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Embryonic Stem Cells, Telomeres and Aging

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ABSTRACT

Embryonic Stem Cells (ESCs) constitute a small elite group of immortal cells with specific biological mission and mechanisms of rejuvenation, self-renewal and maintenance. Successes in nuclear reprogramming, induced pluripotency and other related protocols make these naturally pluripotent cells "gold standard" for comparison, understanding the basic mechanisms of pluripotency and for development of new biotechnologies. Here, we briefly addressed three aspects of ESCs biology that could be directly linked to anti-aging and longevity-promoting interventions: (i) how ESCs maintain the telomere length and why it is so important for pluripotency; (ii) paradoxical combination of superior protection of ESCs with hypersensitivity to apoptosis; and (iii) the role of hypercapnic/hypoxic conditions in ensuring and keeping the ESC features.

Keywords: Embryonic stem cells, Pluripotency, Telomeres, Telomerase, Hypoxia, Hypercapnia, Aging

Abbreviations: ALT: Alternative Lengthening of Telomeres; DSBs: Double Strand Breaks; ESCs: Embryonic Stem Cells; HSCs: Hematopoietic Stem Cells; iPSCs: Induced Pluripotent Stem Cells; LUCA: Last Universal Common Ancestor; MSCs: Mesenchymal Stem Cells; ROS: Reactive Oxygen Species; TERRA RNA: Telomeric Repeat-Containing RNA; Terc (TERC): Telomere RNA Component: Tert (TERT): Telomerase Reverse Transcriptase

INTRODUCTION

Embryonic Stem Cells (ESCs) are a gold standard of cellular pluripotency and immortality. They can generate any cell of over 200 cell types in our body, known thus far and preserve stable karyotype, pluripotency, proliferative capacity and telomere length after hundreds of population doublings during months and years of continuous maintenance in culture [1-3]. The ever-growing interest to this elite group of cells in gerontology is primarily supported by the expectations of new efficient strategies in tissue regeneration and anti-aging. To promote the study on ESC biology, the NIA Mouse ESC Bank, for example, has generated and held 185 ESC lines which can be differentiated into various cell types by specific transcription factors within 48 h [4].

According to intriguing data by Ratajczak et al. [5-8] adult tissues contain ESC-like pluripotent stem cells as a backup for the tissue-committed stem cells. Most likely, these quiescent cells are remnants of ESCs, can be mobilized at stressful conditions to support tissue repair, and presumably have a role in determination of longevity. Comparison of several murine strains differing in their lifespan showed that the longer-lived strains have a more abundant pool of ESC-like cells [6].

Another important aspect of putative anti-aging effects of ECSs and probably other pluripotent (e.g. iPSCs) and multipotent (e.g. MSCs) stem cells is that they may also act in a paracrine manner [9-11]. This gained support from the observation that ESC-conditioned medium suppressed

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cellular senescence, maintained proliferative capacity and accelerated the wound healing [12] – all of which undergo age-related changes and contribute to the aging process [13-17].

Before deployment of the ambitious perspectives in cellular rejuvenation and tissue regeneration based on artificial pluripotent cells, it seems relevant studying as much as possible about their natural counterparts. ESCs could be a perfect choice to fulfill the task. Unfortunately, the expectations for application of pluripotent cells in anti-aging, especially in *in vivo* models [18] remain insufficiently explored, supporting the contentions of gearing up their study. Here, we briefly addressed three aspects of ESC biology that could be directly linked to anti-aging and longevity-promoting interventions: (i) how ESCs maintain the telomere length and why it is so important for pluripotency; (ii) paradoxical combination of superior protection of ESCs with hypersensitivity to apoptosis; and (iii) the role of hypercapnic/hypoxic conditions in ensuring and keeping the ESC features.

ESCs GET TO THE TOP IN MAINTAINING THE TELOMERE LENGTH

Telomeres and telomerase

Each end of a chromosome of eukaryotic organisms contains a special region of repetitive nucleotide sequences called a telomere. In vertebrates, telomeric DNA consists of multiple tandem hexomere repeats – GGGATT for the telomere DNA heavy strand and complementary CCCTAA for the light strand. In complex with six proteins known as shelterin, non-translating (TERRA) RNA and proteins associated with the heavy strand overhang, telomeres create a unique telomeric chromatin for protecting the chromosome ends [19,20]. Telomeres are apparently the most vulnerable elements of the genome and are prone to attrition (a loss of small fragments of DNA at each round of replication), because of the DNA incomplete end-replication problem. Yet, occasional damage to telomeric DNA and especially breaks of the single-stranded overhangs could be a main cause of telomere shortening [21]. Overly short or otherwise impaired telomeres could cause chromosome end-to-end fusion or recombination, thus leading to genome instability, with far-reaching consequences including tumorigenesis [22,23].

