

Review

Sirtuins in Metabolic and Epigenetic Regulation of Stem Cells

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Sirtuins are highly conserved NAD⁺-dependent enzymes that are capable of removing a wide range of lipid lysine acyl-groups from protein substrates in a NAD⁺-dependent manner. These NAD⁺-dependent activities enable sirtuins to monitor cellular energy status and modulate gene transcription, genome stability, and energy metabolism in response to environmental signals. Consequently, sirtuins are important for cell survival, stress resistance, proliferation, and differentiation. In recent years, sirtuins are increasingly recognized as crucial regulators of stem cell biology in addition to their well-known roles in metabolism and aging. This review article highlights our current knowledge on sirtuins in stem cells, including their functions in pluripotent stem cells, embryogenesis, and development as well as their roles in adult stem cell maintenance, regeneration, and aging.

Introduction

First discovered in yeast as key components in gene silencing complexes [1], the Silent Information Regulator 2 (Sir2) family of ancient proteins (sirtuins) is present in all kingdoms of life. Sirtuins bear a unique NAD⁺-dependent protein deacetylase activity through which a variety of lipid acyl-groups, including acetyl, succinyl, malonyl, glutaryl, or long-chain acyl-groups, are transferred from the substrate proteins to the ADP-ribose moiety of NAD⁺ [2–5]. Given the essential role of NAD⁺ in cellular metabolism, this exclusive requirement of NAD⁺ enables sirtuins to monitor the cellular energy status and modulate the function of an array of protein substrates ranging from histones and transcription factors to metabolic enzymes and cell membrane proteins.

Mammals have seven members of sirtuins, Sirtuin 1 (SIRT1) to Sirtuin 7 (SIRT7) [6]. These seven proteins are localized in distinct subcellular localizations and have different tissue expression patterns with differential substrate specificities [7–11] (Box 1). While a large body of literature is centered on the importance of sirtuins in nutrient sensing, aging, and age-associated conditions/diseases [12,13], emerging evidence reveals this family of unique enzymes as pivotal regulators of stem cell biology. In this review article, we summarize the involvement of sirtuins in metabolic and epigenetic regulation of stem cells, with focus on pluripotent stem cells (PSCs) and embryogenesis, as well as adult stem cell maintenance and tissue regeneration.

Metabolic and Epigenetic Regulation of Stem Cells

Stem cells, including PSCs and adult tissue stem cells (ASCs) (Box 2), possess a unique set of metabolic programs and a specific epigenetic status to sustain their unlimited proliferation while maintaining their pluri- and multi-potency [14–17]. Energetically, stem cells share striking similarities with highly proliferating cancer cells, requiring a high glycolytic flux

Highlights

As cellular metabolic and stress sensors, sirtuin family of NAD⁺-dependent deacetylases are pivotal regulators of stem cell biology in addition to their well-known roles in metabolic diseases and aging.

Despite their common dependence on cellular NAD⁺, different sirtuins display cell type-specific and/or stage-dependent impacts on stem cell biology in response to various environmental cues.

Nuclear and cytosolic sirtuins modulate pluripotent stem cells and embryogenesis through regulation of pluripotency factors, metabolism, epigenetics, redox homeostasis, and cellular stress response.

Sirtuins maintain self-renewal, quiescence, and regenerative capacity of adult stem cells and protect against adult stem cell depletion in response to stress and aging.

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Box 1. Mammalian Sirtuins

Mammals have seven sirtuins, Sirtuin 1 (SIRT1) to Sirtuin 7 (SIRT7). Among them, SIRT1, SIRT6, and SIRT7 are nuclear proteins. As the most conserved mammalian sirtuin, SIRT1 predominately resides in the nucleus, but it also retains an ability to shuttle between cytosol and nucleoplasm in response to different environmental signals. By contrast, SIRT6 is a nuclear, chromatin-bound protein, and SIRT7 is highly enriched in the nucleolus. Three additional mammalian sirtuins, SIRT3, SIRT4, and SIRT5, are found in the mitochondrial matrix, where they participate in several metabolic and survival processes associated with the mitochondrial activity. A substantial proportion of SIRT5 is extramitochondrial/cytosolic, where it functions to regulate glycolysis through demalonylation of key glycolytic enzymes. SIRT2 is primarily a cytosolic protein that can also shuttle between the cytosol and nucleus.

The diverse subcellular localizations of sirtuins, together with their distinct tissue expression patterns and differential substrate specificities, lead to wide-ranging impacts of sirtuins on many fundamental biological processes, including gene transcription, genome stability, energy metabolism, stress responses, inflammation, proliferation, and differentiation. Not surprisingly, dysfunction of this family of crucial regulators has been associated with many human diseases, such as metabolic disorders and cancer.

under aerobic conditions ('Warburg effect') and addicting to exogenous glutamine for rapid growth and maintenance of pluripotency [15,18–20]. Stem cells, particularly embryonic stem cells (ESCs), also have increased dependence on one-carbon catabolism [21–24]. These metabolic programs provide anabolic precursors required for rapid cell proliferation. They also produce many intermediate metabolites, such as acetyl-CoA, NAD⁺, α -ketoglutarate, and S-adenosylmethionine (SAM), which function as substrates or cofactors for enzymes that regulate chromatin modification and gene expression [24–29]. Therefore, the unique metabolic state of stem cells directly links to distinctive epigenetics and gene expression profiles, greatly influencing their self-renewal, pluri-, and multipotency [20,30].

