Cell Tissue Res (2008) 331:79–90
DOI 10.1007/s00441-007-0469-4

REVIEW

Telomeres, senescence, and hematopoietic stem cells

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Received: 13 June 2007 / Accepted: 10 July 2007 / Published online: 25 October 2007

Abstract The replicative lifespan of normal somatic cells is restricted by the erosion of telomeres, which are protective caps at the ends of linear chromosomes. The loss of telomeres induces antiproliferative signals that eventually lead to cellular senescence. The enzyme complex telomerase can maintain telomeres, but its expression is confined to highly proliferative cells such as stem cells and tumor cells. The immense regenerative capacity of the hematopoietic system is provided by a distinct type of adult stem cell: hematopoietic stem cells (HSCs). Although blood cells have to be produced continuously throughout life, the HSC pool seems not to be spared by aging processes. Indeed, limited expression of telomerase is not sufficient to prevent telomere shortening in these cells, which is thought ultimately to limit their proliferative capacity. In this review, we discuss the relevance of telomere maintenance for the hematopoietic stem cell compartment and consider potential functions of telomerase in this context. We also present possible clinical applications of telomere manipulation in HSCs and new insights affecting the aging of the hematopoietic stem cell pool and replicative exhaustion.

Keywords Senescence · Aging · Telomerase · Telomere · Stem cells

This work was supported by European Community Grant LSHC-CT-2004-502943 (MOL CANCER MED).

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Introduction

Billions of new blood cells are produced every day in a human being based on the undisturbed function of hematopoietic stem cells (HSCs). These progenitor cells represent only about 0.01% of all bone marrow cells (Lansdorp et al. 1997). A fine balance between self-renewal and differentiation is required by the HSCs in order to maintain the complex hematopoietic system throughout life. However, the proliferative potential and thereby the self-renewal capacity of HSCs appears to be increasingly affected in the aging individual (Lansdorp 1995). A fundamental determinant for proliferation is found in the telomeres, which are specialized nucleoprotein complexes protecting the ends of eukaryotic chromosomes (for a review, see Stewart and Weinberg 2006). Telomeres consist of repeated units of G-rich sequences (TTAGGG in humans) that occur as a single-stranded 3' overhang, which in turn contributes together with telomere-binding proteins to a higher order terminal loop (T loop) structure (Griffith et al. 1999; Blackburn 2001). The number of telomeric repeats varies between different species and within an organism; in humans, the telomere length is in the range of 2 to 15 kb (Martens et al. 1998). During each cell division, about 50–100 bp telomeric DNA are lost mainly because the tip of the chromosome cannot be replicated (Watson 1972). Continuous telomere erosion finally leads to the loss of telomere function, which is associated with replicative senescence or apoptosis. Most eukaryotic cells depend on the enzyme telomerase, a reverse transcriptase, for the de novo synthesis of telomeres (for a review, see Cech 2004). Telomerase is a ribonucleoprotein complex that uses its RNA component (TERC) as a template for the production of telomeric repeats via the catalytic subunit TERT (telomerase reverse transcriptase; Greider and Blackburn

1989). Telomerase activity in normal human cells is generally undetectable leading to successive telomere shortening with each cell division, which ultimately limits their proliferative capacity in vitro and, most probably, in vivo (Harley et al. 1990). Immortal cells such as germline cells (Allsopp et al. 1992), embryonic stem cells (Thomson et al. 1998), and 90% of all tumor cells in humans (Kim et al. 1994) maintain their telomeres by activated telomerase, thereby providing an unrestricted lifespan (Fig. 1). Overexpression of hTERT, the catalytic subunit of telomerase, can restore telomerase activity in various human cells such as fibroblasts and retinal pigment epithelial cells; this leads to telomere maintenance and immortalization (Bodnar et al. 1998; Vaziri and Benchimol 1998). The telomere biology of adult stem cells in highly proliferative tissues such as blood appears however to be more complex, since there is telomere shortening, despite the presence of telomerase activity.

Telomerase activity in human HSCs

In principle, stem cells from adult tissues including skin (Yasumoto et al. 1996; Harle-Bachor and Boukamp 1996), gut (Kolquist et al. 1998), and blood (Broccoli et al. 1995; Chiu et al. 1996; Morrison et al. 1996; Engelhardt et al. 1997) exhibit low levels of telomerase activity. Blood progenitor and stem cells show graduations of these basal telomerase levels (Chiu et al. 1996; Engelhardt et al. 1997; Hiyama et al. 1995; Yui et al. 1998). Higher telomerase activity can be detected in the bone marrow (BM) $CD34^+CD38^+$ committed progenitor cell fraction than in the $CD34^+CD38^{-/low}$ fraction containing primitive hematopoietic progenitor cells (Hiyama et al. 1995). BM $CD34^+CD38^-$ cells show lower telomerase activity compared with corresponding cells isolated from fetal liver (Yui et al. 1998). These initially basal telomerase levels can be transiently up-regulated during in vitro cultures upon stimulation with cytokines such as interleukin 3 (IL-3; Engelhardt et al. 1997; Yui et al. 1998; Zimmermann et al. 2004). The cell cycle and telomerase activity of HSCs is linked as demonstrated by an elevated telomerase level in actively cycling cells and its down-regulation upon differentiation and re-entry into G_0, the phase in which most resting HSCs can be found (Engelhardt et al. 1997; Zhu et al. 1996). Since repopulating HSCs represent only a minor fraction of the $CD34^+CD38^-$ cells (Bhatia et al. 1997), and since candidate human HSCs considered for the repopulation of the BM of non-obese diabetic severe-combined-immunodeficient (NOD/SCID) mice (SRCs) contain heterogeneous subpopulations with distinct engraftment and differentiation capacities (Glimm et al. 2001), the extent of telomerase activity in the rare SRCs is not known. A recent study with an hTERT-reporting adenoviral vector in $CD34^+$ cells in cord blood (CB) has demonstrated up-regulated hTERT expression in proliferating short-term SRCs; this remains relatively high in committed colony-forming progenitor cells but is

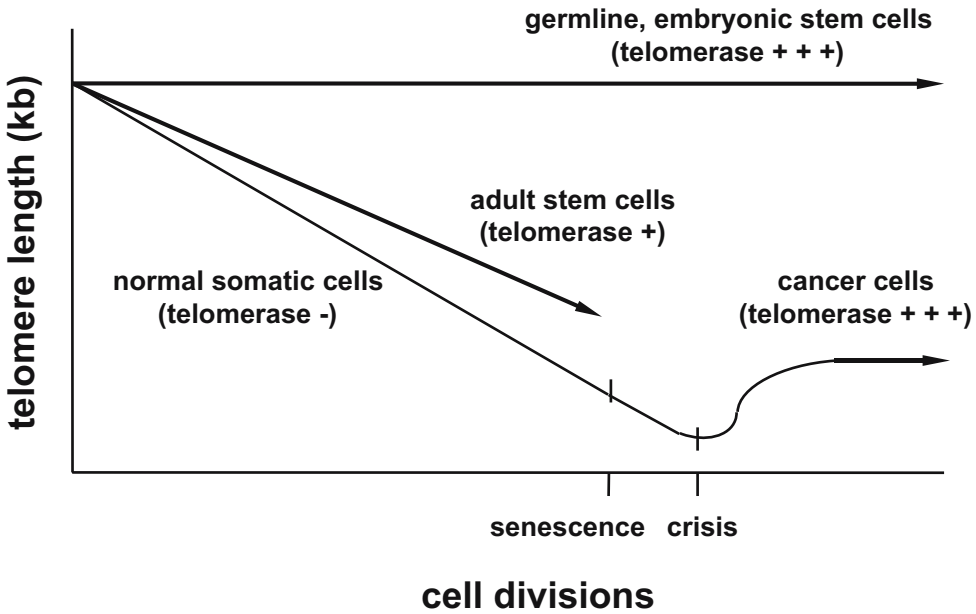

Fig. 1 Telomere-length dynamics in human cells; dependence on telomerase. Telomerase is down-regulated during embryonic development, which leads to telomere shortening with successive cell divisions in normal somatic cells. Critically short telomeres are associated with loss of telomere function eventually leading to senescence and apoptosis. The by-passing of such telomere-dependent growth barriers and the reactivation of telomerase is fundamental for the immortalization of most tumor cells. Adult stem cells from highly proliferative tissues undergo telomere erosion despite marked levels of telomerase activity

down-regulated in mature myeloid cells (Jaras et al. 2006). Nevertheless, a decreased self-renewal capacity is exhibited by SRCs that express the highest hTERT levels, thus indicating the requirement of a tight control of telomerase in these cells.