Telomere length is recovered by a specialized ribozyme – telomerase or by a homologous recombination known as alternative lengthening of telomeres (ALT). Telomerase consists of two functionally diverse subunits – reverse transcriptase (Tert) and telomere RNA template (Terc) [20]. Robust expression of these subunits and especially Tert, is an important attribute of all unlimitedly proliferating pluripotent stem cells (except of early embryo cleavage stages), as they maintain telomere length mostly through telomerase activity. ALT has primarily been found in cells with no or very low telomerase activity – in early embryo cleavage stages (when the number of cells increases without increasing their total mass) [24] and in somatic cells [25].

Telomeres and telomerase activity in ESCs

Efficient telomere homeostasis is critical for proper functionality of both embryonic and resident stem cells and therefore essential for tissue homeostasis throughout the life of an organism [26]. Telomere elongation is crucial for the self-renewal of ESCs [27], while telomere shortening leads to replicative senescence of their differentiated progeny and was suggested as one of the hallmarks of aging [13]. The rate of telomere shortening seems to be in some proportion to the species-specific longevity. For example, the mouse telomeres shorten 100 times faster than human telomeres [28].

As could be expected, ESCs have one of the longest telomeres as compared to other cell types. In humans, telomere size first decreases in early embryo cleavage stage cells but then reaches its maximum length in the blastocysts (8.4 kb and 12.2 kb, respectively) [29]. Notably, the elongation of telomeres in the blastocysts is accompanied by a gradual exacerbation of the intracellular hypoxia/hypercapnia, especially in ESCs of the inner cell mass of the blastocyst.

The most stringent tests for pluripotency – generation of complete pups and germline-competent chimeras – showed that only ESCs with long telomeres possessed authentic developmental pluripotency, whereas ESCs with short telomeres failed the tests [27]. Additionally, ESCs with short telomeres exhibited a lower proliferative rate and germ cell differentiation, as well as a capacity to modify the expression of genes related to embryogenesis and epigenetics. iPSCs with longer telomeres also were superior in generating chimeras over the cells with short telomeres, thus suggesting that telomere length could be a valuable marker of cell pluripotency [27].

TERT overexpression enhanced telomerase activity, proliferative rate and colony-forming capacity of human ESCs [30]. Differentiated progeny of TERT-overexpressing ESCs also showed an enhanced telomerase activity and resistance to oxidative stress, whereas downregulation of TERT decreased the proliferative rate and resulted in loss of pluripotency [30]. The very ends of eukaryotic linear chromosomes are additionally protected by the telomeric overhangs [19]. As telomeres, they are shorter in differentiated progeny of ESCs [31]. The Tert overexpression in mouse ESCs not only increased their telomerase activity but also extended the length of overhangs, simultaneously elevating ESC proliferative capacity and resistance to apoptosis [31].

Epigenetic factors, such as histone modifications and DNA methylation, play an important role in establishing and maintaining pluripotency of ESCs [32,33]. Not surprisingly, the telomere homeostasis is also under epigenetic control

[34,35]. Moreover, this control could be species-specific [36]. As typical for pluripotent stem cells, ESCs have more relaxed chromatin conformation, including telomeric regions [27,37,38]. Accordingly, ESCs have a higher number of open chromatin markers, acetylated and methylated lysine 27 of histone 3, in combination with trimethylated histone 3 lysine in non-expressing genes [39]. Modification of the di/tri methylated lysine ratio at regulatory regions of ESCs was sufficient for acquisition or repression of cell lineage transcriptional program and phenotypes [39]. Methylation of cytosine (5mC) is another powerful tool of epigenetics. Oxidation of 5mC by the Tet protein family results in formation of oxidized derivatives of 5mC. They are selectively recognized and excised by thymine DNA glycosylase, thus leading to DNA demethylation. Tet knockout ESCs exhibit elongated telomeres and elevated telomere-sister chromatid exchange, indicating the direct impact of DNA demethylation on telomere homeostasis [40].

In cell cultures, ESCs exist as a mixture of metastable cells sporadically entering into the 2-cell embryo-like state. The genes Zscan4, Tcstv1 and Tcstv3 were shown to be involved in the formation of this state; they were also responsible for telomere maintenance and genomic stability [41,42]. Ectopic overexpression Tcstv1 or Tcstv3 genes resulted in telomere elongation, whereas their knockdown shortened telomeres of ESC [42].

