Review

Stem cell function and maintenance – ends that matter: Role of telomeres and telomerase

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Stem cell research holds a promise to treat and prevent age-related degenerative changes in humans. Literature is replete with studies showing that stem cell function declines with aging, especially in highly proliferative tissues/organs. Among others, telomerase and telomere damage is one of the intrinsic physical instigators that drive age-related degenerative changes. In this review we provide brief overview of telomerase-deficient aging affects in diverse stem cells populations. Furthermore, potential disease phenotypes associated with telomerase dysregulation in a specific stem cell population is also discussed in this review. Additionally, the role of telomerase in stem cell driven cancer is also briefly touched upon.

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1. Introduction

Stem cells are pluripotent or multi-potent cells, capable of self-renewal and multi-lineage differentiation potential (Zhang *et al*. 2012). A plethora of studies has demonstrated the identification of multi-potent adult stem/progenitor cells in nearly all body tissues/organs (Chen *et al*. 2012). For instance, bone marrow serves as a pool for several stem/progenitor cell populations, including mesenchymal stem/stromal cells, hematopoietic stem cells and endothelial progenitor cells (Ballas *et al*. 2002; Chao and Hirschi 2010). Tissue/organ-resident stem cells possess two unique properties, that is, self-renewal, to maintain their pool, and differentiation, to provide more specialized cells to cater tissue/organ functional and regenerative demands. These unique properties make them indispensable during embryonic development for tissue formation and during adult life to ensure tissue repair and regeneration to maintain tissue/organ homeostasis (Weissman 2000; Rando 2006; Rossi *et al*. 2008).

Tissue homeostasis is exceptionally maintained by a strict balance between cell loss and cell replacement during the course of tissue/organ life (Weissman 2000; Rando 2006). However, with aging and degenerative diseases, this balance declines progressively resulting in reduced supply of new cells to compensate the lost/dead cells, thus compromising tissue integrity and function along with diminished regeneration capacity upon damage (Artegiani and Calegari 2012). Disequilibrium coupled with overall decline in stem/progenitor functions could be due to number of cell intrinsic and extrinsic factors such as DNA damage (Gao *et al*. 2001; von *et al*. 2001), telomere shortening (Beausejour 2011), oxidative stress (Balaban *et al*. 2005), and secretion of growth factors, mortifying enzymes (Krtolica and Campisi 2003) and inflammatory cytokines (Saeed *et al*. 2011) that not only compromise stem/progenitor functions but also enhance their aging. We have recently shown that most of these intrinsic and extrinsic factors were overtly present in telomerase-deficient aging mice having compromised stem cell functions along with alterations in cell extrinsic micro-environment (Saeed *et al*. 2011).

Therefore, this review will focus on telomerase-dependent telomere shortening and its affect on stem/progenitor functions in different cellular compartments, which could provide a rationale for diverse disease and degenerative phenotypes with advanced aging in various telomerase-deficient mouse models or a condition associated with telomerase deficiency.

Keywords. Aging; stem cells; telomerase; telomeres

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2. Telomerase and telomeres

By the end of 20th century,telomerase was discovered by Greider *et al*. (Greider and Blackburn 1985)in the extracts of the protozoan *Tetrahymena thermophila*. Telomeres are chromosomal ends and nucleoproteins that cap the end of all the eukaryotic chromosomes, thus playing an essential role in the maintenance and integrity of the chromosome as well as cell viability. Telomeres are G-rich simple repeat sequences (TTAGGG) that are synthesized by a special reverse transcriptase called *Telomerase* (Blackburn and Chiou 1981; Blackburn and Gall 1978). This enzyme requires a template to act which is the RNA component of telomerase, i.e. *TERC* (Blackburn and Gall 1978; Blackburn 1984). Telomerase is inactive in most somatic cells but active in germ cells, stem cells and actively dividing cells (Kassem *et al*. 2004). Telomerase deficient mice ($Terc^{-/-}$) have been instrumental in delineating the impact of telomere shortening in context of whole organism (Blasco 2005). Disease states that appear in $Terc^{-/-}$ reiterate the disease states, with more or less same etiology, in humans, characterized by short telomere in the cells as a result of excessive proliferation (Lee *et al*. 1998; Vulliamy *et al*. 2002; Ju *et al*. 2007). However, expression of telomerase surpassing endogenous levels has been reported in 80% of human cancers (Satyanarayana *et al*. 2004). Seemingly, intricate balance of telomerase expression is pre-requisite for maintaining healthy trail that neither diverges to aging or to cancer by maintaining steady stem cell functions. Thus, the role of telomerase in diverse stem cell populations, including cancer stem cells, will be discussed briefly in this review.

