

Perspective

Sirt1, notch and stem cell "age asymmetry"

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Almost all complex multicellular organisms on earth utilize oxygen for the production of energy. This strategy carries the risk for damaging ROS to be generated and so these biochemical pathways must be highly regulated. Because of this, regulation of oxidative-phosphorylation is tightly coordinated with every aspect of cellular physiology, including stem cell regulation during embryonic development and in adult organisms. The protein-deacetylase, SIRT1, has received much attention because of its roles in oxygen metabolism, cellular stress response, aging, and has been investigated in various species and cell types including embryonic stem cells. However, there is a dearth of information on SIRT1 in adult stem cells, which have a pivotal role in adult aging processes. Here, we discuss the potential relationships between SIRT1 and the surface receptor protein, Notch, with stem cell self-renewal, asymmetric cell division, signaling and stem cell aging.

Almost every eukaryotic organism on earth uses oxygen as the terminal electron acceptor in the production of adenosine triphosphate (ATP) for its energy needs. Mitochondrial oxidative phosphorylation (OXPHOS) is a more efficient mechanism of ATP production compared to anaerobic glycolysis, but this efficiency comes with a risk. A byproduct of OXPHOS that is sometimes generated is reactive oxygen species (ROS). ROS are toxic and can damage macromolecules and lipids; accumulation of this damage is believed to be important in aging and age-associated pathologies.¹ Given this, it is surprising that a small amount of intrinsically generated ROS is important for proper regulation of proliferation and differentiation in a number of tissues.^{2,3} ROS and the oxidative status (REDOX) of tissues and cells, especially stem cells, play important roles during early metazoan development and in tissue maintenance and organization.¹⁻³ Many hypoxic environments exist in metazoans where the REDOX status allows cells, like stem cells, to adapt, and take advantage of, these specialized niches.

Sirt1 and Self-Renewal

Recently, we have reported that ROS and oxidative stress can have an important role in regulating mouse embryonic stem cell (mESC) apoptosis and self-renewal/pluripotency.⁴ Mild intrinsically-generated ROS induces p53-mediated transcriptional upregulation of antioxidant enzymes GPX1, SESN1 and SESN2 that help suppress the damaging effects of ROS in mESC. At the same time, p53 also suppresses the transcription of Nanog, which is required for pluripotency maintenance and suppression of differentiation.^{4,5} p53 is a multifunctional protein important for proper regulation of apoptosis, cell cycle, DNA-damage/checkpoint responses, and metabolism in addition to its influence on pluripotency/self-renewal (via Nanog) and ROS-defense (via anti-oxidative enzymes).⁶⁻⁸ Therefore, p53 must be tightly controlled to maintain a balance between all its effects to promote survival, pluripotency and self-renewal in mESC. Much of p53's influence is via transcriptional regulation in the nucleus, but it also has potent effects when localized to the cytoplasm such as its ability to directly promote mitochondrial-mediated apoptosis.^{9,10} Thus, regulation of p53 effects is highly dependent on its nuclear or cytoplasmic localization. The nuclear translocation of p53 can be regulated by post-translational modification, especially by acetylation.^{4,11} The aging-related protein, Sirtuin 1 (Sirt1), is a member of a class of protein deacetylases. p53 is an important target substrate of Sirt1.^{4,11} We demonstrated that, in mESC, Sirt1 is essential for proper p53 localization to nuclear or cytoplasmic mitochondria in response to ROS stress.⁴ Normal p53 responses to ROS stress were abrogated in Sirt1^{-/-} mESC, but could be restored by lentiviral vector gene expression of Sirt1 in the Sirt1^{-/-} mESC cell line. We also showed that the influence of Sirt1 on p53 translocation linked it to expression of other p53-activated genes, like p21^{cip1-waf1}, and especially Nanog. The regulation of Nanog expression by Sirt1 via p53 nuclear translocation was then shown to be clearly linked to surface expression of several pluripotency markers during ROS-stress induced differentiation of Sirt1^{-/-} mESC compared to wild type mESC. Together, our data⁴ suggested that Sirt1 is very important in maintaining mESC in an undifferentiated/self-renewing state in-vitro, especially when confronted with changing oxidative conditions. There have been numerous studies of Sirt1 in yeast and ESC, but only recently has there been interest in Sirt1 in adult vertebrate stem cells. To our knowledge, there has only been one limited study of the potential roles of Sirt1 in adult hematopoietic progenitor cells.¹² That study evaluated the effects in-vitro of a phenotypically-defined

