

Extra Views

Functional Link Between Myc and the Werner Gene in Tumorigenesis

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ABSTRACT

We have recently reported a connection between the expression of the Werner syndrome gene (*WRN*), whose loss of function has been implicated in a human progeroid syndrome (WS), and the Myc oncoprotein. Myc overexpression directly elevates transcription of the *WRN* gene, whose presence is required to avoid senescence during Myc proliferative stimuli. Here we discuss several hypotheses to explain why *WRN* might be required to support oncogenic proliferation in light of the known function of *WRN* protein and Myc in genomic instability and transcriptional modulation. In addition, we address the apparent paradox of why patients with WS, lacking *WRN* function, have increased incidence of certain cancers.

ORGANISMAL SENESCENCE AND RECQ HELICASES

Human progeroid syndromes are associated with varied age-associated phenotypes, both at the organismal and cellular level, which mimic some, but not all, aspects of accelerated aging.¹ This suggests that the aging process cannot be recapitulated entirely by a single gene mutation. Nevertheless, it is intriguing that at least two of these diseases, Werner (WS) and Rothmund-Thompson (RTS) syndromes, are the result of homozygous loss of function mutations of RecQ helicases,^{2,3} thus implicating this family of proteins in the aging process. RecQ DNA helicases have been shown to play a role in DNA-recombination and repair, a function that is conserved in yeast,⁴ mammals⁵ and bacteria^{6,7} This raises the possibility that the premature aging phenotype of these human syndromes may result from an accelerated accumulation of genetic changes, that over time impairs the cellular replication capacity. This hypothesis is consistent with the delayed accumulation of aging phenotypes observed in WS, which is manifested only after puberty in tissues and organs that require constant cell turnover such as skin, hair and reproductive organs. In contrast, tissues with a low cell turnover, such as the central nervous system, are not affected in WS patients.¹

There is much debate on whether organismal aging and cellular senescence may share common pathways.^{8,9} In support of this connection, WS derived cells have a diminished lifespan and exhibit signs of accelerated senescence in culture,¹⁰ while immortalized cells exhibit elevated levels of *WRN* relative to mortal cells.¹¹ This observation prompted us to investigate a possible role for *WRN* in the replication potential of cells following oncogenic stimulation.

DOES THE MYC ONCOPROTEIN IMPINGE UPON GENES THAT CONTROL AGING?

Because overexpression of Myc facilitates immortalization in some cellular contexts,¹²⁻¹⁴ we chose to study the effects of Myc on *WRN* expression. Myc is a transcription factor that influences the expression of a broad array of genes that enhance cell growth and promote cell-cycle entry.^{15,16} Myc also induces expression of *hTERT*, the rate limiting enzyme of the telomerase complex.^{14,17} Collectively, these findings implicate Myc as a major player in cell immortalization and raise the possibility that Myc may trigger the expression of other genes, beside *hTERT*, in order to prolong cellular lifespan. Thus, we hypothesized that up-regulation of *WRN*, may occur in Myc overexpressing cells to avoid senescence.

The results of our study indicated that Myc induced transcription of *WRN* in a variety of cell systems that utilized conditional *c-myc* alleles.¹⁸ Furthermore, a direct link between Myc and *WRN* expression was established by chromatin-cross-linking and immunoprecipitation assays (ChIPs), demonstrating that Myc bound to the *WRN* gene promoter through specific DNA consensus sites. Surprisingly, Myc required *WRN* to promote proliferation, as expression of exogenous Myc in WS fibroblasts lacking *WRN* protein induced senescence,¹⁸

