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SIRT1, STEM CELLS, AGING, AND STEM CELL AGING

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Summary

The same thermodynamic and biochemical mechanisms linked to aging in somatic cells may also work in stem cells. Developments in mitochondrial biology and new drug development based on this knowledge, are finding their way into the clinic (i.e. diabetes), and may illuminate new ways of manipulating and using stem cells in medicine.

Keywords

SIRT1; stem cell aging; mitochondria; telomere; lifespan extension

Introduction

Fundamental causes of human aging, at physiological, cellular, and biochemical levels, are essentially unknown. Information obtained from studies of model organisms, such as yeast, flys, worms, and rodents as well as from rare human progeroid diseases such as Werner syndrome and Hutchinson-Gilford syndrome, have led to general hypotheses about the causes of aging. The union of several fields of biological study (mitochondrial metabolism, oxidative stress response, lifespan extension by diet) have begun to produce surprising evidence of important interrelationships of the proteins and signaling pathways in these fields and how they are involved in the aging process. These studies could ultimately lead to the development of new intervention strategies to lessen severity of age-related illness. This review will highlight the latest studies of the aging-related protein, SirT1, and consider how this information may impact our understanding of stem cell aging. We focus on two areas of cellular aging: telomere erosion, and oxidative damage; as relates to SirT1 function.

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Purpose of review

New discoveries focused on mitochondrial metabolism and gene silencing and their regulation by the sirtuin family of protein deacetylases is stimulating new ideas on how to improve geriatric medicine. Information about sertuins in stem cell biology is scarce. We consider recent information on the sirtuin, SirT1, its role in aging and metabolism in several species and tissues, and attempt to anticipate how it might influence stem cell aging.

Recent findings

Calorie-restriction lengthens lifespan, due in part, to mitochondrial metabolism reorganization via SirT1/PGC1-α-regulated mitochondrial biogenesis. This reduces radical oxygen species levels which cause macromolecule damage, a major contributor to aging. Little is known about these processes in stem cells, whose longevity is implicated in human aging. Recent work indicates SirT1 influences growth-factor responses and maintenance of stem cells. SirT1 is required for calorie-restriction-induced lifespan extension in mice, and calorie-restriction upregulates SirT1 in humans. SirT1 also appears to influence lineage/cell-fate decisions of stem cells via redox status.

SirT1, gene silencing, and stem-cell aging

The mammailian sirtuin, SirT1(and its ortholog in other species), appears to have a key role in determining lifespan of yeast, flies, and mice [1–3,4*,5**]. Mechanisms of SirT1 regulation of lifespan is being studied, but information on SirT1 in mammalian embryonic stem cells has, to our knowledge, only been reported by us [6*] and one other group [7] and in adult stem cells by one group [8*]. One mechanism by which SirT1 extends lifespan in the budding yeast, Sachromyces cervisiae, is by modifying the physical structure (deacetylation) of key heterochromatin regions of the genome, especially extrachomosomal rDNA circles (ERCs), which accumulate in the nucleolus [1,9,10]. This results in suppression of replication of ERCs during each cell division. Budding yeasts divide by asymmetric self-renewal where daughter cells receive primarily newly synthesized organelles, while mother cells preferentially retain “old” organelles [9,10]. ERCs accumulate in mother cells in the nucleolus as the mother cell continues to divide until 500 to 1000 copies of ERCs are present in the nucleolus, usually after about 20 divisions. Then the nucleolus disintegrates and mother cells no longer grow/divide and become quiescent. Thus, yeast mother cells have a lifespan of approximately 20 divisions.

Mutations in SirT1 cause disregulation of the promoter regions of ERCs so their numbers are prematurely increased leading to premature nucleolar decomposition and quiescence. Yeast cells containing an extra copy of the SirT1 gene accumulate ERCs more slowly because extra SirT1 stabilizes the chromatin structure of ERCs, suppressing their replication and accumulation allowing more divisions and longer lifespan than wild-type yeast [1,9,10]. Whether stem cells, which can also divide asymmetrically [11*], have an analogous system to define their “division age” is unknown, but recent data from hematopoietic stem cell (HSC) transplantation studies [12] show that HSC have “memory” of their proliferation/ differentiation state, suggested to be linked to their epigenetic programming (i.e. chromatin structure/gene silencing). This behavior is remarkably similar to yeast “age memory”.