Telomerase in cellular senescence, aging and rejuvenation

Aging is often referred as to a progressive decline in tissue homeostasis and repair caused by malfunction of somatic stem cells, accompanied by accumulation of senescent cells [17,43,44]. However, there is a growing consensus that cellular senescence and aging are reversible [45-48]. Somatic cells can be rejuvenated back to the ESC-like state by various procedures of induced pluripotency, nuclear transferring, fusing with ESCs, tetraploid embryo complementation or exposure of somatic cells to ESC extracts. The essential point is that the viable pups could be derived from cell clones rejuvenated by all these procedures [27,41,49,50]. For example, treatment of mouse fibroblasts with ESC protein extracts was sufficient for reprogramming the adult fibroblasts into ESC-like cells [41]. Moreover, these cells were functionally and biologically indistinguishable from ESCs and exhibited complete developmental potency, giving rise to fetal animals. The ESC extract-induced alterations in the global gene expression, DNA methylation and histone modifications were typical for the conversion of somatic cells into pluripotent stem cells. In another study [12], the mouse ESC-conditioned medium supplemented to cultured human dermal fibroblasts was shown to suppress cellular senescence and maintain their proliferative capacity, presumably, through up-regulation of fibroblast growth factor 2 and down-regulation of CS-associated p53.

The microarray analysis showed that the enhanced self-renewal and extended lifespan of cells were associated with activation of a variety of genes. Among them, telomerase was suggested as a “survival enzyme” in ESCs and their differentiated progeny [31]. Cellular rejuvenation is ubiquitously associated with telomerase activation and telomere elongation, supporting the idea of “cause-and-effect” relationships between aging and telomere integrity [51]. Moreover, there is evidence that telomere size established by ESCs could be deterministic for mammalian longevity [52,53].

IT IS BETTER TO DIE THAN TO BE WRONG

This “Samurai Law of Biology” [54] is especially relevant to ESCs. In contrast to the mostly local effects of somatic cell failure, DNA damage and mutations in ESCs could have catastrophic consequences for most tissues and the whole organism and may also be passed to the germline progenies. This assumes that ESCs should evolutionarily be rendered superior defense and selection systems. Indeed, ESCs are characterized by a robust DNA repair and low levels of mutations [55-57]. For example, spontaneous or induced mutation frequency of the reporter genes was several orders of magnitude lower in mouse ESCs than in the embryo fibroblasts. Yet, DNA breaks are rather frequent in ESCs, especially during DNA replication [58]. Homologous recombination is recognized as the main pathway of DNA double strand breaks (DSBs) repair and Rad51 is a key regulator of this process [59]. In mouse ESCs, Rad51 showed a 2-fold increase in mRNA and 15-fold increase in protein expression, compared with the embryo fibroblasts. Moreover, only a small portion of Rad51 protein was recruited to repair DSBs or stalled replication forks in normal conditions, thus indicating substantial reserves of Rad51-dependent DNA repair in stressful conditions [60].

Further supporting superior protection of ESC vs. differentiated cells are lower rates of free radical generation in combination with a higher antioxidant defense [61]. Measurements of 8-OH-G (8-hydroxyguanine), a well-known marker of oxidative stress damage in DNA, showed that ESCs cultured with 300 μM hydrogen peroxide had lower levels of 8-OH-G than more differentiated cells. The better protection of ESC DNA against free radicals was further supported by an enhanced expression of 8-OH-G repair-associated genes [62]. It appears that ESCs and their genome are more resistant to oxidative stress. Degradation of misfolded, aggregated or otherwise damaged proteins via autophagy or proteasomes was also superior in ESCs, thus underpinning their generally ameliorated intracellular environment [63,64].

Apart from a robust DNA repair and a low mutational level, hypersensitivity of ESCs to apoptosis is another important

tool to protect their genome integrity. ESCs with unrepaired DNA damage readily undergo apoptosis or differentiation, thus removing the damaged cells from the pluripotent pool [65,66]. In fact, ESCs and their differentiated progeny adhere to different stress response strategies [67]. For example, sub lethal heating activated apoptosis in human ESCs while induced premature senescence in the ESC-derived fibroblast-like cells [68]. Another example includes hypersensitivity of ESCs to camptothecin, a topoisomerase I inhibitor which induces DNA DSBs and an intensive p53-mediated apoptosis in human ESCs, but to a much lesser degree in differentiated ESCs [69]. The importance of genome integrity in ESCs is further exemplified by mice deficient in Cdk12 (cyclin-dependent kinase 12), a multifunctional protein involved in maintaining the genomic stability and pluripotency of ESCs [58]. The $Cdk12^{-/-}$ embryos displayed a reduced expression of DNA damage response genes and insufficient DNA repair, with subsequent activation of apoptosis. This resulted in abrogated accumulation of the inner cell mass in blastocysts and lethality of the embryos shortly after the implantation [58]. Of note, an increased DNA repair and sensitivity to apoptosis have been observed in several models of lifespan extension [44].