3. Telomeres, telomerase and stem cells

In fact, several lines of evidence suggest that stem cells are required for unrelenting supply of mature and functionally proficient stem cells for normal tissue turnover and tissue regeneration (Bianco *et al*. 2001; Fibbe 2002; Stenderup *et al*. 2003; Endo *et al*. 2004; Abdallah and Kassem 2008; Abdallah and Kassem 2009). In this regard, telomerase deficiency in mice ($Terc^{-/-}$) has been shown to affect the maintenance and regeneration of tissues and organs undergoing extensive proliferation, such as liver, intestine, testis, ovaries and spleen (Blasco *et al*. 1997; Lee *et al*. 1998; Herrera *et al*. 1999; Rudolph *et al*. 1999), the effects that are reminiscent of pre-mature aging. Furthermore, stem cell populations having telomerase deficiency are inclined to lack the capacity to regenerate or compensate the intense demands of tissues/organs, during the course of aging or progression of degenerative diseases (Lee *et al*. 1998; Herrera *et al*. 1999; Rudolph *et al*. 1999; Ju *et al*. 2007; Pignolo *et al*. 2008). Telomerase activity is usually repressed after stem cell differentiation (Sharma *et al*. 1995; Armstrong *et al*. 2000; Forsyth *et al*. 2002). There is a considerable agreement that most normal somatic cells in human exhibit undetectable telomerase activity; however, a low level of telomerase activity has been found in adult stem cells from skin, gut and the hematopoietic system (Fehrer and Lepperdinger 2005). The low level or absence of telomerase activity leads to telomere shortening, impaired cell proliferation, that ultimately leads to permanent cell cycle arrest a phenomenon termed as replicative senescence (Simonsen *et al*. 2002). Surprisingly, tissues that were positive and negative for telomerase activity, when tested for telomerase (mTERT) mRNA expression, showed more or less the similar mTERT expression profile, indicating that tissues lacking telomerase activity are also telomerase competent but do not have activity, possible explanation could be the alternative splicing of the mTERT gene (Martin-Rivera *et al*. 1998). Moreover, telomerase activity is stringently regulated during development and adult age with assorted tissue specificity (Ju and Rudolph 2006). The following is a short description of the role of telomerase in different stem cell populations.

3.1 *Telomerase and hematopoietic stem cells (HSCs)*

Studies have shown that HSCs exhibit telomerase activity and undergo telomere shortening with advancing age in humans (Vaziri *et al*. 1994; Chiu *et al*. 1996; Morrison *et al*. 1996). Similarly, telomere lengths of adult blood leukocytes and adult bone-marrow derived HSCs were found to be shorter than the germ-line counterparts of the same donor and HSCs derived from fetal liver and cord blood, respectively (Vaziri *et al*. 1993; Cooke and Smith 1986). The effect of telomerase deficiency on hematopoiesis is further strengthened by the identification of human diseases associated with mutations in telomerase and its vital components, such as dyskeratosis congenita, Fanconi anemia and aplastic anemia (Vulliamy *et al*. 2002; Vulliamy *et al*. 2004). Similarly, telomerase -eficient mice have been shown to have extramedullary hematopoiesis in the spleen and liver, confirming previous reports vis-à-vis defects in hematopoietic system (Lee *et al*. 1998; Herrera *et al*. 1999; Rudolph *et al*. 1999; Ju *et al*. 2007). Despite detectable telomerase activity in HSCs, their progeny and peripheral blood lymphocytes (Hiyama *et al*. 1995; Morrison *et al*. 1996) exhibit telomere attrition with progressive aging, suggesting that this autogenous activity is inadequate to prevent telomeric loss in HSCs with advanced aging. These results signify the importance of telomerase in HSCs renewal and subsequent differentiation.