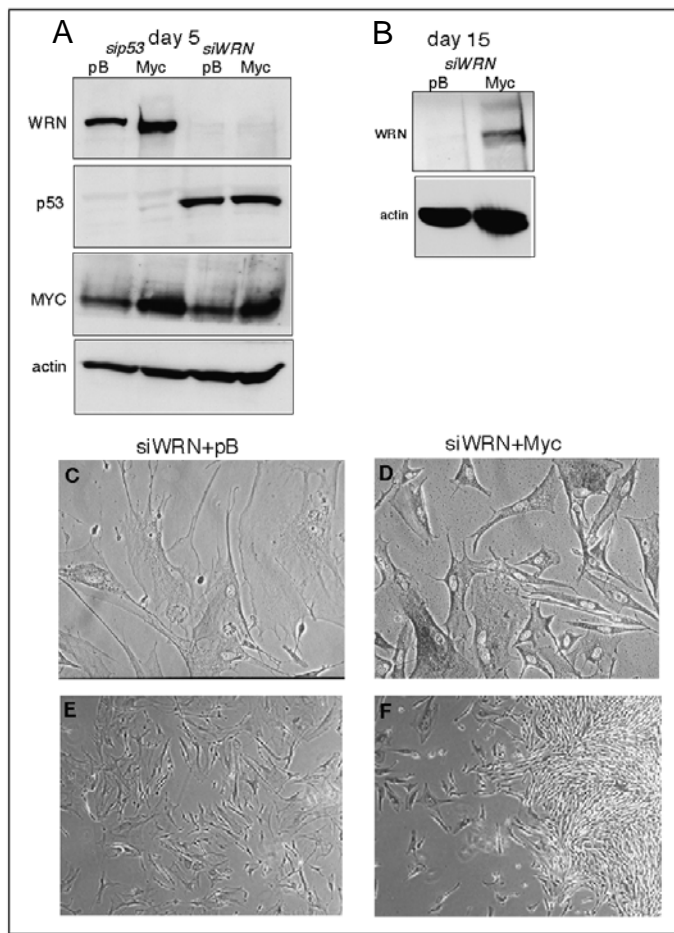


Figure 1. Myc overexpression rapidly selects for colonies that escape the effect of *siWRN*. (A) Western analysis showing the successful silencing of the WRN protein after 5 days from transduction with an *siWRN* expressing retrovirus. *sip53* is shown for control, indicating that the WRN protein was indeed expressed in these cells and it is not knocked down by *sip53*. (B) Western analysis after 15 days in culture indicating the reappearance of WRN protein in Myc overexpressing cells, thus escaping silencing, while in empty vector transduced cells, pB, silencing is maintained. (C and D) Senescence associated (SA) β -gal staining in hTERT-immortalized fibroblasts upon *siWRN* and Myc overexpression after 15 days from transduction. As previously reported depletion of WRN causes senescence of a high percentage of cells when Myc is simultaneously overexpressed (D) but not in control cells with empty vector, pB (C). (E and F) Picture at low power magnification shows the appearance of proliferating colonies in Myc transduced cultures (F) but not in the control (E). These colonies of "escapees" do not stain with SA- β -gal as the surrounding senescent cells. This coincides with the loss of silencing as shown in (B).

an outcome not normally observed following high constitutive Myc expression. This finding was supported by the fact that depletion of WRN protein by RNA interference, using a retroviral vector expressing a short hairpin specific for *WRN* (*siWRN*), caused senescence in response to Myc expression in normal *hTERT* immortalized fibroblasts (ref. 18 and Fig. 1A, C and D). Recent results suggest that some colonies of proliferating cells escape this senescent arrest, by reexpressing WRN, thus evading the effects of *siWRN* (Fig. 1B, E and F). However, "escapees" are not readily detected in cells without Myc. This observation further indicates a strong selective pressure to maintain expression of WRN when Myc levels are elevated.

WHY DO CELLS THAT LACK WRN SENESCE IN RESPONSE TO MYC?

A known major trigger of cellular senescence is genomic instability due either to telomere shortening after extended cell replication or to DNA damage caused by oxidative stress and other DNA damaging agents. Thus, we considered the possibility that senescence was triggered in WS cells following Myc expression because the combination of Myc de-regulation and lack of WRN protein together may exacerbate genomic instability. Indeed, a number of chromosomal abnormalities that include translocations, dicentric chromosomes and tetraploidy have been observed upon Myc overexpression.^{19,20} WS derived fibroblasts are also genetically unstable, exhibiting increased frequency of chromosomal translocations, inversions, and deletions.^{21,22} The Werner helicase, WRN, has been shown to recognize and unwind forked duplex DNA substrates which form during DNA replication and/or recombination.²³ This property of WRN may allow it to stabilize and repair stalled replication forks in S-phase or recombination intermediates during mitosis.²⁴⁻²⁶ Moreover, in human cells WRN protein has been shown to relocate from the nucleolus to the nucleoplasm at sites of DNA damage and associate with other proteins involved in DNA repair such as Rad51,²⁷ Rad52²⁸ and p53.²⁹ These observations suggest that Myc-induced chromosomal abnormalities might go unrepaired in cells lacking WRN. We therefore examined Myc transduced WS fibroblasts for the presence of aggravated genomic instability. The results indicated a similar incidence of genetic changes, with a predominance of chromatid breaks and tetraploidy, accumulating in both normal as well as WS derived cells upon transduction with Myc but not with empty vector, as assessed within ~2-5 cell doublings (data not shown). The predominance of chromatid breaks suggests that the DNA damage caused by Myc occurred in both normal and WS fibroblasts cell types during or after DNA replication. It is clear that Myc rapidly caused signs of genomic instability in our cell system, and we detected enhanced levels of chromatid breaks in the Myc-transduced WS cell. This observation is consistent with the recently reported increase in chromatid breaks in response to activation of a conditional Myc.³⁰ In addition, it is possible that more subtle genetic abnormalities, not detectable by cytogenetic analysis, may accumulate in WS cells upon Myc overexpression. It is also possible that we were unable to capture the earliest chromosomal lesions induced by Myc in the presence or absence of WRN. Future experiments will address this issue. It is also conceivable that a failure to resolve abnormal DNA structures during stimulation of DNA synthesis by Myc may trigger checkpoint mechanisms and eventually lead to proliferative arrest and senescence. These interesting possibilities need further evaluation. Finally, WRN and homologous helicases have been implicated in telomere maintenance,^{31,32,33} consistent with the accelerated shortening of telomere in WS fibroblasts.³⁴ Recently, the colocalization of WRN with telomere associated factors in telomerase-independent immortalized cells raises the interesting possibility for the involvement of WRN in the alternative lengthening of telomere pathway, (ALT).³⁵ However, the exact role played by WRN at telomeres remains to be demonstrated. Because our experiments were carried out in *hTERT* overexpressing WS fibroblasts it seems unlikely, that telomere alterations may be the cause of senescence in our system.