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population highly enriched for hematopoietic stem cells (CD34⁺Flk2⁺Sca1⁺c-kit⁺lineage⁻), but did not evaluate Sirt1 in functional hematopoietic stem cells in-vivo nor did it consider the influence of oxygen in culture. They did report an increase in progenitor cell growth capacity in Sirt1 deficient animals compared to wild type. Armed with our recently gained knowledge of Sirt1 during oxidative stress in ESCs,⁴ we have begun to investigate the potential roles of Sirt1 in mouse hematopoietic stem and progenitor cells.

To evaluate the influence of Sirt1 on hematopoietic progenitor cells (HPC), a type of somatic stem/progenitor cell (instead of an ESC), we assessed cytokine-stimulated colony formation of mouse fetal liver (FL) cells from Sirt1^{-/-}, Sirt1^{+/-} and wild-type (+/+) littermate controls under selected oxidative conditions: normal oxygen tension (=normoxia; ~20% O₂), compared to hypoxic conditions of 5% O₂. We have previously shown enhanced colony formation by murine and human HPC at 5%, compared to ~20%, O₂.¹³⁻¹⁶ Our new data (Broxmeyer HE, Wang RH, Deng C, unpublished information) now confirms this behavior in FL HPC. We found that, under normoxic conditions, Sirt1^{-/-} FL HPC have enhanced colony formation compared to +/+ HPC, when cells were stimulated with a combination of growth factors, under culture conditions similar to those reported previously for growth of murine bone marrow cells.¹⁷ This suggests that Sirt1 has growth-suppressive activity for proliferation of HPC at normoxic O₂ tension, consistent with results of others for mouse bone marrow cells.¹² This suppressive effect is not seen in Sirt1 heterozygous (+/-) HPC, where Sirt1^{+/-} HPC numbers are decreased compared to +/+ cells and to Sirt1^{-/-} cells. Thus, the loss of just one allele results in the opposite effect of the loss of both.

As previously reported for mouse and human bone marrow and human cord blood HPC colony formation,¹³⁻¹⁶ we noted colony formation by FL HPC^{+/+} was higher when cells were grown at 5%, compared with 20%, O₂ tension. However, under hypoxic conditions of 5% O₂, Sirt1^{-/-} HPC manifested decreased colony numbers compared to +/+ HPC; these were numbers approximately equal to colony numbers of +/+ HPC growing under normoxic conditions. This suggests Sirt1 may act as a positive regulator for HPC colony growth at 5% O₂. This growth decrease at 5% O₂ in comparison to +/+ HPC is also seen for Sirt1^{+/-} cells suggesting that even with one missing allele, Sirt1 could act as a positive regulator at 5% O₂.

Further studies are needed to determine if HPC from bone marrow of mice after birth manifest the same behavior as FL HPC when +/+, Sirt1^{-/-} and Sirt1^{+/-} HPC are assessed. Such studies will need to be followed by analysis of intracellular signaling to provide mechanistic insight into a role for Sirt1 in HPC. Some limited studies have been reported on a possible role for Sirt1 in function of HSC,¹² but much more in-depth study is clearly warranted in this area, especially with regards to response of HSC and HPC to stress. However, our new information clearly show that Sirt1 is important for proper proliferative responses to cytokines in somatic stem/progenitor cells and that it appears vital for proper responses to oxidative stress in these adult stem cells in a way similar to its role in embryonic stem cells.⁴

Sirt1 and Aging Hypothesis

Sirt1 is a protein that has received much scientific attention because it appears to have a central role in aging mechanisms, at the level of both cells and whole organisms.¹⁸⁻²² The involvement of Sirt1 in aging has been observed in various model organisms like

