). Thus, to restart stalled replication forks, FANCJ through its MLH1 interaction could normally unwind and eliminate DNA structures bound by MSH2 or displace MSH2 from such structures. Mre11 has been implicated in the restart of stalled replication forks (Bryant et al., 2009; Hashimoto et al., 2010). As proposed for the tumor suppressor and HR factor BRCA2 as well as the DNA damage sensor poly (ADP-ribose) polymerase 1 (PARP1; Schlacher et al., 2011; Ying et al., 2012), FANCJ may help to prevent uncontrolled MRE11-dependent degradation of stalled replication forks by inhibiting its nuclease activity. Such an anti-nuclease role may aid in fork stabilization without excessive single-stranded DNA production to allow for regulated checkpoint signal transduction and effective repair of the replication-blocking lesion.

FANCJ RESOLVES G-QUADRUPLEX DNA TO ENABLE SMOOTH REPLICATION AND PRESERVE GENOMIC INTEGRITY

G-quadruplexes, composed of planar stacks of four guanine residues engaged in Hoogsteen hydrogen bonding, are now believed to form *in vivo* and exert biological effects on DNA replication and transcription, and play unique structural roles in telomere capping (Wu and Brosh, 2010a; Bochman et al., 2012; Maizels and Gray, 2013). FANCJ, and certain other DNA helicases (e.g., PIF1, WRN, BLM), resolve G4 DNA structures *in vitro*; however, the biological significance of G4 resolution by the various human DNA helicases *in vivo* is less clear. Human cells deficient in FANCJ, but not FANCA or FANCD2, were found to be sensitive to the G4 binding drug TMS, suggesting a role of the helicase to preserve genomic stability at G4-forming sequences that is independent of the classic FA DNA repair pathway (Wu et al., 2008); furthermore, a FA-J human patient cell line was found to accumulate deletions at predicted G4-forming sequences in the genome (London et al., 2008). Although a number of DNA helicases have been shown to unwind intermolecular G4 structures, FANCJ is distinct among the Fe-S cluster helicases tested in its ability to resolve entropically favored intramolecular G4 substrates (Bharti et al., 2013). Consistent with the biochemical results, a deficiency in FANCJ, but not the Fe–S helicases DDX11 or XPD, sensitized human cells to TMS as measured by induction of the DNA damage marker γ-H2AX (Bharti et al., 2013). These findings suggest that FANCJ has a specialized function among the Fe–S helicases to facilitate smooth replication of genomic regions prone to form G4s in order to prevent fork breakage (**Figure 7**). A recent study using Xenopus egg extract and single-stranded plasmid DNA template with a predicted G4-forming sequence validated the role of FANCJ to promote DNA synthesis through G4 structures (Castillo et al., 2014). It will be of interest to determine if a putative role of FANCJ at telomeres involves its ability to resolve the G-rich telomeric tail and influence telomere capping events.

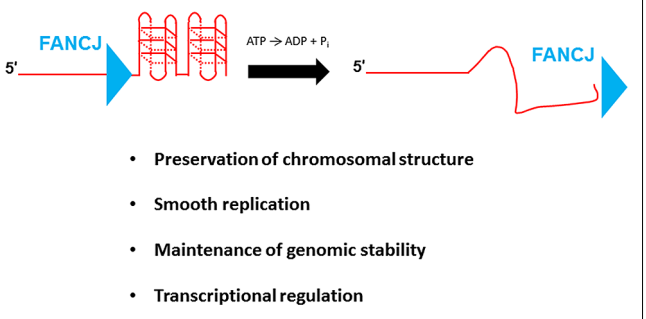


FIGURE 7 | FANCJ resolves G-quadruplex DNA to maintain genomic stability and cellular homeostasis. See text for details.