Telomere length in human HSCs

Independent of the effective degree of telomerase activity in HSCs, there is clear evidence that proliferation in this compartment is accompanied by extensive telomere shortening during cell culture in vitro (Engelhardt et al. 1997; Zimmermann et al. 2004) and aging in vivo (Vaziri et al. 1994). Thus, $CD34^{+}CD38^{low}$ candidate HSCs from adult BM possess telomeres that are about 4 kb shorter than their counterparts from fetal liver (Vaziri et al. 1994). Furthermore, significantly longer telomeres are found in BM $CD34^{+}CD38^{-}$ and, to some extent, in side-population cells compared with the corresponding $CD34^{+}CD38^{+}$ cells; this positively correlates with the known proliferation potential of these highly purified subpopulations representing different HSC candidate populations (Van Ziffle et al. 2003). Telomere-length dynamics of HSCs expanded in vitro for 3–4 weeks indicate an overall loss of 35–75 bp of telomeric repeats per population doubling in the HSC pool (Engelhardt et al. 1997; Vaziri et al. 1994), a value that is lower in relation to other somatic cells, which lose between 50–100 bp/population doubling (Harley et al. 1990; Allsopp et al. 1992; Vaziri et al. 1993). Although the in vitro studies are inconsistent in terms of heterogeneous starting cell populations from different tissues and diverse expansion protocols with various media and additives, telomerase seems generally not to counteract telomere erosion in these cells (Engelhardt et al. 1997; Zimmermann et al. 2004; Vaziri et al. 1994). Nevertheless, studies of actual telomere dynamics in HSCs are complicated because of the lack of definite HSC markers. Even $CD34^{+}CD38^{-}$ purified cells represent a heterogeneous population, which comprise only to a small percentage of real HSCs. Functional studies have revealed an ontogeny-related decline in the hematopoietic capacity of HSCs manifesting in a reduced proliferative and NOD/SCID engrafting potential of stem cells from adult mobilized peripheral blood compared with CB $CD34^{+}$ cells (Lansdorp et al. 1994; Traycoff et al. 1995; Tanavde et al. 2002). Repopulation of the hematopoietic system during BM or HSC transplantations represents an interesting model for studying telomere dynamics of HSCs in vivo. Earlier reports indicated a significantly reduced telomere length in recipients after allogeneic stem cell transplantation compared with their respective donors (Notaro et al. 1997; Wynn et al. 1998; Akiyama et al. 1998, 2000; Lee et al. 1999). Subsequent data suggest a less severe influence of such transplantations on the telomeres, mainly related to the size of the transplanted stem cell pool and the age of the donor (Brummendorf et al. 2001a; Rufer et al. 2001a; Thornley et al. 2002; Roelofs et al. 2003). The impact of the used cell source on telomere shortening also appears to be negligible resulting in the same degree of shortening for BM and peripheral blood stem cell (PBSC) transplants (Robertson et al. 2001). Nevertheless, Pipes et al. (2006) have recently shown that CB HSCs with longer telomeres have a replicative advantage in comparison with PBSCs during allogeneic stem cell transplantions. In a more recent study, the long-term effect of such treatment modalities on the telomeres of lymphoma survivors has been investigated, and a significant and persistent reduction in the telomere length of hematopoietic progenitors has been observed, accompanied by myelopoietic cell abnormalities (Rocci et al. 2007).

Telomere length in mature hematopoietic cells

In addition to HSCs, some mature hematopoietic cells, such as T lymphocytes, have low levels of telomerase activity, which rises transiently in response to antigen stimulation (Weng et al. 1996, 1998; Liu et al. 1999). Since extensive cell divisions and clonal expansion are critical for effective immune function, telomere dynamics are of particular interest in these cells. Again, limited telomerase levels are not sufficient to prevent telomere shortening and replicative senescence in T cells. However, over-expression of hTERT is able to reconstitute a constant high level of telomerase activity and extend the lifespan of this compartment (Hooijberg et al. 2000; Rufer et al. 2001b). Although the rate of telomere shortening is lower in hTERT-transduced T cells, long-term observations indicate progressive telomere shortening that eventually results in even shorter telomeres at senescence than in controls (Roth et al. 2005). Interestingly, the displacement of endogenous hTERT in human T lymphocytes by a dominant-negative mutant (DN-hTERT) leads to a shorter lifespan and cytogenetic abnormalities (Roth et al. 2003). This indicates a major influence of hTERT on the longevity of these cells without preventing overall telomere shortening but possibly with a role in the repair of sporadic telomere attrition. Telomere shortening in mature hematopoietic cells in vivo follows a cubic function over time and is characterized by a significant drop within the first year of life and a slower, more steady decline thereafter (Rufer et al. 1999; for a review, see Ohyashiki et al. 2002). This rapid telomere shortening in the first year after birth has been confirmed in a longitudinal study on granulocytes and lymphocytes from newborn baboons (Baerlocher et al. 2006). The stabilization in telomere length after 1 year in all cell types suggests a switch to a

different functional mode for HSCs characterized by a decreased turnover rate after an initial phase of rapid expansion. Individual replicative histories of lymphocytes are represented by heterogeneous telomere-length distributions in different subpopulations: T cells have a shorter mean telomere length than B cells (Rufer et al. 1999; Martens et al. 2002; Baerlocher and Lansdorp 2003), whereby even B cells are able to induce telomerase after stimulation resulting in telomere maintenance during differentiation from naive to memory B cells (Son et al. 2003). Since granulocytes have a short lifespan and do not replicate, their age-related telomere loss is much less pronounced than in lymphocytes. Therefore, the homogeneous telomere length of granulocytes seems to be a good surrogate marker for HSC proliferation kinetics under the assumption that the HSCs exhibit a constant telomere shortening during replication and differentiation from the HSC to granulocytes (Rufer et al. 1999; for a review, see Verfaillie et al. 2002). In addition to an overall trend for age-related telomere shortening in hematopoietic cells, there is considerable inter- and intra-individual variability of telomere length at any given age (Frenck et al. 1998; Rufer et al. 1998). Nevertheless, twin studies have suggested at least a genetic determination of telomere length (Rufer et al. 1999; Slagboom et al. 1994). The replication rates of HSC in vivo have been estimated in a stochastic simulation method based on granulocyte telomere lengths and have indicated that human HSCs only replicate once per 45 weeks (Shepherd et al. 2004), whereas murine HSCs divide more frequently (1/2.5 weeks; Abkowitz et al. 2000). Such a slow replication rate of human HSCs would relativize the telomere loss calculated above, at least in normal individuals. Despite this, an understanding of these telomere dynamics in vivo might be important for assessment of the consequences of a higher stem cell turnover found after allogeneic stem cell transplantation.