One hypothesis about how humans age is the “stem cell aging” hypothesis [13**]. It suggested that a fundamental mechanism of aging is that as stem cells grow older, they become quiescent and/or die. The human body is dependent on a variety of rare stem cells which function to replace somatic cells and tissues as they get damaged, diseased, die, or otherwise lost. Replacement of these lost cells declines progressively with age because of stem cell aging and attrition. One way in which human stem cells age is by telomere erosion. In the absence of telomerase, telomere shortening occurs and eventually causes a change in chromatin structure.

This is recognized by the DNA-damage response pathway and activates programmed cell death. In humans, telomerase activities is primarily restricted to self-renewing stem cell compartments and telomeres are shorter than in mice [14,15]. Mice with “humanized” telomere length and telomerase gene deletion (and other types of telomere dysfunction) readily display a premature aging /death phenotype characterized by a declining pool of self-renewing stem cells [13**–15].

This phenotype can be rescued by deleting p53, attesting to the role p53 has in the DNAdamage-apoptosis pathway. However, because p53 is essential for tumor suppression, the lifespan of telomerase-deficient mice is not increased due to increased tumorigenesis. In context of a p21cip-1/waf-1 deficient background (p21cip-1/waf-1 is a downstream effector of p53), there is partial restoration of lifespan of telomerase deficient mice [16*–19]. This is thought to be because p21cip-1/waf-1 is a potent suppressor of the cell cycle and other diverse downstream functions of p53, but is not itself a tumor suppressor.

SirT1 could have a role in telomere maintenance during stem cell aging because of its essential role in gene silencing [10]. Yeast SIR proteins are associated with telomeres. [10,20,21].

However, evidence from SirT1 gene-deleted ESC and SirT1gene-deleted mice suggests there was no widespread disruption of heterochromatin maintenance or gene silencing, and no evidence of premature telomere erosion in adult splenic lymphocytes [7,22] from these animals, although, telomere erosion was not measured in any adult stem cells. This conclusion was buttressed by studies in C. elegans [5**] which suggested lifespan-extension of these nematodes is unrelated to gene silencing or suppression of recombination by SirT1, both features of SirT1 lifespan extension mechanisms in yeast [1,9,10]. This raised the possibility that SirT1 may have no significant role in telomere maintenance in mammals. On the other hand, studies in SirT1-gene deleted mice suggest a somewhat different picture [8*,23**]. HSC from SirT1-gene deleted mice were less dependent on growth factors and had increased growth capacity, especially under stress of growth-factor deprivation compared to wild type animals. Again, telomere erosion was not measured in these HSC. However, the same group found that suppressing SirT1 expression with shRNA in a telomerase-immortalized human fibroblast cell line enhanced their growth capacity, but neither overexpression nor suppression of SirT1 had any influence on lifespan (Hayflick limit) in these cell lines [8*]. Interestingly, telomerase was increased by SirT1 suppression in non-immortalized cells. This suggests that SirT1 may be a growth-factor-responsive suppressor of cell growth in certain cell types, but no correlation to stem cell aging was made. Therefore, there could still be an important link between SirT1 genesilencing and stem cell aging. SirT1 gene-deleted mice display infertility, retarded growth rate, reduced survival to adulthood, greatly reduced weight, and significantly slower rate of bone mineralization compared to wild type mice [22]. All of which could potentially be explained by accelerated stem cell aging and attrition. The reason for male infertility was found to be due to a severe developmental defect in spermatogenesis which is dependent on germ-line stem cells.

Telomere erosion has not been studied in stem cells from SirT1-gene deleted mice and therefore effects of SirT1 loss in mice could still be linked to gene-silencing or telomere destabilization, specifically in stem cells. SirT1 is abundant in mouse embryonic stem cells (mESC) [6*,7], but mESC are not compromised in their ability to silence generalized gene expression [7]. Telomere erosion in SirT1 gene-deleted mESC has not been studied, especially in the context of telomerase deficiency. Maintenance of telomere stability via histone de-acetylation and chromatin stability modifications remains a potential mechanism by which SirT1 could influence stem cell aging and ultimately contribute to longer lifespan.