Recent advances using the chicken DT40 cell lines have improved our understanding of the role of FANCJ in DNA repair and G4 DNA metabolism. Human FANCJ can rescue the sensitivity of chicken brip1/fancj mutant cells to agents that induce DNA cross-links (Bridge et al., 2005) or stabilize G4 structures (Wu et al., 2008), indicating a conservation of function in the vertebrate species. In addition to its role in protecting brip1 cells from G4-associated genomic instability, the chicken ortholog of FANCJ serves a more general protective role in chromosomal maintenance that appears to operate outside the FA pathway (Kitao et al., 2011; **Figure 7**). In subsequent work, it was shown that FANCJ, together with the RecQ helicases WRN and BLM, preserve epigenetic stability by helping to efficiently couple histone recycling with replication fork progression through their ability to resolve G4s that impede smooth DNA synthesis (Sarkies et al., 2012). FANCJ seems to be a central player in the maintenance of epigenetic stability by collaborating as well with the REV1 translesion polymerase at G-rich sequences predicted to form quadruplexes. The precise molecular mechanisms of how these processes occur are still not well understood, but the model proposed by the Sale group predicts that FANCJ with its opposite polarity of directional movement may initiate its action on the opposite side of G4 structure(s) as that of the 3′–5′ RecQ heli or the TLS polymerase REV1 (Sarkies et al., 2012).

To examine the role of FANCJ in cellular DNA replication, the Niedzwiedz lab employed a DNA fiber analysis in isogenic fancj null and WT DT40 cells and determined that FANCJ helps to promote replication fork progression at regions prone to stalling, such as G4-forming sequences (Schwab et al., 2013). Furthermore, stabilization of G4 structures in FANCJ-deficient cells by the G4 binder TMS quenched fork progression, resulting in uncoupling of leading and lagging strand synthesis. The ability of FANCJ to enable smooth replication fork progression would help to maintain normal chromatin structure by preventing its condensation and reorganization.

FANCJ-deficient DT40 cells exposed to the G4 ligand TMS displayed stronger staining by a murine monoclonal antibody specific for binding G4 DNA compared to untreated fancj null cells, or normal cells exposed to TMS (Henderson et al., 2013). Enrichment of G4 DNA in FANCJ-deficient cells exposed to TMS

Huppert, J. L., and Balasubramanian, S. (2007). G-quadruplexes in promoters throughout the human genome. *Nucleic Acids Res.* 35, 406–413. doi: 10.1093/nar/gkl1057

Jones, G. D., Le Pla, R. C., and Farmer, P. B. (2010). Phosphotriester adducts (PTEs): DNA's overlooked lesion. *Mutagenesis* 25, 3–16. doi: 10.1093/mutage/gep038

Kamileri, I., Karakasilioti, I., and Garinis, G. A. (2012a). Nucleotide excision repair: new tricks with old bricks. *Trends Genet.* 28, 566–573. doi: 10.1016/j.tig.2012.06.004

Kamileri, I., Karakasilioti, I., Sideri, A., Kosteas, T., Tatarakis, A., Talianidis, I., et al. (2012b). Defective transcription initiation causes postnatal growth failure in a mouse model of nucleotide excision repair (NER) progeria. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2995–3000. doi: 10.1073/pnas.1114941109

Karakasilioti, I., Kamileri, I., Chatzinikolaou, G., Kosteas, T., Vergadi, E., Robinson, A. R., et al. (2013). DNA damage triggers a chronic autoinflammatory response, leading to fat depletion in NER progeria. *Cell Metab.* 18, 403–415. doi: 10.1016/j.cmet.2013.08.011

Kee, Y., and D'Andrea, A. D. (2012). Molecular pathogenesis and clinical management of fanconi anemia. *J. Clin. Invest.* 122, 3799–3806. doi: 10.1172/JCI58321

Kelsall, I. R., Langenick, J., MacKay, C., Patel, K. J., and Alpi, A. F. (2012). The fanconi anaemia components UBE2T and FANCM are functionally linked to nucleotide excision repair. *PLoS ONE* 7:e36970. doi: 10.1371/journal.pone.0036970