ESCs RESIDE IN HYPOXIC/HYPERCAPNIC MICROENVIRONMENT AND RELY ON GLYCOLYSIS

Increased sensitivity to apoptosis and completely de-differentiated status of ESCs apparently require a low metabolic rate associated with less production of ROS. This could be supported by hypoxic/hypercapnic microenvironment which is optimal not only for ESCs but for other stem cells as well [70-72]. ESCs are usually obtained from the inner cell mass of pre-implantation embryos (blastocysts). While moving down the oviduct towards the uterus, embryos continue dividing and reach a considerable size at the pre-implantation blastocysts. At this stage, there are usually several hundreds of the inner mass cells and comparable number of cells of the outer layer of the blastocyst-trophoblast [73]. Lack of vascularization or other mechanisms of O_2/CO_2 active transport, aggravated by the assembly of substantial number of ESCs, means that ESC environment in the blastocyst cavity (blastocoele) is essentially hypoxic/hypercapnic. We hypothesized that the severe hypoxia/hypercapnia in blastocysts is required for suppression of ESC differentiation, thus allowing a highly efficient DNA repair — a crucial event before ESCs start to differentiate. Besides, this temporary suppression allows synchronizing a consequent differentiation of ESCs.

Such hypoxic/hypercapnic microenvironment will decrease the rate of oxidative phosphorylation (and accordingly, ROS generation) and activate glycolysis, in part due to the hypoxia inducible factor 1 alpha (Hif1alpha) which concomitantly promotes telomerase expression and enhance self-renewal of stem cells [74]. Anaerobic metabolism could also be necessary to supply substrates for the anabolic processes, which is typical for intensively dividing cells (Warburg effect) [75,76]. In fact, activated glycolysis and associated higher concentrations of pyruvate and lactate are metabolic hallmarks of apparently all actively dividing cells, ESCs included [77-79]. In the *in vitro* cultured early mouse embryos, glucose consumption was undetectable until the blastocyst stage but became the main source of energy generation in the later stages (embryo's day 6.5 and 7.5), in contrast to the opposite dynamics of pyruvate utilization [80]. Of note, almost the same pattern of metabolic alterations was observed in various models of induced pluripotency which usually started by inhibition of oxidative phosphorylation and activation of glycolysis [81,82]. Remarkably, even at high levels of O_2, ESCs utilized primarily the anaerobic metabolism [83]. This apparently allowed them to survive hyperoxia by reducing the ROS generation. Another important finding is that the main transcription factors of pluripotency, Oct4 and Nanog, can directly induce expression of the key glycolytic enzymes hexokinase 2 and pyruvate kinase M2, thus delaying differentiation and preserving pluripotency of ESCs [84]. In turn, the genes involved in the control of glucose uptake (GLUT3) and metabolism (PKM2) are also involved in regulation of Oct4 expression [85]. It should be emphasized that even though the pluripotent stem cells rely on glycolysis, mitochondria also play an important role in the maintenance of pluripotency [79]. In particular, several mitochondrial genes (e.g. POLG, Gfer, Drp1) were shown to regulate the expression of pluripotent factors.

CONCLUDING REMARKS

ESCs belong to the enigmatic group of immortal, pluripotent cells. Successes in nuclear reprogramming, induced pluripotency and other related protocols make these naturally pluripotent cells "gold standard" for comparison, understanding of the basic mechanisms of pluripotency and for the development of new biotechnologies. The huge potential of ESC application in anti-aging remains insufficiently explored, warranting further research in the field.

Pluripotency of ESCs is supported by complex networks of the growth and transcription factors, primarily by Oct4, Sox2 and Nanog, which expression is under a strict epigenetic control. Survival and proper functionality of ESCs and their differentiated progeny strongly depend on the telomere homeostasis [26], which apparently has wider assignments than just canonical protection of the chromosomes ends. Whatever the case, telomeres and telomerase are key regulators of stem cell proliferation, simultaneously restraining cancer and delaying aging [86].

