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The Role of Telomeres in Stem Cells and Cancer

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Telomere shortening impairs proliferation of transformed cells but also leads to cancer initiation by inducing chromosomal instability. Here, we discuss recent developments in our understanding of the role of telomeres in replication stress and how telomerase expression in somatic stem cells may affect genome integrity control and carcinogenesis.

Telomeres are implicated in genome integrity control and carcinogenesis. Most research over the last decades focused on the role of telomere shortening and telomerase activation in this process. Increasing data indicate that telomeres have additional functions in genome integrity control mediated by its role in sensing replication stress. In addition, the role of telomerase needs to be revisited due to the fact that stem and progenitor cells express detectable levels of telomerase, and there is increasing evidence that these cells are the cell type of origin of cancer formation.

In line with the role of telomere shortening in tumor suppression, cancer cells were shown to depend on telomere maintenance mechanisms in order to gain immortal proliferation capacity and to prevent genetic chaos induced by telomere dysfunction. Two mechanisms of telomere maintenance were identified in mammalian cells. Most human tumors utilize the enzyme telomerase, which can synthesize telomeres de novo. However, 10%–20% of human tumors activate alternative mechanisms of telomere lengthening (ALT), but the molecular mechanisms that control the activation of ALT remain incompletely understood. Inhibition of telomerase can reduce tumor growth in mouse models, but activation of ALT accounts for tumor relapse (Hu et al., 2012).

Aging-associated telomere shortening can also contribute to the evolution of genome instability and cancer formation by inducing chromosome end resection, fusions, and breakage (Figure 1A). As indicated above, genetically unstable tumor cells that arise under such circumstances need to reactivate telomere maintenance mechanisms in order to avoid genetic chaos and to gain immortal growth. Mouse studies demonstrated that transient telomere dysfunction, followed by telomerase reactivation, promotes the development of malignant tumors (Begus-Nahrmann et al., 2012) by selecting for chromosomal instabilities and genetic alterations that enhance tumor progression and metastasis (Ding et al., 2012). Other forms of genome instability are very similar to telomere loss in exhibiting a dual role in carcinogenesis. It appears to be a general theme that tumors rely on genome instability to arise but can also fall victim to it when there is too much of it (Cahill et al., 1999).

The choice of DNA repair pathways represents an important factor determining cellular consequences in response to telomere dysfunction. In this context, the activation of nonhomologous end-joining (NHEJ) pathways leads to chromosomal fusions, whereas the activation of homology-directed repair (HDR) can mediate telomerase-independent lengthening of telomeres by ALT. Accordingly, the choice of repair responses at dysfunctional telomeres could influence the evolution of genomic instability and cancer initiation by promoting end joining but could also contribute to the capacity of transformed cells to gain immortal growth capacity by activating ALT (Figure 1A). Because chromosomal fusions and breakage of fused chromosomes can contribute to cancer initiation, an understanding of the DNA repair pathways that induce chromosomal fusions in response to telomere dysfunction is required to better understand the role of telomere shortening in cancer initiation in aging tissues.

Telomeric DNA acts in concert with telomere-binding proteins to form secondary structures (e.g., G-quadruplexes and T-loops) that suppress the inadequate activation of checkpoints and DNA repair response at chromosome ends. Experiments on the conditional deletion of telomere-binding proteins (Trf1 and Trf2) in mice revealed that these proteins prevent the activation of six DNA damage response (DDR) pathways: ATM-signaling, ATR-signaling, DNA resection, HDR, classical nonhomologous end-joining (c-NHEJ), and alternative NHEJ (alt-NHEJ) (Sfeir and de Lange, 2012). It remains to be defined which of these responses are activated at telomeres that lose functionality in response to telomere shortening. The repair responses that are induced by telomere deprotection in response to the deletion of specific telomere-binding proteins can be different from those induced by telomere dysfunction in response to physiological telomere shortening. For example, the formation of chromosomal fusions in response to Trf2 inhibition is Ligase IV (Lig4) dependent and thus mediated by c-NHEJ, whereas Lig4 and DNA-PK$_{cs}$ (two essential components of c-NHEJ) are dispensable for chromosome end joining in response to telomere shortening (Rai et al., 2010). The prominent role of alt-NHEJ in the formation of chromosomal fusions in response to telomere shortening quests for a more detailed analysis of alternative end joining pathways at naturally shortened, dysfunctional telomeres. Such studies would be important to better understand the evolution of chromosomal instability and cancer initiation in aging tissues.