Kitao, H., Nanda, I., Sugino, R. P., Kinomura, A., Yamazoe, M., Arakawa, H., et al. (2011). FancJ/Brip1 helicase protects against genomic losses and gains in vertebrate cells. *Genes Cells* 16, 714–727. doi: 10.1111/j.1365-2443.2011.01523.x

Kottemann, M. C., and Smogorzewska, A. (2013). Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature* 493, 356–363. doi: 10.1038/nature11863

Kumaraswamy, E., and Shiekhattar, R. (2007). Activation of BRCA1/BRCA2-associated helicase BACH1 is required for timely progression through S phase. *Mol. Cell Biol.* 27, 6733–6741. doi: 10.1128/MCB.00961-07

Kuper, J., Wolski, S. C., Michels, G., and Kisker, C. (2011). Functional and structural studies of the nucleotide excision repair helicase XPD suggest a polarity for DNA translocation. *EMBO J.* 31, 494–502. doi: 10.1038/emboj.2011.374

Le May, N., Mota-Fernandes, D., Velez-Cruz, R., Iltis, I., Biard, D., and Egly, J. M. (2010). NER factors are recruited to active promoters and facilitate chromatin modification for transcription in the absence of exogenous genotoxic attack. *Mol. Cell* 38, 54–66. doi: 10.1016/j.molcel.2010.03.004

Levitus, M., Waisfisz, Q., Godthelp, B. C., de Vries, Y., Hussain, S., Wiegant, W. W., et al. (2005). The DNA helicase BRIP1 is defective in fanconi anemia complementation group *J. Nat. Genet.* 37, 934–935. doi: 10.1038/ng1625

Levran, O., Attwooll, C., Henry, R. T., Milton, K. L., Neveling, K., Rio, P., et al. (2005). The BRCA1-interacting helicase BRIP1 is deficient in fanconi anemia. *Nat. Genet.* 37, 931–933. doi: 10.1038/ng1624

Li, X. L., Lu, X., Parvathaneni, S., Bilke, S., Zhang, H., Thangavel, S., et al. (2014). Identification of RECQ1-regulated transcriptome uncovers a role of RECQ1 in regulation of cancer cell migration and invasion. *Cell Cycle* doi: 10.4161/cc.29419 [Epub ahead of print].

Litman, R., Peng, M., Jin, Z., Zhang, F., Zhang, J., Powell, S., et al. (2005). BACH1 is critical for homologous recombination and appears to be the fanconi anemia gene product FANCJ. *Cancer Cell* 8, 255–265. doi: 10.1016/j.ccr.2005.08.004

London, T. B., Barber, L. J., Mosedale, G., Kelly, G. P., Balasubramanian, S., Hickson, I. D., et al. (2008). FANCJ is a structure-specific DNA helicase associated with the maintenance of genomic G/C tracts. *J. Biol. Chem.* 283, 36132–36139. doi: 10.1074/jbc.M808152200

Machwe, A., Xiao, L., Groden, J., and Orren, D. K. (2006). The Werner and Bloom syndrome proteins catalyze regression of a model replication fork. *Biochemistry* 45, 13939–13946. doi: 10.1021/bi0615487

Maizels, N., and Gray, L. T. (2013). The G4 genome. *PLoS Genet* 9:e1003468. doi: 10.1371/journal.pgen.1003468

Makharashvili, N., Tubbs, A. T., Yang, S. H., Wang, H., Barton, O., Zhou, Y., et al. (2014). Catalytic and noncatalytic roles of the CtIP endonuclease in double-strand break end resection. *Mol. Cell* 54, 1022–1033. doi: 10.1016/j.molcel.2014.04.011

Mori, R., Yoshida, K., Tanahashi, T., Yawata, K., Kato, J., Okumura, N., et al. (2013). Decreased FANCJ caused by 5FU contributes to the increased sensitivity to oxaliplatin in gastric cancer cells. *Gastric Cancer* 16, 345–354. doi: 10.1007/s10120-012-0191-0