3.2 *Telomerase effect on T and B lymphocytes*

T and B cells, during clonal expansion, experience extreme proliferative stress in order to acquire their desire phenotype and functional competence. This has prompted many researchers to study telomerase and telomere dynamics in

these cell populations. Studies have shown that telomere shortening occurs in T and B cells with advancing age in humans (Weng *et al*. 1995; Iwama *et al*. 1998; Son *et al*. 2000). This erosion of telomeres occurs at a rate of 50 bp/year in human CD4+ and CD8+ cells (Rufer *et al*. 1999). Potential explanation of this erosion could be the recurrent activation of T cells or oxidative damage during aging (dda di *et al*. 2003). Additionally, proliferative stress, being the primary stimulus for this attrition, was then observed by comparing telomere lengths of CD4+ and CD8+ memory T cells. Memory CD4+ and CD8+ T cells showed shorter telomeres, in comparison to naive T cells (Weng *et al*. 1996; Plunkett *et al*. 2005). This was further strengthened by the observation that the telomere shortening occurs in chronic diseases with consistent T cell activation such as HIV infection (Effros *et al*. 1996), rheumatoid arthritis (Wagner *et al*. 2004), psoriasis and atopic dermatitis (Wu *et al*. 2000). This shows that antigenic challenge resulted in enhanced clonal expansion and exhaustion of replicative capacity that resulted in the shortening of telomeres. Similarly, telomerase activity has been observed during *in vitro* stimulation of T cells obtained from young and old donors (Son *et al*. 2000).

Similar to T cells, telomere length variations have been observed in B cell populations. Telomerase activity measurements in naive and memory B cells showed that there is little or no activity in these cell types, but high levels of telomerase activity in the B cell germinal centre was observed (Hodes *et al*. 2002). Studies on mice regarding B cell responses showed that, in response to a stimulation, B cells undergo telomere elongation, which is telomerase dependent (Herrera *et al*. 2000). Moreover, recent evidence of reduced T and B lymphopoiesis due to telomerase deficiency came from the studies on telomerase-deficient aging mice, so far the best model for aging studies (Song *et al*. 2010). However, it is pertinent to note that telomere elongation of immune cells is telomerase dependent.

3.3 *Telomerase and epidermal stem cells*

Telomerase-deficient mouse has also been employed in understanding the biological outcome of telomere shortening on epidermal stem cells. Telomere shortening results in marked reduction in the capacity of hair follicle stem cells to regenerate the skin and hair, owing to defective mobilization of stem cells out of their niche (Flores *et al*. 2005). Besides, these mice also exhibit premature aging skin-phenotype such as decreased wound healing, graying and loss of hairs (Lee *et al*. 1998; Herrera *et al*. 1999; Rudolph *et al*. 1999) and are resistant to skin carcinogenesis (Gonzalez-Suarez *et al*. 2000). On the other hand, telomerase over-expressing studies in epidermal stem cells (*K5-mTert* mice) showed increase proliferation of keratinocytes, hair growth and skin hyperplasia (Flores *et al*. 2005). Interestingly, another study using conditional knock-in technique targeting epidermal stem cells (ESC) showed telomerase-dependent switching of ESC from telogen phase (quiescent resting phase) to anagen phase of rapidly dividing cells. Moreover, these effects of telomerase enzyme were independent of its RNA component (*Terc*), and thus independent of its activity, rendering it a non-canonical function of telomerase enzyme (Sarin *et al*. 2005). These results indicate telomerase-dependent and telomerase-independent roles in stem cells compartment; nevertheless, in stem cell compartment, distinct stem cell populations have varying responses to telomerase inflection. However, non-canonical roles of telomerase in other stem cells such as BMSC, HSCs have been poorly studied.

