Cell Stem Cell
Review

Epigenetic Control of Stem Cell Potential during Homeostasis, Aging, and Disease

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Stem cell decline is an important cellular driver of aging-associated pathophysiology in multiple tissues. Epigenetic regulation is central to establishing and maintaining stem cell function, and emerging evidence indicates that epigenetic dysregulation contributes to the altered potential of stem cells during aging. Unlike terminally differentiated cells, the impact of epigenetic dysregulation in stem cells is propagated beyond self; alterations can be heritably transmitted to differentiated progeny, in addition to being perpetuated and amplified within the stem cell pool through self-renewal divisions. This Review focuses on recent studies examining epigenetic regulation of tissue-specific stem cells in homeostasis, aging, and aging-related disease.

Introduction

Tissue-specific stem cells are imbued with self-renewal potential and the capacity for differentiation to generate mature effector cells, and thus they are responsible for sustained function of tissues throughout life. Aging is associated with the progressive inability to maintain tissue homeostasis or robustly regenerate tissue after injury or stress. These processes are mediated by tissue-specific stem cells, suggesting that impaired stem cell function may underlie central cellular pathophysiologies associated with aging. Indeed, mounting evidence indicates that degenerative aging-associated changes in adult stem cells are a central driver of many age-related phenotypes (reviewed in Oh et al., 2014; Liu and Rando, 2011; Behrens et al., 2014; Rossi et al., 2008). The mechanistic basis for aging-associated stem cell decline is not completely understood, but numerous studies have shown that loss of polarity (Florian et al., 2012), mitochondrial dysfunction (Bratic and Larsson, 2013), altered autophagy (Warr et al., 2013), replicative stress (Flach et al., 2014), and accrual of DNA damage (Rossi et al., 2007; Rübe et al., 2011; Yahata et al., 2011; Wang et al., 2012; Beerman et al., 2014) all contribute to stem cell aging. In addition, increasing evidence suggests that epigenetic dysregulation is also an important mechanistic driver of stem cell aging.

Epigenetic regulation is a term used to classify heritable changes of gene expression that are not attributed to changes in DNA sequence (Waddington, 1942; Goldberg et al., 2007; Bird, 2007). Epigenetic marks, including but not restricted to DNA methylation and histone modifications, allow all cells within an organism to possess the same genetic sequence yet carry out vastly different functions. The particular epigenetic landscape of each cell both restricts and permits access to genes that collectively coordinate the transcriptional programs unique to each cell type. In differentiated cells, epigenetic regulation is used not only to coordinate ongoing cellular activity but also to restrict access to lineage-inappropriate gene programs (Hodges et al., 2011; Ji et al., 2010; Bock et al., 2012; Kaaij et al., 2013). Importantly, stem cells have potential beyond self-renewal and can differentiate into cells with distinct potentials and, in some instances, can generate a large repertoire of effector cells with enormous functional diversity. The epigenetic landscape of stem cells not only regulates the transcriptional programs that dictate the function of the stem cells themselves but must also possess the potential to coordinate differentiation toward distinct effector lineages. Stem cells heritably transmit epigenetic marks to their daughter cells, and thus marks set in the stem cell can prime lineage-specific loci for activation or repression in downstream progeny. Epigenetic alterations arising in stem cells can be perpetuated and amplified within the stem cell pool via self-renewal divisions (horizontal transmission) where they may have a direct, autonomous functional consequence in the stem cell compartment. Altered epigenomic marks propagated in this fashion can alter the clonal composition of the stem cell pool, particularly if a selective advantage or disadvantage is conferred. Clones imbued with a competitive advantage can in turn serve as the reservoir in which additional genetic or epigenetic alterations could arise and could eventually lead to malignancy (Figure 1). In addition, heritable alterations of the epigenetic landscape arising in stem cells can be transmitted to differentiated progeny with functional consequences manifest in downstream lineages (vertical transmission) (Figure 1).

This Review will focus on research that establishes the functional importance of epigenetic regulation in multiple tissue-specific stem cells and how dysregulation is associated with aging and disease. These topics will be discussed in the context of altered DNA methylation, changes in histone modifications, and synergistic relationships between epigenetic and genomic integrity.