Mui, T. P., Fuss, J. O., Ishida, J. P., Tainer, J. A., and Barton, J. K. (2011). ATP-stimulated, DNA-mediated redox signaling by XPD, a DNA repair and transcription helicase. *J. Am. Chem. Soc.* 133, 16378–16381. doi: 10.1021/ja207222t

Nakanishi, R., Kitao, H., Fujinaka, Y., Yamashita, N., Iimori, M., Tokunaga, E., et al. (2012). FANCJ expression predicts the response to 5-fluorouracil-based chemotherapy in MLH1-proficient colorectal cancer. *Ann. Surg. Oncol.* 19, 3627–3635. doi: 10.1245/s10434-012-2349-8

Nalepa, G., and Clapp, D. W. (2014). Fanconi anemia and the cell cycle: new perspectives on aneuploidy. *F1000 Prime Rep.* 6, 23. doi: 10.12703/P6-23

Nalepa, G., Enzor, R., Sun, Z., Marchal, C., Park, S. J., Yang, Y., et al. (2013). Fanconi anemia signaling network regulates the spindle assembly checkpoint. *J. Clin. Invest.* 123, 3839–3847. doi: 10.1172/JCI67364

Nguyen, G. H., Dexheimer, T. S., Rosenthal, A. S., Chu, W. K., Singh, D. K., Mosedale, G., et al. (2013). A small molecule inhibitor of the BLM helicase modulates chromosome stability in human cells. *Chem. Biol.* 20, 55–62. doi: 10.1016/j.chembiol.2012.10.016

Nguyen, G. H., Tang, W., Robles, A. I., Beyer, R. P., Gray, L. T., Welsh, J. A., et al. (2014). Regulation of gene expression by the BLM helicase correlates with the presence of G-quadruplex DNA motifs. *Proc. Natl. Acad. Sci. U.S.A.* 111, 9905–9910. doi: 10.1073/pnas.1404807111

Park, J. Y., Zhang, F., and Andreassen, P. R. (2014). PALB2: the hub of a network of tumor suppressors involved in DNA damage responses. *Biochim. Biophys. Acta* 1846, 263–275. doi: 10.1016/j.bbcan.2014.06.003

Pauty, J., Rodrigue, A., Couturier, A., Buisson, R., and Masson, J. Y. (2014). Exploring the roles of PALB2 at the crossroads of DNA repair and cancer. *Biochem. J.* 460, 331–342. doi: 10.1042/BJ20140208

Peng, M., Litman, R., Jin, Z., Fong, G., and Cantor, S. B. (2006). BACH1 is a DNA repair protein supporting BRCA1 damage response. *Oncogene* 25, 2245–2253. doi: 10.1038/sj.onc.1209257

Peng, M., Litman, R., Xie, J., Sharma, S., Brosh, R. M. Jr., and Cantor, S. B. (2007). The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. *EMBO J.* 26, 3238–3249. doi: 10.1038/sj.emboj.7601754

Peng, M., Xie, J., Ucher, A., Stavnezer, J., and Cantor, S. B. (2014). Crosstalk between BRCA-fanconi anemia and mismatch repair pathways prevents MSH2-dependent aberrant DNA damage responses. *EMBO J.* 33, 1698–1712. doi: 10.15252/embj.201387530

Pickering, A., Zhang, J., Panneerselvam, J., and Fei, P. (2013). Advances in the understanding of the fanconi anemia tumor suppressor pathway. *Cancer Biol. Ther.* 14, 1089–1091. doi: 10.4161/cbt.26380

Polato, F., Callen, E., Wong, N., Faryabi, R., Bunting, S., Chen, H.T., et al. (2014). CtIP-mediated resection is essential for viability and can operate independently of BRCA1. *J. Exp. Med.* 211, 1027–1036. doi: 10.1084/jem.20131939

