Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Cell Biology

Consequences of telomere shortening during lifespan

Catarina M Henriques and Miguel Godinho Ferreira

Telomerase expression is restricted in human cells and so telomeres shorten throughout our lives, providing a tumour suppressor mechanism that limits cell proliferation. As a trade-off, continuous telomere erosion results in replicative senescence and contributes to ageing. Recently, telomerase therapies were proposed as a valid approach to rescue degenerative phenotypes caused by telomere dysfunction. However, systemic effects initiated by short telomeres may prove dominant in limiting tissue renewal in the whole organism. Most of our knowledge of telomere biology derives from mouse models that do not rely on telomere exhaustion for controlling cell proliferation and tissue homeostasis. In order to understand the impact of telomere shortening in natural ageing, we need to investigate animal models that, like humans, have evolved to have telomere length as a cell division clock.

Address
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Corresponding author: Ferreira, Miguel Godinho (mgferreira@igc.gulbenkian.pt)

Current Opinion in Cell Biology 2012, **24**:804–808

This review comes from a themed issue on **Cell division, growth and death**

Edited by **Julia Promisel Cooper** and **Richard J Youle**

For a complete overview see the Issue and the Editorial

Available online 2nd November 2012

0955-0674/$ – see front matter,

http://dx.doi.org/10.1016/j.ceb.2012.09.007

Introduction

Ever since the discovery that human primary cells have a limited replicative potential as a direct consequence of telomere shortening, telomere biology has been an intense area of study. Telomere shortening and re-activation of telomerase, the reverse transcriptase responsible for telomere synthesis, stepped into the spotlight as prime candidates to explain the rise of human cancers with age [1].

Studying the consequences of telomere shortening in the lab is a serious challenge. Telomerase fails to neatly provide a single length to all telomeres, so telomeres are distributed over a population of sizes around a given average. It is difficult to acquire *in vivo* information on length distribution and, even more difficult, the number of crucially short telomeres. Since it is the number of crucially short telomeres that determine replicative senescence [2], simply knowing the average telomere length is not enough. It is therefore unwise to compare studies that use different techniques to assess telomere dynamics and this has significantly confused the field [3].

The traditional culprit of ageing has been the accumulation of mutations over our lives associated with reactive oxygen species (ROS). However, recent work unified telomere shortening and consequent p53 activation to the downregulation of mitochondria biogenesis and ROS production [4•,5]. Other studies claim that re-introduction of telomerase may rescue age-related degenerative phenotypes in mice [6•,7••]. However, our knowledge of vertebrate telomere biology derives mainly from mouse models that do not rely on telomere depletion as means for controlling cell proliferation [8]. We consider it is of timely importance to clarify the role of telomere shortening in animals that, like humans, have evolved telomere length as an internal cell division timer [9].

This review offers a synthesis of what is known about telomere shortening in the wild and in model organisms, highlighting crucial discoveries of the past two years. In addition, we point to alternative vertebrate models for studying telomere biology. We favour a view where age-related dysfunctions have evolved as a consequence of different life strategies adopted in nature. Finally, we explore a model where telomere dysfunction is perceived by the organism both in autonomous and non-cell-autonomous ways. Telomere dysfunction may ultimately provide both 'seed' and 'soil' for loss of tissue homeostasis, promoting disease and contributing to the phenomena of ageing.

Telomere shortening in nature

Telomeres constitute the ends of linear chromosomes, comprising DNA (TTAGGG)n and associated proteins, known as shelterin [10]. The enzyme telomerase is responsible for elongating telomeres. However, its expression is restricted in human somatic cells and so telomeres shorten during our lifespan [11]. Telomere erosion triggers a DSB-like DNA damage response that culminates in senescence and/or apoptosis. Depending on the genetic background, cells can overcome this block and enter crisis, leading to genome catastrophe and eventually cell death [12]. If telomerase is re-expressed at this stage *in vitro*, cells escape crisis and become immortalized [13], while *in vivo*, tumour progression is significantly increased [14].

In nature, most species either do not restrict telomerase or die long before telomeres shorten to crucial lengths.