Telomerase manipulation in adult stem cells

As seen above for T cells, modulation of telomerase expression could be a valuable tool for studying its function in various cell types. We and others have shown that hTERT over-expression in hematopoietic progenitors such as $CD34^+$ CB cells results in a significant elevation of telomerase activity that cannot prevent overall telomere shortening. Furthermore, the elevated telomerase activity is incapable of increasing the replicative capacity of these cells (Zimmermann et al. 2004; Elwood et al. 2004; Akimov et al. 2005; Wang et al. 2005). A co-transduction of hTERT with oncogenes HPV16 E6/E7 is required for a continuous proliferation of $CD34^+$ CB cells, which then give rise to permanent cell lines with a myeloerythroid/mast cell progenitor phenotype. Such cell lines exhibit stabilized telomeres and minimal chromosomal aberrations in contrast to CB cell cultures, which express only the oncogenes and eventually go through a crisis period with highly aneuploid cells (Akimov et al. 2005). An eventual stabilization of telomere length without changes in telomerase activity has been observed during the establishment of leukemic cell lines from normal CB cells, indicating that additional genetic or epigenetic alterations are required for telomere maintenance in immortalized human hematopoietic cells (Wang et al. 2005), altogether evidence is accumulating that hTERT functions independently of its enzymatic activity as a pro-survival factor (Elwood et al. 2004; Cao et al. 2002; Yamada et al. 2003; Gorbunova and Seluanov 2003; Folini et al. 2005; Armstrong et al. 2005; Massard et al. 2006). Thus, over-expression of hTERT in a cytokine-dependent human hematopoietic progenitor cell line and in normal CB $CD34^+$ cells results in protection from apoptosis in the absence of cytokine stimulation but does not favour unlimited replicative potential (Li et al. 2006). Extensive CB $CD34^+$ cell expansion for up to 18 weeks in long-term cultures with sustained telomerase activity and minimal telomere loss has been demonstrated only under the conditions of refined culture conditions including special cytokine cocktails and stroma support (Gammaitoni et al. 2004). Similarly, the application of an optimized serum-free and cytokine-limited defined medium during CB $AC133^+$ cell expansion allows early uncommitted HSC proliferation and is accompanied by high levels of telomerase activity to maintain telomere length (Yao et al. 2006). No matter which approach is used, the in vitro expansion of HSCs for a prolonged period without telomere shortening has striking clinical implications for allogeneic transplantation, in view of the limited HSC numbers in individual CB samples and other HSC sources (Moore 2000; Jaroscak et al. 2003).

Telomerase in mesenchymal stem cells

BM harbors another type of adult stem cell, the so-called mesenchymal stem cells (hMSCs), which can differentiate along variable lineages, including those of bone, cartilage, adipose, and muscle cells (Jiang et al. 2002). Surprisingly, unlike HSCs and other adult stem cells, hMSCs exhibit a complete lack of telomerase activity (Simonsen et al. 2002; Zimmermann et al. 2003). Ectopic telomerase expression is able to expand their otherwise limited replicative capacity in tissue culture, although the cells retain their functional characteristics (Simonsen et al. 2002; Shi et al. 2002). This is of particular interest for the application of these cells in tissue engineering (for a recent review, see Satija et al.

2007). Nevertheless, premalignant changes have been observed during long-term cultures of hTERT over-expressing hMSCs (Serakinci et al. 2004) evoking some concerns about such applications (Keith 2004). The finding that stem cells and cancer cells share several common features, such as the same factors regulating self-renewal to those of normal HSCs and leukemic cells (Lessard and Sauvageau 2003), has led to the "cancer stem cell" hypothesis (for a review, see Marx 2003). A stem cell origin for certain cancers would mean that these stem cells possess a telomerase that does not need to be reactivated, although the enzyme activity may still increase in later stages of carcinogenesis (for a review, see Armanios and Greider 2005; Fig. 2). Vice versa, a limited telomerase activity related to a finite lifespan in adult stem cells may promote aging but may prevent cancer. On the other hand, evidence is available that telomere shortening can induce chromosomal instability and cancer initiation.

Side-effects of telomerase inhibition in HSCs?

Once telomeres have become significantly eroded, a DNA damage cascade is activated, and cells usually undergo replicative senescence and/or apoptosis (Harley et al. 1994; Maser and DePinho 2002). Because the majority of tumor cells is reliant on telomerase for telomere stabilization, inhibition of this enzyme represents an attractive concept for cancer therapy (for a review, see Zimmermann and Martens 2007). Proof of principle for such strategies have been provided by studies in which telomerase activity is abolished by the over-expression of DN-hTERT in immortal cancer cell lines, resulting in telomere erosion and the induction of senescence or apoptosis (Zhang et al. 1999; Hahn et al. 1999). In addition to genetic approaches, various synthetic telomerase inhibitors are being tested at present with promising results for the specific killing of tumor cells (Herbert et al. 1999; Damm et al. 2001; Asai et al. 2003; El-Daly et al. 2005, for a review, see Zimmermann and Martens 2007); some of these inhibitors are progressing significantly toward clinical application (Dikmen et al. 2005; Djojosubroto et al. 2005; Gellert et al. 2006). Most of the normal human cells considered as being telomerase-negative should not be affected by telomerase inhibition, but potential side-effects produced by such treatment modalities concern telomerase-positive stem cells such as HSCs. The most likely side-effects are probably not dramatic in the light of the low HSC replication rates and generally much longer telomeres in stem cells than in most tumor cells, so that an anti-telomerase therapy could, in principle, be stopped after the cancer cells had ceased to proliferate and before the stem cells had reached the stage of having critically short telomeres. However, there is evidence that telomerase activity is required in stem cells for health and viability throughout life (Mitchell et al. 1999; Vulliamy et al. 2001). This became evident during investigations of dyskeratosis congenita (DKC), a human disease that is characterized by anemia, immune deficiency, skin and nail lesions, chromosomal instability, and cancer (for a review,

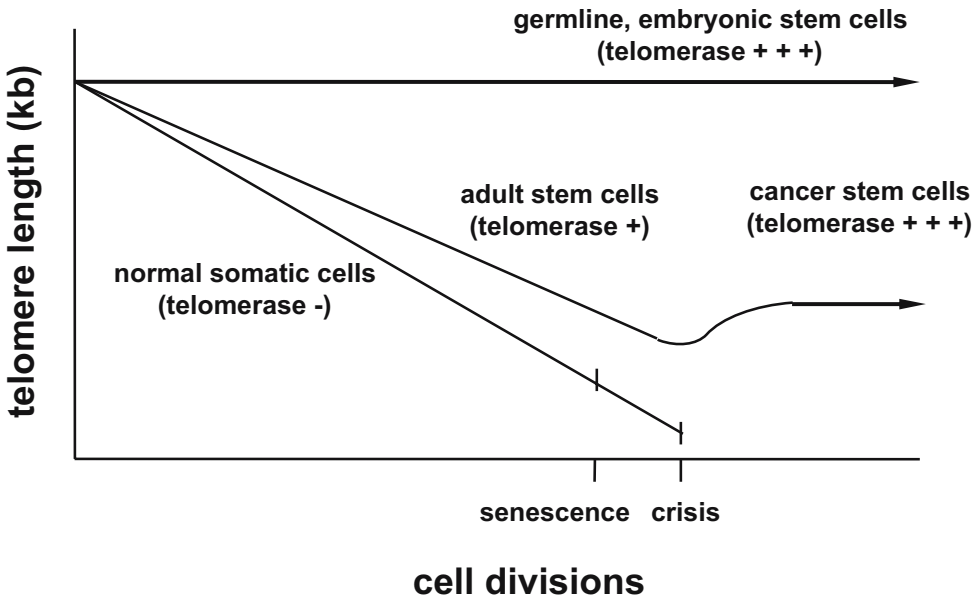

Fig. 2 Telomere-length dynamics as described in Fig. 1. For the development of so-called cancer stem cells, there is no need to reactivate telomerase, since it is available in the original stem cell to various extents. Therefore, a limitation of telomerase in such cells could moderate the risk of tumor development while still allowing for a slightly increased replicative capacity compared with that of normal cells