3.4 *Telomerase and epithelial stem cells*

Studies on telomerase-deficient mice models have demonstrated that the profound effects of telomerase deficiency were mainly observed in tissues/organs having higher load of cell turnover (Lee *et al*. 1998; Herrera *et al*. 1999; Rudolph *et al*. 1999). One such tissue compartment, with high cell turnover, is intestinal epithelia having high proliferative and regenerative demands. Epithelial stem cells of intestinal crypts of both mouse and human have been shown to have telomerase activity, while its deficiency results in atrophy of intestinal crypts in mouse (Espejel *et al*. 2004; Hao *et al*. 2005). Similarly, telomerase activity has also been observed in other epithelial compartments such as endometrium and in the apical region of dental epithelium (Jurgensen *et al*. 1996; Harada *et al*. 2002). Furthermore, studies on humans have shown that telomerase expression is reduced in ulcerative colitis and telomerase activity negatively correlates with the inflammation (Usselmann *et al*. 2001). These studies on epithelial stem cell clearly classify telomerase as an important factor for maintaining the integrity of stem cells in tissues/organs, undergoing continual restoration.

3.5 *Telomerase and cancer stem cells*

Studies on human aging and cancer, in connection with telomerase, revealed that telomerase evolved not only to make humans decrepit but also to prevent humans from cancer. Direct evidence of stem cell association with cancer came from the studies on hematopoietic malignancies, such as leukemia (Lapidot *et al*. 1994). Later, studies directly implicated telomerase RNA (TERC) and TERT mutations in myelodysplasia (MDS) and acute myeloid leukemia (AML) (Kirwan *et al*. 2009). Similarly, inactivation of telomerase has been shown to affect the growth of myeloid leukemia cells (Roth *et al*. 2003). This means that the

proliferative control has purposefully been evolved to prevent the invasion, unrelenting proliferation of cells, and damage to the adjoining tissues. Telomere erosion occurs in most human organs and tissues with aging (Djojosubroto *et al*. 2003). Unlike the presumed notion that telomerase repression and telomere shortening suppresses cancer, it was observed that the risk of cancer increases with telomere shortening amid aging and during chronic diseases (Djojosubroto *et al*. 2003). Yet, studies have shown that telomerase is required for telomere stabilization in cancer-initiating cells (Wright and Shay 1992). This concept was further supported by the observation that 80% of human cancers showed re-activation of telomerase enzyme activity (Satyanarayana *et al*. 2004). Moreover, recent experimental data from many studies showed that the telomere lengths of cancer cells are shorter than the non-transformed surrounding cells (Plentz *et al*. 2003, 2004). Yet, interestingly, studies on telomerase-deficient mice (*Terc*) have shown that telomere shortening act through a complex modus operandi, i.e. enhancing the earliest events of tumor initiation, while concurrently inhibiting the progression and development of advanced macroscopic tumours (Greenberg *et al*. 1999; Gonzalez-Suarez *et al*. 2000; Rudolph *et al*. 2001). Chromosomal instability is a hallmark of cancer in humans, and proof of this concept was obtained from studies on telomerase-deficient mice showing enhanced chromosomal instability (Lengauer *et al*. 1998; Artandi *et al*. 2000; Rudolph *et al*. 2001). From the above-mentioned studies it is quite obvious that cancer cells ought to maintain their telomeres for extensive proliferation and this can be achieved either by re-activation of telomerase (Counter *et al*. 1994) or by another mechanism termed as alternate lengthening of telomeres (ALT) utilizing homologous recombination process (Counter *et al*. 1994; Stewart 2005).