Popuri, V., Bachrati, C. Z., Muzzolini, L., Mosedale, G., Costantini, S., Giacomini, E., et al. (2008). The Human RecQ helicases, BLM and RECQ1, display distinct DNA substrate specificities. *J. Biol. Chem.* 283, 17766–17776. doi: 10.1074/jbc.M709749200

Rafnar, T., Gudbjartsson, D. F., Sulem, P., Jonasdottir, A., Sigurdsson, A., Jonasdottir, A., et al. (2011). Mutations in BRIP1 confer high risk of ovarian cancer. *Nat. Genet.* 43, 1104–1107. doi: 10.1038/ng.955

Ralf, C., Hickson, I. D., and Wu, L. (2006). The Bloom's syndrome helicase can promote the regression of a model replication fork. *J. Biol. Chem.* 281, 22839–22846. doi: 10.1074/jbc.M604268200

Ren, B., Duan, X., and Ding, H. (2009). Redox control of the DNA damage-inducible protein DinG helicase activity via its iron-sulfur cluster. *J. Biol. Chem.* 284, 4829–4835. doi: 10.1074/jbc.M807943200

Rudolf, J., Makrantoni, V., Ingledew, W. J., Stark, M. J., and White, M. F. (2006). The DNA repair helicases XPD and FancJ have essential iron-sulfur domains. *Mol. Cell* 23, 801–808. doi: 10.1016/j.molcel.2006.07.019

Sarkies, P., Murat, P., Phillips, L. G., Patel, K. J., Balasubramanian, S., and Sale, J. E. (2012). FANCJ coordinates two pathways that maintain epigenetic stability at G-quadruplex DNA. *Nucleic Acids Res.* 40, 1485–1498. doi: 10.1093/nar/gkr868

Schlacher, K., Christ, N., Siaud, N., Egashira, A., Wu, H., and Jasin, M. (2011). Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell* 145, 529–542. doi: 10.1016/j.cell.2011.03.041

Schwab, R. A., Nieminuszczy, J., Shin-Ya, K., and Niedzwiedz, W. (2013). FANCJ couples replication past natural fork barriers with maintenance of chromatin structure. *J. Cell Biol.* 201, 33–48. doi: 10.1083/jcb.201208009

Seal, S., Thompson, D., Renwick, A., Elliott, A., Kelly, P., Barfoot, R., et al. (2006). Truncating mutations in the fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nat. Genet.* 38, 1239–1241. doi: 10.1038/ng1902

Shiozaki, E. N., Gu, L., Yan, N., and Shi, Y. (2004). Structure of the BRCT repeats of BRCA1 bound to a BACH1 phosphopeptide: implications for signaling. *Mol. Cell* 14, 405–412. doi: 10.1016/S1097-2765(04)00238-2

Singh,T. R., Ali, A. M., Paramasivam, M., Pradhan, A., Wahengbam, K., Seidman, M. M., et al. (2013). ATR-dependent phosphorylation of FANCM at serine 1045 is essential for FANCM functions. *Cancer Res.* 73, 4300–4310. doi: 10.1158/0008-5472.CAN-12-3976

Sommers, J. A., Banerjee, T., Hinds, T., Wan, B., Wold, M. S., Lei, M., et al. (2014). Novel function of the fanconi anemia group J or RECQ1 helicase to disrupt protein-DNA complexes in a replication protein a-stimulated manner. *J. Biol. Chem.* 289, 19928–19941. doi: 10.1074/jbc.M113.542456

Sommers, J. A., Rawtani, N., Gupta, R., Bugreev, D. V., Mazin, A. V., Cantor, S. B., et al. (2009). FANCJ uses its motor ATPase to disrupt protein-DNA complexes, unwind triplexes, and inhibit Rad51 strand exchange. *J. Biol. Chem.* 284, 7505–7517. doi: 10.1074/jbc.M809019200

Sontz, P. A., Mui, T. P., Fuss, J. O., Tainer, J. A., and Barton, J. K. (2012). DNA charge transport as a first step in coordinating the detection of lesions by repair proteins. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1856–1861. doi: 10.1073/pnas.1120063109

