Cell & Tissue Transplantation & Therapy

Libertas Academica
FREEDOM TO RESEARCH

OPEN ACCESS
Full open access to this and thousands of other papers at http://www.la-press.com.

REVIEW

Regulation of Somatic Stem Cell Function by DNA Methylation and Genomic Imprinting

Mo Li[1], Na Young Kim[1], Shigeo Masuda[1] and Juan Carlos Izpisua Belmonte[1,2]

[1]Salk Institute for Biological Studies, 10010 N Torrey Pines Rd, La Jolla, CA 92037, USA.
[2]Center of Regenerative Medicine in Barcelona, Dr Aiguader, 88, 08003 Barcelona, Spain.
Corresponding author email: mli@salk.edu

Abstract: Epigenetic regulation is essential for self-renewal and differentiation of somatic stem cells, including hematopoietic stem cells (HSCs) and neural stem cells (NSCs). The role of DNA methylation, one of the key epigenetic pathways, in regulating somatic stem cell function under physiological conditions and during aging has been intensively investigated. Accumulating evidence highlights the dynamic nature of the DNA methylome during lineage commitment of somatic stem cells and the pivotal role of DNA methyltransferases in stem cell self-renewal and differentiation. Recent studies on genomic imprinting have shed light on the imprinted gene network (IGN) in somatic stem cells, where a subset of imprinted genes remain expressed and are important for maintaining self-renewal of these cells. Together with emerging technologies, elucidation of the epigenetic mechanisms regulating somatic stem cells with normal or pathological functions may contribute to the development of regenerative medicine.

Keywords: somatic stem cells, epigenetics, DNA methylation, genomic imprinting, hematopoietic stem cells, neural stem cells

Cell & Tissue Transplantation & Therapy 2013:5 19–23

doi: 10.4137/CTTT.S12142

This article is available from http://www.la-press.com.

© the author(s), publisher and licensee Libertas Academica Ltd.

This is an open access article published under the Creative Commons CC-BY-NC 3.0 license.

Introduction

In adult animals, somatic stem cells (also known as adult stem cells) are responsible for maintaining tissue homeostasis and participate in tissue regeneration under injury conditions. Self-renewal and differentiation are two important aspects of somatic stem cell function. Epigenetic mechanisms underlying these processes have been intensively investigated. With the increasing ability to identify and manipulate somatic stem cell populations from diverse tissues, it is possible to dissect the epigenetic pathways that are either unique for a specific tissue or universally important in regulating stemness and differentiation. Epigenetic control of somatic stem cell function exists at various levels, including DNA methylation, histone modification, and higher-order chromatin structure dynamics. Here, we focus on recent progress in our understanding of how DNA methylation regulates somatic stem cell function.

DNA Methylation and Stem Cell Function

The role of DNA methylation in somatic stem cell compartments has gained increasing attention. Recent evidence has shown that DNA methylation is dynamically regulated during somatic stem cell differentiation and aging.[1] A study of methylomes of human hematopoietic stem cells (HSCs) and two mature hematopoietic lineages, including B cells and neutrophils, showed that hypomethylated regions of lineage-specific genes often become methylated in opposing lineages, and that progenitors display an intermediate methylation pattern that is poised for lineage-specific resolution.[2] Another study compared genome-wide promoter DNA methylation in human cord blood hematopoietic progenitor cells (HPCs) with that in mobilized peripheral blood HPCs from aged individuals. It was found that aged HPCs lose DNA methylation in a subset of genes that are hypomethylated in differentiated myeloid cells and gain *de novo* DNA methylation at polycomb repressive complex 2 (PRC2) target sites.[3] It was hypothesized that such epigenetic changes contribute to age-related loss of HSC function, such as a bias toward myeloid lineages. Recently, Beerman et al. studied the global DNA methylation landscape of HSCs in the context of age-associated decline of HSC function.[4] Overall, the DNA methylation landscape remains stable during HSC ontogeny. However, HSCs isolated from old mice display higher global DNA methylation. Interestingly, they observed localized DNA methylation changes in genomic regions associated with hematopoietic lineage differentiation. These methylation changes preferentially map to genes that are expressed in downstream progenitor and effector cells. For example, genes that are important for the lymphoid and erythroid lineages become methylated in "old" HSCs, which is consistent with the decline of lymphopoiesis and erythropoiesis during aging. Additionally, inducing HSC proliferation by 5-fluorouracil treatment or by limiting the number of transplanted HSCs recapitulates the functional decline and DNA methylation changes during physiological aging. A closer examination of the overlapping genes with significant DNA methylation changes during aging or enforced proliferation showed an enrichment of DNA hypermethylation at PRC2 target loci, echoing the observation by Bocker et al. in human HSCs. Interestingly, a recent report showed that epigenetic alterations such as DNA hypermethylation that are accrued during aging, can be fully reset by somatic reprogramming, raising an interesting possibility that these aging-related epigenetic defects may be reserved by small molecules.[5]

Methylation of cytosines at CpG dinucleotides is catalyzed by three key enzymes. DNA (cytosine-5)-methyltransferase 1 (DNMT1) is responsible for maintaining DNA methylation patterns during DNA replication by methylating the newly synthesized hemi-methylated DNA. The other two DNA methyltransferases, DNMT3a and DNMT3b, are not DNA replication-dependent and can methylate fully unmethylated DNA *de novo*. They are responsible for establishing new DNA methylation patterns during development.

