Commentary

Stem Cell INVESTIGATION

# As“SIRT”ing the role of an epigenetic modifier in hematopoietic stem cell homeostasis

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## Sirtuin family of histone deacetylases and their diverse roles

Mammalian Sirtuin protein family consists of SIRT1-SIRT7. Sirtuins are $NAD^+$-dependent histone deacetylases (HDACs) known to regulate metabolic homeostasis, chromatin structure and DNA repair thereby regulating genomic stability. They were first discovered in yeast as silent information regulator 2 (Sir2) that promote longevity (1). Subcellular localization of sirtuins varies depending on the type of cell and the function associated with the sirtuin. For most part, SIRT1 is nuclear and mediates the effects of calorie restriction which reduces tumor formation and stress-induced apoptosis. SIRT1 also mediates cancer drug resistance. SIRT2 is predominantly cytoplasmic and regulates oxidative stress. SIRTs 3, 4 and 5 are in mitochondria and regulate mitochondrial functions such as energy usage. SIRT6 is nuclear and regulates DNA repair and SIRT7 is nucleolar regulating rRNA transcription (2). SIRTs 4 and 5 have weak deacetylase activity but SIRT4 has ADP-ribosylase activity and SIRT5 has demalonylase and desuccinylase activities. Besides deacetylation, SIRT6 also regulates TNFα secretion by modulating its lysine fatty acylation (3).

Sirtuins deacetylate lysine9 or lysine56 on histone 3 thereby forming heterochromatin and reducing the activity of the genes associated with it. p53 is the first non-histone target of sirtuins to be identified and hence inhibition of sirtuins can increase the tumor suppression mediated by p53. Sirtuins also target NF-κB and reduce signaling from these proteins. Through p53 and NF-κB, sirtuins regulate tumor suppression and inflammation respectively (4). Although sirtuins are known to increase longevity (anti-ageing factors) by limiting replicative lifespan and protecting against oxidative stress, they also act as tumor-suppressors and their expression levels go down in several cancer conditions such as colon cancer, glioblastoma, breast cancer and prostate cancer. However, sirtuins can act as oncogenes in some cases (5). Overall, sirtuins regulate numerous cellular processes that are important in maintaining normal cell survival.

## Epigenetic regulation of stem cell function

Maintaining stem cell homeostasis, i.e., self-renewal *vs.* differentiation requires regulation by several epigenetic mechanisms including histone modifications (such as methylation, acetylation and ubiquitination), DNA methylation and chromatin remodeling. Sirtuins regulate cellular processes by modifying histones and thereby regulating expression of genes associated with them. Besides sirtuins, several epigenetic modifiers have been identified to regulate homeostasis of different stem cells. Interestingly, HDAC inhibitors such as valproic acid expand functional human cord blood $CD34^+$ cells in the presence of a cocktail of cytokines (6). Combined inhibition of DNA methylation (5Aza 2’deoxycytidine) and histone deacetylases (trichostatin A) also increased functional human marrow $CD34^+$ HSCs without inhibiting their marrow repopulating ability (7).

Polycomb group proteins (PcG) are epigenetic modifiers that are involved in transcriptional repression.

They regulate HSC maintenance and differentiation in a developmental stage-specific manner where PcGs are required for survival of adult bone marrow HSCs but not fetal liver HSCs (8). Enhancer of Zeste homolog 2 (Ezh2), a PcG binds to DNA methyltransferases and is required for DNA methylation and gene silencing thereby regulating adult HSC self-renewal (9). However, loss of a DNA methyltransferase, Dnmt3a lead to expansion of HSCs with impaired differentiation ability during serial transplantation assays (10) and hence Dnmt3a is required for normal HSC differentiation suggesting that different epigenetic modifiers have differential effects on HSC homeostasis.