Suhasini, A. N., and Brosh, R. M. Jr. (2010). Mechanistic and biological aspects of helicase action on damaged DNA. *Cell Cycle* 9, 2317–2329. doi: 10.4161/cc.9.12.11902

Suhasini, A. N., and Brosh, R. M. Jr. (2012). Fanconi anemia and bloom's syndrome crosstalk through FANCJ-BLM helicase interaction. *Trends Genet.* 28, 7–13. doi: 10.1016/j.tig.2011.09.003

Suhasini, A. N., and Brosh, R. M. Jr. (2013). Disease-causing missense mutations in human DNA helicase disorders. *Mutat. Res.* 752, 138–152. doi: 10.1016/j.mrrev.2012.12.004

Suhasini, A. N., Rawtani, N. A., Wu, Y., Sommers, J. A., Sharma, S., Mosedale, G., et al. (2011). Interaction between the helicases genetically linked to fanconi anemia group J and Bloom's syndrome. *EMBO J.* 30, 692–705. doi: 10.1038/emboj.2010.362

Suhasini, A. N., Sommers, J. A., Mason, A. C., Voloshin, O. N., Camerini-Otero, R. D., Wold, M. S., et al. (2009). FANCJ helicase uniquely senses oxidative base damage in either strand of duplex DNA and is stimulated by replication protein a to unwind the damaged DNA substrate in a strand-specific manner. *J. Biol. Chem.* 284, 18458–18470. doi: 10.1074/jbc.M109.012229

Suhasini, A. N., Sommers, J. A., Muniandy, P. A., Coulombe, Y., Cantor, S. B., Masson, J. Y., et al. (2013). Fanconi anemia group J helicase and MRE11 nuclease interact to facilitate the DNA damage response. *Mol. Cell Biol.* 33, 2212–2227. doi: 10.1128/MCB.01256-12

Suhasini, A. N., Sommers, J. A., Yu, S., Wu, Y., Xu, T., Kelman, Z., et al. (2012). DNA repair and replication fork helicases are differentially affected by alkyl phosphotriester lesion. *J. Biol. Chem.* 287, 19188–19198. doi: 10.1074/jbc.M112.352757

Tanner, N. K., Cordin, O., Banroques, J., Doere, M., and Linder, P. (2003). The Q motif: a newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. *Mol. Cell* 11, 127–138. doi: 10.1016/S1097-2765(03)00006-6

Tu, Z., Aird, K. M., Bitler, B. G., Nicodemus, J. P., Beeharry, N., Xia, B., et al. (2011). Oncogenic RAS regulates BRIP1 expression to induce dissociation of BRCA1 from chromatin, inhibit DNA repair, and promote senescence. *Dev. Cell* 21, 1077–1091. doi: 10.1016/j.devcel.2011.10.010

Walden, H., and Deans, A. J. (2014). The fanconi anemia DNA repair pathway: structural and functional insights into a complex disorder. *Annu. Rev. Biophys.* 43, 257–278. doi: 10.1146/annurev-biophys-051013-022737

Wallace, S. S. (2002). Biological consequences of free radical-damaged DNA bases. *Free Radic. Biol. Med.* 33, 1–14. doi: 10.1016/S0891-5849(02)00827-4

Wang, H., Li, Y., Truong, L. N., Shi, L. Z., Hwang, P. Y., He, J., et al. (2014). CtIP maintains stability at common fragile sites and inverted repeats by end resection-independent endonuclease activity. *Mol. Cell* 54, 1012–1021. doi: 10.1016/j.molcel.2014.04.012

Wolski, S. C., Kuper, J., Hanzelmann, P., Truglio, J. J., Croteau, D. L., Van Houten, B., et al. (2008). Crystal structure of the FeS cluster-containing nucleotide excision repair helicase XPD. *PLoS Biol.* 6:e149. doi: 10.1371/journal.pbio.0060149

