Experimental Gerontology 44 (2009) 75–82

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Review

Cell intrinsic and extrinsic mechanisms of stem cell aging depend on telomere status

Zhangfa Song [a], Zhenyu Ju [a,b,*], K. Lenhard Rudolph [a,*]

[a] Institute of Molecular Medicine and Max-Planck-Research Group on Stem Cell Aging, University of Ulm, 89081 Ulm, Germany
[b] Institute of Laboratory Animal Sciences and Max-Planck-Partner Group on Stem Cell Aging, Chinese Academy of Medical Sciences, China

ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form 24 June 2008
Accepted 25 June 2008
Available online 3 July 2008

Keywords:
Telomere
Stem cell
Aging

ABSTRACT

The function of adult stem cells declines during aging and chronic diseases. An understanding of the molecular mechanisms underlying these processes will help to identify targets for future therapies in order to improve regenerative reserve and organ maintenance. Telomere shortening represents a cell intrinsic mechanism inducing DNA damage in aging cells. Current studies in telomerase knockout mice have shown that telomere dysfunction induces cell intrinsic checkpoints and environmental alteration that limit stem cell function. While these phenotypes differ from wild-type mice with long telomere reserves, they appear to be relevant for human aging, which is associated with an accumulation of telomere dysfunction and DNA damage.

1. Introduction

There is growing evidence for a functional decline of adult stem cells during aging. In aging mammals, the functional reserve of adult stem cells decreases in the hematopoietic system (Waterstrat et al., 2008), in pancreatic islets (Krishnamurthy et al., 2006), in areas of neurogenesis in the brain (Molofsky et al., 2006), in hair follicles (Nishimura et al., 2005), in intestinal crypts (Martin et al., 1998), and in muscle (Brack and Rando, 2007).

Several lines of evidence indicate that the decline in stem cell function during aging can involve both cell intrinsic mechanisms and environmental alterations [for review see: (Ju and Rudolph, 2008)]. In humans, it has been shown that both donor age (Castro-Malaspina et al., 2002; Kollman et al., 2001) and recipient age (Kantarjian et al., 2006; Socie et al., 1999) influence patients' outcomes in response to hematopoietic stem cell (HSC) transplantation. These clinical data indicate that cell intrinsic and cell extrinsic (environmental) mechanisms could contribute to the functional decline of HSCs during human aging. The relative contribution of these mechanisms to the decline in function and maintenance of aging stem cells remains to be defined in different organ systems and genetic modifiers have yet to be identified. Overcoming these obstacles may provide a rational basis to begin therapeutic strategy development, which could improve regenerative reserve during aging. In this review, we focus on the influence of telomere dysfunction on cell intrinsic and cell extrinsic mechanisms limiting stem cell function during aging.

2. Telomere shortening and cellular aging

Telomeres consist of simple tandem DNA repeats and associated telomere binding proteins (de Lange, 2005). The main function of telomeres is to cap chromosome ends thus preventing an activation of DNA damage responses and the evolution of chromosomal instability (Blackburn, 2001). Telomeres shorten with each round of cell division due to the 'end replication problem' of DNA polymerase and due to processing of telomeres during cell cycle (Levy et al., 1992; Sfeir et al., 2005). The enzyme telomerase is required to compensate for telomere shortening. Telomerase synthesizes telomeres *de novo*. In humans, telomerase is active during embryogenesis (Wright et al., 1996), but it is suppressed in most somatic cells after birth. In adults, telomerase remains active in germ cells and certain stem cell compartments, like in intestinal stem cells and hematopoietic stem cells (Broccoli et al., 1995; for review see: Zimmermann and Martens, 2005; Artandi, 2008). In addition, telomerase is transiently up-regulated in stimulated lymphocytes (Hiyama et al., 1995).

Telomere shortening limits the lifespan of primary human cells to a finite number of cell divisions, which is 50–70 for human fibroblast. This limit is called after the discoverer – the 'Hayflick limit' (Hayflick, 1965). Studies on overexpression of telomerase have proven that telomere shortening is the underlying mechanism limiting the lifespan of human cells (Bodnar et al., 1998). Since most primary human cells lack telomerase expression the lifespan of human cells is limited by telomere shortening. Studies on human fibroblasts have shown that telomere shortening induces two

* Corresponding authors. Tel./fax: +86 10 6777 6051/6838 (Z. Ju); tel./fax: +49 731 5036 100/102 (K.L. Rudolph).
E-mail addresses: zhenyuju@hotmail.com (Z. Ju), Lenhard.Rudolph@uni-ulm.de (K.L. Rudolph).

0531-5565/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.exger.2008.06.009

checkpoints. The first checkpoint is activated in response to low levels of telomere dysfunction [3–5 dysfunctional telomeres per cell, (Zou et al., 2004)]. At this stage human fibroblasts enter a permanent cell cycle arrest, which is named replicative senescence (Wright and Shay, 1992). Human fibroblasts can escape the senescence checkpoint when p53 and Rb checkpoints are abrogated (Wright and Shay, 1992). However, during this extended lifespan period (ca. 10–20 cell divisions) telomeres continue to shorten and eventually reach a second checkpoint, which is named crisis (Wright and Shay, 1992). Crisis is characterized by high levels of telomere dysfunction, massive chromosomal instability, and cell death.

3. Telomere shortening and human aging

There is ample evidence that telomeres shorten during human aging (Jiang et al., 2007). This shortening also affects stem cell compartments, e.g., telomeres shorten in CD34+ hematopoietic cells during human aging (Vaziri et al., 1994), although human HSCs exhibit low level of telomerase activity. Together, it appears that telomerase expression contributes to the proliferative capacity of human HSCs but the level of telomerase expression is not sufficient to maintain stable telomeres into old age (Zimmermann and Martens, 2005).