ESCs have superior mechanisms for DNA repair and genome maintenance. Yet, being potentially immortal in cultures, ESCs are predisposed to apoptosis instead of trying

to repair the damage — an additional line of protection of the ESC progeny. Despite the efficient DNA repair, generally well-maintained telomere homeostasis and sensitivity to apoptosis, long-term manipulations with ESCs *in vitro* often lead to genomic and epigenomic abnormalities, with a subsequent decrease in cell viability or acquiring tumorigenicity [32,87,88]. These aspects of ESC and iPSC biology have not yet been fully addressed and definitely require more attention.

An important point is that ESCs live in hypoxic/hypercapnic microenvironment, as apparently once did the last universal common ancestor (LUCA) of all animal species in primordial Earth atmospheres. On the one hand, such microenvironment ensures minimal generation of ROS due to glycolysis-based metabolism. On the other hand, it promotes conditions for efficient DNA repair. This is especially true for ESCs of the pre-implanted blastocyst, when hypoxia/hypercapnia reaches its peak. This short period of ESC life provides a unique opportunity for the "last-minute" correction of the DNA damage, before ESCs start to differentiate. The very fact that the life still exists, and the accumulated damage does not pass on from generation to generation is, to a great extent, guaranteed by that "last-minute" correction of ESCs.

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REFERENCES

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, et al. (1998) Embryonic stem cell lines derived from human blastocysts. Science 282: 1145-1147.
2. Hoffman LM, Carpenter MK (2005) Human embryonic stem cell stability. Stem Cell Rev 1: 139-144.
3. Li F, Ge Y, Liu D, Songyang Z (2019) The role of telomere-binding modulators in pluripotent stem cells. Protein Cell.
4. Yamamizu K, Sharov AA, Piao Y, Amano M, Yu H, et al. (2016) Generation and gene expression profiling of 48 transcription-factor-inducible mouse embryonic stem cell lines. Sci Rep 6: 25667.
5. Ratajczak MZ, Liu R, Ratajczak J, Kucia M, Shin DM (2011) The role of pluripotent embryonic-like stem cells residing in adult tissues in regeneration and longevity. Differentiation 81: 153-161.
6. Ratajczak MZ, Shin DM, Liu R, Mierzejewska K, Ratajczak J, et al. (2012) Very small embryonic/epiblast-like stem cells (VSELs) and their potential role in aging and organ rejuvenation - An update and comparison to other primitive small stem cells isolated from adult tissues. Aging (Albany NY) 4: 235-246.
7. Ratajczak MZ, Zuba-Surma E, Wojakowski W, Suszynska M, Mierzejewska K, et al. (2014) Very small embryonic-like stem cells (VSELs) represent a real challenge in stem cell biology: Recent pros and cons in the midst of a lively debate. Leukemia 28: 473-484.
8. Ratajczak MZ, Bartke A, Darzynkiewicz Z (2017) Prolonged growth hormone/insulin/insulin-like growth factor nutrient response signaling pathway as a silent killer of stem cells and a culprit in aging. Stem Cell Rev 13: 443-453.
9. Balseanu AT, Buga AM, Catalin B, Wagner DC, Boltze J, et al. (2014) Multimodal approaches for regenerative stroke therapies: Combination of granulocyte colony-stimulating factor with bone marrow mesenchymal stem cells is not superior to G-CSF alone. Front Aging Neurosci 6: 130.
10. Gobshtis N, Tfilin M, Wolfson M, Fraifeld VE, Turgeman G (2017) Transplantation of mesenchymal stem cells reverses behavioral deficits and impaired neurogenesis caused by prenatal exposure to valproic acid. Oncotarget 8: 17443-17452.
11. Sandu RE, Balseanu AT, Bogdan C, Slevin M, Petcu E, et al. (2017) Stem cell therapies in preclinical models of stroke. Is the aged brain microenvironment refractory to cell therapy? Exp Gerontol 94: 73-77.
12. Bae YU, Choi JH, Nagy A, Sung HK, Kim JR (2016) Anti-senescence effect of mouse embryonic stem cell conditioned medium through a PDGF/FGF pathway. FASEB J 30: 1276-1286.
13. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153: 1194-1217.
14. Tacutu R, Budovsky A, Yanai H, Fraifeld VE (2011) Molecular links between cellular senescence, longevity and age-related diseases - A systems biology perspective. Aging (Albany NY) 3: 1178-1191.
15. Yanai H, Toren D, Vierlinger K, Hofner M, Nöhammer C, et al. (2015) Wound healing and longevity: Lessons from long-lived αMUPA mice. Aging (Albany NY) 7: 167-176.
16. Yanai H, Budovsky A, Tacutu R, Barzilay T, Abramovich A, et al. (2016) Tissue repair genes: The TiRe database and its implication for skin wound healing. Oncotarget 7: 21145-21155.