Sirtuins in Metabolic and Epigenetic Regulation of PSCs and Development

Different sirtuins have distinct roles in PSCs and development. In mouse ESCs (mESCs), SIRT1 is the most highly expressed sirtuin, followed by SIRT6 and SIRT7 [31]. By contrast, the relative levels of other sirtuins, including mitochondrial sirtuins, are low in mESCs [31]. Consistently, germline knocking out SIRT1, SIRT6, or SIRT7, but not other sirtuins, causes significant developmental defects [8,32–38], highlighting the importance of three nuclear sirtuins in regulation of ESC biology and animal development.

Box 2. Stem Cells

Stem cells, including pluripotent stem cells (PSCs) and adult tissue stem cells (ASCs), are uniquely characterized by their dual capacity for self-renewal and pluri-/multi-potent differentiation. PSCs are stem cells giving rise to all cells of the tissues of the body. There are two types of PSCs: embryonic stem cells (ESCs) and induced pluripotent stem cell (iPSCs). ESCs are derived from the inner cell mass of preimplantation embryos. iPSCs are reprogrammed from adult somatic cells *in vitro* through simultaneous overexpression of four core pluripotent factors: Oct4, Sox2, Klf4, and c-Myc. Both ESCs and iPSCs can be indefinitely maintained and expanded in the pluripotent state *in vitro* and are capable to differentiate into all the derivatives of the three germ layers.

ASCs are the stem cells characterized by their ability to self-renew and differentiate to generate all the cell types in a tissue. Based on their turnover rates in various tissues/organs, ASCs can be categorized into three types: (i) ASCs that continuously cycle themselves in high-turnover tissues, such as intestine stem cells and short-term hematopoietic stem cells (HSCs); (ii) ASCs that are maintained in quiescent state and for which proliferation is strongly induced by injury or other environmental/developmental cues, such as muscle satellite cells, adult neuron stem cells, and long-term HSCs; and (iii) ASCs that periodically alternate between quiescent and proliferative states, such as hair follicle stem cells.

Stem cells are essential for embryogenesis, tissue regeneration, and the maintenance of tissue homeostasis throughout the lifetime of an organism. A better understanding of mechanisms underlying stem cell self-renewal and pluri-/multipotency will therefore improve our knowledge on development, regeneration, and aging.

SIRT1 Is Important for Normal Embryogenesis and Animal Development

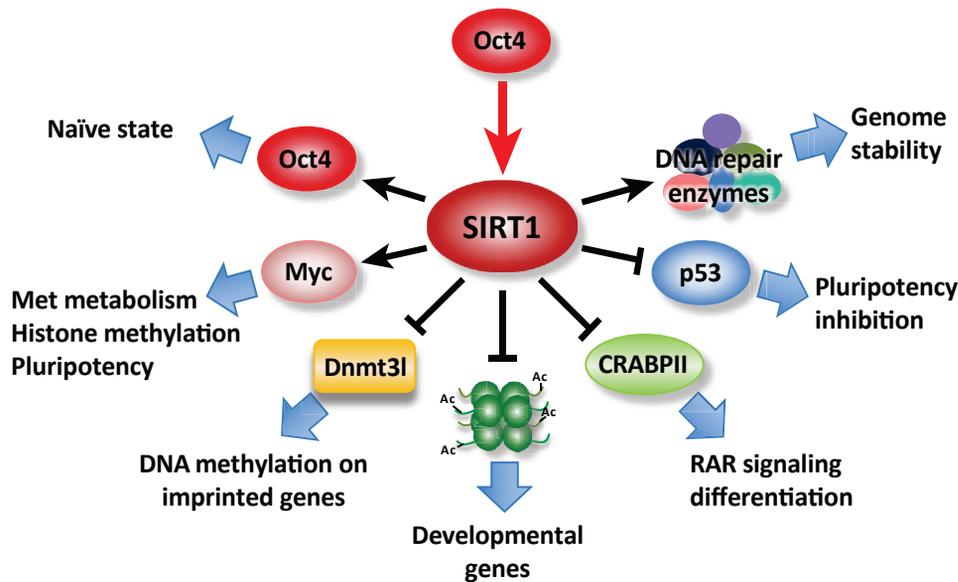
SIRT1 has numerous functions at different stages of animal life. However, one of the primary functions of SIRT1 is to regulate embryogenesis and animal development. SIRT1 is extremely highly expressed in pre-implantation embryos, particularly at the two-cell stage when the embryo starts to activate its own genome [39], indicating that SIRT1 is one of the first activated genes during embryogenesis. Consistently, SIRT1 null zygotes generated through *in vitro* fertilization of SIRT1-deficient sperm and oocytes display a substantially compromised efficiency of developing to the two-cell stage compared to control zygotes [40]. SIRT1 expression is gradually reduced during development of pre-implantation embryos [39]. However, ESCs isolated from the inner cell mass from the blastocyst still express massively higher amounts of SIRT1 than differentiated cells and tissues [39,41], further suggesting that SIRT1 is critical for ESC function and embryogenesis. In support of this notion, germline deletion of SIRT1 in mice leads to severe developmental defects on various genetic backgrounds, including intrauterine growth retardation, developmental defects of the retina and heart, defective germ cell differentiation, bone developmental delay, and neonatal lethality [32,33,42–44]. Although the penetrance and severity of SIRT1 deletion-induced developmental phenotypes vary depending on the genetic background, and mice lacking SIRT1 on the FVB background [45] or a few percentages of SIRT1 null mice on some mixed backgrounds [32,33] can survive into adulthood, growth retardation and sterility with developmental defects are still observed in these adult mice. In addition, SIRT1 instability caused by loss of USP22, a deubiquitinating enzyme that stabilizes SIRT1, is associated with the defective embryogenesis in USP22 null mice [46].