In addition to telomere shortening during aging, various chronic diseases accelerate telomere shortening in humans (Jiang et al., 2007). This has been reported for different forms of anemia, hypertension, coronary heart diseases, chronic HIV infection, ulcerative colitis, chronic liver disease, and others (Wiemann et al., 2002; Jiang et al., 2007). Moreover, telomere shortening during chronic diseases is often associated with disease progression (Jiang et al., 2007; Wiemann et al., 2002). It has also been documented that telomere shortening correlates with age-related pathology and reduced human lifespan (Cawthon et al., 2003; Collerton et al., 2007). Lastly, mutations in human telomerase genes lead to telomere shortening, stem cell defects, impaired organ maintenance and a shortened lifespan (Armanios et al., 2007; Tsakiri et al., 2007; Vulliamy et al., 2004). Together, these data strongly indicate that human telomere reserves are limited. New experimental data indicate that increasing levels of biomarkers of telomere dysfunction and DNA damage characterize human aging and aging-associated disease (Jiang et al., in press). Given the pivotal role of telomeres and adult stem cells for tissues maintenance and repair, it appears to be of utmost importance to understand the functional consequences of telomere dysfunction on stem cell levels. The characterization of stem cell aging in wild type mice cannot address this critical question since laboratory wild type mouse strains have very long telomeres compared to humans (Prowse and Greider, 1995) and inbred laboratory wild-type mice do not experience significant levels of telomere dysfunction during aging (Jiang et al., in press). In fact, there is experimental evidence that the decline in stem cell function in wild type laboratory mouse strains in response to replicative stress is mainly driven by telomere-independent mechanisms (Allsopp et al., 2003).

4. DNA damage signaling in response to telomere dysfunction

Critical telomere shortening leads to uncapping of chromosomal ends. These dysfunctional telomeric ends are recognized as DNA double strand breaks that lead to activation of DNA-damage checkpoints (d'Adda di Fagagna et al., 2003; Takai et al., 2003). DNA damage signals in response to telomere dysfunction are similar to those induced by γ-irradiation. The initial step of this DNA damage signaling is the formation of DNA damage foci at dysfunctional telomeres. These foci consist of proteins involved in DNA damage recognition and repair including γH2AX, 53bp1, NBS1, MDC1, among others (d'Adda di Fagagna et al., 2003; Satyanarayana et al., 2004). These DNA damage foci induce ATM/Chk2 and ATR/Chk1 kinase pathways (d'Adda di Fagagna et al., 2003) activating p53 (Gire et al., 2004; Chin et al., 1999) and its downstream target p21 (Brown et al., 1997) inducing cellular senescence (Brown et al., 1997). In contrast to the senescence checkpoint, the crisis checkpoint is less well understood and may involve an activation of multiple p53-independent checkpoints inducing cell death.

There is a continuous need to investigate checkpoints in response to telomere dysfunction using *in vivo* models. It is conceivable that telomere dysfunction induces different checkpoint responses depending on the tissue type. In addition, checkpoint responses in stem cells may differ from somatic cells. In line with this assumption, it has been shown that embryonic stem cells lack efficient G1 cell cycle checkpoints in response to γ-irradiation (Hong and Stambrook, 2004). It is currently unknown whether similar differences are present in adult stem cells compared to somatic cells. Given the difficulties in culturing undifferentiated, adult stem cells, it is necessary to investigate consequences of telomere dysfunction using *in vivo* models.

5. Telomere shortening and stem cell aging

Inbred laboratory mice have long telomeres (25–60 kb compared to 10–12 kb in humans) and a short lifespan (24–30 months) compared to 75–80 years in humans (Cawthon et al., 2003; Choudhury et al., 2007; Prowse and Greider, 1995). Using an unbiased proteomic approach we have recently identified biomarkers of telomere dysfunction. These markers show an increased expression in various tissues and blood plasma of aging telomere dysfunctional mice but not in wild-type controls. The same biomarkers significantly increase during human aging and aging-associated disease (Jiang et al., in press). Together these results support the hypothesis that accumulation of telomere dysfunction and DNA damage contribute to human aging, but have little impact on aging of wild-type mice with long telomere reserves. Thus, telomerase knockout mice provide a unique experimental model to study the *in vivo* consequences of this important aspect of human aging.

Current experimental evidence indicates that telomere shortening influences stem cell aging. In some wild-type mouse strains (e.g., C57Bl/6J) the number of HSCs increases during aging, whereas other mouse strains, such as DAB2 and BalbC, show a decrease in HSC number during aging (Morrison et al., 1996; Waterstrat et al., 2008). Interestingly, the number of HSCs has been associated with murine lifespan (Henckaerts et al., 2004; Van Zant et al., 1990).

Although HSCs in wild-type C57Bl/6J mice acquire defects in long-term repopulating potential during age-dependent, the total stem cell activity remains fairly constant due to the increase in stem cell number in this strain (Chambers et al., 2007). Moreover, C57BL/6J mice rarely develop hematopoietic failure during aging. This scenario appears to be very different from human aging, which is characterized by a decline in HSC number (Waterstrat et al., 2008), and frequent occurrence of bone marrow failure and anemia (Beghe et al., 2004). It appears likely that shorter telomeres and longer lifespan in humans represent fundamental differences affecting stem cell aging in humans but not in C57BL/6J mice. In agreement with this assumption, heterozygous mutations in telomerase gene impair telomerase activity by haplo-insufficiency and represent risk factors for bone marrow failure in human aplastic anemia and dyskeratosis congenita (DC) (Vulliamy et al., 2004). In contrast, heterozygous mutations of telomerase in C57BL/6J mice with long telomeres have little effect on stem cell function during aging (Choudhury et al., 2007). However, the heterozygous