However, different strategies for body sizes and lifespans were selected throughout evolution. Consequently, some organisms had telomere maintenance under evolutionary scrutiny. For example, telomere length inversely correlates with lifespan in mammalian species [15,16]. Large animals undergo many more cell divisions than smaller ones and, since they live longer, tumourigenesis is more likely due to mutation accumulation. Large animals evolved additional tumour suppressor mechanisms that rely on counting cell divisions, of which telomere shortening is proposed to be one. There are, however, exceptions. For example, the naked mole rat is a small-sized rodent that lives up to 30 years and has evolved robust tumour suppressor mechanisms. Coincidently, these animals have shorter telomeres than most of the closely related rodents [17].

Most rodents and insectivores, such as hedgehogs, mice and rats, have long telomeres. However, both short-telomere and long-telomere species are found separately within at least four mammalian orders (Carnivora, Chiroptera, Rodentia, Lagomorpha) [16]. This suggests that telomere length evolved multiple times in response to different life strategies. The evolutionary history of telomeres is still unravelling and, while there is evidence suggesting short telomeres as a tumour suppressor mechanism, there is still no convincing explanation for selection of long telomeres in short-lived animals.

In humans it is still unclear how different tissues respond to telomere shortening, but premature ageing syndromes such as Werner, Hutchinson-Gilford and Dyskeratosis Congenita (DC) exhibit shorter telomeres, accelerated ageing and reduced lifespan [18]. Accordingly, several studies in human populations point to a negative correlation of telomere length with age [19–21]. In addition, recent studies have identified telomerase gene mutations in a variety of syndromes with short telomeres, such as pulmonary fibrosis [22] and aplastic anaemia [23]. Conversely, longer telomeres have been positively linked with healthy life and longevity [19]. Individuals with longer telomeres have a generally improved health profile and cognitive functions relative to controls.

Teleost fish have emerged as promising vertebrate models. Recent work shows that zebrafish have human length telomeres [24]. Despite detection of telomerase activity in various tissues, zebrafish telomeres shorten with age [25]. This suggests that telomerase expression in some somatic cells is not sufficient to prevent telomere shortening, thus mimicking the human scenario [26]. Telomere shortening was further associated with impaired regenerative responses in wild type aged zebrafish, suggesting a role for telomere maintenance in tissue homeostasis [25]. Accordingly, our work shows that telomerase deficient zebrafish degenerate and die prematurely in the first generation, suggesting that, similarly to humans, telomerase is required for zebrafish lifespan (C. Henriques *et al.*, submitted for publication).

Telomere shortening in the lab

Assessing the natural consequences of telomere shortening in the lab is a serious challenge. Since telomeres shorten slowly with age, and these studies usually take too long. Scientists frequently use short-lived animals, such as lab mice, that either have long telomeres (5–10× longer than humans) or mutants with artificially shortened telomeres. A major drawback of this strategy is that, unlike humans, most short-lived species have long telomeres and telomerase-independent cell division counting mechanisms [8,9,11]. Murine primary cells lack replicative senescence *in vitro* [16]. Instead, they arrest cell proliferation by undergoing stress-induced senescence (or STASIS), a phenomenon directed by either environmental stress or aberrant signalling [8,16]. Furthermore, immortalization occurs at a far higher frequency than human cells [9,27], and is largely telomerase-independent, engaging instead Alternative Lengthening of Telomeres (ALT) recombination-based mechanisms [9]. Accordingly, old lab mice develop a wide range of telomerase-independent tumours that differ substantially from ageing-associated cancers in humans [9].

Most of our knowledge on how vertebrates respond to short telomeres derives from studies using telomerase-deficient mice [28]. Telomerase-deficient mice are viable up to six generations showing no particular phenotypes until later generations [29–31]. Consistent with the idea that short telomeres do not take part of the mouse ageing process, wild type mice grow old and age despite the presence of very long telomeres [9]. The main mechanism directing ageing in the mouse is not totally understood. One possibility is that ageing in mice is primarily dictated by exogenous and endogenous stress, such as ROS [32••,33]. It is wrong, however, to assume that mice that normally lack short telomeres will maintain evolutionary conserved mechanisms to deal with telomere shortening. Thus, the use of current lab mouse strains to model the role of telomeres in human cancer and ageing must be crucially validated.