17. Yanai H, Fraifeld VE (2018) The role of cellular senescence in aging through the prism of Koch-like criteria. Ageing Res Rev 41: 18-33.

18. Abramovich A, Muradian KK, Fraifeld VE (2008) Have we reached the point for *in vivo* rejuvenation? Rejuvenation Res 11: 489-492.

19. Blackburn EH (2001) Switching and signaling at the telomere. Cell 106: 661-673.

20. MacNeil DE, Bensoussan HJ, Autexier C (2016) Telomerase regulation from beginning to the end. Genes (Basel) 7. pii: E64.

21. Frolkis VV, Muradian KHK (1991) Life Span Prolongation. CRC Press, Boca Raton.

22. Engelhardt M, Martens UM (1998) The implication of telomerase activity and telomere stability for replicative aging and cellular immortality (review). Oncol Rep 5: 1043-1052.

23. Blackburn EH, Epel ES, Lin J (2015) Human telomere biology: A contributory and interactive factor in aging, disease risks and protection. Science 350: 1193-1198.

24. Liu L, Bailey SM, Okuka M, Muñoz P, Li C, et al. (2007) Telomere lengthening early in development. Nat Cell Biol 9: 1436-1441.

25. Neumann AA, Watson CM, Noble JR, Pickett HA, Tam PP, et al. (2013) Alternative lengthening of telomeres in normal mammalian somatic cells. Genes Dev 27: 18-23.

26. Batista LF (2014) Telomere biology in stem cells and reprogramming. Prog Mol Biol Transl Sci 125: 67-88.

27. Huang J, Wang F, Okuka M, Liu N, Ji G, et al. (2011) Association of telomere length with authentic pluripotency of ES/iPS cells. Cell Res 21: 779-792.

28. Vera E, Bernardes de Jesus B, Foronda M, Flores JM, Blasco MA (2012) The rate of increase of short telomeres predicts longevity in mammals. Cell Rep 2: 732-737.

29. Turner S, Wong HP, Rai J, Hartshorne GM (2010) Telomere lengths in human oocytes, cleavage stage embryos and blastocysts. Mol Hum Reprod 16: 685-694.

30. Yang C, Przyborski S, Cooke MJ, Zhang X, Stewart R, et al. (2008) A key role for telomerase reverse transcriptase unit in modulating human embryonic stem cell proliferation, cell cycle dynamics and *in vitro* differentiation. Stem Cells 26: 850-863.

31. Armstrong L, Saretzki G, Peters H, Wappler I, Evans J, et al. (2005) Overexpression of telomerase confers growth advantage, stress resistance and enhanced differentiation of ESCs toward the hematopoietic lineage. Stem Cells 23: 516-529.

32. Grafi G (2013) Stress cycles in stem cells/iPSCs development: Implications for tissue repair. Biogerontology 14: 603-608.

33. Paksa A, Rajagopal J (2017) The epigenetic basis of cellular plasticity. Curr Opin Cell Biol 49: 116-122.

34. Liu L (2017) Linking telomere regulation to stem cell pluripotency. Trends Genet 33: 16-33.

35. Hu H, Li B, Duan S (2019) The alteration of subtelomeric DNA methylation in aging-related diseases. Front Genet 9: 697.

36. Dang-Nguyen TQ, Haraguchi S, Furusawa T, Somfai T, Kaneda M, et al.(2013) Downregulation of histone methyltransferase genes SUV39H1 and SUV39H2 increases telomere length in embryonic stem-like cells and embryonic fibroblasts in pigs. J Reprod Dev 59: 27-32.

37. Azuara V, Perry P, Sauer S, Spivakov M, Jørgensen HF, et al. (2006) Chromatin signatures of pluripotent cell lines. Nat Cell Biol 8: 532-538.

38. Grafi G, Rivka Ofir R, Chalifa-Caspi V, Plaschkes I (2011) Illuminating hidden features of stem cells. In: Embryonic Stem Cells - Basic Biology to Bioengineering (ed. Michael Kallos). InTech, pp: 265-282.

39. Juan AH, Wang S, Ko KD, Zare H, Tsai PF, et al. (2016a) Roles of H3K27me2 and H3K27me3 examined during fate specification of embryonic stem cells. Cell Rep 17: 1369-1382.

40. Lu F, Liu Y, Jiang L, Yamaguchi S, Zhang Y (2014) Role of Tet proteins in enhancer activity and telomere elongation. Genes Dev 28: 2103-2119.

