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Modulation of telomere binding proteins: a future area of research for skin protection and anti-aging target

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Summary

Telomere shortening is considered as one of the main characteristics of cellular aging by limiting cellular division. Besides the fundamental advances through the discoveries of telomere and telomerase, which were recognized by a Nobel Prize, telomere protection remains an essential area of research. Recently, it was evidenced that studying the cross-talks between the proteins associated with telomere should provide a better understanding of the mechanistic basis for telomere-associated aging phenotypes. In this review, we discuss the current knowledge on telomere shortening, telomerase activity, and the essential role of telomere binding proteins in telomere stabilization and telomere-end protection. This review highlights the capacity of telomere binding proteins to limit cellular senescence and to maintain skin tissue homeostasis, which is of key importance to reduce accelerated tissue aging. Future studies addressing telomere protection and limitation of DNA damage response in human skin should include investigations on telomere binding proteins. As little is known about the expression of telomere binding proteins in human skin and modulation of their expression with aging, it remains an interesting field of skin research and a key area for future skin protection and anti-aging developments.

Keywords: anti-aging, skin protection, skin tissue homeostasis, telomere binding proteins, telomere-end protection, telosome

Introduction

Telomere is one of the key determinants of life span, and telomere erosion to a critically short stage limits replicative life span of cultured cells, a phenomenon known as Hayflick limit or replicative senescence.[1] The quest for longevity has driven scientists to continuously explore telomere maintenance. The Nobel Prize in physiology or medicine 2009 was awarded for the discovery of telomere and telomerase. Nonetheless, telomere length is not the only contributor to replicative senescence. The length and structure of telomere are tightly controlled by numerous telomere-associated proteins. Understanding the cross-talks between the proteins associated with telomere should provide the mechanistic basis for telomere-associated aging phenotypes.[2] Telomeres are the physical ends of chromosomes, which in humans are composed of tandem repeats of (TTAGGG)n and proteins associated with the telomeric sequence. The length of this terminal repeat region varies between chromosomes, between different cells, and between species and is thought to be dependent on a dynamic equilibrium between loss and addition of repeat units.[3] Although the length of telomere repeats decreases with each round of DNA replication, a minimum number of repeats are essential for proper telomere function and to avoid sustained activation of

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DNA damage pathways that may result in replicative senescence or cell death. To counteract this loss of genomic material, it was evidenced that the telomerase, a ribonucleoprotein containing the reverse transcriptase telomerase protein (hTERT) and the telomerase RNA template (hTERC), could polymerize terminal repeats of DNA *de novo* onto the 3′single-strand end of chromosomes.[4] This enzyme provides an important function within the cell, as it helps maintain the integrity of the chromosomes that could not be completely replicated by the DNA replication machinery.[5] Telomerase is expressed at high levels in proliferative cells such as the germ line and stem cells, allowing maintenance of a constant telomere length and apparent immortal growth of such cells, whereas it appears to be either absent or present at very low levels in many adult somatic tissues and insufficient to balance the loss of telomeric DNA.[6,7] Therefore, the low or missing telomerase activity in replicating cells explains the gradual decrease in the telomere lengths with proliferation and with age. The loss of telomeric function induces a double-strand DNA damage response triggering a p53-dependent G1/S cell cycle checkpoint that may be additive with other forms of acute and accumulated (nontelomeric) DNA damage. As a result, cells could gradually increase their sensitivity to genotoxic stress. Little information on the role of DNA damage signals originating from telomeres *vs.* other triggers of apoptosis or senescence in cells is available. Studies in this area are of interest in relation to the aging of stem cells but are still complicated by difficulties in obtaining information about telomere length and about the replicative history of small numbers of cells.[8]

Telomere length regulation

Telomere length is maintained in the germ line at its maximum length (10–20 kb) for subsequent generations by the expression of telomerase. However, in somatic tissues, where telomerase is repressed in the majority of cells, telomere length is extremely heterogeneous, and this can make difficult the interpretation of telomere length data. The rate of loss observed in humans, typically 50–120 bp/population doubling, is less than that predicted by end replication losses alone. Inherited telomere length remains a critical determinant of the telomere length in somatic cells. For example, the average telomere length in nucleated blood cells from monozygotic twins, even in old age, was found to be similar in contrast to highly variable values observed in the general population.[9,10] Although genetic factors are important determinants of telomere length, various other epigenetic factors are involved in shortening and elongation of telomeres in human cells. Several proteins important for human telomerase assembly in the nucleolus and for its intranuclear localization depending on cell cycle stage are also thought to modulate telomere length.[11] In addition, it was suggested that oxidative damage contributes to telomere erosion and telomere length heterogeneity in cultured human cells.