DNMT3a, a gene required for neurogenesis, is expressed in postnatal neural stem cells (NSCs). In NSCs, DNMT3a methylates non-proximal promoter regions, such as gene bodies and intergenic regions. Surprisingly, rather than silencing gene expression, DNMT3a-mediated DNA methylation in gene bodies antagonizes Polycomb-dependent repression and facilitates the expression of neurogenic genes.[6]

The role of DNMT3a in HSCs has also been investigated. Both *Dnmt3a* and *Dnmt3b* are expressed in HSCs. An earlier study did not identify any defects in

HSC function when *Dnmt3a* or *Dnmt3b* was removed. However, HSCs lacking both of these *de novo* methyltransferases fail to self-renew, yet retain the capacity to differentiate.[7] A more recent study re-examined the consequences of *Dnmt3a* loss in HSCs and uncovered a progressive defect in differentiation that is only manifested during serial transplantation.[8] At the molecular level, while *Dnmt3a* loss results in the expected hypomethylation at some loci, it counterintuitively causes hypermethylation in even more regions.[8] This seemingly paradoxical result echoes the unconventional role of *Dnmt3a* in transcriptional activation in NSCs (as discussed above). Both cases suggest a more complex regulatory function of DNMT3a that is beyond simply methylating DNA. In contrast, the loss of *Dnmt1* produces more dramatic and immediate phenotypes in HSCs, manifested in premature HSC exhaustion and block of lymphoid differentiation, highlighting the distinct requirements for different DNA methyltransferases in HSCs.[9,10]

Genomic Imprinting and Stemness

DNA methylation also underlies genomic imprinting, which is an evolutionarily conserved epigenetic mechanism of ensuring appropriate gene dosage during development. One allele of the imprinted genes is epigenetically marked by DNA methylation to be silenced according to the parental origin. The pattern of imprinting is established in germ cells and maintained in somatic cells. Imprinted genes are thought to play critical roles in organismal growth and are relatively downregulated after birth.[11] Recently, a series of reports demonstrated that a subset of imprinted genes belonging to the purported imprinted gene network (IGN)[12] remain expressed in somatic stem cells and are important for maintaining self-renewal of these cells. Through gene expression profiling, one group identified that several members of the IGN are expressed in murine muscle, epidermal, and long-term hematopoietic stem cells as well as in human epidermal and hematopoietic stem cells.[13] In particular, the paternally expressed gene 3 (Peg3) gene was shown by another group to mark cycling and quiescent stem cells in a wide variety of mouse tissues.[14] The role of imprinted genes in regulating somatic stem cell function has been examined in two types of tissues. In bronchioalveolar stem cells (BASCs), a lung epithelial stem cell population, expression of IGN members is required for their self-renewal. *Bmi1*, a polycomb repressive complex 1 (PRC1) subunit, is essential for controlling the expression of imprinted genes in BASCs without affecting their imprinting status.[15] In *Bmi1* mutant BASCs, many members of the IGN become derepressed, including *p57, H19, Dlk1, Peg3, Ndn, Mest, Gtl2, Grb10, Plagl1*, and *Igf2*. Knockdown of *p57*, which is the most differentially expressed imprinted gene between normal and mutant BASCs, partially rescues the self-renewal defect of lung stem cells. Interestingly, insufficient levels of *p57* also inhibit self-renewal of lung stem cells. Because *p57* expression remains monoallelic in *Bmi1* knockdown cells, *Bmi1* is thought to maintain an appropriate level of expression from the expressed allele of *p57*.[15] Another IGN member-delta-like homologue 1 (*Dlk1*) has been shown to be important for postnatal neurogenesis. Interestingly, in this context, *Dlk1* loses its imprinting in postnatal neural stem cells and niche astrocytes.[16] These studies suggest that modulating IGN may represent another epigenetic mechanism for balancing self-renewal and differentiation in somatic stem cells. Thus, somatic stem cells either co-opt or remodel these developmental pathways involving the IGN to fulfill the needs of tissue homeostasis during the adult stage.