What other factors could contribute to the senescence of Myc expressing cells in the absence of WRN? An additional hypothesis is derived from the proposed role of WRN protein in both RNA

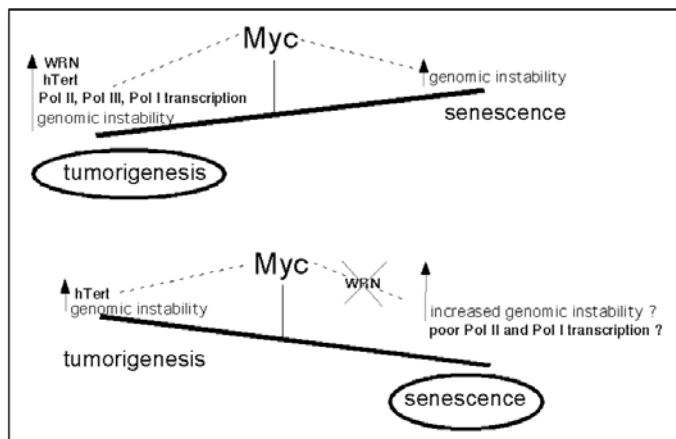


Figure 2. WRN function may change the rapidly shifting balance between tumorigenic conversion versus senescence caused by Myc oncogenic stimulation. In normal cells Myc drives proliferation and growth through elevation of a broad set of genes, which include *hTERT* and *WRN*, thus promoting extended cell renewal. Myc also causes increased genomic instability, which could favor mutation of tumor suppressor genes. This, together with Myc transcriptional rewiring of the cell, which includes Pol II, Pol III and Pol I, may contribute to tumorigenesis. However, if WRN protein is lacking, cells may accumulate excessive genomic damage or may fail to carry out transcriptional processes, and the balance is shifted toward senescence.

Polymerase II (Pol II) and Pol I transcription. Both mRNA and rRNA synthesis are indeed downregulated in cells from WS patients.^{36,37} The exonuclease and unwinding activity of the WRN helicase can also act upon RNA-DNA heteroduplexes, which may explain its role in transcription and subsequent cell proliferation and survival.³⁸ A defect in overall RNA levels and synthesis in WS fibroblasts may not be rescued by hTERT, despite the ability of hTERT to extend the replication capacity of WS cells.³⁹ In fact, hTERT expression in WS cells does not prevent characteristic alteration of gene expression⁴⁰ nor certain growth and morphological features of these cells (CG personal observation). Therefore, we hypothesize that overexpression of Myc, which stimulates transcription of Pol II,⁴¹ Pol III⁴² and Pol I (CG, manuscript in preparation), in cells which lack WRN protein, may trigger senescence because global RNA transcription fails. The notion that a defect in transcription may be linked to senescence has been recently shown by the generation of mice carrying a mutation in the Xeroderma pigmentosum D gene (*XPD*), which encodes a DNA helicase involved in a dual function of DNA repair as well transcription initiation. These mutant mice exhibit characteristics of premature aging such as premature graying of hair, osteoporosis, infertility and reduced lifespan.⁴³

In conclusion, it is conceivable that a combination of increased genomic damage as well as defects in transcriptional processes may explain the inability of cells that lack WRN to survive the increased demand imposed by Myc proliferative and growth stimuli and enter senescence (see diagram in Fig. 2).

IS UPREGULATION OF WRN RELEVANT TO MYC-INDUCED TUMORIGENESIS?