DNA Methylation and the Regulation of Stem Cell Function and Aging

In mammalian cells, DNA methylation predominantly occurs at CpG dinucleotides. Methylated cytosine (mC) is found throughout the genome at high frequency, predominantly located at promoter regions of housekeeping and developmental regulation genes, though it is underrepresented at CpG islands (regions with a high occurrence of CpGs). DNA methylation is catalyzed

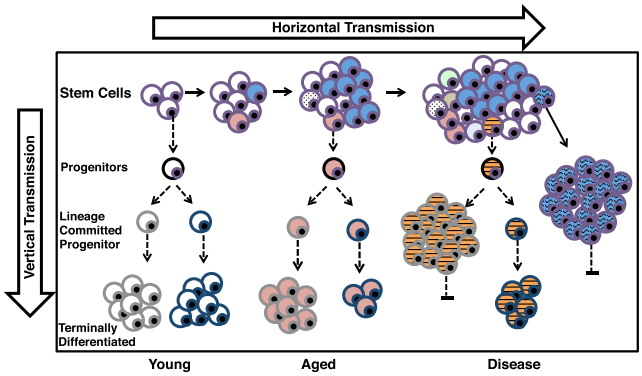

Figure 1. Stem Cell Aging and Epigenetic Dysregulation
Proper epigenetic regulation of stem cells is critical for the maintenance of tissue and is of particular importance given stem cells' capacity to heritably transmit epigenetic marks to their progeny (Vertical Transmission) and to perpetuate and amplify alterations through self-renewal divisions (Horizontal Transmission). Vertical transmission of aging-associated altered epigenetic marks can drive functional consequences in downstream progenitor or effector cells (red fill), while stem cell clones that acquire aging-associated epigenetic alterations providing a selective advantage (blue fill) can lead to an expanded stem cell pool that can serve as the cellular reservoir in which addition events—epigenetic (color fill) or genetic (pattern)—can be accrued that may eventually lead to malignancy.

by DNA methyltransferases, which coordinate the establishment (DNMT3A and DNMT3B) and maintenance (DNMT1) of methylated nucleotides, and together with regulated DNA demethylation, they generate tissue- and cell-type-specific marks that regulate gene expression to coordinate cellular function.

Dynamic Regulation of DNA Methylation and Demethylation Orchestrates Stem Cell Function

DNMT1 maintains methylation marks by binding hemi-methylated sites generated after replication and adding a methyl group to cytosines on the newly synthesized daughter strand. Compelling evidence indicates that DNMT1 coordinates the balance between self-renewal and differentiation in multiple adult stem cell compartments. In the skin, knockdown of *Dnmt1* leads to diminished self-renewal and premature differentiation of epidermal progenitor cells, ultimately leading to tissue loss (Sen et al., 2010). Similarly, conditional knockout of *Dnmt1* leads to decreased proliferation, abnormal differentiation, and compromised self-renewal of bulge stem cells (Li et al., 2012). In neural stem cells (NSCs), loss of DNMT1 prematurely drives differentiation toward astroglia (Fan et al., 2001, 2005), while intestinal stem cells lacking DNMT1 display inhibited differentiation potential (Sheaffer et al., 2014). In hematopoietic stem cells (HSCs), loss of DNMT1 leads to defects in both self-renewal and differentiation potential, leading to a skewed lineage output biased toward myelopoiesis (Trowbridge et al., 2009; Bröske et al., 2009). The altered lineage output of DNMT1-deficient stem cells also suggests that at least a subset of methylation marks present in the stem cell compartment establish the lineage potential of stem cells manifest in downstream lineages. Interestingly, many of the phenotypes associated with loss of DNMT1 mirror those associated with aging, including myeloid lineage skewing of the aged hematopoietic compartment, abnormal bulge cell differentiation, and age-related increases in astrocytes. Together, these data suggest that altered DNA methylation may play a role in underwriting the functional decline associated with stem cell aging in these tissues.

In recent years, numerous studies have demonstrated that unique DNA methylation marks are established as adult stem cells undergo differentiation (Hodges et al., 2011; Ji et al., 2010; Bock et al., 2012; Kaaij et al., 2013), suggesting that de novo methylation is critical for stem cell differentiation. Indeed, impaired differentiation is seen in postnatal *Dnmt3a* knockout mouse NSCs (Wu et al., 2010). Similarly, conditional knockout of *Dnmt3a* in HSCs leads to increased self-renewal at the expense of differentiation (Challen et al., 2012), a phenotype that is exacerbated upon ablation of both *Dnmt3a* and *Dnmt3b* (Challen et al., 2014). These studies demonstrate that tissue-specific stem cells require de novo DNA methylation to restrict self-renewal potential and direct differentiation.

DNA demethylation can occur through various processes, including active demethylation via progressive oxidation of mC catalyzed by ten-eleven translocation (*Tet*) family enzymes (Tahiliani et al., 2009; Ito et al., 2010). Demethylation by TET enzymes can be a multi-step process, in which the initial step is generation of 5-hydroxymethylcytosine (5-hmC). Interestingly, 5-hmC is quite prevalent in the brains of mice and humans (Kriaucionis and Heintz, 2009; Münzel et al., 2011), prompting analysis of the role of TETs in neural progenitor cells (NPCs). Loss of TET1 in NPCs decreases their self-renewal potential without affecting their differentiation potential. TET1-deficient mice display impaired learning and poor memory, which correlates with increased methylation and decreased expression of genes involved in the proliferation of NPCs (Zhang et al., 2013). In vitro studies using NPCs derived from *Tet3* knockout ESCs further establish the role for 5mC-oxidases in primitive neural cells, as loss of TET3 led to dysregulation of the maintenance of the NPC population associated with increased apoptosis (Li et al., 2015).