see Collins and Mitchell 2002). Several genes of the telomerase complex have been found to be mutated in this disease, resulting in partial telomerase inhibition, abnormally short telomeres, and a dramatically limited proliferative capacity of hematopoietic and epithelial tissues in DKC patients. Therefore, this disease appears to be a suitable model for studying the role of telomerase function in vivo (Mitchell et al. 1999; Vulliamy et al. 2001; Goldman et al. 2005; for a review, see Vulliamy and Dokal 2006). Haploinsufficiency of hTERC most likely results in an anticipation of autosomal dominant DKC disease forms correlated with an increase in telomere shortening in successive generations of affected families (Vulliamy et al. 2004). The hematological abnormalities that develop in the vast majority of DKC patients indicate that hematopoietic progenitor cells require telomerase, irrespective of its obscure relationship to telomere maintenance in these cells. Another human disease possibly associated with telomerase is aplastic anemia (AA), a BM failure syndrome that is characterized by pancytopenia with reduced or absent immature hematopoietic cells (Marsh et al. 1990; Maciejewski et al. 1994; Scopes et al. 1994). The higher turnover of HSCs is here expressed in significantly shorter telomeres in mature blood cells, such as peripheral lymphocytes and granulocytes, than in those in age-adjusted healthy controls (Ball et al. 1998; Lee et al. 2001; Brummendorf et al. 2001b). As in DKC, mutations in telomerase hTERC (Vulliamy et al. 2002) might be responsible for the comprised telomerase activity resulting in the observed phenotypes. Recently, evidence for a direct link between hTERC sequence variants found in AA patients and abolished telomerase activity has been described (Ly et al. 2005). Analogously, mutations in hTERT have been identified among AA patients, again associated with short telomeres and low telomerase enzymatic activity (Yamaguchi et al. 2005). BM failure of variable severity attributable to DKC may be present in otherwise phenotypically normal adults and can masquerade as AA, whereas common mutations in hTERC link the two diseases (Dokal and Vulliamy 2003; Fogarty et al. 2003; Marrone et al. 2004). Interestingly, hTERC haploinsufficiency in autosomal dominant DKC cases is associated with a modest reduction of telomerase activation of around 50% and is sufficient to induce the severe phenotypes of the disease described above. Overall, this indicates the need of a tight control of telomerase levels throughout life, not only in human HSCs (for a review, see Collins and Mitchell 2002).

Recent studies suggest that even normal human cells such as primary fibroblasts harbor some previously undetected telomerase activity (Masutomi et al. 2003). These basal telomerase levels seem to be important for the maintenance of the 3' overhang, cell proliferation, and cellular lifespan without any consequences on overall telomere loss (Masutomi et al. 2003). The demonstration of a similar function of telomerase in the stem cell compartment would be of interest. We have found that telomerase ablation in CB $AC133^+$ cells by DN-hTERT over-expression is accompanied by a reduction in clonogenic growth without changing the mean telomere length, supporting the hypothesis above that telomerase, even in HSCs, might have additional functions beyond simple telomere lengthening (Zimmermann et al. 2004). In addition, we have observed that high concentrations of the small molecule telomerase inhibitor, BIBR1532, specifically kill malignant cells of the hematopoietic system and do not harm the proliferation and clonogenic growth of normal $CD34^+$ cells (El-Daly et al. 2005). This might be because of differences between genetic and pharmacological approaches of telomerase inhibition and might not reflect a different composition of telomere-binding proteins in stem cells and leukemic cells.

Telomeres in mice

Telomeres in mice are far longer than those in humans, which complicates any direct comparisons of telomere-dependent replicative capacities in cells from these species. Nevertheless, valuable insights can be obtained from telomerase-deficient mice in which both alleles of mTERC, the murine RNA component of telomerase, are deleted. The primary four to five generations of $mTERC^{-/-}$ mice have unobtrusive phenotypes until telomeres become critically short. In the following generations, cytogenetic aberrations, a comprised BM, immune deficiency, tumor formation, and an overall reduced lifespan appear, as in DKC patients (Blasco et al. 1997; Hande et al. 1999; Herrera et al. 1999; Rudolph et al. 1999). HSCs of wild-type mice have a finite replicative lifespan, based upon their limitation to 4–7 serial transplantations into irradiated mice (Siminovitch et al. 1964; Harrison et al. 1978; Harrison and Astle 1982). Like in human HSCs, there seems to be telomere shortening, despite telomerase activity in murine HSCs, resulting in more than a 40% loss of total telomeric DNA after four rounds of serial transplantation (Allsopp et al. 2001, 2003a). Similar to the human situation, the way that telomere shortening in mouse HSCs has an impact on the more differentiated offspring is not as yet clear. However, stimulated T cells isolated from HSC transplant recipients can rejuvenate their telomeres by activation of telomerase (Allsopp et al. 2002). HSCs from telomerase-deficient mice, including the $mTERC^{-/-}$ mice mentioned above and $mTERT^{-/-}$ mice (Liu et al. 2000), can only be serially transplanted for two rounds because of an accelerated telomere loss compared with wild-type controls (Allsopp et al. 2003b). This indicates a role of telomerase in HSCs in at least limiting the rate of telomere shortening during cell

divisions to allow extended proliferation, the prerequisite of (hematopoietic) stem cell function throughout life. In contrast to the wild-type, transgenic mice over-expressing mTERT exhibit a four-fold elevated level of telomerase activity and stable telomeres during serial transplantations (Allsopp et al. 2003a). However, the transplantation capacity cannot be increased for mTERT over-expressing HSCs, which indicates telomere-independent barriers for the transplantation of mice HSC, possibly reflecting a form of premature senescence or being related to a dilution and loss of real stem cells during serial transplantation (Allsopp et al. 2003a; Wright and Shay 2002). In a similar transgenic mice strain over-expressing mTERT, the proliferation rate of hematopoietic cells is not elevated, although these mice are also characterized by increased telomerase activity and maintain telomere length in hematopoietic and many other tissues compared with non-transgenic control mice (Artandi et al. 2002). Interestingly, robust TERT expression is accompanied by an increased susceptibility of breast cancer in aging females of these mice, which possibly indicates a direct oncogenic role of the enzyme (Artandi et al. 2002). Short telomeres themselves and not telomerase *per se* seem to limit the tissue renewal capacity, thus making uncertain the importance of telomerase dosage effects on telomere length and disease phenotypes as seen above for DKC (Hao et al. 2005). In this context, a recent report involving the use of $mTERC^{-/-}$ mice has shown that telomere dysfunction induces alterations of the environment that can have implications for organismal aging and cell transplantation therapies (Ju et al. 2007a). Finally, the cell cycle inhibitor p21 impairs stem cell function and the survival of aging telomere dysfunctional mice (Choudhury et al. 2007), whereas it protects adult stem cells from acute genotoxic stress by preventing the inappropriate cycling of acutely damaged stem cells (Ju et al. 2007b).

Other factors determining self-renewal and aging in HSCs

The self-renewal process is crucial for maintaining HSC pool size throughout life, but the factors guiding this were previously unknown. Comprehensive transcriptional profiling studies have elucidated the molecular background behind many stem cell attributes, including their self-renewal capacity (Ramalho-Santos et al. 2002; Ivanova et al. 2002). Subsequently, several genetic regulatory programs have been identified as playing important roles in self-renewal decisions of HSCs. Among them are HOXB4 (Sauvageau et al. 1995), Notch1 (Carlesso et al. 1999), Bmi-1 (Lessard and Sauvageau 2003; Park et al. 2003), and the β-catenin pathway (Reya et al. 2003). More recently, some of these diverse intracellular pathways have been shown to be integrated probably by NF-Ya, the regulatory and DNA-binding subunit of the trimeric transcription factor NF-Y. Overexpression of NF-Ya in primitive hematopoietic cells activates the transcription of not only multiple HOX4 paralogs, Notch-1, and LEF-1, but also telomerase RNA. HSCs overexpressing NF-Ya are biased toward primitive hematopoiesis in vitro and show strikingly increased in vivo repopulating abilities, making NF-Ya a potent cellular regulator of HSC self-renewal (Zhu et al. 2005). Previously, NF-Y binding to the CCAAT region of the hTERC promoter had been demonstrated to be decisive for promoter activity (Zhao et al. 2000). Essential attributes of "stemness", as defined by Ramalho-Santos et al. (2002), also involve high resistance to stress with up- regulated DNA repair and detoxifier systems, interaction with the extracellular matrix, engagement in the cell cycle, and a remodeled chromatin , which all or in part could be affected in aging stem cells. Thus, studies of mice deficient for the integral nucleotide excision repair protein ERCC1 indicate that the accumulation of DNA damage leads to the exhaustion of HSC activity (Prasher et al. 2005). In addition, the dependence of the HSC self-renewal capacity on the inhibition of oxidative stress has been shown in studies with mice deficient for the "ataxia telangiectasia mutated" (*Atm*) gene resulting in progressive BM failure (Ito et al. 2004). In general, detoxification of reactive oxygen species is crucial to prevent protein carbonylation, which is associated with age-related human diseases such as Parkinson's disease, Alzheimer's disease, and cancer (for reviews, see Dalle-Donne et al. 2003; Nystrom 2005). Nevertheless, whether detoxification systems are disturbed in aging stem cells eventually leading to an accumulation of carbonylated proteins remains to be demonstrated. Interestingly, oxidative stress has also been discussed as an additional telomere-eroding factor (von Zglinicki 2002) that might explain the rapid telomere loss observed in some cultures (not only of HSCs).