In human Burkitt's lymphomas, *c-myc* is typically translocated downstream of the immunoglobulin enhancer and it is thought to be the main transformation event in these neoplasms.⁴⁴ The levels of WRN protein in different lines established from Burkitt's

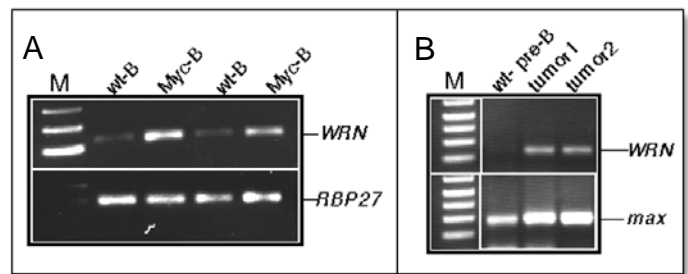


Figure 3. *WRN* expression is elevated in lymphomas and transgenic $\text{E}\mu\text{-c-myc-B}$ cells. (A) Comparison of *WRN* mRNA levels in splenic B-cells isolated from wild type and transgenic mice. Two independent pairs of animals were assayed. Total RNA was reverse transcribed using oligodT primers and PCR was performed with mouse *WRN* specific primers. (B) Comparison of *WRN* expression in wt-preB cells versus two independent lymphoma derived samples. Ribosomal RBP27 and mouse *max* were used as loading controls.

lymphomas indeed indicated that *WRN* was upregulated and that knock-down of *WRN* by RNA interference impaired cell proliferation and increased apoptosis (CG unpublished observations). These preliminary experiments are consistent with a potential important role of *WRN* in Myc induced tumorigenesis.

In order to understand the role of *WRN* in Myc tumorigenesis in vivo, it will also be important to cross *WRN*^{-/-} mice⁴⁵ with the $\text{E}\mu\text{-c-myc}$ transgenic mice.⁴⁶ The latter mice develop aggressive lymphomas within 12–16 weeks of age. Preliminary findings indicate a clear upregulation of *WRN* mRNA in the preneoplastic $\text{E}\mu\text{-c-myc}$ as well as lymphoma cells (Fig. 3). It will be interesting to see if lymphomas arising in *WRN*^{-/-}/ $\text{E}\mu\text{-c-myc}$ will show an increased propensity to senesce. Indeed, $\text{E}\mu\text{-c-myc}$ lymphoma cells can be induced to senesce in vivo, as revealed by the appearance of the senescence associated $\beta\text{-gal}$ staining, following treatment with cyclophosphamide. This response is dependent upon the elimination of apoptosis through overexpression of *bcl-2* and upon intact p53 signaling pathways.⁴⁷

IS IT A PARADOX THAT WS PATIENTS HAVE INCREASED INCIDENCE OF CANCERS?

WS patients are prone to develop neoplasia in the 3rd and 4th decades of life in comparison with the 5th–7th decades in the general population. The most frequent tumors detected in WS patients derive from mesenchymal tissues, and include soft tissue sarcomas, osteosarcomas and meningiomas. Additional tumors are melanomas and thyroid follicular carcinomas.⁴⁸ These types and locations of tumors are rare in the general population. It is possible that tumor development in WS occurs because of the pronounced genetic instability, which, over time, results in the loss of tumor suppressors and activation of oncogenes. In this respect, it is important to note that Myc has not been implicated in the types of tumors typical of WS. Perhaps, the types of cancers observed in WS may tolerate a high degree of genomic instability relative to tumors from other tissues. Another important contribution to tumorigenesis in WS patients could be made by the senescent stromal tissues, which through secretion of growth factors and extracellular matrix remodeling enzymes, may provide a permissive ground for tumor establishment.⁴⁹ Finally, alteration of telomere replication may also play a role in WS cancer development, although at present there is no information on the telomere replication pathway adopted by tumors in WS patients.

CONCLUSIONS

In summary, the WRN helicase may play apparent opposing roles in cancer development, by participating in both oncogenic proliferation through the avoidance of senescence, as well as protection of the genome from mutations that will eventually promote tumor establishment. This is a paradigm that has been reported for telomerase as well. Similarly, activation of telomerase promotes cell immortalization and thus tumorigenesis, while its absence protects cancer prone mice from tumor development.^{50,51} However, the exacerbated genomic instability, occurring after several generations in telomerase negative mice, eventually contributes to an increased incidence of cancers.⁵²

Several questions still remain unanswered about the role the WRN helicase plays in Myc driven tumorigenesis or cancer in general. Finding the answer to these questions will certainly provide new clues on how senescence occurs and how tumor cells have devised strategies to avoid it.

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