Bone Marrow Stromal Cell (BMSC) and skeletal aging: Role of telomerase enzyme

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Abstract: Telomere shortening and telomerase deficiency have been linked with several age related degenerative diseases. Moreover, degenerative changes in various tissues/organs have been attributed to derangement of stem cell functions causing regenerative tragedy. Bone marrow stromal cells (BMSCs) are considered the ideal candidates for regenerative approaches owing to their beneficial effects in numerous clinical applications. Thus, the effect of telomerase deficiency in perpetrating age related changes in BMSC functions during *in vitro* culture; their morphology, proliferation and differentiation, that can be extrapolated and reasoned for skeletal aging is conversed in this review. Besides, information regarding pertinent molecular and biochemical markers that can be employed to examine the earliest events, during the course of BMSC aging, is also provided. Additionally, impact of telomerase deficiency in enacting skeletal aging phenotype and its associated microenvironment is also discussed. In the end, further studies, using tissue specific models of telomerase deficiency, are recommended as a future research strategy to advance our understanding of tissue specific telomerase regulation.

Keywords: Telomeres, telomerase, bone marrow stromal cells, aging.

INTRODUCTION

Regenerative medicine holds a promise for future therapies, for patients with chronic degenerative diseases and severe injuries such as, Osteoporosis, Alzheimer´s and Parkinson´s disease and spinal cord injuries, respectively (Abdallah and Kassem, 2009; Sugaya *et al*., 2007; Vidaltamayo *et al*., 2010). Regenerative capacity of our body decreases with aging. Regeneration of tissues and organs with aging depend upon resident tissue/organ specific stem cells (Wagner *et al*., 2009). Bone marrow stromal cells (BMSCs), because of their ease of culture, adaptable growth and multi-lineage differentiation potential (Peister *et al*., 2004; Pittenger *et al*., 1999), are ideal candidates for regenerative approaches and are currently being employed in clinical trials (Abdallah and Kassem, 2008; Abdallah and Kassem, 2009). Plausible regenerative applications of BMSCs include stem cell transplantation (Devine and Hoffman, 2000), stem cell based strategies for the repair of damaged organs (Bruder *et al*., 1998) and gene therapy (Fukuda *et al*., 2000). Moreover, beneficial effects of BMSCs, alone or in combination, in regenerative approaches have been reported in humans and mice studies, i-e., they can enhance the engraftment of hematopoietic stem cells after co-transplantation (Anklesaria *et al*., 1987), regenerate the marrow environment after myelo-ablative therapy(Koc *et al*., 2000) and as immuno-modulators (Lu *et al*., 2009; Yagi *et al*., 2010). BMSCs have been considered as the primary source of osteoblasts for skeletal repair (Fibbe, 2002). However with aging, owing to number of factors, including telomerase deficiency, functional and numerical decline of BMSCs resulted in the compromised ability of BMSCs to repair the skeleton and maintain skeletal homeostasis (Bergman *et al*., 1996; D'Ippolito *et al*., 1999; Saeed *et al*., 2011).

Skeletal homeostasis is maintained by a highly precise and intricate process termed as bone re-modeling, which allows a unique micro-environment to facilitate coupled osteoclast reosption and osteoblast bone formation, a process termed as *coupling* (Andersen *et al.,* 2009). Disruption in the coupling process with aging initiates a bone related pathological condition termed as *osteoporosis* most prevalent pathological condition in humans with advanced age (Ahlborg *et al*., 2010; Khosla, 2010; Manolagas, 2000; Reginster and Burlet, 2006). One of the primary patho-physiological mechanisms of osteoporosis is the impairment of osteoblastic bone formation during bone remodeling with aging (Cohen-Solal *et al*., 1991). Number of studies have shown that age/osteoporosis-related impairment of bone formation is caused by accumulation of senescent osteogenic stem cells, which not only impair bone regeneration capacity but also create a "senescent microenvironment" that inhibits osteogenic stem cell differentiation (Justesen *et al*., 2002; Kveiborg *et al*., 2000; Stenderup *et al*., 2003). Telomere shortening owing to telomerase deficiency is one of the factors contributing to senescence associated replicative arrest in cultured stem cells (Simonsen *et al*., 2002; Stenderup *et al*., 2003; Kassem *et al*., 1997). While telomerase over-expression in human bone marrow stromal stem cells (hBMSC-TERT) resulted in long telomeres and improved self-renewal capacity. Importantly, telomerase not only inhibited the cellular

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senescence but also maintains cell proliferation potential with improved *in vivo* bone formation capacity (Simonsen *et al*., 2002; Abdallah *et al*., 2005).