yeast, rodents, and also recently, humans, and may be conserved in all eukaryotic cells.¹⁸⁻²² In addition to a more direct involvement in aging, Sirt1 also seems to be involved in age-related pathological conditions, i.e., diabetes.²³ It is hoped that a deeper understanding of Sirt1 involvement in diseases of aging, coupled with a new generation of small molecule Sirt1 modulators (i.e., resveratrol, stimulates Sirt1 activities), could help advance geriatric medicine. There is a close relationship between Sirt1 involvement in aging and the ROS hypothesis of aging.²⁴⁻²⁶ The ROS hypothesis of aging is perhaps the most durable and well-studied mechanism of aging. Simply, it proposes that ROS generation, a natural byproduct of oxygen metabolism in eukaryotes, causes macromolecular damage, some of which is irreparable. This damage, especially to DNA and long-lived proteins, accumulates over time, eventually causing the cell or tissue to die. The stem cell hypothesis of aging postulates that, with age, stem cells lose their self-renewing proliferative capacity, and this attrition finally disrupts the organization/function of the tissue/organ.^{27,28} Combining the cellular-based ROS hypothesis with the tissue/organ-based stem cell hypothesis results in a compelling and testable model of aging. Our recent studies of Sirt1, ROS, p53 and Nanog present a potential molecular link between the ROS- and stem cell-hypotheses of aging. Moreover, our unpublished information noted above on FL HPC now links Sirt1 function to response to different oxidative conditions in one kind of tissue stem/progenitor cell. We thus propose that stem cell self-renewal and pluripotency are also linked, via Nanog in ESC and possibly other transcription factors in hematopoietic stem cells (HSC), to ROS and Sirt1.

Sirt1 and Asymmetric Stem Cell Division

While Sirt1 has a role in the function of many diverse cells and tissues, its function in stem cells may play a direct role in organismic aging. Sirt1 has been studied primarily in embryonic stem cells and much less so in adult stem cells. The role of adult (somatic, or tissue) stem cells in aging is seminal, according to the stem cell aging hypothesis discussed earlier. The consequences and mechanisms of stem cell aging have recently been appreciated and studied.²⁷ We have also recently pointed out the potential significance of Sirt1 in stem cell aging.²⁸

The generation of cellular diversity during development and for diversity maintenance in metazoan organisms relies heavily on asymmetric stem cell division.²⁹ Stem cell maintenance in adult organisms requires self-renewal (the capacity to make more of itself). To achieve both of these requirements, adult stem cells can undergo a symmetric division giving rise to two similar cells, both with stem cell function, or asymmetric division with two daughter cells having different fates; one can remain a stem cell, while the other can differentiate into a functional tissue or organ cell. So a stem cell could be characterized as a cell capable of a mitotic division where the two daughter cells are identical or not identical. Asymmetric division may present itself immediately (intrinsically) or later because of a change in environmental conditions such as the influence of hormones or signaling from nearby cells (extrinsically).³⁰ Asymmetric stem cell division is often influenced or regulated by the "niche" in which the stem cell resides, but asymmetry can also be achieved in single-celled organisms like yeast by breaking cellular symmetry and polarization. In the case of most higher organisms, asymmetry is manifested by the unequal segregation of cell fate determinants to different sides of

the cell as defined by the metaphase spindle and cleavage furrow.²⁹ This tightly locks into coordinated regulation of cell cycle mechanisms with stem cell self-renewal and asymmetric division. Stem cell mitotic spindle positioning relative to niche stem cell positioning is often a crucial cueing mechanism; we have previously discussed the role of the spindle checkpoint in asymmetric cell division and self-renewal.³¹ In an interesting twist, the spindle checkpoint does not appear to be completely functional in mESCs or in human (h) ESCs,³² but does appear to be functional in mouse adult stem cells.³³ The reasons for this and the influence this has on development and asymmetric stem cell division is unknown.

Asymmetric stem cell division and/or self-renewal has been most rigorously studied in lower organisms like *Saccharomyces cerevisiae* and in invertebrates like *Crenorhabditus elegans* and *Drosophila melanogaster*, but has more recently been investigated in vertebrate brain, kidney, skin, hair and pancreatic islet β -cells.²⁹ An interesting body of evidence suggests that the fundamental principles and mechanisms of asymmetric stem cell division and self-renewal are conserved in metazoan evolution. Of note, HSC from mouse bone marrow are of particular interest. This is because they can easily be purified to relative homogeneity. There is already a large body of information about their function and regulation; they undergo asymmetric cell division and self-renewal.³⁴ Knowledge about them can more easily be applied in a clinical setting. HSCs are considered to reside in a bone marrow niche, but can also circulate throughout the body and even embed and influence extra-bone marrow tissues. Like all the other self-renewing compartments, they are susceptible to a repeatable pattern of aging and decline.^{25,34}

The investigation of Sirt1's influence on aging, self-renewal, proliferation and survival in HSCs is in its infancy.²⁸ However, the data from our recent investigations in mESC,⁴ our unpublished information in FL HPC, studies from Sirt1^{-/-} mouse ESC,²⁰ and information obtained in other systems,^{25,35} are already remarkably consistent and provide beginning evidence for Sirt1's potential role in stem cell aging and stem cell self-renewal. Because self-renewing divisions can involve asymmetric division, and because Sirt1 has been linked to self-renewal, we have begun to develop a proposal that Sirt1 might be important in stem cell asymmetric division.