In summary, several factors participate in regulating the epigenome of somatic stem cells. Perturbations in the epigenome of somatic stem cells, either during organismal aging or under pathological conditions, will tip the balance between self-renewal and differentiation of somatic stem cells (Fig. 1). A detailed understanding of the mechanisms underlying these changes will likely result in novel therapeutic approaches targeting somatic stem cells.

Future Perspectives

The epigenetic mechanisms governing self-renewal and differentiation of somatic stem cells are likely to be complex because of the diverse needs of different tissues. It would be interesting to determine whether a common mechanism, such as the IGN, exists across different somatic stem cells. Additionally, studying epigenetic pathways that are specific to one type of somatic stem cell requires the isolation of these cells and their differentiated progeny, which is more practical in model organisms than in humans. Along these lines, developing robust *in vitro* culture methods for human somatic stem cells and protocols for

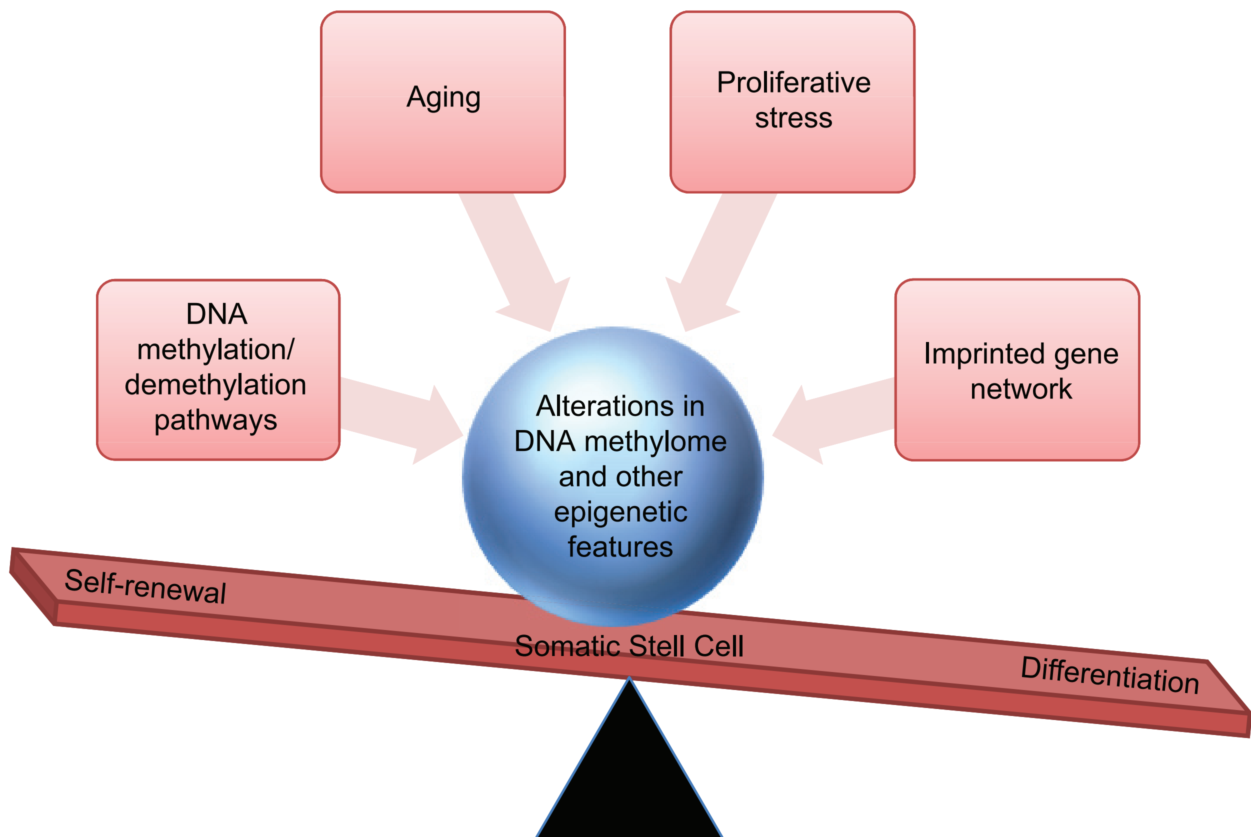

Figure 1. The epigenome of somatic stem cells is regulated by diverse factors.