In contrast, loss of TET2 in the HSC compartment leads to a profound increase in HSC self-renewal (Ko et al., 2011; Moran-Crusio et al., 2011; Shide et al., 2012). In addition, *Tet2* knockouts also have abnormally high levels of myeloid lineage output (Ko et al., 2011; Moran-Crusio et al., 2011; Shide et al., 2012) and develop a chronic myelomonocytic leukemia (CMML)-like disease (Moran-Crusio et al., 2011). Interestingly, *Tet1* deletion also leads to enhanced self-renewal of HSCs, but *Tet1* null HSCs also have an increased bias toward B cell production and develop B cell malignancies (Cimmino et al., 2015). Consistent with demonstrated functional overlap of these TET family proteins during development (Dawlaty et al., 2011), these studies suggest a conserved role for Tet proteins in regulating HSC self-renewal, yet each protein also plays unique roles in regulating the differentiation of distinct lineages from HSCs.

In addition to TET-mediated DNA demethylation, studies have reported that growth arrest and DNA-damage-inducible (GADD45) family members can also mediate the active removal of 5mC (Barreto et al., 2007; Ma et al., 2009; Schmitz et al., 2009). GADD45 family members are best known for their role in DNA damage response. Though they do not have known enzymatic demethylase activity, they are proposed to demethylate DNA through interactions with other proteins involved in base excision and/or nucleotide excision repair (Sen et al., 2010). Together with the recruited DNA repair proteins, GADD45

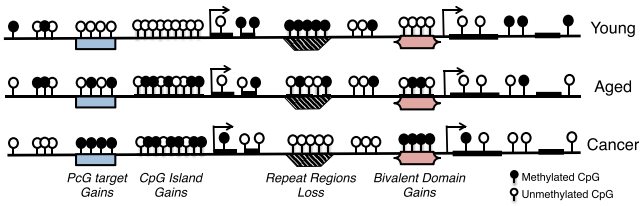

Figure 2. DNA Methylation Patterns in Stem Cells during Aging and Cancer

A representation of DNA methylation patterns of HSCs during aging (Young and Aged) and those reported for multiple types of cancers (Cancer). Though these regions illustrated are not necessarily restricted to a single category (for example bivalent domains can also be PcG targets), we present a simple overview of the aging-associated gains of methylation that are predominantly found at CpG islands near promoter regions, bivalent domains, and on PcG target regions and the loss of methylation that occurs throughout the genome and at repeat elements in HSCs.

proteins are thought to mediate demethylation of a limited set of genes to promote their transcriptional activation and mediate differentiation in several tissue-specific stem cell compartments.

Consistently, combinatory loss of GADD45A/GADD45B in epidermal progenitor cells prevented differentiation, which was attributed to impaired removal of DNA methylation at loci that are typically hypo-methylated during differentiation (Sen et al., 2010). Conversely, overexpression of *Gadd45A* or *Gadd45B* prompted premature differentiation at the expense of epidermal progenitor cell expansion, supporting a role for these proteins in regulating differentiation. In mesenchymal stem cells, demethylation of promoter regions in osteogenic lineage genes correlated with upregulation of *Gadd45A,* and knockdown of *Gadd45A* led to hyper-methylation of these regions, loss of osteogenic gene expression, and inhibition of differentiation (Zhang et al., 2011). *Gadd45B* has also been implicated to play a critical role in adult, activity-driven neurogenesis (Ma et al., 2009). Together, these studies suggest that GADD45 proteins play a role in DNA demethylation necessary for tissue-specific stem cell differentiation, though it is yet unclear what underwrites target specificity.