Recently, a wild-derived inbred mouse strain (Cast/EiJ) that displays shorter, human-like telomeres has been advanced as a more suitable model to study telomere dysfunction [34•]. Telomerase deficiency in this strain gives rise to first generation defects similar to the ones observed human DC syndromes [34•]. Thus telomere length may be limiting for Cast/EiJ longevity, making it a promising alternative to the current mouse models. Our recent work using zebrafish suggests this species also evolved to depend on telomerase to regulate lifespan. This vertebrate will offer an important counterweight to our current knowledge.

Tissue communication in response to telomere shortening

How an organism responds to telomere shortening will depend on the choice between senescence and apoptosis in different tissues [35]. While stem cells are more sensitive to apoptosis and resistant to senescence [36], somatic cells tend to enter replicative senescence in culture [37]. Whether this is the case *in vivo* is still not clear, but removing p16-positive senescent cells from a premature ageing mouse model partially rescued tissue degeneration [38••]. Some organs were preferentially rescued over others, suggesting different contributions of senescence in the whole body.

Tissues with high cell turnover are more sensitive to telomere shortening than more quiescent tissues [30]. Telomere shortening can impact tissue homeostasis in a cell-autonomous manner by impairing cell proliferation and giving rise to genome instability, providing the 'seed' for disease development. Accordingly, short telomeres significantly promote and enhance tumour progression *in vivo* when telomerase is re-expressed at a time when cells have already entered crisis and genome instability [14].

Telomere shortening may also contribute to the 'soil' in which old age diseases set in. Short telomeres trigger cell senescence and senescent cells accumulate in aged tissues. These cells produce factors that affect not only the local tissue but also the whole organism. Senescence-associated secretory phenotype (SASP) is characterized by the secretion of growth factors, tissue remodelling enzymes and pro-inflammatory cytokines, capable of influencing tumour development [39].

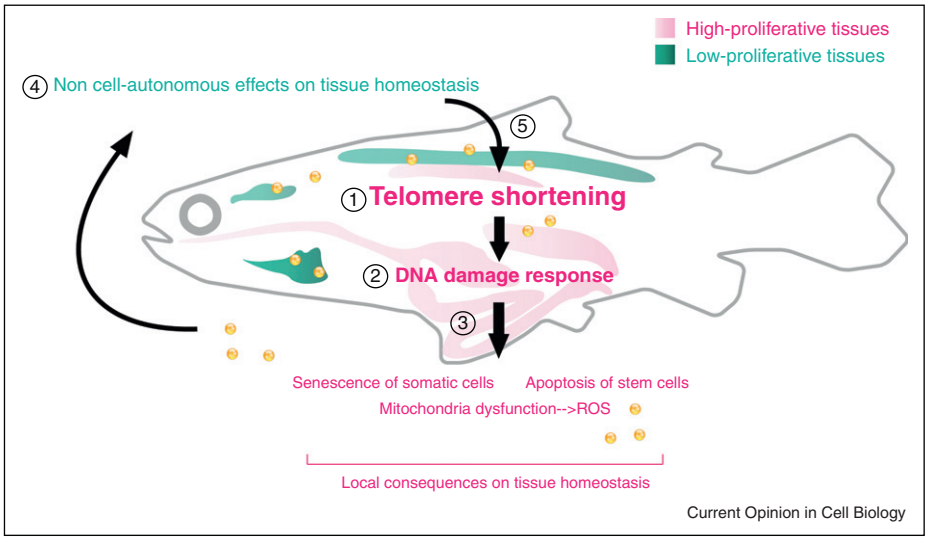
Telomere shortening can therefore affect whole body homeostasis by both cell-autonomous and non-cell-autonomous mechanisms (Figure 1). Understanding which of these phenomena limits tissue homeostasis in the old is crucial, particularly when considering possible telomerase 'therapies'. Crucially, systemic microenvironment in late generation telomerase knockout mice irreversibly impairs haematopoietic stem cell homeostasis [40••]. This suggests that short telomeres may cause a global inhibitory growth response. Given this, whether organ rejuvenation can be sustained within a surrounding senescent environment remains to be clarified. This knowledge will have profound implications, if we are to design therapies that rely on the transplantation of rejuvenated cells, such as iPS [41].