The $p16^{INK4a}$ protein inhibits cell-cycle progression and induces cellular senescence, and its expression increases with age in many tissues, in line with the accumulation of senescent cells (for reviews, see Collins and Sedivy 2003; Campisi 2005). A recent study has found that $p16^{INK4a}$ levels accumulate with age in mouse BM HSCs and modulate specific age-associated HSC functions, whereas knocking-out $p16^{INK4a}$ diminishes HSC repopulating defects and apoptosis and is associated with an improved stress tolerance and survival of cells (Janzen et al. 2006).

In addition, novel data suggest that chromatin structure and its modifications regarded as cellular memory play an important role during HSC aging (for a review, see Kamminga and de Haan 2006). Possibly, a spread of heterochromatin, which represents transcriptionally silent

parts of the genome, occurs upon HSC aging, and differentiation is reflected by a higher number of distinct transcripts in HSCs than in committed progenitors (Terskikh et al. 2003). Although the extensive formulation of heterochromatin, expressed as characteristic senescence-associated heterochromatin foci (SAHF), is now considered as a hallmark of cellular senescence (Zhang et al. 2005), the role of such epigenetic changes for the aging of stem cells has to be investigated.

Concluding remarks

Hematopoiesis based on HSCs is maintained through tightly regulated layers of self-renewal, differentiation, and cell death during the entire lifespan of vertebrates. The reason that telomere shortening as a hallmark of replicative senescence proceeds in HSCs remain unclear, since conventional correlations between telomere maintenance and telomerase activity appear to be only partially valid in these cells. Obviously, telomerase activity and telomere dynamics seem to play different roles in embryonic stem cells and adult stem cells. Although stem cell proliferation is required for tissue repair and regeneration, it also bears the risk of cancer. Tumor suppressor mechanisms should impair this risk by either eliminating potential cancer cells (apoptosis) or at least limiting their proliferation (senescence). Factors ensuring stem cell proliferation as a prerequisite of relative longevity must presumably be well balanced with respect to mechanisms that prevent cancer (for a review, see Beausejour and Campisi 2006). Thus, the coexistence of telomerase expression and telomere shortening might appear less contradictory in the HSC compartment, especially if the telomerase here is seen more as an anti-apoptotic factor.

The relevance of cellular senescence to in vivo aging is difficult to evaluate not only in stem cells since there is a paucity of markers for this phenomenon. Recently, the combinatory use of three biomarkers (telomere dysfunction, activation of the ATM DNA-damage response, and SAHF formation) in aging baboons has provided evidence that senescent cells exist in vivo and can account for more than 15% of skin fibroblasts in aged animals (Herbig et al. 2006). Whether the stem cell compartment is affected to the same extent remains elusive. Hence, the identification of further senescence markers could facilitate a better monitoring of senescence phenotypes in vivo, not least in HSCs. Furthermore, elucidation of the particular mechanisms of telomerase expression and telomere maintenance in HSCs might contribute to strategies that focus on the rejuvenation of stem cell populations for gene therapy and transplantation and on the repopulation capacity of HSC after myeloablative therapies.

Acknowledgements Grant support: European Community Grant LSHC-CT-2004-502943 (MOL CANCER MED).

References

Abkowitz JL, Golinelli D, Harrison DE, Guttorp P (2000) In vivo kinetics of murine hemopoietic stem cells. Blood 96:3399–3405

Akimov SS, Ramezani A, Hawley TS, Hawley RG (2005) Bypass of senescence, immortalization, and transformation of human hematopoietic progenitor cells. Stem Cells 23:1423–1433

Akiyama M, Hoshi Y, Sakurai S, Yamada H, Yamada O, Mizoguchi H (1998) Changes of telomere length in children after hematopoietic stem cell transplantation. Bone Marrow Transplant 21:167–171

Akiyama M, Asai O, Kuraishi Y, Urashima M, Hoshi Y, Sakamaki H, Yabe H, Furukawa T, Yamada O, Mizoguchi H, Yamada H (2000) Shortening of telomeres in recipients of both autologous and allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 25:441–447

Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, Greider CW, Harley CB (1992) Telomere length predicts replicative capacity of human fibroblasts. Proc Natl Acad Sci USA 89:10114–10118

Allsopp RC, Cheshier S, Weissman IL (2001) Telomere shortening accompanies increased cell cycle activity during serial transplantation of hematopoietic stem cells. J Exp Med 193:917–924

Allsopp RC, Cheshier S, Weissman IL (2002) Telomerase activation and rejuvenation of telomere length in stimulated T cells derived from serially transplanted hematopoietic stem cells. J Exp Med 196:1427–1433

Allsopp RC, Morin GB, Horner JW, DePinho R, Harley CB, Weissman IL (2003a) Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. Nat Med 9:369–371

Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL (2003b) Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. Blood 102:517–520

Armanios M, Greider CW (2005) Telomerase and cancer stem cells. Cold Spring Harb Symp Quant Biol 70:205–208

Armstrong L, Saretzki G, Peters H, Wappler I, Evans J, Hole N, von Zglinicki T, Lako M (2005) Overexpression of telomerase confers growth advantage, stress resistance, and enhanced differentiation of ESCs toward the hematopoietic lineage. Stem Cells 23:516–529

Artandi SE, Alson S, Tietze MK, Sharpless NE, Ye S, Greenberg RA, Castrillon DH, Horner JW, Weiler SR, Carrasco RD, DePinho RA (2002) Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc Natl Acad Sci USA 99:8191–8196

Asai A, Oshima Y, Yamamoto Y, Uochi TA, Kusaka H, Akinaga S, Yamashita Y, Pongracz K, Pruzan R, Wunder E, Piatyszek M, Li S, Chin AC, Harley CB, Gryaznov S (2003) A novel telomerase template antagonist (GRN163) as a potential anticancer agent. Cancer Res 63:3931–3939

Baerlocher GM, Lansdorp PM (2003) Telomere length measurements in leukocyte subsets by automated multicolor flow-FISH. Cytometry A 55:1–6

Baerlocher GM, Rice K, Vulto I, Lansdorp PM (2006) Longitudinal data on telomere length in leukocytes from newborn baboons