3.6 *Telomerase and neural stem cells*

Telomerase has been shown to play an indispensable role in the maintenance of neural stem cells (NSCs), while its deficiency results in the exhaustion of NSCs pool, leading to compromised olfactory bulb neurogenesis (Ferron *et al*. 2004). Telomerase expression in neural progenitors start to decline upon differentiation into neurons (Ferron *et al*. 2009), and correspondingly, neural stem cells loose telomerase activity upon differentiation into astroyctes (Miura *et al*. 2001). Interestingly, telomerase over-expression in neural cell lines inhibits neural differentiation (Richardson *et al*. 2007), suggesting that a fine balance in telomerase activity and telomere length is stringently regulated in neural stem cell compartment compared to other adult stem cell compartment such as BMSCs. This fact was further demonstrated by a study on telomerase deficient aging mice – despite reduced adult neurogenesis and short-term memory loss, progression of Alzheimer-disease-associated amyloid pathology was delayed in these mice, possibly owing to telomere-dependent effects on micro-glia activation (Rolyan *et al*. 2011), and chiefly due to impaired IGF-1 signalling (Freude *et al*. 2009; Killick *et al*. 2009). More recently, an elegant study by Jaskeiloff *et al*. showed that *in vivo* telomerase reactivation reversed neuro-degeneration in aged telomerase-deficient mice with restoration of $Sox2^+$ neural progenitors, DCX^+ newborn neurons and $Olig2^+$ oligo-dendrocyte populations (Jaskelioff *et al*. 2011). These studies also suggest that telomere length and telomerase activity in different stem cell compartments are cell autonomous relying more on intrinsic features of stem cells rather than tissue/organ micro-environment.

3.7 *Telomerase and bone marrow stromal stem cells (BMSCs)*

One of the major biological outcomes of telomerase deficiency and telomeres attrition in BMSC is cellular senescence (Hayflick and Moorhead 1961). Emerging evidence in the literature suggests that senescence occurs via different pathways but primarily due to telomere dysfunction and DNA damage, entailing p53 and p16/Rb signalling pathways in response to oncogenes, chromatin re-arrangements and stresses (Campisi 2005). It is also assumed that accumulation of senescent cells not only results in defective regeneration but they also secrete factors such as degenerative enzymes, inflammatory cytokines and growth factors that enhance senescence and tumorigenesis (Knapowski *et al*. 2002; Krtolica and Campisi 2003). A positive co-relation has been demonstrated between proliferative capacity of human BMSCs and telomere length, both in culture and with donor age (Sharpless and DePinho 2004). Similarly, cells obtained from adult donors showed telomere attrition at the rate of 17 bp per year; moreover, telomere length of 10 kb in human BMSCs have been shown to be a critical point, at which cells stop to divide (Baxter *et al*. 2004). BMSCs lack telomerase activity (Simonsen *et al*. 2002) and exhibit telomere shortening that ultimately resulted in replicative senescence phenotype in long-term culture (Stenderup *et al*. 2003). In highly sensitive assays no telomerase activity has been found in asynchronous hBMSCs during *ex vivo* culturing (Zimmermann *et al*. 2003), but when cells were synchronized to S phase during *ex vivo* culturing, positive telomerase activity was detected (Zhao *et al*. 2008). Morphologically, BMSCs undergoing senescence, due to telomere shortening, are large in size with loss of spindle-shaped morphology, as observed by comparative analysis of BMSC obtained from young and old donors (Dimri *et al*. 1995; Baxter *et al*. 2004). Additionally, γ-H2AX foci, the earliest events in DNA damage response, were more apparent in telomerase-deficient BMSCs compared to control BMSCs (Saeed *et al*. 2011). Age-related decline in telomere length is

observed in osteoblasts and chondrocytes (Martin and Buckwalter 2001; Yudoh *et al*. 2001). Interestingly, telomeres lengths of chondrocytes and ostoeblasts were found to be shorter in comparison to BMSCs from which they were derived (Parsch *et al*. 2002; Schieker *et al*. 2004). Besides, adult proliferative chondrocytes, pre-adipocytes, ostoeblasts precursors and fetal osteoblasts showed telomerase activity *in vitro* (Darimont *et al*. 2002, 2003; Montjovent *et al*. 2004; Parsch *et al*. 2004).