Telomerase is inactive in most somatic cells but active in germ cells, stem cells and actively dividing cells (Kassem *et al*., 2004). This enzyme requires a template to act, which is the RNA component of telomerase i.e., *TERC*. RNA component (Blackburn, 1984; Blackburn and Gall, 1978). Telomerase deficient mice (Terc-/-) have been extensively employed to study the impact of telomere shortening in context of whole organism (Blasco, 2005). Disease conditions in *Terc*$^{-/-}$ aging mice were similar to several aging conditions in humans, with more or less same etiology, characterized by short telomere in the cells as a result of excessive proliferation (Ju *et al*., 2007; Lee *et al*., 1998; Vulliamy *et al*., 2002). Telomerase deficient mice (*Terc*$^{-/-}$) exhibited compromised maintenance and regeneration in tissues/organs undergoing extensive proliferation, like liver, intestine, testis, ovaries and spleen, and their stem cell populations are inclined to lack and compensate the intense regenerative demands of these tissues/organs, during the course of aging or progression of degenerative diseases (Lee *et al*., 1998; Herrera *et al*., 1999; Blasco *et al*., 1997;) Ju *et al*., 2007; Pignolo *et al*., 2008; Rudolph *et al*., 1999). We have recently provided a proof of this concept in *Terc*$^{-/-}$ mice (G3) in another dynamic organ that is bone (Saeed *et al*., 2011).

This review, therefore, will entail about BMSCs, telomerase dependent age-related decline in the functional capacities of BMSCs, impact of telomerase deficiency on bone architecture and its microenvironment, conclusion and future perspective.

Bone Marrow Stromal Cells (BMSCs)

Friendestein (1973) first demonstrated the osteogenic potential of cells obtained from bone marrow and could be distinguished from the others, such as hematopoietic cells, by selective adherence to tissue culture dishes, fibroblast like morphology and exhibit density insensitive growth (Owen and Friedenstein, 1988; Friedenstein *et al*., 1970). Later it was confirmed that these are multi-potent cells - capable of differentiating into different mesodermal lineages, such as osteoblasts, adipocytes and chondrocytes (Bianco and Robey, 1999; Friedenstein *et al*., 1987; Kuznetsov *et al*., 1997). However, only small numbers of these cells (MSCs) can be obtained from the bone marrow accounting for about 0.01 – 0.001% of the total bone marrow cells (Pittenger *et al*., 1999b). This led to the identification of BMSCs from variety of other sources, including peripheral blood (Kuznetsov *et al*., 2001), adipose tissue (Zuk *et al*., 2001), umbilical cord blood (Rosada *et al*., 2003), synovial membranes (De *et al*., 2001), deciduous teeth (Miura *et al*., 2003) and recently, amniotic fluid (De *et al*., 2007). Despite, similarities among various BMSC populations e.g., surface markers they exhibited differences in gene expression profiles and differentiation potential when compared side-by-side (Wagner *et al*., 2005).

BMSCs are characterized by the identification of surface antigens (CD markers) to distinguish them from other cell types. Generally, BMSCs are negative for hematopoietic surface markers: CD34, CD45, CD14 and positive for: Stro-1, CD29, CD73, CD90, CD105, CD106, CD166, CD146 and CD44 (Abdallah *et al*., 2005; Dominici *et al*., 2006; Foster *et al*., 2005). Additionally, several other markers have been employed to attain more homogenous population of BMSCs with clonogenic potential such as, STRO-1 (which give a more clonogenic identification) (Simmons and Torok-Storb, 1991) or MCAM (CD146) (Sacchetti *et al*., 2007). Furthermore, these surface markers are used alone or in combinations for enhanced enrichment, for example: using STRO-1 alone or in combination with CD106 (VCAM-1) or CD146 (MUC18) (Gronthos *et al*., 2003), CD271 (low affinity nerve growth factor receptor) (Quirici *et al*., 2002), CD18 (b-2 integrin) (Miura *et al*., 2005) and embryonic stem cell marker: SSEA-4 (Gang *et al*., 2007). However, none of the markers are reliable and specific to identify their *in vivo* location and to isolate a prospective true multi-potent BMSC population. More recently, several other markers were employed, alone or in combination to document their *in vivo* location and nature, such as PDGFR-α, Nestin and α-SMA (Grcevic *et al*., 2012; Mendez-Ferrer *et al*., 2010; Morikawa *et al*., 2009). Yet, there are other unresolved barriers that need to be resolved to exploit their remarkable potential in the clinic.

Un-resolved barriers for clinical use of BMSCs

Despite extensive research there are two major barriers to use BMSCs in the clinic, such as *in vitro* expansion and likely cell intrinsic and extrinsic (micro-environment) alterations with aging that makes overall picture of their pertinent use in cell based therapies, a bit murky. Studies have shown that *in vitro* expansion of BMSC during the long term culture is sub-optimal as they exhibit stunted proliferation rates and enter a state of growth arrest called replicative senescence (Hayflick, 1965; Kassem *et al*., 1997). Similarly, we and others have shown that cells obtained from aging mice exhibit cell autonomous intrinsic alterations with advancing aging (fig. 1A & B) (Rossi *et al*., 2005; Song *et al*., 2009; Saeed *et al*., 2011) thus, age dependent cell intrinsic alterations pose a major challenge to autologous regenerative approaches in elderly, which require the most of regenerative medical interventions. Above-mentioned studies clearly state that cells (BMSC/Adult stem cells) exhibit aging under both, *in vitro* and *in vivo,* conditions. Therefore, it is imperative to understand cellular aging and factors that control replicative senescence or cellular aging, particularly of BMSC.