Notch, Sirt1, Asymmetric Stem Cell Division and Stem Cell "Age-Asymmetry"

Notch is an ancient and very highly conserved family of cell surface receptors that help coordinate and regulate development.³⁶ Notch signaling, via the gamma-secretase-cleaved intracellular domain of the receptor, is implicated in stem cell self-renewal, proliferation and differentiation.³⁶⁻³⁹ The cleavage is facilitated by binding of ligands which are expressed on companion cells commonly found in the "niche" of stem cell systems.^{29,34,36,37} Notch is highly implicated in asymmetric (differential) segregation of cell-fate determinants during stem cell self-renewing divisions.³⁶ Expression of constitutively activated Notch1 in hematopoietic stem and progenitor cells (HSPC) induces immortalized cell lines that can reconstitute myeloid and lymphoid lineages in long-term mouse reconstitution assays^{35,37} and also delays differentiation of human HSPC in-vitro.⁴⁰ Overexpression of Notch1 in HSCs also cause increased production of HSC in-vitro and in-vivo.³⁷ Human bone-marrow cell populations enriched with HSC that are cultured with the soluble Notch1

ligand, Jagged1, have enhanced engraftment in-vivo.³⁸ Also, human CD34⁺ umbilical cord blood cells (cell populations enriched for human HSC) cultured with immobilized Notch ligand, Delta-1, enhanced their marrow-repopulating ability in immunodeficient mice.⁴¹ These studies suggest that Notch1 is important for HSPC and HSC self-renewal and can act as a suppressor of stem-cell differentiation. However, a recent study found that by suppressing Notch signaling by dominant-negative forms or gene-deletion of key Notch-mediated transcription factors in HSC, the cells could still support long-term hematopoietic reconstitution of animals, even after secondary competitive transplantation.³⁹ This is strong evidence supporting the idea that Notch is dispensable for HSC self-renewal. Still other studies demonstrated that overexpressing the major Notch1 target, Hes1, in human HSPC inhibits cell cycling in-vitro and cell expansion in-vivo.⁴² Interestingly, it was also shown in these studies that short-term repopulation was reduced in Hes1-transduced mouse and human HSPC, but long-term repopulation was maintained. So, while some uncertainty remains regarding the precise role of Notch1, via its transcription-regulating target, Hes1, in self-renewal and differentiation of HSC, there is an abundance of evidence in other model systems to support an important role for Notch and Notch-signaling in asymmetric stem cell division which is an important component for stem cell self-renewal and function. Notch and receptors like it are thought to function in asymmetric cell division by binding to their ligands which are expressed on the surface of companion or niche cells resulting in segregation of these receptors to one side of the stem cell before cell division. The orientation of the mitotic spindle relative to the Notch-niche axis directs which proteins (cell fate determinants) are sequestered into which, or both, daughter cells resulting in one cell remaining a stem cell and the other beginning a program of differentiation.²⁹ Thus, receptors like Notch are linked to asymmetric cell division in stem cells which is necessarily linked to self-renewing stem cell divisions, which are integral to the stem cell-aging hypothesis.

So how might the self-renewal- and aging-linked effects of Sirt1 be related to Notch and asymmetric stem cell division? To our knowledge, there are no direct studies of Sirt1 linkage to Notch signaling or to asymmetric division. However, an intriguing and likely connection is via the major Notch effector protein, Hes1, which is also an important substrate and effector of Sirt1.⁴³ Figure 1 suggests how aging, hypoxia and ROS, and metabolism might all influence Hes1 activities, and both Sirt1 and Notch are implicated in these processes.⁴⁴⁻⁴⁷ Moreover, there are two Nanog binding sites in the HES1 promoter, potentially implicating Nanog in HES1 regulation. Based on the pathways implicated in Figure 1, it seems possible that Sirt1 may act as an attenuating influence on Notch effects and vice-versa via HES1, and therefore Sirt1 could influence the role of Notch pathway in asymmetric stem cell division and self-renewal divisions. In this context, it is also interesting to note that Sirt1 suppresses the co-repressor for ppar-gamma, (NCoR), while Notch signaling enhances NCoR.⁴⁸ ppar-gamma is considered a master regulator of mitochondrial biogenesis and so the same kind of balancing regulation observed for Hes1 might also be at work for metabolic/energy regulation by Sirt1 and Notch. Also of note, there is experimental evidence supporting an influence of nanog, wnt/beta-catenin pathway, and sonic hedgehog pathways on Hes1 and its family members.^{4,37,49} These pathways are considered a part of