Most adult tissues have low levels of SIRT1 compared to pre-implantation embryos and ESCs [32,39]; however, SIRT1 maintains a high expression level in germ cells, particularly in male germ cells [32,47]. Consistently, whole-body SIRT1 deficiency reduces the spermatogenic stem cell number in embryonic day 15.5 embryos, abrogating spermatogenesis, but not oogenesis [40]. The action of SIRT1 in germ cells is cell autonomous, as germ cell-specific deletion of SIRT1 disrupts spermatogenesis [43,47]. Taken together, the high expression of SIRT1 in pre-implantation embryos, ESCs, and germ cells, combined with SIRT1 deficiency-induced defects in embryogenesis and germ cell development, strongly indicates that SIRT1 is a key regulator of embryogenesis and animal development.

SIRT1 Maintains Pluripotent ESCs through Multilevel Mechanisms

SIRT1 is a multifaceted regulator in the pluripotent ESCs (Figure 1). First, SIRT1 interacts with several master transcription factors to directly modulate the pluripotency of ESCs. Specifically, SIRT1 interacts with Oct4, a key component of the core pluripotency network. In human ESCs (hESCs), SIRT1 is under the transcriptional control of Oct4 and mediates Oct4-dependent pluripotency through repression of p53, a well-known SIRT1 deacetylation substrate [48]. In mESCs, SIRT1 is required for the maintenance of the naïve state through direct deacetylation of Oct4 [49]. During the naïve-to-primed transition, the activity of SIRT1 is reduced and Oct4 becomes hyperacetylated. Acetylated Oct4 binds to an enhancer to induce the expression of *Otx2* that, in turn, interacts with acetylated Oct4 to induce the primed pluripotency gene network [49].

Second, SIRT1 directly and indirectly modulates epigenetics of ESCs. We recently reported that SIRT1 interacts with Myc to metabolically and epigenetically maintain pluripotent mESCs [39]. Deletion of SIRT1 in mESCs leads to hyperacetylation of both N-Myc and c-Myc, decreasing their stability or reducing their recruitment to the promoter of methionine adenosyltransferase 2a. This defect impairs the conversion of methionine to SAM and markedly decreases methylation levels of histones, especially H3K4me3, thereby altering gene



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Figure 1. SIRT1 Is a Multifaceted Regulator in the Pluripotent ESCs. Sirtuin 1 (SIRT1) is critically involved in the maintenance of pluripotent embryonic stem cells (ESCs) and animal development through multilevel mechanisms. The expression of SIRT1 in ESCs is under transcriptional control of Oct4, and the interactions between SIRT1, Oct4, and Myc are important to metabolically and epigenetically maintain pluripotent ESCs. SIRT1 also actively represses the expression of developmental and differentiation genes through deacetylation of histones and inhibition of cellular retinoic acid signaling. Finally, SIRT1 enhances stress response of ESCs by repressing p53 and promoting DNA repair. Abbreviations: Ac, acetylation; RAR, retinoic acid receptor.

expression profiles, comprising the pluripotency, and sensitizing mESCs to methionine restriction-induced differentiation and apoptosis [39]. This SIRT1-Myc-regulated metabolic and epigenetic regulation is physiologically important, as SIRT1 knockout (KO) embryos are sensitive to maternal methionine restriction-induced lethality, whereas maternal methionine supplementation increases the survival of SIRT1 KO newborn mice [39]. In addition, SIRT1 interacts with DNA methyltransferase 3-like (Dnmt3l) to modulate DNA methylation, the expression of imprinted and germline genes, and the differentiation potential of mESCs [50]. Heo *et al.* showed that Dnmt3l, a catalytically inactive DNA methyltransferase that interacts with Dnmt3a and Dnmt3b to stimulate *de novo* methylation, is regulated by SIRT1 at both transcriptional and post-translational levels; consequently, SIRT1 deficiency delayed neurogenesis and spermatogenesis [50]. Therefore, SIRT1 metabolically and epigenetically regulates ESC maintenance and animal development.