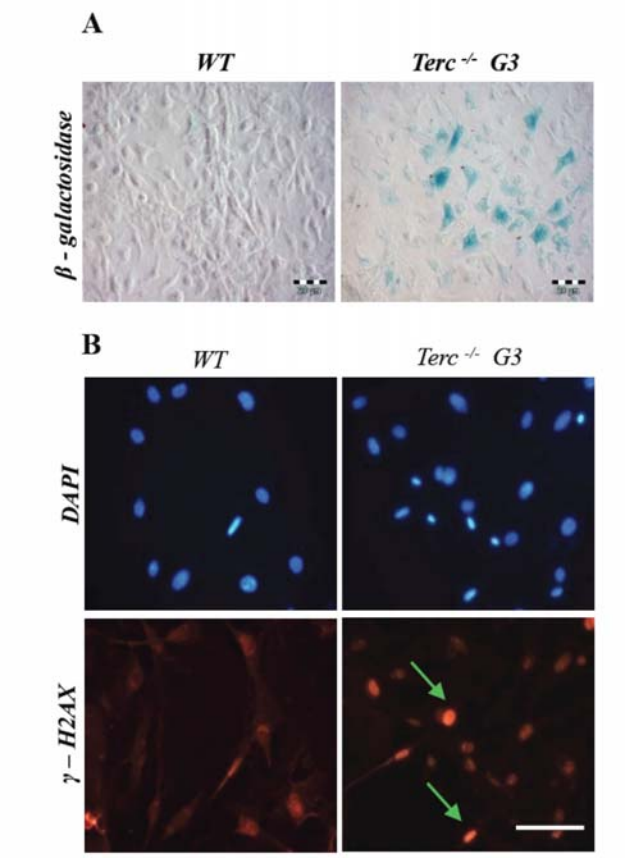

Fig. 1: Telomerase deficient BMSCs exhibited enhanced senescence (β-gal+) and DNA damage (γ-H2AX+). A) Increased number of β-gal+ cells (blue) in *G3 - Terc-/-* BMSCs. B) *G3 - Terc-/-* BMSCs accumulated DNA damage (γ-H2AX+) shown by staining, pointed by green arrows. Taken from, Saeed et al., 2011.

Cellular Aging

In biological terms, aging is defined as a functional decline of an organism in terms of one's ability to cope with stresses, rising homeostatic imbalance and increased threat of diseases that are influenced by one´s genetics and environment. However, aging might theoretically be different from cellular aging that is often related to senescence associated replicative arrest. A term replicative senescence was first used by Hayflick (Hayflick and Moorhead, 1961), defined as irreversible arrest of cell division. Senescence differs from apoptosis as senescent cells remain alive with derangement of functions (Itahana *et al*., 2001). This has further been supported by clinical and animal studies showing that the regenerative capacity of bone and other tissues decline with age(Rauscher *et al*., 2003). Emerging evidence suggest that senescence may be one of the basis of compromised regeneration of tissues that, subsequently, can result in organ failure and death (Knapowski *et al*., 2002). Besides, senescent cells secrete number of factors that can affect the neighboring cells thus alter the micro-environment (Ju *et al*., 2007) such as, growth factors that enhance aging phenomenon in tissues (Krtolica and Campisi, 2003), alter differentiation potential(Abdallah *et al*., 2006), inflammatory cytokines (Saeed *et al*., 2011) and mortifying enzymes (Krtolica and Campisi, 2003). However, our understanding of BMSC per se is still not absolute, therefore; it's even more cumbersome to define aging of BMSCs. Thus, obvious questions are, does BMSC aging *in vitro* reflect factual aging or it is an outcome of sub-optimal *in vitro* culture conditions that contribute to this sham aging phenotype. To our knowledge of cellular aging, among others, one of the contributing factors involved in accelerated senescence and intrinsic stem cell alterations, is the deficiency of the telomerase enzyme that results in progressive shortening of telomeres after every round of cell division. Numerous studies have shown that telomerase deficiency plays an important role in the aging of tissues and organs by affecting their functional cellular machinery (Simonsen *et al*., 2002). Therefore, we would like to focus on peculiar characteristics of telomerase dependent BMSCs aging.