a core set of signaling pathways needed for proper stem cell maintenance and self-renewal.³⁰ This further suggests the possibility of a role for Sirt1 and Notch in stem cell self-renewal and asymmetric divisions.

Finally, how can Sirt1, Notch and asymmetric stem cell division relate to stem cell aging? That stem cells age in an autonomous manner has been investigated and discussed,²⁵ and ROS figures prominently in this process.⁴⁵ There is also considerable crosstalk between notch and other pathways that can regulate stem cell self-renewal which influences stem cell aging.⁵⁰ Aging in some lower eukaryotes like budding yeast have also been studied, and their age is measured in terms of “divisional age”, not chronological age. Every division is tabulated by accumulation of specialized extra-chromosomal circular DNAs (ERCs) that are replicated once per division and sequestered in the nucleolus. This process depends on segregating the ERCs asymmetrically into the mother cell and not the budding daughter cell,⁵¹⁻⁵³ and Sir2, the yeast homologue of Sirt1, has been implicated in this process.^{22,54} An analogous process of tracking divisional age in metazoan stem cells has not been identified, but structures similar to ERCs have been identified in human lymphocytes and fibroblasts and are associated with in-vitro and in-vivo aging.^{55,56} So it remains a possibility that mechanisms of divisional-age accounting in yeast could have similarities with other eukaryotes. It therefore seems likely (and logical) that stem cell asymmetric division will have an important role in metazoan stem cell aging as it appears to have in more primitive single cell eukaryotes. The concept of “age-asymmetry” has been discussed in yeast where mother cells are divisionally older than newly budded daughter cells. As stated earlier, this divisional age is “tracked” and most budding yeast cells live about 20 divisions.^{51,52} Caloric-restriction and other metabolic modification can lengthen this life-span and Sirt1 is highly implicated in this, but to our knowledge, Notch has not been linked to caloric-restriction-induced lifespan extension. We have recently commented on the potential role of Sirt1 in stem cell aging,²⁸ and how Sirt1 is likely to be involved in the same way in adult stem cells as it is in ESCs, especially as relates to oxidation-, and energy-status, where mitochondrial function is extremely important. Mitochondria can be segregated asymmetrically in yeast and we have speculated about potential roles of this behavior in stem cell aging.²⁸ Based on the involvement of Notch in self-renewal and asymmetric division, the role of Sirt1 in life-span extension, and the overlapping signaling pathways of Notch and Sirt1 (through Hes1), we propose that adult, and possibly fetal stem cells, in particular HSC, may be constrained by a similar kind of age-asymmetry. If correct, this could have implications for stem cell biology. For example, if HSCs are constrained by an intrinsically determined divisional age, does it influence ex-vivo expansion of HSCs? Ex-vivo expansion of HSCs is considered as a potential means to enhance the use of collected samples whose HSC numbers can be limiting (e.g., in umbilical cord blood).⁵⁷ What influence would intrinsically determined divisional age have on HSC transplantation? Could this behavior help explain the phenotypic changes occurring in HSCs during aging? Does dysregulation of this process contribute to progeroid diseases? In this context, a mutant form of presenilin-1, a component of gamma-secretase (the enzyme complex that cleaves the Notch intracellular domain), is already implicated in Alzheimer’s