Third, SIRT1 actively represses differentiation of ESCs. Highly expressed SIRT1 has been reported to repress the transcription of several developmental genes in ESCs through deacetylation of histones [51]. Differentiation of ESCs reduces both SIRT1 mRNA and protein post-transcriptionally [41,51], either by microRNAs-mediated inhibition of SIRT1 protein translation [41] or by CARM1-HuR-dependent destabilization of SIRT1 mRNA [51,52]. Reduction of SIRT1 then reactivates key developmental genes [51]. Moreover, SIRT1 represses mESC differentiation through inhibition of cellular retinoic acid (RA) signaling [44]. In mESCs, SIRT1 interacts with and deacetylates CRABP II, a cellular RA binding protein that is acetylated and translocated into the nucleus to activate retinoic acid receptor signaling upon RA treatment. SIRT1-mediated deacetylation recycles CRABP II back to the cytosol, inactivating the RAR-mediated activation

of differentiation genes. Notably, SIRT1 deficiency induced development defects in mice, particularly bone development delay, is associated with increased RA signaling [44].

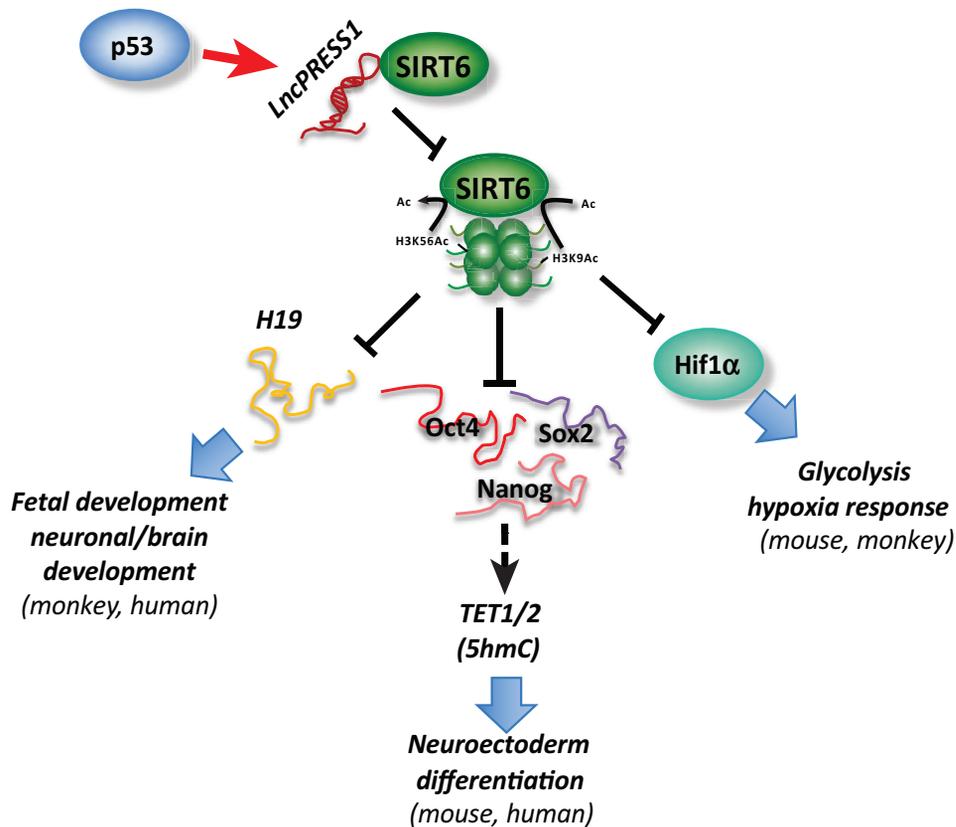
Finally, SIRT1 is important to determine cell fates in response to stress in ESCs. In response to endogenous reactive oxygen species (ROS), SIRT1 functions to maintain healthy pluripotent ESCs by sensitizing mESCs to mitochondrial p53-induced apoptosis while inhibiting nuclear p53-mediated suppression of *Nanog* expression [53]. In contrast, SIRT1 protects ESCs from apoptosis induced by high concentrations of exogenous ROS (e.g., 1 mM H₂O₂), in part, through the class III phosphatidylinositol 3-kinase/Beclin 1 and mammalian target of rapamycin (mTOR)-mediated autophagy [54]. Therefore, the function of SIRT1 in protection against stress-induced apoptosis in ESCs is dependent on the degree of oxidative stress. In addition, SIRT1 strongly enhances the survival of hESCs by promoting DNA repair [55] and promotes telomere elongation and genome stability after reprogramming in induced pluripotent stem cells (iPSCs) [56]. Collectively, SIRT1 is critically involved in the maintenance of pluripotent ESCs and animal development through regulation of pluripotency factors, metabolism, epigenetics, redox homeostasis, and cellular stress response.

SIRT7 Regulates Embryogenesis and Lifespan through Maintenance of Genome Stability

SIRT7, a nuclear sirtuin enriched in the nucleolus, also plays a role in animal development [38]. SIRT7-deficient pups display defective embryogenesis and growth retardation and are born at sub-Mendelian ratios. More than 20% of SIRT7 null mice die with the first month of life, and the remaining KO mice show signs of accelerated aging with dramatically reduced lifespan [38]. Mechanistically, SIRT7 is recruited to sites of DNA damage to deacetylate H3K18, which is important for efficient recruitment of damage response factors to DNA double-strand breaks [38]. Therefore, SIRT7 directly promotes DNA non-homologous end joining repair and is important for the maintenance of genome stability.