In vitro Characteristics of telomerase dependent BMSC Aging

Salient features of BMSC aging or cellular aging include aberrant morphology (Baxter *et al*., 2004; Stenderup *et al*., 2003), stunted growth (senescence)(Kveiborg *et al*., 2001; Stenderup *et al*., 2003), reduced colony size and number (De *et al*., 2007), short telomeres (Simonsen *et al*., 2002), accumulation of DNA damage and derangement of functions in terms of compromised self-renewal, differentiation (Baxter *et al*., 2004) and migratory potential (Flores *et al*., 2005).

Morphology

Peculiar cellular morphology of aging cell is an important bio-marker of replicative senescence. Studies have shown that, morphologically, BMSCs undergoing senescence are large in size with loss of spindle shaped morphology, as observed by comparative analysis of BMSCs obtained from young and old donors (Baxter *et al*., 2004; Dimri *et al*., 1995; Hayflick and Moorhead, 1961) along with more actin stress fibers (Baxter *et al*., 2004; Stenderup *et al*., 2003) while cells immortalized by telomerase (Tert) are smaller and showed spindle shape morphology compared to non-transfected counterpart (Simonsen *et al*., 2002). Moreover, because senescent cells are larger, cellular density of confluent senescent culture is lower than the cellular density of confluent young culture also because senescent cells are more sensitive to contact inhibition, suggesting anti-cancer strategy (Seluanov *et al*., 2009). Very few studies looked to the causes of these morphological changes, specifically, pointing to the one study demonstrating that caveolin-1 regulates senescence associated morphology by modulating focal adhesion kinase (FAK) activity and by formation of actin stress fibers (Cho *et al*., 2004)

Senescence & DNA damage

However with increasing interest of scientific community in senescent associated cellular aging, markers other than morphological changes have been identified and were found to be linked with these overt morphological changes. It has been shown that senescent cells exhibit not only enlarged flattened morphology but also have increased lysosomal activity assessed by beta galactosidase (β-gal^{+}) blue staining, now considered as one of the well established senescent markers (Dimri *et al*., 1995). Similarly, BMSCs from old donors have more β-gal^{+} BMSCs, during *in vitro* cultures, compared to young donors (Stenderup *et al*., 2003). It is also assumed that accumulation of senescent cells not only resulted 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). These secretary factors along with accumulation of senescent cells *in vivo* affects the behavior of neighboring cells, suggesting that senescence phenomenon may influence tissue homeostasis (Krtolica and Campisi, 2003; C.Beausejour, 2011).

Similarly, one of the retrogressive features of senescence is the accumulation of DNA damage (Gao *et al*., 2001; von *et al*., 2001). Accumulation of DNA damage in aging hematopoietic stem cells (HSC) owing to telomerase deficiency have been reported previously (Rossi *et al*., 2007). Similarly, Wang *et al*., has shown that activation of DNA damage response and formation of DNA damage foci at either uncapped telomeres or DNA strand breaks is crucial to elicit cell senescence (Wang *et al*., 2009). We have also shown that BMSCs and osteo-progenitors obtained from *Terc*$^{-/-}$ mouse exhibited senescence associated β-gal^{+} staining and DNA damage (γ-H2AX^{+}) accumulation (fig. 1A & B) coupled with enhanced expression of cell cycle inhibitors, *p21* and *p16,* compared to WT BMSCs (Saeed *et al*., 2011). These effects are mostly mediated through the shortening of telomeres owing to lack of telomerase activity.

Telomere length shortening and Telomerase activity

After every round of cell division telomere gets shortened owing to inability of DNA polymerase to replicate the ends (C.Beausejour, 2011). 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). Cells obtained from adult donors showed telomere attrition at the rate of 17 bp per year, moreover telomere length of 10kb in human BMSCs have been shown to be a critical point, at this point cells stop to divide (Baxter *et al*., 2004). Age related decline in telomere length is observed in osteoblasts and chondrocytes (Martin and Buckwalter, 2001; Yudoh *et al*., 2001). Interestingly, telomeres lengths

Table 1: Summary of studies describing mean telomere lengths, telomere shortening and telomerase activity during *in vitro* culture

Studied age group/specie	Mean telomere length	Telomerase activity	*In vitro* telomere loss	Reference
Human (19-57 yrs)	-	Positive even at passage 12	-	(Pittenger *et al.,* 1999)
Human 0-18p (10) 57-79p (5)	11.5kb (16PD) 10.4kb (16PD)	-	88bp/PD ±10 $hMSC_{p0-18}$ & 78bp/PD ±34 $hMSC_{p59-75}$	(Baxter *et al.,* 2004)
Fetal	-	Positive	-	(Fu *et al*., 2001)
Mouse (8 weeks) Rat (8 weeks)	27kb 27 kb	- Positive	No loss (40PD) No loss (42PD)	(Jiang *et al*., 2002)
Human (2-50 years)	11-15 kb (35PD)	-	Unchanged till 30 PD	(Reyes *et al*., 2001)
Human (22 and 46 yrs)	9.2 kb (15PD)	Negative	-	(Simonsen *et al*., 2002)
Human	-	Very subtle activity	-	(Zimmermann *et al*., 2003)
Human (4-74 yrs)	7.3kb (p3)	-	7.8bp in young samples, 6.1bp in oldest at p3	(Lee *et al*., 2003)
Rat	-	Positive	-	(Seruya *et al*., 2004)
Human (18-29 yrs)	10.4kb	Negative	100bp/PD	(Stenderup *et al*., 2003)
Human (healthy donors)	15kb	Positive	No loss	(Yoon *et al.,* 2005)
Human	8 kb	-	50bp/PD	(Serakinci *et al.,* 2007)
Human	-	Negative	-	(Graakjaer *et al.,* 2007)
Human Rhesus monkey	10-15 kb 21 kb	Positive Positive	- -	(Izadpanah *et al.,* 2006)