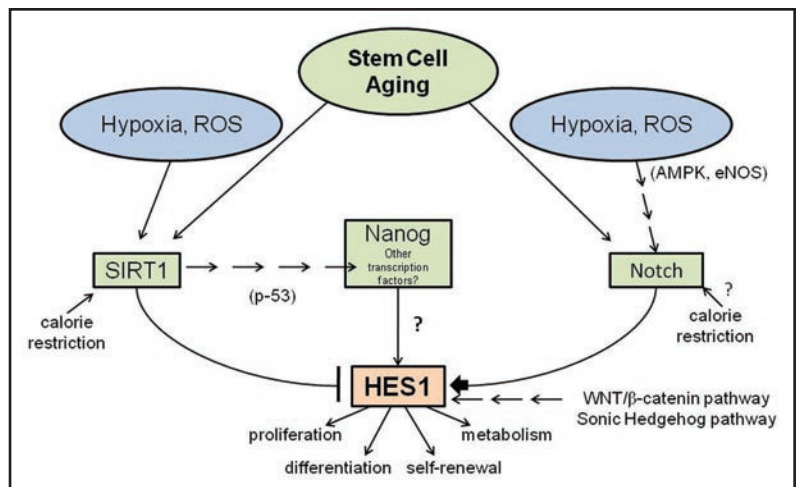


Figure 1. Potential model of intersecting pathways of Sirt1 and Notch. See text for detail. ROS is reactive oxygen species; AMPK is AMP-activated protein kinase; eNOS is endothelial nitric oxide synthase.

disease.⁵⁸⁻⁶⁰ Also, a mutant form of Lamin-A, called progerin, causes Hutchinson-Gilford Progeria Syndrome, a disease of premature aging, and it also influences the Notch/Hes pathways.⁶¹

The seminal experiments needed to test our proposal will need to demonstrate the maintenance of divisional age-asymmetry between two daughters of a stem cell division. While simple to envision, this would be very difficult from a technical perspective. Perhaps a kind of “age-determinant”, in addition to a fate-determinant, might be found that is asymmetrically sequestered in the two stem cell daughters. It would seem that the best place to start looking for such kinds of age-determinants (other than accumulated ROS- and epigenetic-damage) might be in the same pathways that are already implicated in stem cell self-renewal, like wnt/beta-catenin, nanog, Notch, hedgehog and potentially Sirt1 pathways. Exploring these possibilities could reveal new opportunities for intervention and preventive medicine for the aged.

References

- Kamata H, Hirata H. Redox regulation of cellular signalling. *Cell Signal* 1999; 11:1-14.
- Haddad JJ. Oxygen sensing and oxidant/redox-related pathways. *Biochem Biophys Res Commun* 2004; 316:969-77.
- Porwol T, Ehleben W, Brand V, Acker H. Tissue oxygen sensor function of NADPH oxidase isoforms, an unusual cytochrome aa3 and reactive oxygen species. *Respir Physiol* 2001; 128:331-48.
- Han MK, Song EK, Guo Y, Ou X, Mantel C, Broxmeyer HE. SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. *Cell Stem Cell* 2008; 2:241-51.
- Lin T, Chao C, Saito S, et al. p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat Cell Biol* 2005; 7:165-71.
- Hussain SP, Amstad P, He P, et al. p53-induced upregulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. *Cancer Res* 2004; 64:2350-6.
- Tan M, Li S, Swaroop M, Guan K, Oberley LW, Sun Y. Transcriptional activation of the human glutathione peroxidase promoter by p53. *J Biol Chem* 1999; 274:12061-6.
- Budanov AV, Sablina AA, Feinstein E, Koonin EV, Chumakov PM. Regeneration of peroxiredoxins by p53-regulated sestrins, homologs of bacterial AhpD. *Science* 2004; 304:596-600.
- Leu JI, Dumont P, Hafey M, Murphy ME, George DL. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol* 2004; 6:443-50.
- Mihara M, Erster S, Zaika A, et al. p53 has a direct apoptogenic role at the mitochondria. *Mol Cell* 2003; 11:577-90.
- Vaziri H, Dessain SK, Ng Eaton E, et al. hSIR2(SIRT1) functions as a NAD-dependent p53 deacetylase. *Cell* 2001; 107:149-59.
- Narala SR, Allsopp RC, Wells TB, Zhang G, Prasad P, Coussens MJ, Rossi DJ, Weissman IL, Vaziri H. SIRT1 acts as a nutrient-sensitive growth suppressor and its loss is associated with increased AMPK and telomerase activity. *Mol Biol Cell* 2008; 19:1210-9.