SIRT6 Epigenetically Promotes Proper Lineage Commitment of ESCs and Animal Development

In contrast, SIRT6 is a nuclear sirtuin evolutionally important for proper differentiation of ESCs (Figure 2). SIRT6 KO mice show developmental delay and premature death, and SIRT6-deficient ESCs form small embryoid bodies (EBs) and exhibit skewed differentiation toward neuroectoderm when induced to differentiation [57]. However, although SIRT6 represses glycolysis that is important for ESC self-renewal [14–17] through deacetylation of histones [58], elevated glycolysis is not accounting for the observed developmental phenotypes in SIRT6 KO ESCs [57]. Etchegaray *et al.* recently found that SIRT6 epigenetically represses the expression of the core pluripotency genes (*OCT4*, *SOX2*, and *NANOG*) by targeting H3K56ac and H3K9ac [57]. Deletion of SIRT6 in mouse promotes persistent expression of *OCT4*, *SOX2*, and *NANOG* in ESCs and EBs. *OCT4* and *SOX2* then enhance the expression of *TET1* and *TET2*, increasing 5-hydroxymethylcytosine, particularly on neural genes [57]. SIRT6 therefore functions as a chromatin regulator to epigenetically regulate the hierarchical expression of the core pluripotency genes and *TET* genes, safeguarding the balance between pluripotency and differentiation [57]. Consistent with its role in promoting proper differentiation and development in mice, SIRT6 is inhibited by a p53-targeted long noncoding RNA (lncRNA), *lncPRESS1*, in hESCs [59]. *lncPRESS1* physically interacts with SIRT6 and prevents its chromatin localization, maintaining high levels of H3K56ac and H3K9ac at promoters of pluripotency genes [59]. Importantly, SIRT6 is an evolutionally conserved regulator of ESC differentiation and development *in vivo* (Figure 2). Deletion of SIRT6 in cynomolgus monkey leads to hyperacetylation of histone, activating the lncRNA *H19* and resulting in prenatal developmental retardation and neonatal death [60]. A



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Figure 2. SIRT6 Epigenetically Regulates Proper Lineage Commitment of ESCs and Animal Development.

In mouse, monkey, and human, Sirtuin 6 (SIRT6) represses expression of metabolic genes (particularly glycolytic genes), core pluripotent genes, and maternal imprinted long noncoding RNA (lncRNA) H19 through deacetylation of H3K9ac and K3K56ac. These actions of SIRT6 metabolically and epigenetically safeguard the balance between pluripotency and differentiation. In human embryonic stem cells (hESCs), a p53-targeted lncRNA, lncPRESS1, physically interacts with SIRT6 and prevents its chromatin localization that, in turn, maintains high levels of H3K56ac and H3K9ac at promoters of pluripotency genes. Therefore, in contrast to SIRT1, SIRT6 is an evolutionally conserved regulator of ESC differentiation and animal development both *in vitro* and *in vivo*. Abbreviations: 5hmC, 5-hydroxymethylcytosine; Ac, acetylation; Hif1α, hypoxia-inducible factor 1α.

homozygous SIRT6 inactive mutation in humans also leads to hyperacetylation of H3K9 and H3K56, dramatically elevating pluripotent genes and causing severe congenital anomalies and perinatal lethality [61].

SIRT2 Promotes Differentiation of ESCs *In Vitro*

SIRT2, a predominately cytosolic sirtuin, is not essential for embryonic viability and postnatal development in mice [37]. However, it plays a role in PSCs *in vitro*, but again in an opposing direction compared to SIRT1. In contrast to SIRT1, the level of SIRT2 is low in hESCs and high in differentiated somatic cells [62], and its induction during RA-induced differentiation of mESCs represses glycogen synthase kinase 3β and promotes differentiation [63]. Cha *et al.* recently showed that SIRT2 is the primary deacetylase that deacetylates and inhibits glycolytic enzymes in hESCs [62]. The expression of SIRT2 is under control of miR-200c-5p in hESCs, and its induction/overexpression promotes differentiation through repression of glycolysis.

Conversely, inhibition of SIRT2 in fibroblasts promotes the reprogramming of fibroblasts to iPSCs [62]. Therefore, SIRT2 is critical in controlling metabolic switch between PSCs and differentiated cells *in vitro*. It will be interesting to investigate whether other sirtuin-mediated deacetylations that regulate glycolysis, SIRT5 mediated demalonylation, and activation of glycolytic enzymes, for example [64], also play a role in mediating metabolic transition between PSCs and differentiated cells in response to stress.

Sirtuins in Metabolic and Epigenetic Regulation of ASCs

ASCs are the stem cells that persist throughout the life, replacing cells lost due to homeostatic turnover, disease, and injury [65,66]. Different tissue ASC populations have varying proliferative plasticity and distinct turnover patterns (Box 2). However, the quiescence, proliferation, self-renewal, and differentiation of ASCs are delicately balanced by common mechanisms [65,66]. Disruption of ASC homeostasis, either by declines in ASC number and/or functions or by aberrant activation of ASCs, has been linked to many human disorders, including degenerative disease, aging, and cancer. Much data have been accumulated to show that sirtuins can protect against ASC depletion in response to stress and aging through maintenance of their self-renewal, quiescence, and regenerative capacity.