of chondrocytes and ostoeblasts were found to be shorter in comparison to BMSC from which they were derived (Parsch *et al*., 2002; Schieker *et al*., 2004). However, stem cells possess unique features that could cause diminished telomere shortening, such as asymmetrical cell division and immortal strand segregation; mechanisms by which stem cell manages to protect their genome from replication errors and telomere shortening (Serakinci *et al*., 2008). Studies on BMSC describing mean telomere lengths, telomere shortening and telomerase activity during *in vitro* culture is summarized in table 1.

Telomere lengths are maintained by telomerase enzyme. There is 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). Telomerase activity is usually repressed after the differentiation of stem cells (Armstrong *et al*., 2000; Forsyth *et al*., 2002; Sharma *et al*., 1995). Utilizing highly sensitive assays no telomerase activity has been found in asynchronous hBMSCs during *ex vivo* culturing (Zimmermann *et al*., 2003), however when cells were synchronized to S phase during *ex vivo* culturing positive telomerase activity was detected (Zhao *et al*., 2008). Adult proliferative chondrocytes, pre-adipocytes, ostoeblasts precursors and fetal osteoblasts showed telomerase activity *in vitro* (Darimont *et al*., 2002; Darimont *et al*., 2003; Montjovent *et al*., 2004; Parsch *et al*., 2004). Whether this low or absence of telomerase activity in BMSC is a consequence of sub-optimal culture conditions or an innate feature of BMSC is still un-resolved. Moreover, absence of telomerase activity and shortening of telomeres affects functional capacity of stem cells, thus contribute to the aberrant biological functions and pathways.

Proliferation

As mentioned above, irreversible growth arrest is distinctive trait of cellular aging, in this context, plethora of studies have demonstrated the role of telomerase and telomere shortening in stem and progenitors proliferative potential (Campisi *et al*., 2001; Choi *et al*., 2008; Kassem *et al*., 1997; Lee *et al*., 1998; Saeed *et al*., 2011). Normal human cells can only divide a limited number of population doublings (PD) termed as Hayflick limit, which varies depending upon the cell type, donor age and culture conditions (Campisi *et al*., 2001; Shay and Wright, 2000). Mouse cells are explicit than human MSC in terms of telomerase expression (Prowse and Greider, 1995; Weng and Hodes, 2000) and have long telomeres of about 60 kb in comparison to 10-15kb in humans (Sharpless and DePinho, 2004). Human BMSCs from young donors can undergo 24-40 PD *in vitro* and that was quite reduced in hBMSC obtained from old donors (Stenderup *et al*.,2003). However, murine BMSC, reportedly, seems to proliferate for a much longer time (Meirelles and Nardi, 2003).

Self-renewal

Another peculiar feature of BMSC is their self-renewal capacity which is an outcome of asymmetric cell division and plays an important role in the maintenance of stem cell pool (Bonyadi *et al*., 2003). As a consequence of asymmetric cell division stem cells can give rise to large number of differentiated daughter cells without themselves going through extensive cell divisions, thus maintaining their telomere lengths (Wai, 2004). Therefore, self renewal property helps in maintaining a constant pool of undifferentiated stem cells throughout the life time, suggesting that the ability of cells to produce the progeny either undifferentiated or differentiated is dependent on proficient self renewal capacity of a stem cell (Dykstra and de, 2008). Self-renewal capacity of BMSC is generally assessed by generation of colony forming units (CFU-F) whereas, senescent associated reduction in self-renewal capacity effects the frequency of CFU-F/CFU-ALP in aging or in age-related pathological conditions such as, osteoporosis (Bonyadi *et al*., 2003; Wang *et al*., 2006). Similarly, reduced colony size and number were observed when osteoprogenitors were cultured from aging women and men in comparison to young donors (Muschler *et al*., 2001). More recently, we have demonstrated that the self-renewal ability of *Terc-/-* BMSCs, compared to WT, to generate sufficient number of CFU-F/CFU-ALP colonies declines significantly during *in vitro* cultures (Saeed *et al*., 2011)