Discussion

Even though telomere biology has been the target of intense study for the past twenty years, we still do not understand the consequences of telomere shortening in different tissues and how these affect whole organism in naturally aged animals. Most studies have a modular approach, studying a particular response in a particular tissue, forgetting the need to integrate consequences of telomere dysfunction in the whole body.

Ageing is a peculiar phenomenon in nature, in the sense that it is actually rarely seen in most animals. Animals

Figure 1



Tissue communication in response to telomere dysfunction: (1) Restriction of telomerase expression leads to telomere erosion throughout lifetime. (2) Organs with high-proliferative capacity, such as gonads and gut, become rate-limiting over time and initiate DNA damage responses (DDRs). (3) DDRs culminate in apoptosis of stem cells and senescence of somatic cells. Tissue homeostasis is locally perturbed both in a cell-autonomous and non-cell-autonomous manner. (4) Non-cell-autonomous signals spread to produce a systemic effect that extends to low-proliferating organs, such as the brain and muscle. (5) Systemic tissue damage leads to metabolic disorders that give rise to further ROS and cellular damage, thus creating a positive feedback loop capable of impairing whole body homeostasis.

reproduce and die mostly before ageing sets in. Reproductive success is the most important driver of natural selection. So, it is conceivable that specific organs, such as the reproductive system, may play a rate-limiting role in affecting whole body homeostasis. Revealing studies in animal models as different as *Caenorhabditis elegans* [42] and mice [43] show how the reproductive system directly impacts on lifespan. Tissue communication is at the heart of transmitting information between different organs, be it in initiating (e.g. gonads), interpreting (e.g. brain) or effecting the response (e.g. gut) [44].

In species, such as humans, that evolved to regulate cell proliferation based on telomere length, high-turnover tissues will be affected with age. Telomere shortening in these tissues may be responsible for initiating whole body responses in a non-cell-autonomous way (Figure 1). Identifying these rate-limiting tissues will provide targets on which to direct telomerase therapies thus correcting defects locally and possibly rescue whole body degeneration [45].

Future studies will offer an integrative view and test whether cell-autonomous telomerase rejuvenation can recover whole body degeneration. Understanding the impact that individual tissues have in a systemic response will allow us to evaluate the contribution of telomere shortening to the ageing process as a whole.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Prescott J, Wentzensen IM, Savage SA, De Vivo I: **Epidemiologic evidence for a role of telomere dysfunction in cancer etiology**. *Mutat Res* 2012, **730**:75-84.

2. Kaul Z, Cesare AJ, Huschtscha LI, Neumann AA, Reddel RR: **Five dysfunctional telomeres predict onset of senescence in human cells**. *EMBO Rep* 2012, **13**:52-59.

3. Aubert G, Hills M, Lansdorp PM: **Telomere length measurement-caveats and a critical assessment of the available technologies and tools**. *Mutat Res* 2012, **730**:59-67.

4. • Sahin E, Depinho RA: **Linking functional decline of telomeres, mitochondria and stem cells during ageing**. *Nature* 2010, **464**:520-528.

This paper attempts to unify two major theories of ageing: telomere shortening and mitochondria dysfunction. It gives short telomeres a central role in a telomere-p53-mitochondria-mediated ageing process.

5. Sahin E, Depinho RA: **Axis of ageing: telomeres, p53 and mitochondria**. *Nat Rev Mol Cell Biol* 2012, **13**:397-404.

6. • Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, Blasco MA: **Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer**. *EMBO Mol Med* 2012.

Report showing that telomerase gene therapy delays onset of age-associated phenotypes and improves longevity in wild type mice

7. •• Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadinanos J *et al.*: **Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice**. *Nature* 2011, **469**:102-106.