SIRT1 Is Important for the Maintenance of Diverse ASC Pools

SIRT1 is an essential factor for preserving ASC pools in multiple tissues. For example, SIRT1 inactivation has been shown to largely account for the premature phenotype in progeroid syndrome, a group of rare genetic disorders characterized with severe early-onset premature aging [67]. Genetically, progeroid syndrome is caused by progerin, a truncated Lamin A protein. Liu *et al.* showed that both progerin and unprocessed prelamin A protein are defective in binding and activation of SIRT1, leading to development of progeroid or progeroid-like syndrome [67]. Several ASCs, including mesenchymal stem cells (MSCs), hair follicle progenitor cells, and hematopoietic stem cells (HSCs), are rapidly depleted in mice deficient of *Zmpste24*, a metalloproteinase responsible for prelamin A maturation. They further showed that resveratrol promotes the interaction between SIRT1 and Lamin, activating SIRT1 and rescuing ASC decline in *Zmpste24*^{-/-} mice [67]. Although direct *in vivo* evidence is needed to substantiate the role of SIRT1 in these ASCs, maintaining ASC pool appears to be one of the mechanisms through which SIRT1 promotes longevity.

Mechanistically, SIRT1 maintains different types of ASCs through diverse mechanisms. In ASCs that continuously divide asymmetrically in high-turnover tissues, such as intestinal stem cells (ISCs), SIRT1 is necessary and sufficient to promote their self-renewal and expansion in response to caloric restriction (CR) [68]. It has been previously shown that CR augments ISC functions by reducing mTORC1 signaling, thereby increasing the production of a paracrine factor cyclic ADP ribose (cADPR) in Paneth cells, a key constituent of the mammalian ISC niche, but not in ISCs [69]. The increased release of cADPR from Paneth cells then acts on ISCs to activate calmodulin-dependent protein kinase kinase and AMP-activated protein kinase (AMPK) [68,69]. Igarashi and Guarente recently showed that CR-mediated activation of AMPK in ISCs stimulates the transcription of *Nampt*, the rate-limiting enzyme in a NAD⁺ salvage pathway, enhancing the activity of SIRT1. Enhanced SIRT1 then deacetylates S6K1, facilitating mTORC1 to phosphorylate S6K1 and stimulating protein synthesis [68]. Although it is surprising that mTORC1 signaling is activated in ISCs in response to CR, this study indicates that mTORC1 inhibition in Paneth cells cooperates with mTORC1 activation in ISCs to stimulate ISC expansion in response to CR, further supporting the notion that SIRT1-mediated preservation of the ASC pool is a part of the longevity response to CR.

In ASCs that are maintained in quiescent state but strongly induced by injury, such as the skeletal muscle stem cells satellite cells (SCs) and adult neuron stem cells (NSCs), SIRT1 is required for the maintenance of their quiescence and regeneration capacity [70–72]. Ryall *et al.* showed the transition of SCs from quiescence to proliferation is accompanied with a metabolic switch from fatty acid oxidation to glycolysis despite an increased mitochondrial content [70]. This metabolic switch decreases intracellular NAD⁺ levels and the activity of SIRT1, leading to elevated H4K16ac and activation of muscle gene transcription. Therefore, decreased activity of SIRT1 is associated with proliferation and differentiation of SCs [70]. Consistently, skeletal muscle-specific ablation of SIRT1 deacetylase domain deregulates activation of the myogenic program in SCs, leading to premature differentiation, and impairs muscle growth during development and regeneration upon injury [70]. In adult NSCs, SIRT1 also suppresses their proliferation, as specific deletion of SIRT1 in adult NSCs enhances their self-renewal [71,72] and promotes lineage specification to oligodendrocyte progenitor cells/oligodendrocytes [73]. This impact of SIRT1 is also sensitive to metabolic switch/redox stress and involves inhibition of Hes1 transcription factor [71], Notch signaling [72], or cell metabolism and growth factor signaling [73]. It would be interesting to determine whether SIRT1 similarly impacts long-term maintenance of the NSC pool and neurogenesis in response to injury as in SCs and skeletal muscle in the future.

SIRT1 is also important for the maintenance of quiescence and regeneration capacity of HSCs in response to environmental stress and aging [74] (Figure 3). SIRT1 KO mice do not exhibit any abnormalities in their hematopoietic compartment under normal conditions [75]. However, SIRT1-deficient mESCs are compromised in their hematopoietic commitment *in vitro* and SIRT1 KO mice have decreased survival of hematopoietic progenitors *ex vivo*, particularly under hypoxia condition or under conditions of delayed growth factor addition [76]. Moreover, conditional ablation of SIRT1 in adult hematopoietic stem/progenitor cells (HSPCs) cell autonomously induces HSPC expansion and loss of long-term repopulation capacity under stress [77]. This stress-induced loss of HSC function is associated with genomic instability, p53 activation, and increased DNA damage in SIRT1-deficient HSPCs [77]. SIRT1 loss also causes a significant increase of H4K16ac and upregulates the expression of Hoxa9, a key regulator of HSPC function and proliferation [77]. Collectively, SIRT1 is critical to maintain diverse ASC pools through maintenance of self-renewal or quiescence and regeneration capacity, particularly in response to stress and injury.