## Sirtuins and stem cells

Sirtuins are involved in regulating different stages of hematopoietic development and their functions. SIRT1 is the most studied sirtuin and is required for hematopoietic differentiation from mouse ESCs (11). It is expressed in low levels in HSCs and its expression increases upon differentiation. However, SIRT1 does not play a role in regulating adult HSC function under normal or stress conditions but fetal HSCs require SIRT1 for their maintenance under stress (12). Besides regulating fetal HSCs, SIRT1 is required for survival of chronic myeloid leukemia (CML) stem cells and the expression of SIRT1 increases in CML. Hence inhibition of SIRT1 which activates p53 along with imatinib in combination therapy reduces survival and proliferation of leukemic stem cells (13). SIRT3 is down-regulated during ageing and overexpression of SIRT3 improves regenerative capacity of aged HSCs, however, SIRT3 is dispensable in young mice under homeostatic conditions (14).

On the other hand, SIRT6 is nuclear and is involved in regulating functions of different stem cell types including embryonic stem cells (ESCs), hematopoietic stem cells (HSCs) and human mesenchymal stem cells (hMSCs). In the current study, Wang and colleagues identified an important role for SIRT6 in regulating HSC self-renewal (15). They used 3 different inducible mouse models (Mx-Cre *vs.* cre-ERT *vs.* hematopoietic specific Vav-cre) to knock out SIRT6 and show that SIRT6 maintains quiescence of HSCs by suppressing Wnt signaling through its direct binding to TCF/LEF1 transcription factor. Interestingly, the authors also show that H3K56 but not H3K9 is the target of SIRT6 in HSCs similar to the target site identified in ESCs (15). Since the defects observed in engraftment with Sirt6$^{\Delta/\Delta}$ murine HSCs were not apparent early after the transplantation but are only seen at the later stages (16 weeks), Sirt6$^{\Delta/\Delta}$ murine HSCs might be deficient in the long term HSCs but not in short term stem cell/progenitors involved in the initial engraftment.

SIRT6 also regulates ESC homeostasis by repressing pluripotent genes Oct4, Nanog and Sox2 through deacetylation of H3K56. Loss of SIRT6 reduces ESC proliferation (16), derepresses the pluripotent genes, increases TET expression and subsequent 5-hydroxymethylcytosine leading to skewed differentiation of ESCs towards neural ectoderm (17). SIRT6 is involved in base excision repair and suppresses genomic instability and acts as an anti-ageing factor. SIRT6$^{-/-}$ mice show premature ageing like symptoms and die 4 weeks after birth (16). SIRT6 also protects hMSCs from oxidative stress by inducing heme oxygenase-1 through nuclear factor erythroid 2-related factor 2 (18). Overall SIRT6 maintains genomic stability and regulates homeostasis of HSCs and ESCs.

## Conclusions and future directions

In conclusion, the authors show that SIRT6 is required in maintaining functional self-renewing HSCs in mice and this regulation requires suppression of Wnt signaling (15). Since activation of Wnt signaling combined with TOR inhibition was previously shown to maintain functional self-renewing HSCs (19), it will be interesting to check whether SIRT6 loss also activates mTOR signaling along with activation of Wnt signaling. Although loss of SIRT6 increases Wnt signaling thereby making HSCs functionally defective, inhibition of Wnt signaling upon SIRT6 loss only partially rescued the repopulating capacity of HSCs suggesting that there are additional pathways regulating HSC homeostasis upon loss of SIRT6 in murine HSCs. Furthermore, inhibition of Wnt signaling did not alter the repopulating ability of Sirt6$^{+/+}$ cells suggesting that inhibition of Wnt signaling is only important under the conditions of SIRT6 loss in murine HSCs.

Interestingly, there are more number of phenotypic HSCs in Sirt6$^{\Delta/\Delta}$ mice or mice lacking Dnmt3a but are functionally defective suggesting that there is a precise epigenetic mechanism that balances proliferation *vs.* differentiation. The current study addresses the role of SIRT6 only in murine HSCs. Further studies are required to confirm the importance of SIRT6 in regulating human HSC homeostasis and its localization in HSCs. However, this study places SIRT6 among the critical epigenetic modifiers that regulate HSC homeostasis and furthers our understanding of the signaling events that contribute to HSC self-renewal.

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## Footnote

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