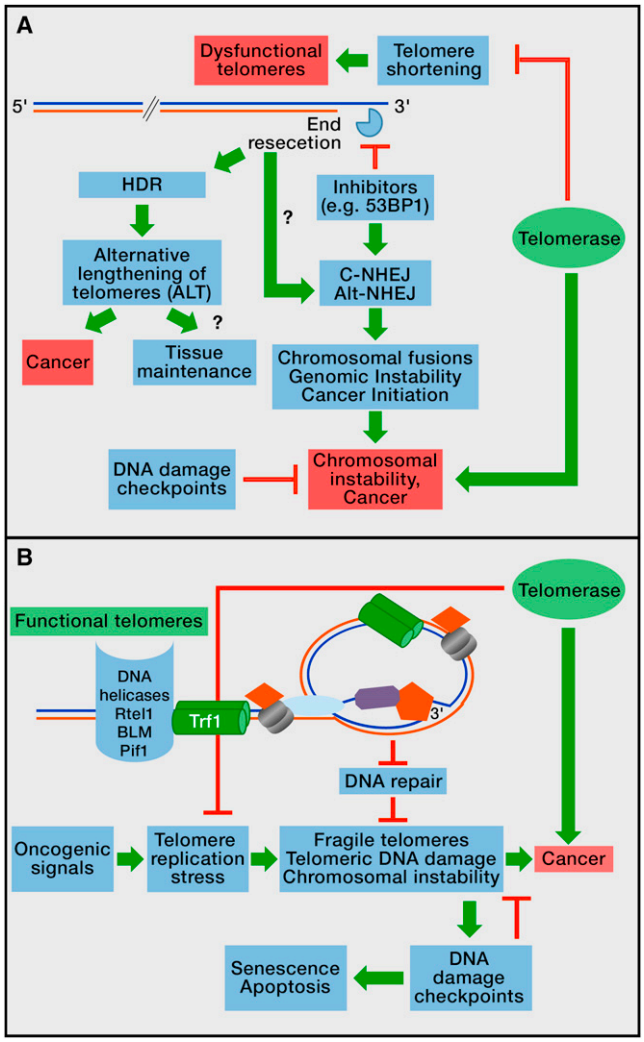

Figure 1. The Role of Chromosome Ends in Genome Integrity Control Depends on Telomere Functionality and Telomerase Expression

(A) During aging and chronic diseases, telomeres lose capping function due to telomere shortening. Dysfunctional telomeres are largely devoid of telomeric DNA and telomere-binding proteins, thus inducing DDR, including DNA repair and checkpoint responses. HDR can contribute to ALT, mediating immortal proliferation capacity in telomerase-negative human cancers. It is currently unknown whether HDR and ALT can contribute to maintenance of non-transformed cells and tissues during aging. Activation of NHEJ leads to generation of chromosomal fusions, thereby initiating chromosomal instability and cancer formation, especially when DDR checkpoint responses are defective. In mammalian cells, chromosome fusions in response to telomere shortening are mediated by alt-NHEJ. This pathway involves microhomology-mediated end-joining and is independent of components involved in c-NHEJ. The choice between different repair pathways is regulated by DNA end resection at dysfunctional telomeres. 5′–3′ end resection generates single-stranded DNA overhangs that inhibit c-NHEJ and activate HDR. 5′–3′ end resection may also promote microhomology-dependent alt-NHEJ, leading to fusion of chromosomes with shortened telomeres.

(B) Young cells and stem cells have long telomeres that cap chromosome ends by forming secondary structures (e.g., G-quadruplexes and T-loops) in concert with telomere-binding proteins. Telomere capping impairs the inappropriate activation of DDR at chromosome ends that would lead to chromosomal instability. However, the same structures that protect the chromosome ends (e.g., G-quadruplexes and T-loops) also represent fragile sites that are difficult to replicate during S phase of the cell cycle. Specific DNA helicases have evolved and cooperate with telomere-binding proteins (e.g., Trf1) to facilitate telomere replication.

In both scenarios (telomere shortening and telomere replication stress), telomerase activation can restore telomere function. Thus, telomerase activity in stem cells contributes to stabilize stem cell genomes, but it may also increase the risk of clonal growth when stem cells accumulate mutations.