SIRT7 Regulates Quiescence and Regenerative Capacity of HSCs

SIRT7 is also highly expressed in HSCs to control their quiescence and regenerative capacity through regulating mitochondrial proteostasis [78] (Figure 3). HSCs are maintained in a metabolically inactive state, but they are readily transformed into a metabolically active state associated with a dramatic increase of mitochondrial contents. Mohrin *et al.* showed that mitochondrial protein folding stress (PFSmt) induces SIRT7 that then interacts with nuclear respiratory factor 1 (NRF1), a master regulator of mitochondria, to inhibit the expression of mitochondrial ribosomal proteins and mitochondrial translation factors. Consistently, *Sirt7*^{-/-} HSCs exhibit phenotypes of aging, including increased PFSmt and apoptosis, loss of quiescence, reduced repopulation capacity, and myeloid-biased differentiation. Conversely, up-regulation of SIRT7 improves the regenerative capacity of aged HSCs [78].

SIRT6 Controls Regeneration and Stress Resistance in HSCs and Mesenchymal Stem Cells

SIRT6 is another nuclear sirtuin that inhibits cell proliferation in HSCs (Figure 3). Specific depletion of SIRT6 in adult mouse HSCs leads to a 2-fold expansion of HSPCs due to increased

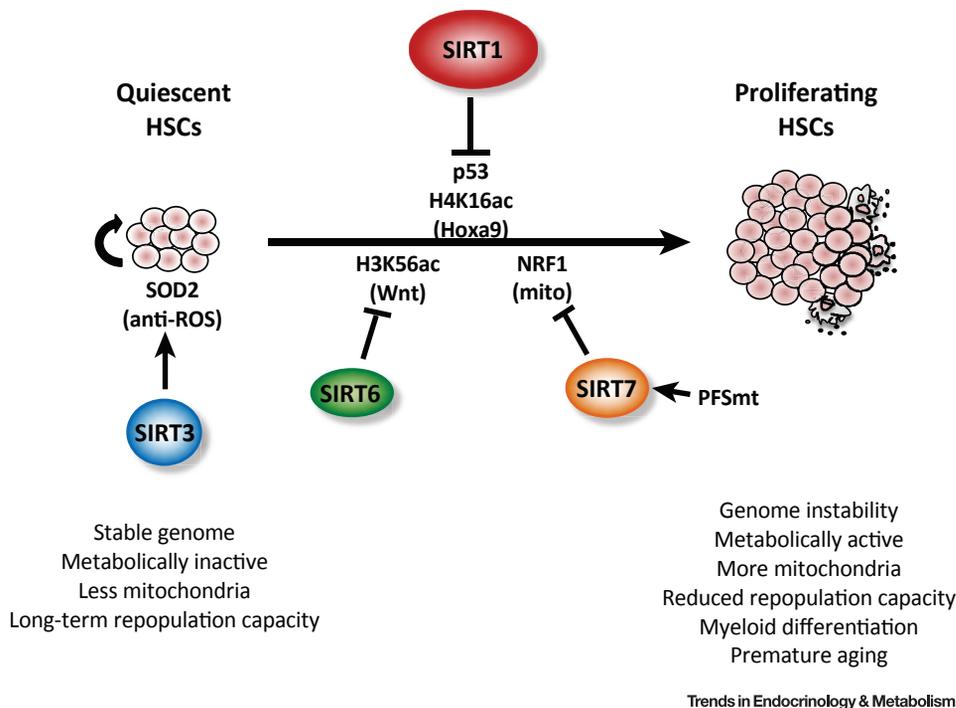


Figure 3. Sirtuins Are Crucial Regulators of HSC Functions in Response to Stress and Aging. Three nuclear sirtuins, SIRT1, SIRT6, and SIRT7, are actively involved in controlling hematopoietic stem cell (HSC) quiescence, which is important to maintain their regenerative capacity and prevent premature aging. Mitochondrial SIRT3 is critical to protect HSCs from age-induced oxidative stress and is therefore indispensable for HSC self-renewal. Abbreviations: NRF1, nuclear respiratory factor 1; PFSmt, mitochondrial protein folding stress; ROS, reactive oxygen species.

H3K56ac and increased transcription of factors in the Wnt pathway [79]. Enhanced proliferation further renders HSCs less quiescent and more prone to depletion. Consequently, SIRT6-deficient HSCs exhibit severe impairment in their long-term repopulation capacity [79]. Together, all three nuclear sirtuins are actively involved in controlling HSC quiescence, which is important to maintain their regenerative capacity and prevent premature aging (Figure 3).

SIRT6-mediated stress resistance also helps to maintain the functionality of MSCs *in vitro* [80]. SIRT6-deficient human MSCs have increased ROS levels and elevated vulnerability to oxidative injury, and they exhibit accelerated cell attrition after implantation to the muscles of immunodeficient mice [80]. This action of SIRT6, again, has been attributed to its deacetylation activity on H3K56ac, which directly upregulates several NRF2-regulated antioxidant genes by recruiting RNA polymerase II to their promoters [80]. Although it remains unclear how a deacetylase that usually represses gene expression through histone deacetylation acts as a co-activator to promote expression of antioxidant genes in MSCs, this study indicates that SIRT6 is important for MSC stress resistance and delay of aging.