Compromised differentiation

There is inadequate literature evidence regarding the primary role of telomerase enzyme in the differentiation of stem cells. Initial evidence of telomerase role in the differentiation of stem cells came from the studies on human hematopoietic stem cells proliferation and differentiation-where telomerase activity increases during proliferation and differentiation while reduction of telomerase activity with aging co-related with reduction in hematopoiesis(Hiyama *et al*., 1995). This concept was further supported by a study on B cell maturation and differentiation in the germinal centre (GC) a stage where GC-B cells showed 128 fold higher telomerase activity compared to naive or memory B cells(Weng *et al*., 1997). Direct evidence of differentiational control of mTERT was provided through the use of mouse erythroleukemic (MEL) system where induction of differentiation was characterized by a biphasic change in mTERT mRNA and telomerase activity levels - telomerase activity was initially low, then up-regulated and maintained at high levels and lastly complete loss of activity(Greenberg *et al*., 1998). Noticeably, role of telomerase in proliferation and differentiation of stem cells in another cellular compartment, such as bone marrow derived BMSCs was shown lately MSCs derived from mice lacking telomerase activity completely failed to differentiate into osteoblasts, adipocytes and chondrocytes, even at early passages (Liu *et al*., 2004), (Pignolo *et al*., 2008, Saeed *et al*., 2011).

Above mentioned literature evidence, provides fine association between telomerase deficiency and decline in stem cells functions during aging. However, telomerase over expression not only circumvent the senescence but also enhanced the proliferation and differentiation of hBMSC into bone(Simonsen *et al*., 2002; Saeed *et al*., 2011), thus offer challenging prospect of partially rescuing some of the biological features associated with aging.

Telomerase and skeletal aging

Skeleton is a highly dynamic and specialized organ undergoing continuous regeneration and therefore, needs a continuous supply of highly specialized cells. During the process of growth and development, the skeleton is fashioned and molded into shape and size by removal of bone from one site while deposition occurs on another in a process known as bone modeling (Manolagas, 2000). Re-modeling most likely occurs to repair fatigue, damage and to prevent aging and its consequences. Therefore, in conclusion, re-modeling occurs to prevent accumulation of old bone. Studies on animals and humans have shown that changes either in the endurance of bone precursors (Bone marrow stromal stem cells) or differentiated osteoblastic cells in the bone marrow provide an explanation of the close link between senescence and bone loss (Jilka *et al*., 1996; Kassem *et al*., 1997). Evidence of a close link between senescence and bone loss came from studies on murine model of age-related osteopenia i-e., SAMP6 mice. Despite sex steroid sufficiency and intact reproductive functions, SAMP6 mouse showed reduced osteoblast progenitors, bone formation rate and bone mass(Jilka *et al*., 1996). This has further been supported by clinical and animal studies showing that the regenerative capacity of bone and other tissues decline with age(Rauscher *et al*., 2003). Skeletal aging in telomerase context has been poorly studied. However, studies on human subjects suggest that leukocyte telomere lengths could be employed as a marker of biological aging of bone (Valdes *et al*., 2007), furthermore replicative and working life span of osteoblasts decrease in rheumatoid arthritis patients with advancing age(Yudoh and Matsuno, 2001). Our recent observations that telomerase deficiency resulted in bone loss phenotype in aging mice, reflects the subtle connection between the telomerase dependent telomere shortening and deterioration of bone architecture with advancing age(Saeed *et al*., 2011). Results obtained in this study demonstrated that *Terc*$^{-/-}$ mice exhibited an age-related bone loss phenotype starting at 8 weeks of age, leading to an osteoporotic phenotype with kyphosis and deteriorated bone architecture (fig. 2). Interestingly, the decreased bone mass was more evident in the trabecular bone than in the cortical bone compartment due to higher bone turnover in *Terc*$^{-/-}$ mice. As bone surface/volume ratio is higher in trabecular bone compared to cortical bone, and trabecular bone loss is usually observed in high turnover states (Parfitt *et al*., 1995). Similar fine relationship between telomere length, bone mineral density and osteoporosis in humans has been suggested before, reinforcing the fact that the reduction in telomere length with age is associated with osteoporotic bone fragility (Valdes *et al*., 2007; Bekaert *et al*., 2005). Moreover, *Terc*$^{-/-}$ mice showed more rod like structures in trabecular region (proximal tibia) in comparison to more plate like structures in the trabecular region of WT mice (Saeed *et al*., 2011). This is consistent with observation in human subjects where existence of qualitative radical change in trabeculae from plate-like to rod-like structures was observed with aging (Ding and Hvid, 2000). These effects can best be explained at cell and molecular levels discussed above (section, Telomerase dependent BMSC aging).