The choice of repair responses at DNA breaks is to a great extent controlled by resection of DNA ends. Resection of the 5′ DNA strand inhibits NHEJ and directs repair toward HDR by generating 3′ overhangs. The MRN complex (consisting of Mre11, NBS, and Rad50), CtIP, and Dna2/BLM initiate resection followed by progressive resection, which is mediated by Exo1 and BLM. The deletion of inhibitors of end resection (e.g., Ku, 53Bp1, and MDC1) reduces the formation of chromosomal fusions in mouse models of telomere deprotection induced by the deletion of telomere-binding proteins (see for example Dimitrova et al. [2008]). Given the differences in the induction of NHEJ pathways at deprotected telomeres (in response to the deletion of telomere-binding proteins) compared to telomere-free ends (in response to critical telomere shortening), it will be important to evaluate the role of these genes in model systems of physiological telomere shortening. Exo1 deletion prolonged survival of telomerase knockout mice by impairing the formation of single-stranded DNA and the induction of ATR-dependent DNA damage signals (Schaetzlein et al., 2007). Of note, Exo1 deletion impaired the rate of anaphase bridges in telomere-dysfunctional mice, suggesting that Exo1-dependent DNA resection may contribute to the formation of chromosomal fusions in response to telomere shortening, possibly involving the activation of microhomology-mediated alt-NHEJ. Interestingly, CtIP-dependent end resection was shown to mediate microhomology-dependent alt-NHEJ in mouse cells, but it remains to be seen whether this mechanism is involved in fusion formation of dysfunctional telomeres in response to telomere shortening (Zhang and Jasin, 2011).

Telomeres in Stem-Cell-Derived Carcinogenesis

There is growing evidence that stem cells often represent the cell type of origin of cancer formation. A series of recent papers showed that human stem cells exhibit an age-dependent increase in mutations showing patterns of clonal evolution toward tumor formation (see for example Welch et al. [2012]). In humans, most somatic cells lack telomerase activity, but somatic stem and progenitor cells express low levels of telomerase. Telomere shortening limits the replicative life span of telomerase-negative cells, but low levels of telomerase in somatic stem cells likely contribute to the prolonged proliferative capacity of these cells compared to differentiated somatic cells. Considering stem cells as the cell type of origin of cancer formation, the "classical" concept of telomere dysfunction during cancer initiation followed by telomerase activation needs to be revisited because somatic stem and progenitor cells express telomerase to start with.

Several lines of evidence indicate that stem cells have evolved more stringent mechanisms of genome integrity protection compared to differentiated proliferating cells. For example, it was shown that mutation frequencies and frequencies of mitotic

recombination in embryonic stem cells are about 100-fold lower than in adult somatic cells or in isogenic mouse embryonic fibroblasts (Cervantes et al., 2002). It is possible that the expression of telomerase protects aging somatic stem cells from telomere dysfunction leading to chromosomal instability and an accumulation of procarcinogenic gene mutations. Telomere shortening leads to an accumulation of chromosomal imbalances in somatic stem cells, especially when p53-dependent checkpoint responses are defective (Sperka et al., 2011). There is evidence that the level of telomerase activity in somatic stem cells is not sufficient to completely prevent telomere shortening during aging. Telomere shortening and DNA damage accumulation occur in human hematopoietic stem and progenitor cells during aging (for review see Sperka et al. [2012]). According to these observations, transient telomere dysfunction may occur in aging stem cells despite low levels of telomerase activity. Compared to other somatic cells, stem cells may then carry a higher intrinsic risk to promote immortal growth in response to transient telomere dysfunction, leading to the accumulation of oncogenic mutations (Figure 1A). The amplification of pre-existing telomerase expression in stem cells may be simpler compared to activation of telomerase in differentiated somatic cells that have completely silenced the expression of the catalytic subunit of the enzyme (TERT). Thus, stem cells may carry an increased capacity to continue to proliferate, to transform, and to gain immortal growth capacity when exposed to genome instability. Therefore, more efficient checkpoints may have evolved in stem cells compared to other somatic cells in order to assure the elimination of damaged stem cells and to prevent stem-cell-derived tumorigenesis. Recent studies revealed experimental evidence that DNA damage induces differentiation of somatic stem cells (Wang et al., 2012). DNA-damage-induced differentiation of adult stem cells limits self-renewal and removes damaged cells from the stem cell pool. The contribution of DNA-damage-induced stem cell differentiation to tumor suppression remains yet to be investigated.

It is conceivable that the carcinogenic role of telomere dysfunction followed by telomerase reactivation could proceed in a different way in stem cells that express telomerase compared to other somatic cells that are telomerase negative. Along these lines, it would be important to analyze molecular mechanisms that cooperate with telomerase expression in allowing clonal evolution in response to oncogenic mutation (see below and Figure 1B).

Replication Stress in Tumor Biology

There is increasing evidence that telomeres can influence aging and carcinogenesis independent of telomere shortening. This new function of telomeres involves the fragility of telomeres (Figure 1B). Due to their specific sequence composition, telomeres can form G-quadruplex structures (G4). These structures are highly stable and are difficult to resolve during replication, which can cause replication fork stalling and chromosome fragility. Fragile sites are particularly prone to chromosomal breakage and recombination events as a result of replication stress, which may result from inappropriate proliferation signals such as oncogene activation. There is evidence that telomere-binding proteins are required for telomere replication. For example, the telomere-binding protein Taz1 and its mammalian ortholog Trf1 ensure efficient replication of telomeres in *Schizosaccharomyces pombe* and mice (Miller et al., 2006; Sfeir et al., 2009). A current model indicates that Trf1 acts epistatically of DNA helicases that have the potential to resolve G4 DNA structures such as Bloom (BLM), Rtel1, and Pif1.

