



Commentary

Predicting Risk at the End of the End: Telomere G-tail as a Biomarker



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Telomeres are essential DNA–protein complexes at the ends of eukaryotic chromosomes, which keep the cell's genomic stability and integrity. Because conventional DNA polymerases cannot replicate the very ends of linearized DNA, telomeric sequence (TTAGGG repeat arrays) is gradually lost during cell division and aging. For example, normal peripheral blood mononuclear cells lose up to about 100-base pair telomeric sequence per year (Iwama et al., 1998). Deregulation of telomere length is associated with various diseases: accelerated telomere shortening exacerbates premature aging in the patients with Werner and Hutchinson–Gilford progeria syndromes, whereas inefficient telomere maintenance in the patients with dyskeratosis congenita causes bone marrow failure. Premalignant hyper-proliferative lesions give rise to shortened telomeres, which are eventually stabilized by telomerase-mediated telomere replenishment in cancer. Accordingly, telomere length is referred to not only as a “mitotic clock”, which counts the number of normal cell division, but also as a biological marker of aging. Recently, telomere length has also been established as a surrogate marker for disease and lifestyle: shortened leukocyte telomeres are associated with a wide range of threat to life, including cardiovascular disease and psychological stresses (Haycock et al., 2014; Lin et al., 2012).

Strictly speaking, however, telomeric TTAGGG repeats *per se* are not the caps for stabilizing the chromosome ends. In fact, the farthest end of the double-stranded telomeric DNA consists of a 50–300-nucleotide single-stranded tract, called 3′-overhang or G-tail (guanine-rich strand-tail), which provides a molecular basis for the chromosome end capping. Telomere G-tail folds back to form a protective “t-loop” by means of strand invasion at the double-stranded telomere DNA (Griffith et al., 1999). This lariat structure masks the chromosome end from being recognized as damage. Genetic manipulation that disrupts telomere G-tails (e.g., knockout of telomeric repeat-binding factor 2) promptly compromises the end protection, eliciting DNA damage response even if the cells retain long telomeres (Celli and de Lange, 2005). This suggests that G-tail length rather than the total telomere length, more directly contributes to or correlates with pathogenesis. But until recently, whether or not this is the case in the clinical settings has remained elusive. In this issue of *EBioMedicine*, using a highly sensitive and quantitative method, Nezu, Tahara and colleagues quantitated both G-tail and total telomere length of leukocytes in patients with a

history of cerebrovascular disease or atypical neurological problems (Nezu et al., 2015). Cross-sectional analyses revealed that G-tail length, rather than total telomere length, is associated with endothelial function and severity of age-related white matter changes. Thus, shorter G-tail predicts higher disease risk.

Nezu et al.'s paper highlights three aspects. First, the authors discovered a non-invasive surrogate marker that could predict an increased risk of future cardiovascular disease, stroke, dementia, and death. Second, while telomere dysfunction in late generations of telomerase knockout mice gives a severe deleterious impact on highly regenerative tissues (Lee et al., 1998), this study focused on the diseases associated with non-regenerative/post-mitotic tissues (*i.e.*, heart and brain) and identified G-tail, the telomere-related parameter, as the predictive disease marker. Third, their observations are consistent with the established concept in telomere biology that G-tail is a sticking tab for t-loop and therefore, plays more fundamental role in the end capping than the double-stranded telomere DNA. In laboratories, total telomere length has been more commonly measured than G-tail length by using conventional methods, such as Southern blotting, polymerase chain reaction, and fluorescence *in situ* hybridization. Relative uncommonness of G-tail studies might be in part due to technical issues such that G-tail measurement needs to be performed under the non-denatured experimental conditions that detect only the single-stranded DNA. Nezu and colleagues successfully employed the hybridization protection assay (HPA) method (Tahara et al., 2005), which allows sensitive and quantitative detection of G-tail.

According to their observations, G-tail length decreases with age (Nezu et al., 2015). This would be in agreement with previous observations that telomere shortening is accompanied by G-tail shortening. Strikingly, however, their analyses demonstrate that G-tail length rather than total telomere length is an independent biomarker for endothelial dysfunction and white matter lesions. This fact suggests that G-tail is a nimbler sensor than the entire telomeres for pathological stresses. In other words, G-tail length could fluctuate upon exposure to various stresses. Indeed, G-tail is also shortened in hemodialysis patients (Hirashio et al., 2014). Mechanistically, factors that could modulate G-tail length include telomerase-mediated G-strand extension, end resection of the antisense C-strand, formation and resolution of higher-order structure called G-quadruplexes, loading of the shelterin protein complexes and innate hypersensitivity to oxidative stress. Meanwhile, a recent study indicates that telomere DNA is transcribed to a non-coding RNA called TERRA, which is implicated in genome-wide alteration of gene expression in human cancer cells *in vivo* (Hirashima and Seimiya,

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2015). It is of interest how these telomere-related parameters affect G-tail dynamics and cell fate under pathological stress conditions. Because the sample size in Nezu et al.'s study is small, it would be necessary to perform large-scale, prospective studies to measure G-tail length in both patient and control populations. Such studies will further elucidate cryptic linkages between G-tail length and health problems.

Conflicts of Interest

Hiroyuki Seimiya is a non-paid Scientific Advisor of MiRTel Co. LTD.

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