Telomere shortening and aging

The pronounced decline in telomere length observed early in life presumably reflects a high turnover of (stem) cells, increasing cell numbers, and body mass. The explanation for the accelerated decline late in life is not clear. It can reflect an overall increase in proliferative activity, perhaps because the proportion of cells that exit the proliferative compartment as a result of accumulated genetic damage increases in a nonlinear fashion. It could also reflect the selective expansion of abnormal cells that can grow or survive with short telomeres because they express higher levels of telomerase or because apoptotic pathways are suppressed (e.g., by overexpression of Bcl-2). It is also possible that the age-related decline in mitochondrial function increases the production of oxygen radicals and increases the oxidative damage to telomeres.[12] Correlation between shorter telomeres in blood DNA and mortality in 143 normal, unrelated individuals 60 years or older was reported.[13] These results support the hypothesis that telomere shortening in human beings contributes to mortality in many age-related diseases and that measurements of telomere length in blood cells provide a surrogate marker for the telomere length in the stem cells of various tissues.[14]

Telomerase activity and *in vitro* replicative senescence

Since Hayflick first described the *in vitro* replicative senescence of human fibroblasts,[1] models have been proposed to extrapolate this observation to organismal aging. Correlations have been reported between the *in vitro* population doublings of human skin fibroblasts and the age of the donor from which they were derived.[15] Fibroblasts from patients with accelerated aging syndromes have shown greatly reduced proliferative capacity in culture.[16] It appeared that the number of population doublings that a telomerase-negative human cell can undergo is determined by telomere shortening.[17] As *in vitro* replicative senescence has been linked to a reduction in telomere size (loss of 50 bp of telomere length during each round of replication) in

cultured human fibroblasts, a role for telomerase in human aging was suggested.[18] A study of cells obtained from donors aged between 0 and 93 demonstrated a relatively weak correlation between replicative capacity and donor age, but a strong correlation between the proliferative capacities of cell cultures and the telomere length of the starting cell population.[19] These observations made it clear that telomere length is a biomarker of cellular aging and led to the hypothesis that telomere erosion could account for the cell division counter that determined the Hayflick limit. However, other authors reported that considering telomere length as a biomarker of aging in humans was equivocal.[14] Neither the number of telomeres nor the critical telomere length at which senescence is triggered has yet been defined, but the minimum telomere length in senescent cells currently stands at around 1 kb. To complete previous observations, it was also reported that transfection of telomerase activity into human cell cultures halted telomere loss and prevented the subsequent proliferation block, which characterizes *in vitro* replicative senescence.[20] Reports have demonstrated that transient telomerase activity can be detected during S phase (in cells that were previously considered not to express hTERT). For example, lymphocytes, initially quiescent and telomerase negative, have their telomerase reactivated and reproliferate upon mitogenic stimulation.[21]

Telomerase activity *in vivo*

As many of the cells in an adult tissue are either quiescent or postmitotic, their telomeres will not therefore shorten significantly during the life of the individual, but the cells still age. Fibroblasts and epithelial cells, formed from their telomerase-positive stem cells, are telomerase negative and do not undergo sufficient numbers of cell divisions during the life of an individual to be affected adversely by shortening of their telomeres.[22] Human fibroblasts taken from elderly individuals are still capable of between 20 and 50 population doublings in culture,[16] which is 40–70% of their replicative capacity as defined by the Hayflick limit.[1] In other words, *in vitro* replicative senescence is not relevant to the *in vivo* situation, because cells in the adult body never reach the Hayflick limit. It has been suggested that declines in physiological function during aging may be the result of telomere-dependent *in vivo* replicative senescence among certain cell types. For example, wound healing is less efficient in the elderly,[23] and age-related changes in collagen synthesis have been reported in fibroblasts.[24] There is certainly evidence that a small cohort of genes show consistent age-related changes in their expression both in cultured fibroblasts and in tissues.[25]