Altered DNA Methylation in Aged Stem Cells

As noted above, dysregulation of DNA methylation leads to aberrant stem cell function with phenotypes frequently mirroring those observed with aging, supporting the possibility that alterations of this epigenetic mark may underlie certain aspects of the aging process. Early studies examining global DNA methylation patterns in aged somatic tissues largely demonstrate age-associated hypo-methylation (Wilson and Jones, 1983; Gonzalo, 2010; Heyn et al., 2012), which was also observed in cultured cells that reached their replicative limit (Wilson and Jones, 1983). Upon more detailed analysis, several studies uncovered locus-specific DNA hyper-methylation associated with aging tissues (Maegawa et al., 2010; Grönniger et al., 2010; Rakyan et al., 2010; Zykovich et al., 2014). Regions that gain methylation with age (regardless of tissue) were commonly found in CpG islands (Christensen et al., 2009; Yuan et al., 2015; Heyn et al., 2012) and were over-represented for targets of polycomb group (PcG) proteins (discussed below) (Maegawa et al., 2010). Interestingly, certain aging-associated hyper-methylation changes universally correlate with age regardless of tissue type (Bocklandt et al., 2011; Koch and Wagner, 2011; Rakyan et al., 2010; Horvath et al., 2012), suggesting some level of coordinated control of the DNA methylome during aging. Furthermore, several studies examining DNA methylation in human peripheral blood have revealed altered profiles associated with aging (Heyn et al., 2012; Christensen et al., 2009; Bell et al., 2012). However, as the frequency distribution of blood cell types dramatically changes with age, with increased preponderance of myeloid cells and diminished lymphoid cells, analyses of whole blood may reflect altered effector cell frequencies rather than aging-specific DNA methylation differences per se. To address this, algorithms have been developed to account for differences in cell composition (Koestler et al., 2013; Houseman et al., 2012) or make reference-free adjustments using a bootstrap method to estimate error (Houseman et al., 2014). Using such approaches, recent analysis of human blood samples has indeed established that the majority of hyper-methylation seen with aging was independent of changes in cellular composition and was instead linked directly to cellular aging (Yuan et al., 2015). Because peripheral blood is composed of diverse cell types originating from a pool of stem cells, these data therefore suggest the observed aging-associated DNA methylation changes originated in the stem cell compartment with all differentiated progeny inheriting the altered methylation marks.

To more directly address the impact of aging on the DNA methylome of stem cells, recent studies have examined the global DNA methylation profiles of purified stem cells isolated from young and old mice (Figure 2). These studies show that HSCs display global hyper-methylation during aging (Beerman et al., 2013, Sun et al., 2014) concomitant with decreased 5-hmC levels (Sun et al., 2014). As has been observed with aging in other systems (Maegawa et al., 2010), aging-associated gains of DNA methylation were over-represented at loci associated with PcG binding in pluripotent stem cells (Beerman et al., 2013; Sun et al., 2014). Similarly, PcG targets were also hyper-methylated in mesenchymal stromal cells during aging or after extended passaging, though a predominance of aging-associated hypo-methylation was reported (Bork et al., 2010; Fernández et al., 2015).

These studies also assayed the correlation between age-associated DNA methylation changes and the transcriptional alterations associated with aging. Consistently, the altered DNA methylation profiles that were identified did not have a direct effect on transcription in stem cells—increased DNA methylation did not correlate with decreased gene expression, and conversely, decreased DNA methylation was not associated with increased expression of genes (Hodges et al., 2011; Ji et al., 2010; Bock et al., 2012; Kaaij et al., 2013; Beerman et al., 2013; Sun et al., 2014). That being said, these studies largely correlated gene expression to methylation changes near transcription start sites or within gene bodies. Thus, it remains possible that altered methylation marks at more distal regulation regions such as enhancers would reveal a more direct regulatory role on gene transcription (Cabezas-Wallscheid et al., 2014), though addressing this possibility is challenging given that distal regulatory regions remain poorly defined in most stem cell populations.

Another possibility unique to stem cells is that altered DNA methylation marks established in the stem cell compartment may not have a direct, autonomous effect on transcription of

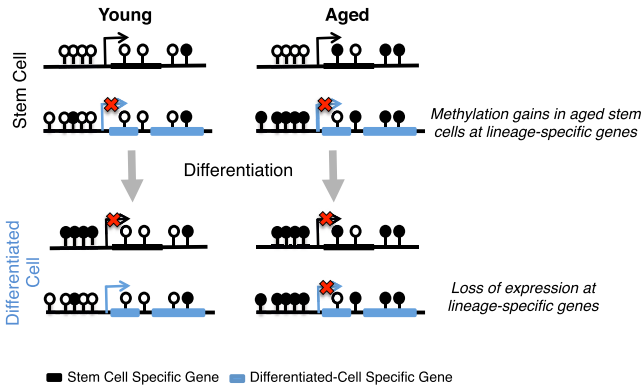

Figure 3. Aberrant Age-Associated DNA Methylation in Stem Cells Can Influence Gene Expression in Downstream Progeny
A mechanism in which DNA methylation changes in aged stem cells do not directly affect the transcription of genes at the stem cell level but instead affect the transcriptional profiles of downstream cells that inherit altered DNA methylation marks acquired in the aged stem cell compartment.

genes in the stem cell compartment, but rather may alter gene expression in downstream progeny because these heritable marks are transmitted during the process of differentiation (Figure 3). Consistent with this idea, one study showed that many of the genes associated with altered DNA methylation (both gains and losses) during HSC aging were not expressed in the stem cell compartment, but instead were exclusively transcribed in downstream lineages (Beerman et al., 2013). These data suggest that these heritable epigenetic marks acquired in the stem cell compartment during aging might alter stem cell differentiation potential by either restricting or allowing access to key lineage-specific genes, and the effects of these altered marks may only be manifest in the transcriptional programs of differentiated progeny (Beerman and Rossi, 2014).