support a marked drop in stem cell turnover around 1 year of age. Aging Cell 6:121–123
Ball SE, Gibson FM, Rizzo S, Tooze JA, Marsh JC, Gordon-Smith EC (1998) Progressive telomere shortening in aplastic anemia. Blood 91:3582–3592
Beausejour CM, Campisi J (2006) Ageing: balancing regeneration and cancer. Nature 443:404–405
Bhatia M, Wang J, Kapp U, Bonnet D, Dick JE (1997) Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc Natl Acad Sci USA 94:5320–5325
Blackburn EH (2001) Switching and signaling at the telomere. Cell 106:661–673
Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW (1997) Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91:25–34
Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279:349–352
Broccoli D, Young JW, de Lange T (1995) Telomerase activity in normal and malignant hematopoietic cells. Proc Natl Acad Sci USA 92:9082–9086
Brummendorf TH, Rufer N, Baerlocher GM, Roosnek E, Lansdorp PM (2001a) Limited telomere shortening in hematopoietic stem cells after transplantation. Ann Acad Sci 938:1–8
Brummendorf TH, Maciejewski JP, Mak J, Young NS, Lansdorp PM (2001b) Telomere length in leukocyte subpopulations of patients with aplastic anemia. Blood 97:895–900
Campisi J (2005) Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 120:513–522
Cao Y, Li H, Deb S, Liu JP (2002) TERT regulates cell survival independent of telomerase enzymatic activity. Oncogene 21:3130–3138
Carlesso N, Aster JC, Sklar J, Scadden DT (1999) Notch1-induced delay of human hematopoietic progenitor cell differentiation is associated with altered cell cycle kinetics. Blood 93:838–848
Cech TR (2004) Beginning to understand the end of the chromosome. Cell 116:273–279
Chiu CP, Dragowska W, Kim NW, Vaziri H, Yui J, Thomas TE, Harley CB, Lansdorp PM (1996) Differential expression of telomerase activity in hematopoietic progenitors from adult human bone marrow. Stem Cells 14:239–248
Choudhury AR, Ju Z, Djojosubroto MW, Schienke A, Lechel A, Schaetzlein S, Jiang H, Stepczynska A, Wang C, Buer J, Lee HW, von Zglinicki T, Ganser A, Schirmacher P, Nakauchi H, Rudolph KL (2007) Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. Nat Genet 39:99–105
Collins K, Mitchell JR (2002) Telomerase in the human organism. Oncogene 21:564–579
Collins CJ, Sedivy JM (2003) Involvement of the INK4a/Arf gene locus in senescence. Aging Cell 2:145–150
Dalle-Donne I, Giustarini D, Colombo R, Rossi R, Milzani A (2003) Protein carbonylation in human diseases. Trends Mol Med 9:169–176
Damm K, Hemmann U, Garin-Chesa P, Hauel N, Kauffmann I, Priepke H, Niestroj C, Daiber C, Enenkel B, Guilliard B, Lauritsch I, Muller E, Pascolo E, Sauter G, Pantic M, Martens UM, Wenz C, Lingner J, Kraut N, Rettig WJ, Schnapp A (2001) A highly selective telomerase inhibitor limiting human cancer cell proliferation. EMBO J 20:6958–6968
Dikmen ZG, Gellert GC, Jackson S, Gryaznov S, Tressler R, Dogan P, Wright WE, Shay JW (2005) In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor. Cancer Res 65:7866–7873
Djojosubroto MW, Chin AC, Go N, Schaetzlein S, Manns MP, Gryaznov S, Harley CB, Rudolph KL (2005) Telomerase antagonists GRN163 and GRN163L inhibit tumor growth and increase chemosensitivity of human hepatoma. Hepatology 42:1127–1136
dDokal I, Vulliamy T (2003) Dyskeratosis congenita: its link to telomerase and aplastic anaemia. Blood Rev 17:217–225
El-Daly H, Kull M, Zimmermann S, Pantic M, Waller CF, Martens UM (2005) Selective cytotoxicity and telomere damage in leukemia cells using the telomerase inhibitor BIBR1532. Blood 105:1742–1749
Elwood NJ, Jiang XR, Chiu CP, Lebkowski JS, Smith CA (2004) Enhanced long-term survival, but no increase in replicative capacity, following retroviral transduction of human cord blood CD34+ cells with human telomerase reverse transcriptase. Haematologica 89:377–378
Engelhardt M, Kumar R, Albanell J, Pettengell R, Han W, Moore MA (1997) Telomerase regulation, cell cycle, and telomere stability in primitive hematopoietic cells. Blood 90:182–193
Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, Read EJ, Lansdorp PM, Young NS (2003) Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet 362:1628–1630
Folini M, Brambilla C, Villa R, Gandellini P, Vignati S, Paduano F, Daidone MG, Zaffaroni N (2005) Antisense oligonucleotide-mediated inhibition of hTERT, but not hTERC, induces rapid cell growth decline and apoptosis in the absence of telomere shortening in human prostate cancer cells. Eur J Cancer 41:624–634
Frenck RW Jr, Blackburn EH, Shannon KM (1998) The rate of telomere sequence loss in human leukocytes varies with age. Proc Natl Acad Sci USA 95:5607–5610
Gammaitoni L, Weisel KC, Gunetti M, Wu KD, Bruno S, Pinelli S, Bonati A, Aglietta M, Moore MA, Piacibello W (2004) Elevated telomerase activity and minimal telomere loss in cord blood long-term cultures with extensive stem cell replication. Blood 103:4440–4448
Gellert GC, Dikmen ZG, Wright WE, Gryaznov S, Shay JW (2006) Effects of a novel telomerase inhibitor, GRN163L, in human breast cancer. Breast Cancer Res Treat 96:73–81
Glimm H, Eisterer W, Lee K, Cashman J, Holyoake TL, Nicolini F, Shultz LD, von Kalle C, Eaves CJ (2001) Previously undetected human hematopoietic cell populations with short-term repopulating activity selectively engraft NOD/SCID-beta2 microglobulin-null mice. J Clin Invest 107:199–206
Goldman F, Bouarich R, Kulkarni S, Freeman S, Du HY, Harrington L, Mason PJ, Londono-Vallejo A, Bessler M (2005) The effect of TERC haploinsufficiency on the inheritance of telomere length. Proc Natl Acad Sci USA 102:17119—17124
Gorbunova V, Seluanov A (2003) Telomerase as a growth-promoting factor. Cell Cycle 2:534–537
Greider CW, Blackburn EH (1989) A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. Nature 337:331–337
Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T(1999) Mammalian telomeres end in a large duplex loop. Cell 97:503–514
Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JH, Meyerson M, Weinberg RA (1999) Inhibition of telomerase limits the growth of human cancer cells. Nat Med 5:1164–1170
Hande MP, Samper E, Lansdorp P, Blasco MA (1999) Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. J Cell Biol 144:589–601

Hao LY, Armanios M, Strong MA, Karim B, Feldser DM, Huso D, Greider CW (2005) Short telomeres, even in the presence of telomerase, limit tissue renewal capacity. Cell 123:1121–1131

Harle-Bachor C, Boukamp P (1996) Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. Proc Natl Acad Sci USA 93:6476–6481

Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. Nature 345:458–460

Harley CB, Kim NW, Prowse KR, Weinrich SL, Hirsch KS, West MD, Bacchetti S, Hirte HW, Counter CM, Greider CW, et al (1994) Telomerase, cell immortality, and cancer. Cold Spring Harb Symp Quant Biol 59:307–315

Harrison D, Astle C (1982) Loss of stem cell repopulating ability upon transplantation. Effects of donor age, cell number, and transplantation procedure. J Exp Med 156:1767–1779

Harrison DE, Astle CM, Delaittre JA (1978) Loss of proliferative capacity in immunohemopoietic stem cells caused by serial transplantation rather than aging. J Exp Med 147:1526–1531

Herbert B, Pitts AE, Baker SI, Hamilton SE, Wright WE, Shay JW, Corey DR (1999) Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. Proc Natl Acad Sci USA 96:14276–14281

Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006) Cellular senescence in aging primates. Science 311:1257

Herrera E, Samper E, Blasco MA (1999) Telomere shortening in mTR$^{-/-}$ embryos is associated with failure to close the neural tube. EMBO J 18:1172–1181

Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, Shay JW, Ishioka S, Yamakido M (1995) Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. J Immunol 155:3711–3715

Hooijberg E, Ruizendaal JJ, Snijders PJ, Kueter EW, Walboomers JM, Spits H (2000) Immortalization of human CD8+ T cell clones by ectopic expression of telomerase reverse transcriptase. J Immunol 165:4239–4245

Ito K, Hirao A, Arai F, Matsuoka S, Takubo K, Hamaguchi I, Nomiyama K, Hosokawa K, Sakurada K, Nakagata N, Ikeda Y, Mak TW, Suda T (2004) Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature 431:997–1002

Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR (2002) A stem cell molecular signature. Science 298:601–604

Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, DePinho RA, Sharpless NE, Scadden DT (2006) Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. Nature 443:421–426

Jaras M, Edqvist A, Rebetz J, Salford LG, Widegren B, Fan X (2006) Human short-term repopulating cells have enhanced telomerase reverse transcriptase expression. Blood 108:1084–1091

Jaroscak J, Goltry K, Smith A, Waters-Pick B, Martin PL, Driscoll TA, Howrey R, Chao N, Douville J, Burhop S, Fu P, Kurtzberg J (2003) Augmentation of umbilical cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase 1 trial using the AastromReplicell System. Blood 101:5061–5067

Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41–49

Ju Z, Jiang H, Jaworski M, Rathinam C, Gompf A, Klein C, Trumpp A, Rudolph KL (2007a) Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. Nat Med 13:742–747