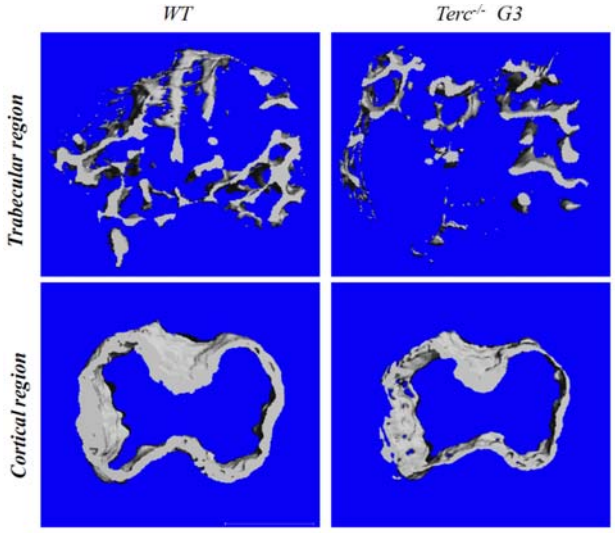

Fig. 2: Telomerase deficiency and bone micro-architecture. Micro-CT scans showing deterioration of bone micro-architecture in telomerase deficient mice (trabecular and cortical). Taken from Saeed *et al.,* 2011.

Telomerase deficiency and bone microenvironment with aging

Literature evidence suggests that senescent cells can alter microenvironment by aberrant secretion of different growth factors, cytokines and degenerative enzymes (Sethe *et al*., 2006). Studies on aged rodent brain have shown homeostatic imbalance of inflammatory mediators, resulting in increased steady-state levels of pro-inflammatory cytokines (Sparkman and Johnson, 2008; Venneti *et al*., 2009). Similarly, studies on human cells having persistent DNA damage signaling resulted in increased secretion of pro-inflammatory cytokines, such as, *IL-6*(Rodier *et al*., 2009). Further studies, in this direction - implicating telomerase enzyme per se, showed that tissue micro-environmental alterations owing to telomerase deficiency resulted in impair stem cell function and organ homeostasis. Ju *et al*. in his report, has demonstrated that these extrinsic alterations caused poor

engraftment of transplanted wild type hematopoietic stem cells due to skewed expression of several cytokines such as, granulocyte colony stimulating factor (G-CSF) (Ju *et al*., 2007). Our recent findings concerning the affects of telomerase deficiency on bone micro-environment showed osteoclast (OC) supportive pro-inflammatory micro-environment (Saeed *et al*., 2011). OC cell generation and functions are regulated by a large number of cytokines and systemic hormones (Grigoriadis *et al*., 1994; Kim *et al*., 2002; Kurihara *et al*., 1990; Zhang *et al*., 2009; Kenner *et al*., 2004; Yamashita *et al*., 2007; Lovibond *et al*., 2003). We observed that aging bones, obtained from *Terc-/-G3* mice, exhibit increased expression of pro-inflammatory cytokines (*Il1-β* and *Tnf-α*) supportive of osteoclastogenesis (Saeed *et al*., 2011). Results showed that, both *RANKL* was up regulated (1.1 fold) and OPG down regulated (1.4 fold) suggesting increased *RANKL/OPG* (2 fold). Additionally, several signaling pathways known to affect osteoclastogensis were up-regulated e.g. NF-kB (Clohisy *et al*., 2004) and IL6 signaling (Kurihara *et al*., 1990b). Similar to our findings employing aging mice, Sierra *et al*., has reported increased expression of pro-inflammatory cytokines, such as, *Il1-β*, *Tnf-α* and *Il-6* by aging microglia (Sierra *et al*., 2007). Furthermore, sera from *Terc*$^{-/-}$ mice contained high levels of several inflammatory molecules related to the innate immune response (Jiang *et al*., 2008). Inhibitory effect of pro-inflammatory cytokines such as, *TNFα* and *IL1β*, on osteogenic potential of BMSCs has also been observed (Lacey *et al*., 2009), therefore, effect of these cytokines on the survival and differentiation of bone forming cells cannot be ruled out with advanced aging. Seemingly, pro-inflammatory changes in the bone microenvironment point towards the close association of bone and immune system. However, it could also be possible that general telomerase deficiency resulted in dys-regulation of hormones such as, sex steroids, or other serum circulating factors affecting bone turnover (Abdallah *et al*., 2006; Mayack *et al*., 2010). Thus, identification of humoral factors, endocrine and exocrine, and their contribution to the bone loss phenotype in *Terc*$^{-/-}$ mice can lead to the development of novel strategies to avert aging bone phenotype.