Regulation of Stem Cell Function by Histone Modifications

An additional layer of epigenetic regulation that does not directly alter the nucleotide chemistry of DNA is mediated by histone modifications. While DNA methylation can be thought of as a binary switch with two options, methylated or unmethylated, histone modifications are substantially more complex. Four core histone proteins, H2A, H2B, H3, and H4, form an octamer that DNA is wound around, thereby compacting the genome. The unstructured N-terminal tails of histones are accessible for post-translational modifications, including acetylation, methylation, phosphorylation, sumoylation, ubquitination, and others, that change chromatin structure and accessibility. These modifications can act in concert either cooperatively or antagonistically to regulate transcriptional activity. This Review will focus mainly on histone acetylation and methylation, as these are two of the most well-studied modifications in stem cells.

Histone Acetylation in Stem Cells and during Aging

Acetylation of histone tails alters the charge of the histone, loosening compacted chromatin and allowing a more open and permissive transcriptional state. Histone acetylation is catalyzed by enzymes that either transfer an acetyl group onto a lysine—histone acetyltransferases (HATs)—or remove the mark—histone deacetylases (HDACs). The activity of HDACs and HATs is dynamic and tightly regulated, facilitating the ability of these transient marks to precisely regulate cell-specific expression.

The critical role of HAT activity in stem cell function has been revealed by numerous genetic studies. In the hematopoietic system, the striking phenotypes associated with hemizygosity of the CREB binding protein (CBP), including splenomegaly, decreased bone marrow cellularity, and diminished B cell production, suggest an important role for this HAT in HSC function (Kung et al., 2000). Indeed, conditional ablation of *Cbp* led to overall loss of HSCs, increased differentiation, and higher levels of apoptosis (Rebel et al., 2002; Chan et al., 2011). Similarly, conditional knockout of the HAT cofactor *Trrap* also led to loss of the primitive compartment and rapid death due to severe anemia and pancytopenia (Loizou et al., 2009). Mice in which the monocytic leukemia zinc-finger protein MOZ, a HAT translocated in human acute myeloid leukemia, has been genetically ablated die during embryonic development with severe loss of HSCs and other lineage-restricted progenitors (Katsumoto et al., 2008). Together these results strongly suggest that proper histone acetylation is required for HSC self-renewal.

Histone acetyltransferase activity has also been shown to be important for homeostatic maintenance and function of neural stem and progenitor cell compartments. CBP is necessary for differentiation of NPCs because it acetylates promoters of neuronal, astrocytic, and oligodendroglial genes, and loss of CBP ultimately leads to cognitive deficits (Wang et al., 2010). Loss of *Trrap* in NPCs leads to premature differentiation (Tapias et al., 2014), whereas other acetyltransferases such as *Moz* and Moz-related factor (MORF) have also been implicated in maintaining NSCs (Perez-Campo et al., 2014; Sheikh et al., 2012) suggesting that HATs can regulate both differentiation and self-renewal of NPCs.

Eighteen mammalian HDACs have been identified and are categorized into four families. Class I HDACs play a role in differentiation and appear to have a high level of functional redundancy. In intestinal stem cells, simultaneous ablation of *Hdac1* and *Hdac2* leads to loss of differentiated cells, altered polarization, and increased cell death (Turgeon et al., 2013; Zimberlin et al., 2015). Similar phenotypes occur in the hematopoietic system where the loss of class I HDACs leads to decreased bone marrow cellularity (Wilting et al., 2010) and in certain instances, loss of stem and progenitor cells (Heideman et al., 2014). In both the intestine and bone marrow, these phenotypes were not attributed to loss of self-renewal potential of the stem cell compartments, but rather to an increased level of apoptosis. Drug-induced inhibition of HDACs in NPCs also leads to altered differentiation associated with elevated apoptosis (Hsieh et al., 2004), similar to the phenotypes seen in blood and intestinal stem cells. Additionally in NSCs, simultaneous knockdown of HDAC3, HDAC5, and HDAC7 (class I and II HDACs) leads to upregulation of the cell-cycle regulator *Cdkn1a* (p21) and inhibits proliferation (Sun et al., 2007), similar to the p21 induction and cell-cycle arrest in human mesenchymal stem cells after drug-induced inhibition of HDAC activity (Lee et al., 2009).