Furthermore, telomerase deficiency has been shown to compromise the differentiation of BMSCs into adipocytes, chondrocytes and osteoblasts during *in vitro* differentiation assays (Liu *et al*. 2004; Saeed *et al*. 2011). Also, BMSC cultures established from elderly donors exhibited impaired cell proliferation, accumulation of senescent cells and shorter replicative lifespan *in vitro* (Abdallah *et al*. 2006). Conversely, 're-telomerization' of BMSC through over-expression of hTERT leads to elongation of telomeres of BMSC and extends their *in vitro* lifespan. In addition, telomerized cells maintain their 'stemness' characteristics and their bone forming abilities are enhanced based on *in vitro* and *in vivo* criteria (Simonsen *et al*. 2002). These data suggest an important role of telomerase enzyme in the maintenance of apposite stem cell functions.

4. Conclusion and future prospects

These above-mentioned studies clearly implicate telomerase-dependent telomere damage as a key player in perpetrating age-associated decline in the functional capacities of an organ/tissue and disease progression related to stem cell functions (figure 1) (Sharpless and DePinho 2007; Sahin and DePinho 2010). It is beyond doubt that maintenance of telomere lengths by telomerase is important for stem cell functions and tissue homeostasis while its dysregulation results in two major shifts, that is, aging and cancer. There can be three plausible propositions regarding the functional decline in different stem cell compartments with aging and malignant transformations: (a) Degree of telomere shortening/length determines the nature of cell intrinsic alterations favouring aberrant stem cell functions, which further leads to either pro-apoptotic or pro-malignant signals down the road. (b) Telomere shortening can result in conformational changes in the chromatin, i.e. from heterochromatin (inactive) to euchromatin (active) – altering gene expression profiles that in turn amends tissue/organ milieu favouring either functional decline with aging or cancer. (c) Combination of cell intrinsic and extrinsic alterations supporting either pro-apoptotic or malignant signals establishes the final outcome. However, assuming that aging and cancer is a stem cell phenomenon, its still unresolved that at what point, during the course of degenerative aging, stem cells decide to transform and what level of cell intrinsic and extrinsic alterations associated with telomere shortening and telomerase activity determines the transformational course, which, therefore, begs further investigations.

Furthermore, endogenous telomerase inflection in diverse stem cell compartments, to maintain tissue/organ homeostasis, seems different and needs to be determined by gain and loss of function studies at tissue/organ level. These studies would not only provide valuable information regarding compartment specific telomerase regulation but also help devising drug-based strategies for maintaining telomerase competent tissue/organ environment with advanced aging.

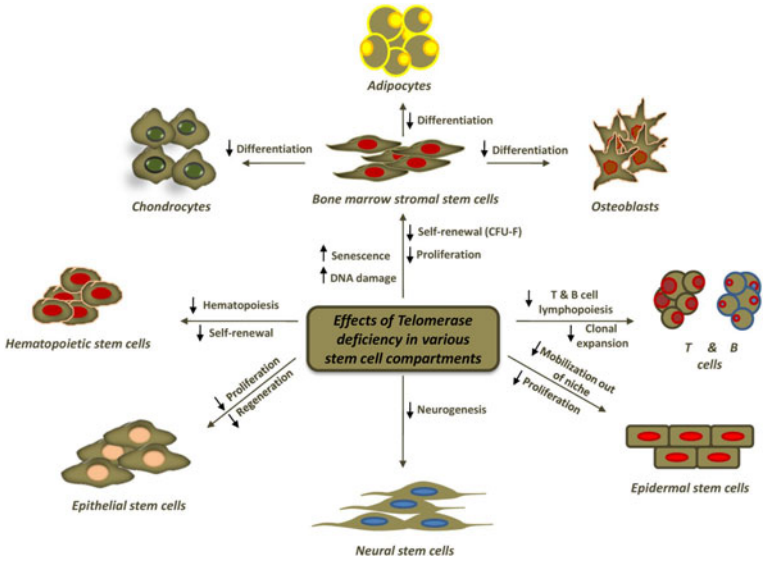

Figure 1. A summary of diverse biological effects of telomerase deficiency on tissue/organ resident stem cell populations.

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