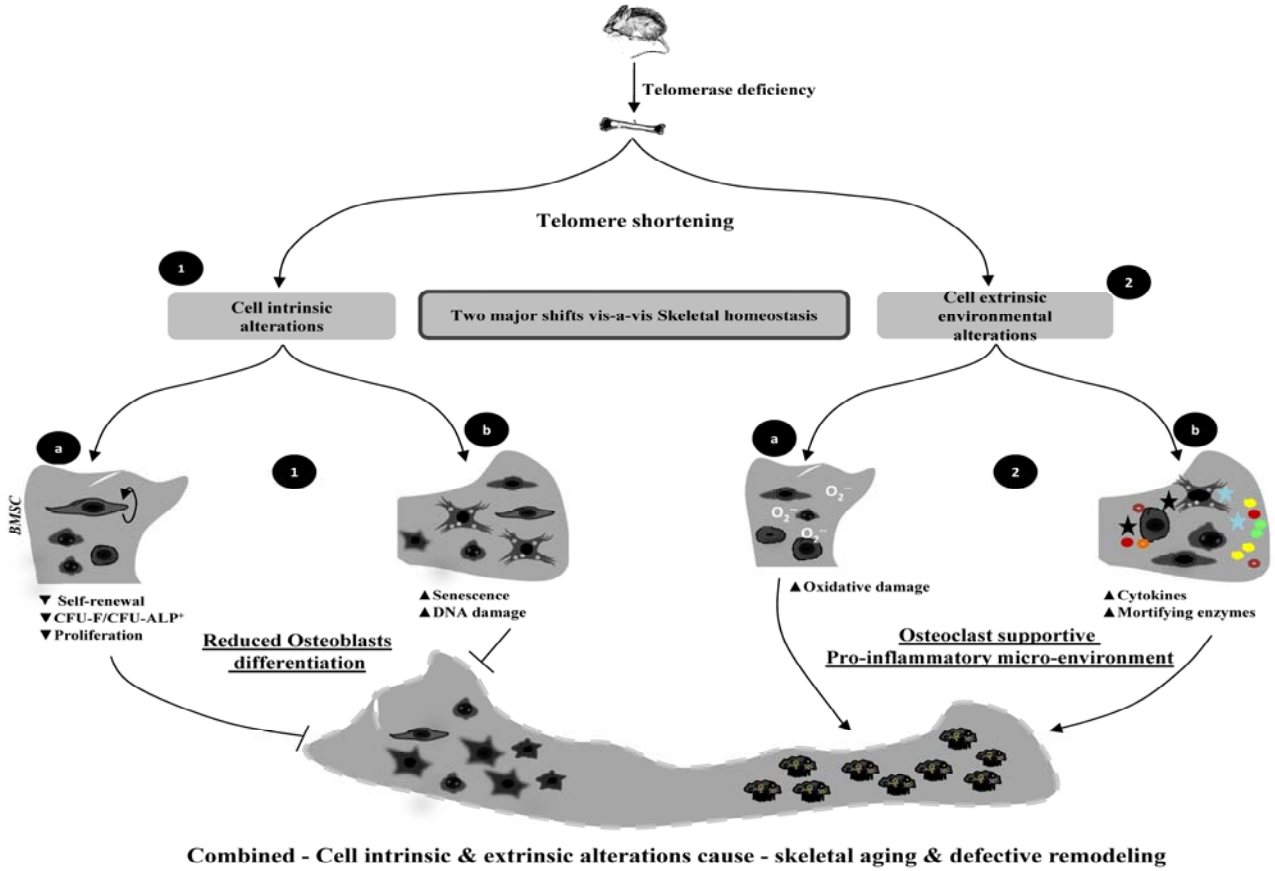

Fig. 3: Model describing Telomerase deficiency dependent skeletal aging; bone micro environmental changes affecting bone remodeling. Telomerase deficiency resulted into two major shifts vis-à-vis skeletal homeostasis. 1. Cell intrinsic alterations (senescence and DNA damage) affecting bone forming cells, proliferation and differentiation. 2. Cell extrinsic bone microenvironment alterations (oxidative damage, cytokines and mortifying enzymes), supporting osteoclast dependent bone resorption.

Conclusion and future perspectives

In conclusion, above-mentioned studies clearly implicate telomerase in age related bone loss by enhancing age dependent mortifying events, that is accumulation of oxidative stress, DNA damage, enhanced senescence, which not only affect functional competence of bone forming cells but also contribute to the up-regulation of several pro-inflammatory cytokines that alter the bone micro-environment, favoring skewed re-modeling cycles. Thus, telomerase deficiency resulted in two major shifts considering bone homeostasis a) cell intrinsic alterations; affecting bone marrow stromal cell functions b) cell extrinsic alterations, that is, altered bone micro-environment teeming with several growth factors and cytokines enhancing bone resorption (fig. 3).

However, it is still not clear if these changes in the bone microenvironment owing to telomerase deficiency are secondary to other, yet unknown, humoral changes, since in most of the studies whole body knock out approach was employed. Therefore, it would be imperative and a reasonable strategy to use conditional/tissue specific telomerase knock out mouse models to delineate tissue specific affect of telomerase deficiency along with further studies on human cell/organotypic culture models. This approach not only helps in understanding the tissue specific contribution of telomerase to a specific patho-physiological condition associated with its function and regulation, during the course of aging, but also helps in developing plausible drug targets towards telomerase enzyme based on its regulation in different tissues and organs with advanced aging.

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