Class III HDACs encompass the Sirtuin family, which are uniquely NAD^+ dependent, whereas the other HDAC families require Zn^{2+} as a cofactor. In mesenchymal stem cells, SIRT1 is linked to differentiation toward bone and cartilage (Simic et al., 2013). In adult NSCs, loss of SIRT1 leads to increased

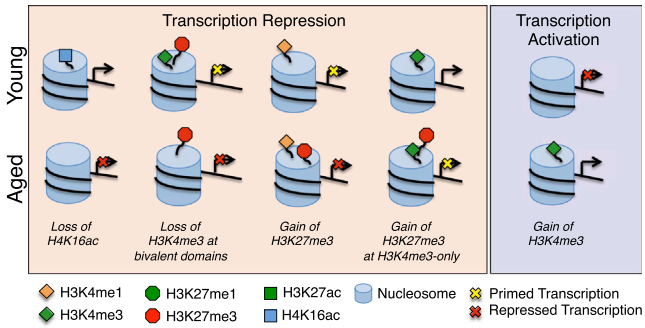

Figure 4. Histone Modifications in an Adult Stem Cell Compartment during Aging Cooperate to Influence Transcriptional Competence

A schematic of histone modification dynamics in stem cells as a consequence of aging and their influence on gene expression. The complex histone code presents many combinations to repress, activate, or prime expression of transcripts.

self-renewal and proliferation with an increased production of oligodendrocytes (Rafalski et al., 2013), and similarly SIRT2 also acts to impede differentiation toward oligodendroglia (Li et al., 2007). Interestingly, though these two HDACs inhibit oligodendrocyte differentiation, glial cells have less global acetylation compared to NPCs or neurons (Hsieh et al., 2004); thus, acetylation of specific SIRT targets must be important for astrocyte and oligodendroctye differentiation. Similarly, SIRT1 also seems to play a role in directing epidermal stem cell differentiation by promoting keratinocyte production (Ming et al., 2014). In HSCs and muscle satellite cells, loss of SIRT1 leads to premature differentiation, thereby implicating SIRT1 as a regulator of self-renewal in these cells (Ryall et al., 2015; Rimmelé et al., 2014; Matsui et al., 2012). Additionally, SIRT1 regulates quiescence of HSCs, with loss of SIRT1 leading to certain phenotypes reminiscent of aging (Rimmelé et al., 2014; Singh et al., 2013). Robust SIRT1 activity is also implicated in maintaining quiescence of muscle satellite cells, and decreased activity of SIRT1—as measured by increased H4K16ac—is associated with decreased NAD^+ levels in the activated muscle satellite cells (Ryall et al., 2015). The elevated level of H4K16ac in these activated stem cells was attributed to the metabolic shift away from fatty acid oxidation to glycolysis during activation. Interestingly, though quiescent HSCs utilize glycolysis rather than oxidative phosphorylation and thus have low levels of available NAD^+, SIRT1 activity still appears to be required to regulate histone acetylation to maintain proper HSC function and possibly aging (Rimmelé et al., 2014).

Direct histone modification analysis in adult stem cell populations during aging is challenging, as conventional chromatin IP technology requires a sizeable number of cells to analyze and most adult stem cell populations are quite rare. To circumvent this hurdle and globally examine acetylation at H4K16 (H4K16ac), immunostaining was used to assess levels of H4K16ac in HSCs during aging (Florian et al., 2012) (Figure 4). Interestingly, a sub-population of aged HSCs showed decreased levels and markedly altered cellular distribution of H4K16ac, in contrast to young HSCs with high levels of polarized H4K16ac expression. The altered H4K16ac in these aged HSCs was reversed by pharmacological inhibition of *Cdc42* concomitant with partial restoration of HSC function (Florian et al., 2012). Although the precise role that altered H4K16ac plays in aged HSCs is unknown, H4K16 hypo-acetylation has been shown to impede DNA damage response and repair of double-strand breaks (Sharma et al., 2010; Li et al., 2010), and thus H4K16 hypo-acetylation in aged HSCs could contribute to the accumulation of DNA damage observed in the aged HSC compartment (Rossi et al., 2007; Rübe et al., 2011; Yahata et al., 2011; Wang et al., 2012; Beerman et al., 2014).

Histone Methylation in Stem Cells and during Aging

In contrast to histone acetylation, methylation can serve as a context-dependent repressive or permissive mark, and it generally regulates gene expression indirectly by altering interactions with other proteins. Although methylation occurs on both lysine and arginine residues, most stem cell studies examine the methylation at lysine residues, catalyzed by histone methyltransferases (HMTs) containing a SET (Su(var)3-9, Enhancer of Zeste, Trithorax) domain. We will focus on the role of the largely activating marks of methylation on H3K4, the repressive methylation at H3K27, and bivalent domains in the regulation of tissue-specific stem cells and aging.