Report showing that deterioration induced by telomere shortening in mice could be reversed by re-expression of telomerase. Importantly, even low-turnover tissues such as the brain benefited from telomerase expression.

8. Itahana K, Campisi J, Dimri GP: **Mechanisms of cellular senescence in human and mouse cells**. *Biogerontology* 2004, **5**:1-10.

9. Wright WE, Shay JW: **Telomere dynamics in cancer progression and prevention: fundamental differences in human and mouse telomere biology**. *Nat Med* 2000, **6**:849-851.

10. de Lange T: **Shelterin: the protein complex that shapes and safeguards human telomeres**. *Genes Dev* 2005, **19**:2100-2110.

11. Forsyth NR, Wright WE, Shay JW: **Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again**. *Differentiation* 2002, **69**:188-197.

12. Shay JW, Wright WE, Werbin H: **Defining the molecular mechanisms of human cell immortalization**. *Biochim Biophys Acta* 1991, **1072**:1-7.

13. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE: **Extension of lifespan by introduction of telomerase into normal human cells**. *Science* 1998, **279**:349-352.

14. Ding Z, Wu CJ, Jaskelioff M, Ivanova E, Kost-Alimova M, Protopopov A, Chu GC, Wang G, Lu X, Labrot ES *et al.*: **Telomerase reactivation following telomere dysfunction yields murine prostate tumors with bone metastases**. *Cell* 2012, **148**:896-907.

15. Gomes NM, Shay JW, Wright WE: **Telomere biology in Metazoa**. *FEBS Lett* 2010, **584**:3741-3751.

16. Gomes NM, Ryder OA, Houck ML, Charter SJ, Walker W, Forsyth NR, Austad SN, Venditti C, Pagel M, Shay JW *et al.*: **Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination**. *Aging Cell* 2011, **10**:761-768.

17. Gorbunova V, Seluanov A: **Coevolution of telomerase activity and body mass in mammals: from mice to beavers**. *Mech Ageing Dev* 2009, **130**:3-9.

18. Kirwan M, Dokal I: ***Dyskeratosis congenita*, stem cells and telomeres**. *Biochim Biophys Acta* 2009, **1792**:371-379.

19. Atzmon G, Cho M, Cawthon RM, Budagov T, Katz M, Yang X, Siegel G, Bergman A, Huffman DM, Schechter CB *et al.*: **Evolution in health and medicine Sackler colloquium: Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians**. *Proc Natl Acad Sci U S A* 2010, **107(Suppl. 1)**:1710-1717.

20. Njajou OT, Cawthon RM, Damcott CM, Wu SH, Ott S, Garant MJ, Blackburn EH, Mitchell BD, Shuldiner AR, Hsueh WC: **Telomere length is paternally inherited and is associated with parental lifespan**. *Proc Natl Acad Sci U S A* 2007, **104**:12135-12139.

21. Njajou OT, Hsueh WC, Blackburn EH, Newman AB, Wu SH, Li R, Simonsick EM, Harris TM, Cummings SR, Cawthon RM: **Association between telomere length, specific causes of death, and years of healthy life in health, aging, and body composition, a population-based cohort study**. *J Gerontol A: Biol Sci Med Sci* 2009, **64**:860-864.

22. Tsang AR, Wyatt HD, Ting NS, Beattie TL: **hTERT mutations associated with idiopathic pulmonary fibrosis affect telomerase activity, telomere length, and cell growth by distinct mechanisms**. *Aging Cell* 2012, **11**:482-490.

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

24. Elmore LW, Norris MW, Sircar S, Bright AT, McChesney PA, Winn RN, Holt SE: **Upregulation of telomerase function during tissue regeneration**. *Exp Biol Med (Maywood)* 2008, **233**:958-967.

25. Anchelin M, Murcia L, Alcaraz-Perez F, Garcia-Navarro EM, Cayuela ML: **Behaviour of telomere and telomerase during aging and regeneration in zebrafish**. *PLoS One* 2011, **6**:e16955.