Wu, Y., and Brosh, R. M. Jr. (2010a). G-quadruplex nucleic acids and human disease. *FEBS J.* 277, 3470–3488. doi: 10.1111/j.1742-4658.2010.07760.x

Wu, Y., and Brosh, R. M. Jr. (2010b). Helicase-inactivating mutations as a basis for dominant negative phenotypes. *Cell Cycle* 9, 4080–4090. doi: 10.4161/cc.9.20.13667

Wu, Y., Shin-Ya, K., and Brosh, R. M. Jr. (2008). FANCJ helicase defective in fanconia anemia and breast cancer unwinds G-Quadruplex DNA to defend genomic stability. *Mol. Cell Biol.* 28, 4116–4128. doi: 10.1128/MCB.02210-07

Wu, Y., Sommers, J. A., Loiland, J. A., Kitao, H., Kuper, J., Kisker, C., et al. (2012). The Q motif of FANCJ DNA helicase regulates its dimerization, DNA binding, and DNA repair function. *J. Biol. Chem.* 287, 21699–21716. doi: 10.1074/jbc.M112.351338

Wu, Y., Sommers, J. A., Suhasini, A. N., Leonard, T., Deakyne, J. S., Mazin, A. V., et al. (2010). Fanconi anemia group J mutation abolishes its DNA repair function by uncoupling DNA translocation from helicase activity or disruption of protein-DNA complexes. *Blood* 116, 3780–3791. doi: 10.1182/blood-2009-11-256016

Wu, Y., Suhasini, A. N., and Brosh, R. M. Jr. (2009). Welcome the family of FANCJ-like helicases to the block of genome stability maintenance proteins. *Cell Mol. Life Sci.* 66, 1209–1222. doi: 10.1007/s00018-008-8580-6

Xie, J., Litman, R., Wang, S., Peng, M., Guillemette, S., Rooney, T., et al. (2010). Targeting the FANCJ-BRCA1 interaction promotes a switch from recombination to poleta-dependent bypass. *Oncogene* 29, 2499–2508. doi: 10.1038/onc.2010.18

Xie, J., Peng, M., Guillemette, S., Quan, S., Maniatis, S., Wu, Y., et al. (2012). FANCJ/BACH1 acetylation at lysine 1249 regulates the DNA damage response. *PLoS Genet.* 8:e1002786. doi: 10.1371/journal.pgen.1002786

Ying, S., Hamdy, F. C., and Helleday, T. (2012). Mre11-dependent degradation of stalled DNA replication forks is prevented by BRCA2 and PARP1. *Cancer Res.* 72, 2814–2821. doi: 10.1158/0008-5472.CAN-11-3417

Yu, X., Chini, C. C., He, M., Mer, G., and Chen, J. (2003). The BRCT domain is a phospho-protein binding domain. *Science* 302, 639–642. doi: 10.1126/science.1088753

Zhang, F., Fan, Q., Ren, K., Auerbach, A. D., and Andreassen, P. R. (2010). FANCJ/BRIP1 recruitment and regulation of FANCD2 in DNA damage responses. *Chromosoma* 119, 637–649. doi: 10.1007/s00412-010-0285-6

Zou, J., Tian, F., Li, J., Pickner, W., Long, M., Rezvani, K., et al. (2013). FancJ regulates interstrand crosslinker induced centrosome amplification through the activation of polo-like kinase 1. *Biol. Open.* 2, 1022–1031. doi: 10.1242/bio.20135801

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 05 September 2014; paper pending published: 18 September 2014; accepted: 05 October 2014; published online: 21 October 2014.

*Citation: Brosh RM Jr. and Cantor SB (2014) Molecular and cellular functions of the FANCJ DNA helicase defective in cancer and in Fanconi anemia. Front. Genet. **5**:372. doi: 10.3389/fgene.2014.00372*

This article was submitted to Genetics of Aging, a section of the journal Frontiers in Genetics.