H3K4me Primes Gene Expression in Stem Cells

Proper regulation of H3K4me has been implicated in HSC self-renewal (Stewart et al., 2015) and in regulating proper differentiation (Kerenyi et al., 2013; Cellot et al., 2013). Interestingly, mono- and di-methylation of H3K4 at putative enhancer regions in HSCs do not directly regulate active transcription in the stem cell compartment, but they instead have been implicated in priming genes for expression in downstream progeny by establishing a permissive state that only becomes transcriptionally active upon differentiation (Attema et al., 2007; Cui et al., 2009; Orford et al., 2008). Similarly, loci marked with H3K4me1 in NPCs denote genes poised for expression, while those containing both H3K4me1 and K3H27ac are actively transcribed (Creyghton et al., 2010; Rada-Iglesias et al., 2011). In NPCs, specific H3K4me1 marks found in concert with H3K27ac were predominantly present near actively expressed genes important for NPC function, whereas marks of H3K4me1 alone were present in enhancer regions of genes that only become expressed in differentiated neural cells (Creyghton et al., 2010). Similar to H3K4me1, H3K4me3 alone also does not always predict gene expression, but it instead marks initiation of transcription (Guenther et al., 2007). For example, quiescent muscle satellite cells show marks of H3K4me3 at genes not expressed during quiescence but which subsequently become actively transcribed when the satellite cells are activated (Liu et al., 2013). These studies indicate that stem cells use histone methylation to establish a poised transcriptional state, priming genes for expression upon activation or differentiation.

The importance of H3K4me gene expression priming in adult stem cells suggests that altered methylation of H3K4 may be involved in stem cell aging. To explore this, H3K4me3 was recently examined in HSCs isolated from young and old mice, revealing that this mark increases with age and covers broader regions, as compared to those found in young HSCs (Sun et al., 2014) (Table 1). Furthermore, these permissive H3K4me3 marks were correlated, to a certain extent, with age-associated gene expression changes in the HSC compartment (Sun et al., 2014), though how these altered marks correlated

Table 1. Histone Modifications in Adult Stem Cell Compartments during Aging Cooperate to Influence Transcriptional Competence

Primary Mark	Secondary Mark	Stem Cell Population	Transcriptional State	Aging	Reference
H3K4me1		NSC	primed	N.S	Creyghton et al., 2010
	H3K27ac	NSC	active	N.S	Creyghton et al., 2010
	H3K27me3/H3K4me3	HSC	primed	N.S	Cui et al., 2009
		HSC	active	N.S	Cui et al., 2009
H3K4me2		HSC	primed	N.S	Attema et al., 2007: Orford et al., 2008
H3K4me3		satellite SC	primed/active	no sig. change	Liu et al., 2013
	H3K27me3	satellite SC	primed	increase of H3K27me3	Liu et al., 2013
		HSC	primed/active	increase	Sun et al., 2014
	H3K27me3	HSC	primed	increase of H3K27me3	Sun et al., 2014
		hair follicle SC	primed/active	N.S	Lien et al., 2011
	H3K27me3	hair follicle SC	primed	N.S	Lien et al., 2011
		mesenchymal SC	primed/active	N.S	Noer et al., 2009
	H3K27me3	mesenchymal SC	primed	N.S	Noer et al., 2009
H3K27me1		HSC	active	N.S	Cui et al., 2009
H3K27me3		HSC	repressive	no sig. change	Sun et al., 2014
		satellite	repressive	increase	Liu et al., 2013
H4K16ac		HSC	active	decrease	Florian et al., 2012

Histone modifications in stem cells, their effect, alterations associated with aging, and references. N.S, not studied.

with gene expression in downstream progeny was not explored. In contrast to what is observed in HSCs, examination of H3K4me3 in muscle satellite cells showed few differences between cells isolated from young and aged animals (Liu et al., 2013) (Table 1). However, unlike HSCs, which give rise to a very large repertoire of effector cell types, satellite cells only give rise to one effector cell type, and thus the importance of H3K4 priming and how this is altered during aging may reflect this difference.

H3K27 Methylation Controls Stem Cell Function during Homeostasis and Aging

Another extensively studied histone mark is methylation of lysine 27 on histone 3 (H3K27). The mono-methylated form of H3K27 is the least well characterized, but it has recently been demonstrated to be associated with permissive gene expression, while the other two forms of H3K27me (H3K27me2/me3) are most often negatively correlated with gene expression.