26. Aubert G, Lansdorp PM: **Telomeres and aging**. *Physiol Rev* 2008, **88**:557-579.

27. Sherr CJ, DePinho RA: **Cellular senescence: mitotic clock or culture shock?** *Cell* 2000, **102**:407-410.

28. Kakuo S, Asaoka K, Ide T: **Human is a unique species among primates in terms of telomere length**. *Biochem Biophys Res Commun* 1999, **263**:308-314.

29. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW: **Telomere shortening and tumor formation by mouse cells lacking telomerase RNA**. *Cell* 1997, **91**:25-34.

30. Lee HW, Blasco MA, Gottlieb GJ, Horner JW 2nd, Greider CW, DePinho RA: **Essential role of mouse telomerase in highly proliferative organs**. *Nature* 1998, **392**:569-574.

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

32. •• Fumagalli M, Rossiello F, Clerici M, Barozzi S, Cittaro D, Kaplunov JM, Bucci G, Dobreva M, Matti V, Beausejour CM *et al.*: **Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation**. *Nat Cell Biol* 2012, **14**:355-365.

Work showing that persistent DNA damage response accumulates at telomeres *in vitro* and *in vivo*, particularly in tissues of old primates. This highlights telomeres as sites of persistent DNA damage, providing a signal for senescent cells and triggering senescence-associated secretory phenotypes.

33. Hewitt G, Jurk D, Marques FD, Correia-Melo C, Hardy T, Gackowska A, Anderson R, Taschuk M, Mann J, Passos JF: **Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence**. *Nat Commun* 2012, **3**:708.

34. • Armanios M, Alder JK, Parry EM, Karim B, Strong MA, Greider CW: **Short telomeres are sufficient to cause the degenerative defects associated with aging**. *Am J Hum Genet* 2009, **85**:823-832.

In vivo study showing that a wild-derived inbred mouse strain, Cast/EiJ, has short telomeres and that, when critically shortened, are sufficient to cause ageing-associated phenotypes.

35. Teplyuk NM: **Near-to-perfect homeostasis: examples of universal aging rule which germline evades**. *J Cell Biochem* 2012, **113**:388-396.

36. Prigione A, Fauler B, Lurz R, Lehrach H, Adjaye J: **The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells**. *Stem Cells* 2010, **28**:721-733.

37. Hayflick L: **The limited in vitro lifetime of human diploid cell strains**. *Exp Cell Res* 1965, **37**:614-636.

38. •• Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM: **Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders**. *Nature* 2011, **479**:232-236.

In vivo study demonstrating that clearance of senescent cells from an ageing mouse model can partially rescue tissue degeneration. Different organs are rescued to different extents, highlighting variable contributions of senescence for ageing.

39. Campisi J, Andersen JK, Kapahi P, Melov S: **Cellular senescence: a link between cancer and age-related degenerative disease?** *Semin Cancer Biol* 2011, **21**:354-359.

40. •• Song Z, Zhang J, Ju Z, Rudolph KL: **Telomere dysfunctional environment induces loss of quiescence and inherent impairments of hematopoietic stem cell function**. *Aging Cell* 2012, **11**:449-455.

In vivo study demonstrating a direct link between telomere shortening in affecting tissue homeostasis in a non-cell-autonomous manner.

41. Lengner CJ: **iPS cell technology in regenerative medicine**. *Ann N Y Acad Sci* 2010, **1192**:38-44.

42. McCormick M, Chen K, Ramaswamy P, Kenyon C: **New genes that extend *Caenorhabditis elegans*' lifespan in response to reproductive signals**. *Aging Cell* 2012, **11**:192-202.

43. Mason JB, Cargill SL, Anderson GB, Carey JR: **Transplantation of young ovaries to old mice increased life span in transplant recipients**. *J Gerontol A: Biol Sci Med Sci* 2009, **64**:1207-1211.

44. Hsin H, Kenyon C: **Signals from the reproductive system regulate the lifespan of *C. elegans***. *Nature* 1999, **399**:362-366.

45. Durieux J, Wolff S, Dillin A: **The cell-non-autonomous nature of electron transport chain-mediated longevity**. *Cell* 2011, **144**:79-91.