Interestingly, telomere fragility in response to Trf1 deletion led to an increase in chromosomal instability and cancer initiation in the skin epithelium of p53-deficient mice (Martínez et al., 2009). These data suggested that telomere replication stress could contribute to genome instability and cancer initiation in the context of defective p53 checkpoints. In contrast to the potential contribution of telomere replication stress to genome instability and cancer initiation, telomere replication stress may also serve as a sensor to limit growth of abnormally proliferating cells in response to oncogenic stress. Overexpression of oncogenic H-RASV12 leads to aberrant proliferation, which, in primary human cells, results in oncogene-induced senescence. Recent studies revealed that the activation of the RAS oncogene induces replication fork stalling, fragile telomeres, and an accumulation of DNA damage foci at telomeres in telomerase-negative human fibroblasts (Suram et al., 2012). Importantly, human cancer precursor lesions exhibit features of replication stress, telomeric DDR foci, and senescence, suggesting that replication stress at telomeres may contribute to the activation of tumor suppressor checkpoints in response to oncogene activation at early stages of carcinogenesis. However, invasive cancers exhibit a diminished accumulation of replication-stress-induced DDR foci, suggesting that progressive tumor growth selects for mechanisms that alleviate replication stress.

Together, it is tempting to speculate that telomere fragility represents yet another type of genome instability exhibiting a dual role in cancer initiation and suppression. The loss of shelterin components can lead to replication stress at telomeres and cancer initiation, whereas the induction of telomere replication stress in response to oncogene activation contributes to induction of senescence and the impairment in cancer progression. However, this hypothesis remains speculative at the moment, and the recent observations raise several questions.

First, it remains to be elucidated why DNA damage in response to oncogenic replication stress specifically accumulates at telomeres. G-quadruplexes are present throughout the genome, and it is unexplained why replication stress specifically leads to telomere fragility. One possible explanation for the accumulation of telomeric DNA damage in response to replication stress could be the inhibition of DNA repair response at telomeres. As discussed above, telomere-binding proteins interact with various DDR components and suppress their activity at telomeres to prevent illegitimate recombination and repair events (see above and Sfeir and de Lange [2012]). Recent studies revealed that the end protection of telomeres comes with the drawback that DNA damage inside telomeres is difficult to repair, leading to prolonged persistence of DDR in response to DNA damage induction at telomeres compared to the rest of the genome (Fumagalli et al., 2012; Hewitt et al., 2012). It is possible that DNA damage, induced by replication stress, is also affected by the antirepair activity of telomere-binding proteins and may thus persist over prolonged periods of time at telomeric sequences compared

to other G-quadruplex-containing sequences (Figure 1B). In addition, replication timing (a subset of telomeres is replicating late in S phase) could contribute to the relative sensitivity of telomeres to replication stress, although this remains to be analyzed.

Second, aside from the question of telomere-specific vulnerability to replication stress, it will also be important to delineate the role of telomerase in this context. Telomerase expression did not suppress the fragile telomere phenotype of human cells in response to RAS oncogene expression. However, telomerase activity suppressed the accumulation of intratelomeric DNA damage foci in response to RAS expression and thus allowed escape from oncogene-induced senescence (Suram et al., 2012). These data indicate that telomerase positivity could enhance the risk of a cell to escape OIS activated by replication stress at telomeres (Figure 1B). How telomerase expression may rescue the accumulation of telomeric DNA damage in the setting of replication stress remains to be defined. Of note, studies in yeast revealed that telomerase expression could rescue replication stress at telomeres, induced by the overexpression of Pif1 (Chang et al., 2009). These data suggest that the right stoichiometry of telomerase and G-quadruplex-resolving helicases may determine the resistance of telomeres to replication stress.

Together, it appears that the most prominent features of telomeres, the specific, repetitive nature of G-rich sequences, and the association of these structures with telomere-binding proteins also disclose the major drawbacks in repair and replication of these structures. Whereas the shelterin components suppress illegitimate recombination and repair of DNA events at the ends of linear chromosomes, they also suppress efficient repair of telomeric DNA damage. In addition to its role in limiting the proliferative life span of human cells, telomeres appear to serve as sensors of inappropriate replication signals, such as oncogene activation.

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