Telomerase access to telomeres and telomere binding proteins

Important mechanisms that regulate telomerase action and telomere length are related to the interaction of telomerase with its substrate at the 3′ single strand of chromosome ends. As T-loops are poor substrate for telomerase, telomerase interacts with its substrate during DNA replication when the T-loop is opened by the replication machinery. A complex set of proteins have been identified to stabilize the T-loop and mediate some of the functions of telomeres. Both the double- and single-stranded telomeric DNA are bound and protected by DNA-binding proteins that in turn associate with other signaling proteins/complexes to achieve telomere-end protection and length control. The telomere-associated proteins TRF1, TRF2, POT1, RAP1, TIN2, and TPP1 form a complex known as the telosome, or shelterin complex, which is essential for telomere function, telomere maintenance, and connections with intracellular signaling pathways.[26–28] POT1, RAP1, TIN2, and TPP1 are assembled into two major complexes formed around the telomere repeat binding factors TRF1 and TRF2 that, in dimeric form, directly bind TTAGGG repeats.[29] TRF1 homodimers monitor telomere length via a telomere repeat counting mechanism that regulates the access of telomerase to the terminus, creating a negative feedback loop. Thus, overexpression of TRF1 in telomerase-expressing cells results in telomere shortening, whereas a dominant negative TRF1 inhibiting the binding of endogenous TRF1 results in telomere elongation. TRF2 homodimers stabilize T-loop formation and protect telomere end. Dominant negative TRF2 induces end-to-end telomeric fusions independently of telomere length. The hPOT1 protein interacting with the 3′ single-strand end is a prime candidate to mediate interactions between telomeric DNA and telomerase. Different splice forms (with different affinities for telomeric DNA) of hPOT1 have been described, indicating another possible layer of telomere length regulation.[30] TRF1 binds long tracts of double-strand DNA, whereas TRF2 binds the double-strand/single-strand DNA junction.[31] Both TRF1 and TRF2 have carboxy-terminal Myb domains, which are essential for binding directly to telomere duplex DNA.[32] Human TRF1 and TRF2 differ from each other at their amino terminus, which comprises an acidic region in TRF1 and a basic region in TRF2. The basic amino-terminal domain of TRF2 is important for binding of the

double-strand/single-strand DNA junction and for the supercoiling of telomeric DNA, and it may regulate the formation and stabilization of the T-loop structure.[33] TRF2 also recruits a variety of other DNA damage–sensing and DNA repair proteins to the telomere, such as Apollo,[34] the DNA repair MRN complex,[35] Ku70/Ku86,[36] and PARP1.[37] The recruitment of these proteins presumably prevents telomere ends being recognized as DNA breaks or to sensitize the cell to damages to the telomeres. Although both TRF2 and POT1 bind telomere DNA and are required for telomere capping, recent studies indicated that they regulate distinct signaling pathways.[38] Loss of function of TRF2 in a number of mammalian cell types (tumor and primary cell lines), and in cells from conditional TRF2-knockout mice, elicits DNA damage responses mediated mainly through the ATM pathway, whereas POT1 knockout triggers the DNA damage response pathway initiated by the protein kinase ataxia telangiectasia related (ATR).[38]

Discussion

Despite the high heterogeneity in humans, information has been gained regarding telomere length and aging. The majority of human somatic cells display no detectable telomerase activity, the activity in humans being restricted to the stem cell compartments of actively proliferating tissues and at least 85% of malignancies.[39] It is believed that *in vitro* replicative senescence, observed in cultured somatic cells, is attributable to a loss of telomere length in those cells, caused by inactivity of telomerase. Numerous cross-sectional surveys of telomere length in various human tissues have evidenced telomere loss, which varies between tissues and appears to correlate with levels of cellular turnover. Tissues with negligible cell turnover display no apparent telomere loss with age, in contrast to highly regenerative tissues such as the epidermis. Telomere shortening was demonstrated in various human tissues during aging with increased senescence-associated b-galactosidase activity in aged human skin.[18,40] Compounds capable of maintaining the functionality of telomere binding proteins should prevent telomere shortening and limit the number of cells entering into senescence. Senescent cells, while in a nondividing state, remain biologically active but display a change in their gene expression profile toward a more catabolic and proinflammatory phenotype degrading the tissue microenvironment.[24] Thus, in addition to the simple cessation of cell division, an age-related accumulation of senescent cells has the potential to disrupt tissue homeostasis and underlie age-related diseases and cancer. In line with numerous reports that have demonstrated the essential role of telomere binding proteins in telomere-end protection, this review highlights their essential capacity to limit cellular senescence and to maintain skin tissue homeostasis, which is of key importance to limit accelerated tissue aging. Consistent with these data, future studies addressing telomere protection and limitation of DNA damage response in human skin should include investigations on telomere binding proteins. As little is known about the expression of telomere binding proteins in human skin, and the modulation of their expression with aging, it remains an interesting field of skin research and a key area for future skin protection and anti-aging developments.

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