Ju Z, Choudhury AR, Rudolph KL (2007b) A dual role of p21 in stem cell aging. Ann N Y Acad Sci 1100:333–344

Kamminga LM, de Haan G (2006) Cellular memory and hematopoietic stem cell aging. Stem Cells 24:1143–1149

Keith WN (2004) From stem cells to cancer: balancing immortality and neoplasia. Oncogene 23:5092–5094

Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015

Kolquist KA, Ellisen LW, Counter CM, Meyerson M, Tan LK, Weinberg RA, Haber DA, Gerald WL (1998) Expression of TERT in early premalignant lesions and a subset of cells in normal tissues. Nat Genet 19:182–186

Lansdorp PM (1995) Telomere length and proliferation potential of hematopoietic stem cells. J Cell Sci 108:1–6

Lansdorp PM, Dragowska W, Thomas TE, Little MT, Mayani H (1994) Age-related decline in proliferative potential of purified stem cell candidates. Blood Cells 20:376–380

Lansdorp PM, Poon S, Chavez E, Dragowska V, Zijlmans M, Bryan T, Reddel R, Egholm M, Bacchetti S, Martens U (1997) Telomeres in the haemopoietic system. Ciba Found Symp 211:209–222

Lee J, Kook H, Chung I, Kim H, Park M, Kim C, Nah J, Hwang T (1999) Telomere length changes in patients undergoing hematopoietic stem cell transplantation. Bone Marrow Transplant 24:411–415

Lee JJ, Kook H, Chung IJ, Na JA, Park MR, Hwang TJ, Kwak JY, Sohn SK, Kim HJ (2001) Telomere length changes in patients with aplastic anaemia. Br J Haematol 112:1025–1030

Lessard J, Sauvageau G (2003) Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature 423:255–260

Li S, Ferguson MJ, Hawkins CJ, Smith C, Elwood NJ (2006) Human telomerase reverse transcriptase protects hematopoietic progenitor TF-1 cells from death and quiescence induced by cytokine withdrawal. Leukemia 20:1270–1278

Liu K, Schoonmaker MM, Levine BL, June CH, Hodes RJ, Weng NP (1999) Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. Proc Natl Acad Sci USA 96:5147–5152

Liu Y, Snow BE, Hande MP, Yeung D, Erdmann NJ, Wakeham A, Itie A, Siderovski DP, Lansdorp PM, Robinson MO, Harrington L (2000) The telomerase reverse transcriptase is limiting and necessary for telomerase function in vivo. Curr Biol 10:1459–1462

Ly H, Calado RT, Allard P, Baerlocher GM, Lansdorp PM, Young NS, Parslow TG (2005) Functional characterization of telomerase RNA variants found in patients with hematologic disorders. Blood 105:2332–2339

Maciejewski JP, Anderson S, Katevas P, Young NS (1994) Phenotypic and functional analysis of bone marrow progenitor cell compartment in bone marrow failure. Br J Haematol 87:227–234

Marrone A, Stevens D, Vulliamy T, Dokal I, Mason PJ (2004) Heterozygous telomerase RNA mutations found in dyskeratosis congenita and aplastic anemia reduce telomerase activity via haploinsufficiency. Blood 104:3936–3942

Marsh JC, Chang J, Testa NG, Hows JM, Dexter TM (1990) The hematopoietic defect in aplastic anemia assessed by long-term marrow culture. Blood 76:1748–1757

Martens UM, Zijlmans JM, Poon SS, Dragowska W, Yui J, Chavez EA, Ward RK, Lansdorp PM (1998) Short telomeres on human chromosome 17p. Nat Genet 18:76–80

Martens UM, Brass V, Sedlacek L, Pantic M, Exner C, Guo Y, Engelhardt M, Lansdorp PM, Waller CF, Lange W (2002) Telomere maintenance in human B lymphocytes. Br J Haematol 119:810–818

Marx J (2003) Cancer research. Mutant stem cells may seed cancer. Science 301:1308–1310

Maser RS, DePinho RA (2002) Connecting chromosomes, crisis, and cancer. Science 297:565–569

Massard C, Zermati Y, Pauleau AL, Larochette N, Metivier D, Sabatier L, Kroemer G, Soria JC (2006) hTERT: a novel endogenous inhibitor of the mitochondrial cell death pathway. Oncogene 25:4505–4514

Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, Brooks MW, Kaneko S, Murakami S, DeCaprio JA, Weinberg RA, Stewart SA, Hahn WC (2003) Telomerase maintains telomere structure in normal human cells. Cell 114:241–253

Mitchell JR, Wood E, Collins K (1999) A telomerase component is defective in the human disease dyskeratosis congenita. Nature 402:551–555

Moore MA (2000) Umbilical cord blood: an expandable resource. J Clin Invest 105:855–856

Morrison SJ, Prowse KR, Ho P, Weissman IL (1996) Telomerase activity in hematopoietic cells is associated with self-renewal potential. Immunity 5:207–216

Notaro R, Cimmino A, Tabarini D, Rotoli B, Luzzatto L (1997) In vivo telomere dynamics of human hematopoietic stem cells. Proc Natl Acad Sci USA 94:13782–13785

Nystrom T (2005) Role of oxidative carbonylation in protein quality control and senescence. EMBO J 24:1311–1317

Ohyashiki JH, Sashida G, Tauchi T, Ohyashiki K (2002) Telomeres and telomerase in hematologic neoplasia. Oncogene 21:680–687

Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, Morrison SJ, Clarke MF (2003) Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature 423:302–305

Pipes BL, Tsang T, Peng SX, Fiederlein R, Graham M, Harris DT (2006) Telomere length changes after umbilical cord blood transplant. Transfusion 46:1038–1043

Prasher JM, Lalai AS, Heijmans-Antonissen C, Ploemacher RE, Hoeijmakers JH, Touw IP, Niedernhofer LJ (2005) Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient Ercc1$^{-/-}$ mice. EMBO J 24:861–871

Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA (2002) "Stemness": transcriptional profiling of embryonic and adult stem cells. Science 298:597-600

Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R, Weissman IL (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature 423:409–414

Robertson JD, Testa NG, Russell NH, Jackson G, Parker AN, Milligan DW, Stainer C, Chakrabarti S, Dougal M, Chopra R (2001) Accelerated telomere shortening following allogeneic transplantation is independent of the cell source and occurs within the first year post transplant. Bone Marrow Transplant 27:1283–1286

Rocci A, Ricca I, Dellacasa C, Longoni P, Compagno M, Francese R, Lobetti Bodoni C, Manzini P, Caracciolo D, Boccadoro M, Ferrero D, Ladetto M, Carlo-Stella C, Tarella C (2007) Long-term lymphoma survivors following high-dose chemotherapy and autograft: evidence of permanent telomere shortening in myeloid cells, associated with marked reduction of bone marrow hematopoietic stem cell reservoir. Exp Hematol 35:673–681

Roelofs H, de Pauw ES, Zwinderman AH, Opdam SM, Willemze R, Tanke HJ, Fibbe WE (2003) Homeostasis of telomere length rather than telomere shortening after allogeneic peripheral blood stem cell transplantation. Blood 101:358–362

Roth A, Yssel H, Pene J, Chavez EA, Schertzer M, Lansdorp PM, Spits H, Luiten RM (2003) Telomerase levels control the lifespan of human T lymphocytes. Blood 102:849–857

Roth A, Baerlcher GM, Schertzer M, Chavez E, Duhrsen U, Lansdorp PM (2005) Telomere loss senescence and genetic instability in CD4+ T lymphocytes overexpressing hTERT. Blood 106:43–50

Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, DePinho RA (1999) Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96:701–712

Rufer N, Dragowska W, Thornbury G, Roosnek E, Lansdorp PM (1998) Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. Nat Biotechnol 16:743–747

Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M, Lansdorp PM (1999) Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J Exp Med 190:157–167

Rufer N, Brummendorf TH, Chapuis B, Helg C, Lansdorp PM, Roosnek E (2001a) Accelerated telomere shortening in hematological lineages is limited to the first year following stem cell transplantation. Blood 97:575–577