SirT1, oxidative stress, and stem cell aging

Another way SirT1 could be involved in stem cell aging is via its central role in calorierestriction (CR)-induced lifespan extension [5**,24,25] and its link to age-related reactive oxygen species (ROS) generation [5**,13**,26*]; both are highly dependent on mitochondrial metabolism. ROS can damage macromolecules and lipids [27] and accumulation of these damaged molecules over time is believed to result in age-related decline in cell and tissue function. The primary source of ROS in cells is mitochondria. ROS, a by-product of ATP generation via the electron transport chain (ETC) and oxidative metabolism, ROS, is toxic and damaging at higher concentrations, but is essential for proper oxygen-sensing, maintenance of cellular redox state [27–29], and regulation of proliferation and differentiation [30–32] at lower concentrations. CR, known to extend lifespan in several model organisms including yeast, flys, worms, and rodents [5**,24,26*], appears to decrease ROS generation in several mouse tissues by forcing mitochondrial reprogramming to preferentially generate ATP by β-oxidation of fatty acids instead of carbohydrate catabolism. Despite an increase in overall mitochondrial biogenesis caused by CR [33*,34], switch to β-oxidation likely results in less ROS being produced because β-oxidation bypasses electron entry into the ETC via chain complex I [5**], a main site of mitochondrial ROS generation when electrons get stalled down the ETC. Increased biogenesis of mitochondria during CR increases overall number of electron

entry points and likely improves electron flow, thus lessening stalling at complex I. SirT1 has been implicated in CR-induced lifespan extension in mice because SirT1-gene deleted mice fail to display at least some of the phenotypes of CR mice [22], the most important of which is failure of CR to extend lifespan in SirT1 gene-deleted mice [23**]. Also, SirT1 transgene-overexpressing mice resemble CR mice [4*]. Furthermore, CR upregulates SirT1 in several mouse tissues and, importantly, in human muscle tissue [33*,35]. SirT1 deacetylates peroxisome proliferatoractivated receptor gamma-coactivator-1a (PGC-1α) and increases its transcriptional activities [36–38*]. PGC-1α transcriptionally regulates genes controlling gluconeogenesis and fatty acid oxidation and other mitochondrial genes such as UCPs (uncoupling proteins) which are involved in thermogenesis in neonates [24]. CR may extend lifespan via SirT1/PGC-1α upregulated mitochondrial biogenesis and metabolic reprogramming leading to decreased ROS generation [5**,10,24–25,26*,38*].

Effects of ROS on ESCs [6*] and adult stem cells [39,40] have been studied. These studies indicate that intracellular ROS is a major contributor to cell damage, but ROS regulates proliferation, differentiation, and survival. Evidence on the influence of CR on stem cell function is scarce. CR can ameliorate age-related decline in mouse HSC function [41,42*], but ROS generation or SirT1 was not studied. This offers a clue supporting the idea that the same metabolic forces are at work in stem cells as in other cells during aging. Because mouse aging does not necessarily cause reduction in HSC numbers, but instead causes decline in HSC functions such as mobilization, homing, transplantation, and differentiation, HSC appear to undergo “cell-intrinsic aging” [43–48]. One possible way that ROS and SirT1 may be involved in HSC aging is through FOXO transcription factors. FOXOs are implicated in regulation of cell cycle, differentiation, apoptosis, DNA-repair, and oxidation damage (ROS). FOXO-deficient mouse HSCs have diminished self-renewal capacity along with other progeroid phenotypes during conditions of elevated ROS [39]. SirT1 has not been studied in this system, but it deacetylates FOXOs and suppresses their nuclear transcriptional activities in a manner analogous to the influence of SirT1 on p53 nuclear translocation [6*,49]. SirT1 could influence HSC aging through mechanisms conserved in somatic cells. Other model examples of stem cell aging include neural stem cells, melanocyte stem cells, and pancreatic islet β-cell self-renewal [13**].

ROS damage and telomere dysfunction are implicated in age-related decline of these stem cells [13**]. SirT1 has yet to be linked, either directly (via gene deletion studies) or indirectly (via CR or SirT1 activity modifying drugs) to longevity of these stem cells. One report demonstrated that activation of SirT1 directly, or indirectly through upregulation of intrinsic ROS, potently influences proliferation and cell fate decisions in neural progenitor cells (NPC) [50*]. Mild ROS treatment caused a shift in NPC fate from neuronal to astroglial differentiation, mediated by SirT1, which suppresses expression of pro-neuronal transcription factor, MASH1. This is accomplished by specific deacetyation of histone H3K9 in the MASH1 promotor with subsequent chromatin stabilization. Mechanisms of SirT1 upregulation by mild intracellular ROS is unknown, but these findings have the potential to lay a new framework where CR, ROS, SirT1 gene silencing, mitochondrial reprogramming, and stem cell aging can be merged into a comprehensive picture of organismal aging. Mild intracellular ROS and SirT1 mediated lineage shift is particularly interesting in light of lineage shifts in aging HSC being well documented [45–47]. It will be important to investigate effects of CR, SirT1-gene deletion, and their combination on HSC lineage choices during aging in animal models. A potential clue to these effects is that SirT1-gene deleted mice have abnormal proportions of certain lymphocytes [22]. A similar shift (decreased lymphoid and increased myeloid cells) occurs in aged animals [45–47].