41. Kwon YW, Paek JS, Cho HJ, Lee CS, Lee HJ, et al. (2015) Role of Zscan4 in secondary murine iPSC derivation mediated by protein extracts of ESC or iPSC. Biomaterials 59: 102-115.

42. Zhang Q, Dan J, Wang H, Guo R, Mao J, et al. (2016) Tcstv1 and Tcstv3 elongate telomeres of mouse ES cells. Sci Rep 6: 19852.

43. Beltrami AP, Cesselli D, Beltrami CA (2011) At the stem of youth and health. Pharmacol Ther 129: 3-20.

44. Moskalev AA, Shaposhnikov MV, Plyusnina EN, Zhavoronkov A, Budovsky A, et al. (2013) The role of DNA damage and repair in aging through the prism of Koch-like criteria. Ageing Res Rev 12:661-684.

45. Lapasset L, Milhavet O, Prieur A, Besnard E, Babled A, et al. (2011) Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state.

46. Rohani L, Johnson AA, Arnold A, Stolzing A (2014) The aging signature: A hallmark of induced pluripotent stem cells? Aging Cell 13: 2-7.

47. Lemey C, Milhavet O, Lemaitre JM (2015) iPSCs as a major opportunity to understand and cure age-related diseases. Biogerontology 16: 399-410.

48. Trokovic R, Weltner J, Noisa P, Raivio T, Otonkoski T (2015) Combined negative effect of donor age and time in culture on the reprogramming efficiency into induced pluripotent stem cells. Stem Cell Res 15: 254-262.

49. Eggan K, Rode A, Jentsch I, Samuel C, Hennek T, et al. (2002) Male and female mice derived from the same embryonic stem cell clone by tetraploid embryo complementation. Nat Biotechnol 20: 455-459.

50. Zhao XY, Li W, Lv Z, Liu L, Tong M, et al. iPS cells produce viable mice through tetraploid complementation. Nature 2009; 461: 86-90.

51. Tam WL, Ang YS, Lim B (2007) The molecular basis of ageing in stem cells. Mech Ageing Dev 128: 137-148.

52. Gomes NM, Ryder OA, Houck ML, Charter SJ, Walker W, et al. (2011) Comparative biology of mammalian telomeres: Hypotheses on ancestral states and the roles of telomeres in longevity determination. Aging Cell 10: 761-768.

53. Lehmann G, Muradian K, Fraifeld E (2013) Telomere length and body temperature-independent determinants of mammalian longevity? Front Genet 4: 111.

54. Skulachev VP (2001) The programmed death phenomena, aging and the Samurai law of biology. Exp Gerontol 36: 995-1024.

55. Tichy ED, Stambrook PJ (2008) DNA repair in murine embryonic stem cells and differentiated cells. Exp Cell Res 314: 1929-1936.

56. Xiong J, Todorova D, Su NY, Kim J, Lee PJ, et al. (2015) Stemness factor Sall4 is required for DNA damage response in embryonic stem cells. J Cell Biol 208: 513-520.

57. Fu X, Cui K, Yi Q, Yu L, Xu Y (2017) DNA repair mechanisms in embryonic stem cells. Cell Mol Life Sci 74: 487-493.

58. Juan HC, Lin Y, Chen HR, Fann MJ (2016b) Cdk12 is essential for embryonic development and the maintenance of genomic stability. Cell Death Differ 23: 1038-1048.

59. Hine CM, Li H, Xie L, Mao Z, Seluanov A, et al. (2014) Regulation of Rad51 promoter. Cell Cycle 13: 2038-2045.

60. Tichy ED, Pillai R, Deng L, Tischfield JA, Hexley P, et al. (2012) The abundance of Rad51 protein in mouse embryonic stem cells is regulated at multiple levels. Stem Cell Res 9: 124-134.

61. Saretzki G, Armstrong L, Leake A, Lako M, von Zglinicki T (2004) Stress defense in murine embryonic stem cells is superior to that of various differentiated murine cells. Stem Cells 22: 962-971.

62. Kuboyama A, Tanaka S, Kawai K, Kasai H, Morii H, et al. (2011) 8-Hydroxyguanine levels and repair capacity during mouse embryonic stem cell differentiation. Free Radic Res 45: 527-533.

63. Vilchez D, Simic MS, Dillin A (2014) Proteostasis and aging of stem cells. Trends Cell Biol 24: 161-170.

64. Lee HJ, Gutierrez-Garcia R, Vilchez D (2017) Embryonic stem cells: A novel paradigm to study proteostasis? FEBS J 284: 391-398.

65. Geijsen N (2013) Primed to perish: Heightened mitochondrial priming explains hESC apoptosis sensitivity. Cell Stem Cell 13: 371-372.