13. Lu L, Broxmeyer HE. Comparative influences of phytohemagglutinin-stimulated leukocyte conditioned medium, hemin, prostaglandin E and low oxygen tension on colony formation by erythroid progenitor cells in normal human bone marrow. *Exp Hematol* 1985; 13:989-93.
14. Smith S, Broxmeyer HE. The influence of oxygen tension on the long term growth in vitro of haematopoietic progenitor cells from human cord blood. *Brit J Haematol* 1986; 63:29-34.
15. Broxmeyer HE, Cooper S, Gabig T. The effects of oxidizing species derived from molecular oxygen on the proliferation in vitro of human granulocyte-macrophage progenitor cells. *New York Acad Sci* 1989; 554:177-84.
16. Broxmeyer HE, Cooper S, Lu L, Miller ME, Langefeld CD, Ralph P. Enhanced stimulation of human bone marrow macrophage colony formation in vitro by recombinant human macrophage colony stimulating factor in agarose medium at low oxygen tension. *Blood* 1990; 76:323-929.
17. Broxmeyer HE, Sehra S, Cooper S, Toney LM, Kusam S, Aloor JJ, Marchal CC, Dinauer M, Dent AL. BAZF-deficient mice have unusual alterations in hematopoietic progenitor cell activity similar to that of BCL-6 deficient mice. *Mol Cell Biol* 2007; 27:5275-85.
18. Guarente L. Sirtuins in aging and disease. *Cold Spring Harb Symp Quant Biol* 2007; 72:483-8.
19. Imai S, Armstrong CM, Kaerberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 2000; 403:795-800.
20. Chen D, Steele AD, Lindquist S, Guarente L. Increase in activity during calorie restriction requires Sirt1. *Science* 2005; 310:1641.
21. Bordone L, Cohen D, Robinson A, et al. SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* 2007; 6:759-67.
22. Guarente L, Picard F. Calorie restriction—the SIR2 connection. *Cell* 2005; 120:473-82.
23. Bordone L, Motta MC, Picard F, et al. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol* 2006; 4:31.
24. Guarente L. Mitochondria—a nexus for aging, calorie restriction and sirtuins? *Cell* 2008; 132:171-6.
25. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* 2007; 8:703-13.
26. Westphal CH, Dipp MA, Guarente L. A therapeutic role for sirtuins in diseases of aging? *Trends Biochem Sci* 2007; 32:555-60.
27. Rossi DJ, Jamieson CH, Weissman IL. Stem cells and the pathways to aging and cancer. *Cell* 2008; 132:681-96.
28. Mantel C and Broxmeyer HE. SIRT1, stem cells, aging and stem cell aging. *Current Opinion in Hematology* 2008; 15:326-31.
29. Gonczy P. Mechanisms of asymmetric cell division: flies and worms pave the way. *Nat Rev Mol Cell Biol* 2008; 9:355-66.
30. Zon LI. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. *Nature* 2008; 453:306-13.
31. Mantel C, Broxmeyer HE. A new connection between the spindle checkpoint, asymmetric cell division and cytokine signaling. *Cell Cycle* 2007; 6:144-6.
32. Mantel C, Guo Y, Lee MR, et al. Checkpoint-apoptosis uncoupling in human and mouse embryonic stem cells: a source of karyotypic instability. *Blood* 2007; 109:4518-27.
33. Rohrabough S, Mantel C, Broxmeyer HE. Mouse hematopoietic stem cells, unlike human and mouse embryonic stem cells, exhibit checkpoint-apoptosis coupling. *Stem Cells and Development* 2008; In Press.
34. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. *Nat Rev Mol Cell Biol* 2008; 9:11-21.
35. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nat Rev Immunol* 2006; 6:93-106.
36. Chenn A, McConnell SK. Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. *Cell* 1995; 82:631-41.
37. Duncan AW, Rattis FM, DiMascio LN, et al. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. *Nat Immunol* 2005; 6:314-22.
38. Karanu FN, Murdoch B, Gallacher L, et al. The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. *J Exp Med* 2000; 192:1365-72.
39. Maillard I, Koch U, Dumortier A, et al. Canonical notch signaling is dispensable for the maintenance of adult hematopoietic stem cells. *Cell Stem Cell* 2008; 2:356-66.