Rufer N, Migliaccio M, Antonchuk J, Humphries RK, Roosnek E, Lansdorp PM (2001b) Transfer of the human telomerase reverse transcriptase (TERT) gene into T lymphocytes results in extension of replicative potential. Blood 98:597–603

Satija NK, Gurudutta GU, Sharma S, Afrin F, Gupta P, Verma YK, Singh VK, Tripathi RP (2007) Mesenchymal stem cells: molecular targets for tissue engineering. Stem Cells Dev 16:7–23

Sauvageau G, Thorsteinsdottir U, Eaves CJ, Lawrence HJ, Largman C, Lansdorp PM, Humphries RK (1995) Overexpression of HOXB4 in hematopoietic cells causes the selective expansion of more primitive populations in vitro and in vivo. Genes Dev 9:1753–1765

Scopes J, Bagnara M, Gordon-Smith EC, Ball SE, Gibson FM (1994) Haemopoietic progenitor cells are reduced in aplastic anaemia. Br J Haematol 86:427–430

Serakinci N, Guldberg P, Burns JS, Abdallah B, Schrodder H, Jensen T, Kassem M (2004) Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 23:5095–5098

Shepherd BE, Guttorp P, Lansdorp PM, Abkowitz JL (2004) Estimating human hematopoietic stem cell kinetics using granulocyte telomere lengths. Exp Hematol 32:1040–1050

Shi S, Gronthos S, Chen S, Reddi A, Counter CM, Robey PG, Wang CY (2002) Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. Nat Biotechnol 20:587–591

Siminovitch L, Till JE, McCulloch EA (1964) Decline in colony-forming ability of marrow cells subjected to serial transplantation into irradiated mice. J Cell Physiol 64:23–31

Simonsen JL, Rosada C, Serakinci N, Justesen J, Stenderup K, Rattan SI, Jensen TG, Kassem M (2002) Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat Biotechnol 20:592–596

Slagboom PE, Droog S, Boomsma DI (1994) Genetic determination of telomere size in humans: a twin study of three age groups. Am J Hum Genet 55:876–882

Son NH, Joyce B, Hieatt A, Chrest FJ, Yanovski J, Weng NP (2003) Stable telomere length and telomerase expression from naive to memory B-lymphocyte differentiation. Mech Ageing Dev 124:427–432

Stewart SA, Weinberg RA (2006) Telomeres: cancer to human aging. Annu Rev Cell Dev Biol 22:531–557

Tanavde VM, Malehorn MT, Lumkul R, Gao Z, Wingard J, Garrett ES, Civin CI (2002) Human stem-progenitor cells from neonatal cord blood have greater hematopoietic expansion capacity than those from mobilized adult blood. Exp Hematol 30:816–823

Terskikh AV, Miyamoto T, Chang C, Diatchenko L, Weissman IL (2003) Gene expression analysis of purified hematopoietic stem cells and committed progenitors. Blood 102:94–101

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147

Thornley I, Sutherland R, Wynn R, Nayar R, Sung L, Corpus G, Kiss T, Lipton J, Doyle J, Saunders F, Kamel-Reid S, Freedman M, Messner H (2002) Early hematopoietic reconstitution after clinical stem cell transplantation: evidence for stochastic stem cell behavior and limited acceleration in telomere loss. Blood 99:2387–2396

Traycoff CM, Kosak ST, Grigsby S, Srour EF (1995) Evaluation of ex vivo expansion potential of cord blood and bone marrow hematopoietic progenitor cells using cell tracking and limiting dilution analysis. Blood 85:2059–2068

Van Ziffle JA, Baerlocher GM, Lansdorp PM (2003) Telomere length in subpopulations of human hematopoietic cells. Stem Cells 21:654–660

Vaziri H, Benchimol S (1998) Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr Biol 8:279–282

Vaziri H, Schachter F, Uchida I, Wei L, Zhu X, Effros R, Cohen D, Harley CB (1993) Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. Am J Hum Genet 52:661–667

Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM (1994) Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. Proc Natl Acad Sci USA 91:9857–9860

Verfaillie CM, Pera MF, Lansdorp PM (2002) Stem cells: hype and reality. Hematology Am Soc Hematol Educ Program 2002:369–391

von Zglinicki T (2002) Oxidative stress shortens telomeres. Trends Biochem Sci 27:339–344

Vulliamy T, Dokal I (2006) Dyskeratosis congenita. Semin Hematol 43:157–166

Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, Dokal I (2001) The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature 413:432–435

Vulliamy T, Marrone A, Dokal I, Mason PJ (2002) Association between aplastic anaemia and mutations in telomerase RNA. Lancet 359:2168–2170

Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I (2004) Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. Nat Genet 36:447–449

Wang JC, Warner JK, Erdmann N, Lansdorp PM, Harrington L, Dick JE (2005) Dissociation of telomerase activity and telomere length maintenance in primitive human hematopoietic cells. Proc Natl Acad Sci USA 102:14398–14403

Watson JD (1972) Origin of concatemeric T7 DNA. Nat New Biol 239:197–201

Weng NP, Levine BL, June CH, Hodes RJ (1996) Regulated expression of telomerase activity in human T lymphocyte development and activation. J Exp Med 183:2471–2479

Weng NP, Hathcock KS, Hodes RJ (1998) Regulation of telomere length and telomerase in T and B cells: a mechanism for maintaining replicative potential. Immunity 9:151–157

Wright WE, Shay JW (2002) Historical claims and current interpretations of replicative aging. Nat Biotechnol 20:682–688

Wynn RF, Cross MA, Hatton C, Will AM, Lashford LS, Dexter TM, Testa NG (1998) Accelerated telomere shortening in young recipients of allogeneic bone-marrow transplants. Lancet 351:178–181

Yamada O, Akiyama M, Kawauchi K, Adachi T, Yamada H, Kanda N, Aikawa E (2003) Overexpression of telomerase confers a survival advantage through suppression of TRF1 gene expression while maintaining differentiation characteristics in K562 cells. Cell Transplant 12:365–377

Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, Lansdorp PM, Young NS (2005) Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med 352:1413–1424

Yao CL, Feng YH, Lin XZ, Chu IM, Hsieh TB, Hwang SM (2006) Characterization of serum-free ex vivo-expanded hematopoietic stem cells derived from human umbilical cord blood CD133(+) cells. Stem Cells Dev 15:70–78

Yasumoto S, Kunimura C, Kikuchi K, Tahara H, Ohji H, Yamamoto H, Ide T, Utakoji T (1996) Telomerase activity in normal human epithelial cells. Oncogene 13:433–439

Yui J, Chiu CP, Lansdorp PM (1998) Telomerase activity in candidate stem cells from fetal liver and adult bone marrow. Blood 91:3255–3262

Zhang X, Mar V, Zhou W, Harrington L, Robinson MO (1999) Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. Genes Dev 13:2388–2399

Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, Pehrson JR, Berger JM, Kaufman PD, Adams PD (2005) Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 8:19–30

Zhao JQ, Glasspool RM, Hoare SF, Bilsland A, Szatmari I, Keith WN (2000) Activation of telomerase RNA gene promoter activity by NF-Y, Sp1, and the retinoblastoma protein and repression by Sp3. Neoplasia 2:531–539

Zhu X, Kumar R, Mandal M, Sharma N, Sharma HW, Dhingra U, Sokoloski JA, Hsiao R, Narayanan R (1996) Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc Natl Acad Sci USA 93:6091–6095

Zhu J, Zhang Y, Joe GJ, Pompetti R, Emerson SG (2005) NF-Ya activates multiple hematopoietic stem cell (HSC) regulatory genes and promotes HSC self-renewal. Proc Natl Acad Sci USA 102:11728–11733

Zimmermann S, Martens UM (2007) Telomeres and telomerase as targets for cancer therapy. Cell Mol Lif Sci 64:906–921

Zimmermann S, Voss M, Kaiser S, Kapp U, Waller CF, Martens UM (2003) Lack of telomerase activity in human mesenchymal stem cells. Leukemia 17:1146–1149

Zimmermann S, Glaser S, Ketteler R, Waller CF, Klingmüller U, Martens UM (2004) Effects of telomerase modulation in human hematopoietic progenitor cells. Stem Cells 22:741–749