66. Liu JC, Guan X, Ryan JA, Rivera AG, Mock C, et al. (2013) High mitochondrial priming sensitizes hESCs to DNA-damage-induced apoptosis. Cell Stem Cell 13: 483-491.

67. Aladjem MI, Spike BT, Rodewald LW, Hope TJ (1998) ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. Current Biology 8: 145-155

68. Alekseenko LL, Zemelko VI, Zenin VV, Pugovkina NA, Kozhukharova IV, et al.2012) Heat shock induces apoptosis in human embryonic stem cells but a premature senescence phenotype in their differentiated progeny. Cell Cycle 11: 3260-3269.

69. García CP, Videla Richardson GA, Romorini L, Miriuka SG, Sevlever GE, et al. (2014) Topoisomerase I inhibitor, camptothecin, induces apoptogenic signaling in human embryonic stem cells. Stem Cell Res 12: 400-414.

70. Vlaski M, Negroni L, Kovacevic-Filipovic M, Guibert C, Brunet de la Grange P, et al. (2014) Hypoxia/hypercapnia-induced adaptation maintains functional capacity of cord blood stem and progenitor cells at 4°C. J Cell Physiol 229: 2153-2165.

71. Hamad M, Irhimeh MR, Abbas A (2016) Hypercapnia slows down proliferation and apoptosis of human bone marrow promyeloblasts. Bioprocess Biosyst Eng 39: 1465-1475.

72. Shojaei F, Rahmati S, Banitalebi Dehkordi M (2019) A review on different methods to increase the efficiency of

mesenchymal stem cell-based wound therapy. Wound Repair Regen.

73. Gilbert SF (2000) Developmental Biology. 6th Edn. Sunderland (MA): Sinauer Associates. Early Mammalian Development. Available from: http://www.ncbi.nlm.nih.gov/books/NBK10052/
74. Mathews J, Davy PM, Gardner LH, Allsopp RC (2016) Stem cells, telomerase regulation and the hypoxic state. Front Biosci (Landmark Ed) 21: 303-315.
75. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science 324: 1029-1033.
76. Krisher RL, Prather RS (2012) A role for the Warburg effect in pre-implantation embryo development: Metabolic modification to support rapid cell proliferation. Mol Reprod Dev 79: 311-320.
77. Karja NW, Kikuchi K, Fahrudin M, Ozawa M, Somfai T, et al. (2006) Development to the blastocyst stage, the oxidative state and the quality of early developmental stage of porcine embryos cultured in alteration of glucose concentrations *in vitro* under different oxygen tensions. Reprod Biol Endocrinol 4: 54.
78. Takahashi M (2012) Oxidative stress and redox regulation on *in vitro* development of mammalian embryos. J Reprod Dev 58: 1-9.
79. Xu X, Duan S, Yi F, Ocampo A, Liu GH, et al. (2013) Mitochondrial regulation in pluripotent stem cells. Cell Metab 18: 325-332.
80. Houghton FD, Thompson JG, Kennedy CJ, Leese HJ (1996) Oxygen consumption and energy metabolism of the early mouse embryo. Mol Reprod Dev 44: 476-485.
81. Folmes CD, Nelson TJ, Terzic A (2011) Energy metabolism in nuclear reprogramming. Biomark Med 5: 715-729.
82. Bukowiecki R, Adjaye J, Prigione A (2014) Mitochondrial function in pluripotent stem cells and cellular reprogramming. Gerontology 60: 174-82.
83. Powers DE, Millman JR, Huang RB, Colton CK (2008) Effects of oxygen on mouse embryonic stem cell growth, phenotype retention and cellular energetics. Biotechnol Bioeng 101: 241-254.
84. Kim H, Jang H, Kim TW, Kang BH, Lee SE, et al. (2015) Core pluripotency factors directly regulate metabolism in embryonic stem cell to maintain pluripotency. Stem Cells 33: 2699-2711.
85. Christensen DR, Calder PC, Houghton FD (2015 GLUT3 and PKM2 regulate OCT4 expression and support the hypoxic culture of human embryonic stem cells. Sci Rep 5: 17500.
86. Flores I, Blasco MA (2010) The role of telomeres and telomerase in stem cell aging. FEBS Lett 584: 3826-3830.
87. Ben-David U, Benvenisty N (2011) The tumorigenicity of human embryonic and induced pluripotent stem cells. Nat Rev Cancer 11: 268-277.
88. Bar S, Benvenisty N (2019) Epigenetic aberrations in human pluripotent stem cells. EMBO J 38: pii: e101033.