40. Carlesso N, Aster JC, Sklar J, Scadden DT. Notch1-induced delay of human hematopoietic progenitor cell differentiation is associated with altered cell cycle kinetics. *Blood* 1999; 93:838-48.
41. Ohishi K, Varnum-Finney B, Bernstein ID. Delta-1 enhances marrow and thymus repopulating ability of human CD34(+)CD38(-) cord blood cells. *J Clin Invest* 2002; 110:1165-74.
42. Yu X, Alder JK, Chun JH, et al. HES1 inhibits cycling of hematopoietic progenitor cells via DNA binding. *Stem Cells* 2006; 24:876-88.
43. Takata T, Ishikawa F. Human Sir2-related protein SIRT1 associates with the bHLH repressors HES1 and HEY2 and is involved in HES1- and HEY2-mediated transcriptional repression. *Biochem Biophys Res Commun* 2003; 301:250-7.
44. Prozorovski T, Schulze-Topphoff U, Glumm R, et al. Sirt1 contributes critically to the redox-dependent fate of neural progenitors. *Nat Cell Biol* 2008; 10:385-94.
45. Gustafsson MV, Zheng X, Pereira T, et al. Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev Cell* 2005; 9:617-28.
46. Ito T, Udaka N, Yazawa T, et al. Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. *Development* 2000; 127:3913-21.
47. Katoh M. Integrative genomic analyses on HES/HEY family: Notch-independent HES1, HES3 transcription in undifferentiated ES cells, and Notch-dependent HES1, HES5, HEY1, HEY2, HEYL transcription in fetal tissues, adult tissues or cancer. *Int J Oncol* 2007; 31:461-6.
48. Perissi V, Scafoglio C, Zhang J, et al. TBL1 and TBLR1 phosphorylation on regulated gene promoters overcomes dual CtBP and NCoR/SMRT transcriptional repression checkpoints. *Mol Cell* 2008; 29:755-66.
49. Ingram WJ, McCue KI, Tran TH, Hallahan AR, Wainwright BJ. Sonic Hedgehog regulates Hes1 through a novel mechanism that is independent of canonical Notch pathway signaling. *Oncogene* 2008; 27:1489-500.
50. Sengupta A, Banerjee D, Chandra S, et al. Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. *Leukemia* 2007; 21:949-55.
51. Sinclair DA, Guarente L. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* 1997; 91:1033-42.
52. Sinclair DA, Mills K, Guarente L. Molecular mechanisms of yeast aging. *Trends Biochem Sci* 1998; 23:131-4.
53. Sinclair DA, Lin SJ, Guarente L. Life-span extension in yeast. *Science* 2006; 312:195-7.
54. Anderson RM, Latorre-Esteves M, Neves AR, Lavu S, Medvedik O, Taylor C, Howitz KT, Santos H, Sinclair DA. Yeast life-span extension by calorie restriction is independent of NAD fluctuation. *Science* 2003; 302:2124-6.
55. Lumpkin CK Jr, McGill JR, Riabowol KT, Moerman EJ, Reis RJ, Goldstein S. Extrachromosomal circular DNA and aging cells. *Adv Exp Med Biol* 1985; 190:479-93.
56. Kunisada T, Yamagishi H, Ogita Z, Kirakawa T, Mitsui Y. Appearance of extrachromosomal circular DNAs during in vivo and in vitro ageing of mammalian cells. *Mech Ageing Dev* 1985; 29:89-99.
57. Broxmeyer HE and Smith FO. *Cord Blood Hematopoietic Cell Transplantation*. 4th Edition. Ed: Appelbaum FR, Forman SJ, Negrin S and Blume KG. Blackwell Scientific Publications, Cambridge MA 2008; 39:In Press.
58. Lee MK, Borchelt DR, Kim G, Thinakaran G, Slunt HH, Ratovitski T, Martin LJ, Kittur A, Gandy S, Levey AI, Jenkins N, Copeland N, Price DL, Sisodia SS. Hyperaccumulation of FAD-linked presenilin 1 variants in vivo. *Nat Med* 1997; 3:756-60.
59. Davis JA, Naruse S, Chen H, Eckman C, Younkin S, Price DL, Borchelt DR, Sisodia SS, Wong PC. An alzheimer's disease-linked PS1 variant rescues the development abnormalities of PS1-deficient embryos. *Neuron* 20:603-9.
60. Fortini ME. Notch and presenilin: a proteolytic mechanism emerges. *Curr Opin Cell Biol* 2001; 13:627-34.
61. Scaffidi P, Misteli T. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. *Nat Cell Biol* 10:452-9.