differentiating these cells into specific lineages are critical for uncovering epigenetic pathways that are unique to human somatic stem cells. In recent years, the field has seen a great improvement in methods of directed differentiation of human embryonic stem cells and induced pluripotent stem cells (iPSCs). For example, it is relatively straightforward to produce high-purity cell populations that resemble neural stem cells or mesenchymal stem cells from iPSCs.[17] These methodologies not only are useful for studying the normal function of somatic stem cells, but also provide an exciting opportunity for understanding the role of somatic stem cells in disease pathology and a platform to screen for drugs. A recent study underscored the usefulness of this approach. Liu et al. studied neural stem cells derived from Parkinson's disease human iPSCs and uncovered previously unknown defects in nuclear morphology and epigenetic regulation in these derived NSCs.[18] The cellular defects only menifest in "aged" neural stem cells, which is consistent with the fact that Parkinson's disease primarily manifests in old age. More importantly, this study identified neural stem cell as a potential target of therapeutic intervention for Parkinson's disease. Targeted modification of the human genome is another technological advancement that is on the horizon to greatly facilitate the dissection of epigenetic pathways in somatic stem cells. Although gene targeting in somatic stem cells has been historically challenging, there have been encouraging successful reports following development of new genome-editing technologies, such as Helper-dependent adenoviral vectors, TALENs, and CAS9/CRISPR. With the development of these new technologies, it seems that the stage has been set for a new wave of discoveries in epigenetic mechanisms of somatic stem cells.

Author Contributions

Wrote the first draft of the manuscript: ML, NYK, SM. Made critical revisions and approved final version: JCIB, ML. All authors reviewed and approved of the final manuscript.

Funding

ML is supported by a CIRM fellowship. JCIB is supported by TERCEL-ISCIII-MINECO, CIBER,

Fundacion Cellex, G. Harold and Leila Y. Mathers Charitable Foundation, Sanofi, The Leona M. and Harry B. Helmsley Charitable Trust and The Ellison Medical Foundation.

Competing Interests

Authors disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests. Provenance: the authors were invited to submit this paper.

References

1. Li M, Liu GH, Izpisua Belmonte JC. Navigating the epigenetic landscape of pluripotent stem cells. *Nat Rev Mol Cell Biol.* 2012;13(8):524–535.
2. Hodges E, Molaro A, Dos Santos CO, et al. Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment. *Mol Cell.* 2011;44(1):17–28.
3. Bocker MT, Hellwig I, Breiling A, Eckstein V, Ho AD, Lyko F. Genome-wide promoter DNA methylation dynamics of human hematopoietic progenitor cells during differentiation and aging. *Blood.* 2011;117(19):e182–e189.
4. Beerman I, Bock C, Garrison BS, et al. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem Cell.* 2013;12(4):413–425.
5. Wahlestedt M, Norddahl GL, Sten G, et al. An epigenetic component of hematopoietic stem cell aging amenable to reprogramming into a young state. *Blood.* 2013;121(21):4257–4264.
6. Wu H, Coskun V, Tao J, et al. Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. *Science.* 2010; 329(5990):444–448.
7. Tadokoro Y, Ema H, Okano M, Li E, Nakauchi H. De novo DNA methyltransferase is essential for self-renewal, but not for differentiation, in hematopoietic stem cells. *J Exp Med.* 2007;204(4):715–722.
8. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet.* 2011;44(1):23–31.
9. Broske AM, Vockentanz L, Kharazi S, et al. DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nat Genet.* 2009;41(11):1207–1215.
10. Trowbridge JJ, Snow JW, Kim J, Orkin SH. DNA methyltransferase 1 is essential for and uniquely regulates hematopoietic stem and progenitor cells. *Cell Stem Cell.* 2009;5(4):442–449.
11. Wood AJ, Oakey RJ. Genomic imprinting in mammals: emerging themes and established theories. *PLoS Genet.* 2006;2(11):e147.
12. Lui JC, Finkielstain GP, Barnes KM, Baron J. An imprinted gene network that controls mammalian somatic growth is down-regulated during postnatal growth deceleration in multiple organs. *Am J Physiol Regul Integr Comp Physiol.* 2008;295(1):R189–R196.
13. Berg JS, Lin KK, Sonnet C, et al. Imprinted genes that regulate early mammalian growth are coexpressed in somatic stem cells. *PLoS One.* 2011; 6(10):e26410.
14. Besson V, Smeriglio P, Wegener A, et al. PW1 gene/paternally expressed gene 3 (PW1/Peg3) identifies multiple adult stem and progenitor cell populations. *Proc Natl Acad Sci U S A.* 2011;108(28):11470–11475.
15. Zacharek SJ, Fillmore CM, Lau AN, et al. Lung stem cell self-renewal relies on BMI1-dependent control of expression at imprinted loci. *Cell Stem Cell.* 2011;9(3):272–281.
16. Ferron SR, Charalambous M, Radford E, et al. Postnatal loss of Dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis. *Nature.* 2011;475(7356):381–385.
17. Li W, Sun W, Zhang Y, et al. Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors. *Proc Natl Acad Sci U S A.* 2011;108(20):8299–8304.
18. Liu GH, Qu J, Suzuki K, et al. Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. *Nature.* 2012;491(7425):603–607.