SIRT3 Maintains the Pool and Regenerative Capacity of HSCs during Aging

In addition to three nuclear sirtuins, SIRT3, a mitochondrial sirtuin, is also highly enriched in HSCs [81] (Figure 3). Brown *et al.* showed that deletion of SIRT3 reduces the HSC pool in aged mice and compromises HSC self-renewal upon serial transplantation stress, in part due to hyperacetylation of SOD2 and subsequent increase in oxidative stress [81]. Consistently, SIRT3 is reduced with age, and overexpression of SIRT3 in aged HSCs reduces oxidative stress and

rescues their reconstitution capacity [81]. Taken together, by linking diverse cellular stresses to distinct signaling pathways, sirtuins transcriptionally and post-translationally maintain HSC functions in response to stress and aging.

Concluding Remarks and Future Perspectives

Emerging evidence reveals crucial functions of the sirtuin family of metabolic sensors in regulation of self-renewal, maintenance, pluri-/multi-potency, and differentiation of both PSCs and ASCs. Dysfunction and dysregulation of sirtuins in stem cells have been linked to many human diseases, including developmental defects, degenerative diseases, and cancer. Therefore, it is clear that stem cell sirtuins are at the crossroads of development, aging, and cancer.

Given the essential role of NAD⁺ in diverse cellular processes including energy metabolism, redox sensing, DNA repair, and genome stability [82], it is not surprising that stem cells harness sirtuin family of NAD⁺-dependent enzymes to tightly link their metabolic plasticity to epigenetics, transcription, genome stability, and stress resistance in response to developmental and environmental cues. However, despite their common dependence on cellular NAD⁺, different sirtuins display cell type-specific and/or stage-dependent impacts on stem cell biology. On the one hand, different sirtuins often have distinct, even contrasting, functions in the same stem cell type. For example, as nuclear sirtuins in ESCs, SIRT1 maintains pluripotency and represses differentiation genes, whereas SIRT6 suppresses pluripotency genes and is critical for their proper lineage commitment upon differentiation. This phenomenon is likely more consistent with their expression dynamics and/or substrate specificities than NAD⁺ sensing. On the other hand, the same sirtuin may elicit differential functional impacts in different stem cells. For example, SIRT6 maintains the quiescent state and long-term regeneration capacity of ASCs but suppresses the stemness of ESCs. Much work is still needed to comprehend how different members of sirtuins integrate distinct environmental cues via their differential dosages, distinct sensitivities to NAD⁺, and diverse substrates and interacting partners at various subcellular compartments to coordinately mediate environmental influence on stem cell activities.

It is also worth noting that mitochondrial sirtuins, SIRT3 in particular, are crucial defenders from oxidative stress in various cell types including HSCs during the process of aging, yet evidence for their contribution to the maintenance of PSCs is still lacking. This is likely due to the fact that stem cells have evolved a variety of mechanisms to protect themselves from ROS, including their preferential use of glycolysis instead of oxidative phosphorylation to reduce ROS production [30,83] and their use of several alternative mechanisms to protect against oxidative stress [84]. Consistently, PSCs such as ESCs have relatively low expression of mitochondrial sirtuins [31]. Nevertheless, given the prevalence of obesity and metabolic syndrome in the reproductive population as well as the increasing parental age in modern society, it will be interesting to study whether mitochondrial sirtuins can regulate the maintenance and survival of PSCs, particularly iPSCs and germline stem cells, under these oxidative stress-associated conditions.

Recent advancements in the field have shown that lysine acetylation is just one type of a widespread protein modification known as lipid lysine acylation. These short-chain and long-chain lipids are generated by completely different metabolic pathways, and distinct protein acylations are associated with differential regulation of gene transcription, particularly during germ cell development [85,86]. Intriguingly, sirtuins have been shown to have deacylation activities to lipid lysine acylations in addition to acetylation [85,87–89]. Further studies on sirtuin-mediated protein deacylation in stem cells will elucidate the functions of these newly discovered protein modifications in regulating stem cell biology.

Outstanding Questions

What determines the specificities of different sirtuins at the same stage of a stem cell?

How is the expression/activity of a sirtuin regulated at the different stages of a stem cell?

How is the expression/activity of a sirtuin regulated in different types of stem cells?

Are there any NAD⁺-insensitive functions of sirtuins in stem cells?

How do different members of sirtuins integrate distinct environmental cues via their differential dosages, distinct sensitivities to NAD⁺, and diverse substrates and interacting partners at various subcellular compartments to coordinately mediate environmental influence on stem cell activities?

Do mitochondrial sirtuins regulate the maintenance and survival of iPSCs and germline stem cells under oxidative stress-associated pathological conditions?

Are there any cell type-specific and/or stage-dependent impacts of protein acylations other than acetylation on stem cell functions? Do sirtuins mediate these impacts through their deacylase activity?

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