Furthermore, NF-κB is a SirT1 target [51] and is essential for differentiation of bone marrow precursor cells into osteoclasts, also regulated by ROS [52]. This might tie SirT1 to stem cell

differentiation and aging because osteopeania is a hallmark of aging. Our recent report [6*] also highlights the important distinction between mild and severe ROS damage and intrinsic and extrinsic ROS generation in mESC self-renewal. We showed that mild, endogenously generated ROS regulates apoptosis in mESCs, but this control is lost in mESC with SirT1 gene deletion, demonstrating its requirement in this system. We also demonstrated that ROS-induced apoptosis was controlled by SirT1 through de-acetylation of p53, causing p53 localization to mitochondria and initiation of Bax-dependent apoptotic cascade. In contrast, when the potent exogenous oxidant, H2O2, was added to mESC, much more apoptosis was observed in the SirT1 gene-deleted cells than in wildtype cells. SirT1 was found to regulate self-renewal/proliferation and differentiation in mESC by controlling access of p53 (by de-acetylation) into the nucleus where it suppresses transcritption of NANOG, which is required to maintain mESC in an undifferentiated state. Therefore, SirT1 in mESC maintains a balance between appropriate levels of ROS and self-renewal/proliferative capacity, findings that could have implications for studies determining conditions of specific invitro lineage-directed differentiation. Our findings are remarkably consistent with recent studies of HSC from SirT1 gene-deleted mice, where SirT1 was also suggested to be a growthsuppressor under growth-factor withdrawal stress conditions [8*]. Thus, SirT1 plays a role in stem cell metabolism by maintaining proper ROS levels, and by limiting ROS accumulation and promoting longer stem cell life.

Finally, linkage of redox status and mild ROS to SIRT1 in NPGs and mESCs is consistent with the importance of ROS signalling in human HSC [40]. These cells have a low basal level of ROS generated by the NADPH oxidase, NOX, which may be involved in mitochondrial biogenesis via activation of the PGC-1α pathway and also by serving as an oxygen sensor. Redox status and mitochondrial ETC status in human HSC is considered important for HSC selfrenewal and differentiation, analagous to that in NPCs [50*]. There is an inverse relationship of mitochondria numbers/function per cell and surface density of HSC marker CD34 [40,53]. The mitochondrial marker dye, rhodamine 123, which measures mitochondrial activity and membrane polarity, is used to enhance purity of self-renewing, repopulation-competent, mouse HSC, which are rhodamine 123 dim, indicating lower mitochondrial activity and membrane polarity, and is consistent with the CD34 marker relationship in human HSC [40,53]. There is also an age-linked relationship between mitochondrial dye efflux and ability to repopulate the hematopoietic system of irradiated animals [53]. These studies support the notion that SIRT1 might influence mitochondrial biogenesis/function in HSC via its regulation of PGC-1α together with mild ROS and thus influence HSC aging. One study [40] proposed, based on bipolar segregation of mitochondria, that this morphology might be conducive to asymmetric segregation of mitochondria during cell division. If true, this would have striking similarity to budding yeast where newly formed organelles are primarily segregated into the budding daughter cell and the mother cell keeps the “old” organelles [10]. This would have profound implications for HSC aging where more active mitochondria are preferentially segregated to daughter cells that are destined to initiate a differentiation program and the “older” organelles are retained by the pluripotent (mother) HSC, allowing damage to accumulate and promote aging/attrition. This concept could be considered based on knowledge of asymmetry found in the HSC niche [11*]. Thus, SirT1 could influence stem cell aging by controlling mitochondrial biogenesis and turnover which may be required for self-renewal.

Conclusion

We propose the following:

1. While the role of SirT1 in telomere maintenance remains an open question, function of SirT1in metabolically active tissues (muscle, liver) may be very similar to that in stem cells, especially SirT1’s role in mitochondria and ROS generation.

2. Genetic, hormonal, or drug manipulation of stem cell mitochondria may be useful as a research tool or to prolong longevity/stability of stem cells prior to clinical use or even to enhance their function (proliferation, engraftment, etc.), findings that may be applicable to HSC transplantation.

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