H3K27 methylation marks are added by the polycomb repressive complex 2 (PRC2), a complex that includes EED, SUZ12, and the catalytically active subunit EZH2. Numerous studies have shown that PRC2 is required for the proper function of multiple stem cell compartments. In HSCs, overexpression of *Ezh2* preserves stem cell potential during serial transplantation (Kamminga et al., 2006) and loss of *Eed* leads to HSC exhaustion (Xie et al., 2014a). Similarly, EZH2 is required for muscle satellite cell self-renewal (Woodhouse et al., 2013; Juan et al., 2011) and conditional knockout of *Ezh2* leads to loss of muscle regenerative potential and an ineffective return to quiescence after stimulation (Woodhouse et al., 2013). *Ezh2* is only expressed in active NPCs, not in quiescent NSCs, and loss of *Ezh2* drives overall decrease of neurogenesis (Zhang et al., 2014), suggesting that H3K27 methylation is important for proper differentiation in adult neural progenitors but may not be required for self-renewal.

Ezh1 can also provide enzymatic activity for the PRC2 complex through binding of EED and SUZ12, and in some instances it can compensate for loss of *Ezh2* (Shen et al., 2008); however, EZH1 and EZH2 appear to have distinct chromatin binding properties (Margueron et al., 2008). EZH1 regulation of mono- and di-methylation at H3K27 in adult HSCs is necessary for both differentiation and self-renewal (Hidalgo et al., 2012), and EZH1 has the capacity to maintain H3K27me1 in an EED-dependent manner (Shen et al., 2008). In the absence of EZH2, EZH1-mediated H3K27me3 is sufficient for normal adult HSC potential (Xie et al., 2014a). Combined loss of both *Ezh1* and *Ezh2* has functional consequences in skin stem cells as well, and it is associated with global loss of H3K27me3 (Ezhkova et al., 2011). *Suz12* knockdown also leads to loss of HSC maintenance and enhanced differentiation toward both lymphoid and myeloid lineages upon transplantation (Kinkel et al., 2015). These results are phenocopied after knockdown of JARID2, a protein associated with the core PRC2 complex. Interestingly, the number of H3K27me3 marks is mostly unchanged in aged HSCs compared to young, but there is a broadening of the coverage and intensity of the H3K27me3 signal in aged HSCs (Sun et al., 2014). Similarly, aged quiescent satellite cells also display increased coverage and intensity of H3K27me3 (Liu et al., 2013). How alteration of H3K27me3 during aging impacts stem cell function in muscle and blood is still unresolved, though it is possible that this repressive mark restricts the regenerative potential of these stem cells, which diminishes with age.

Resolution of Bivalent Domains in Adult Stem Cells during Differentiation and with Aging

Loci marked by both the active H3K4me3 and repressive H3K27me3 in ESCs are termed bivalent domains (Bernstein et al., 2006). Many of these loci are critical for lineage commitment, and bivalent status is thought to serve as a priming

mechanism that allows ESCs to either activate or repress key lineage genes upon differentiation (Bernstein et al., 2006; Mikkelsen et al., 2007). Many bivalent domains resolve to either H3K4me3- or H3K27me3-only states in NPCs derived from ESCs, yet interestingly a substantial fraction of bivalent domains persist in NPCs (Mikkelsen et al., 2007). Similarly, unique bivalent domains are found in both hair follicle stem cells and muscle satellite cells (Liu et al., 2013; Lien et al., 2011), suggesting a potential role for these domains in tissue-specific stem cells. Given that tissue-specific stem cells have the potential to differentiate into all effector cells of their respective tissues, it has been proposed that bivalent domains can facilitate the activation of cell-specific programs and repression of lineage-inappropriate programs during differentiation. Indeed, bivalent domains are present at lineage-specific genes in satellite cells, HSCs, and mesenchymal stem cells (Liu et al., 2013; Sun et al., 2014; Noer et al., 2009), and it is possible that during differentiation, bivalent domains present in the stem cell compartment are resolved to H3K4me3- or H3K27me3-only states, thereby restricting lineage commitment. Consistent with this, the frequency of bivalent domains is highest in HSCs compared to downstream multi-potent progenitors, lineage-restricted progenitors, and terminally differentiated cells, with a progressive diminution of bivalent domains during differentiation (Weishaupt et al., 2010). These data suggest an important mechanistic role for the resolution of bivalency in the orchestration of hematopoietic lineage commitment. Whether such a mechanism governs differentiation of other tissue-specific stem cells that give rise to multiple effector cell types is currently unknown.

Dysregulation of lineage potential is associated with aging of most tissues, and thus examination of bivalent domains in aged populations of stem cells could provide insight into loss of lineage potential. Interestingly, in aged HSCs, there are both gains and losses of bivalent domains compared to those found in young HSCs (Sun et al., 2014) (Figure 4). The emergence of novel bivalent domains in aged HSCs consisted mostly of gains of repressive H3K27me3 marks on regions that only had H3K4me3 activation marks in young HSCs. The age-associated acquisition of new bivalent domains occurred at an ~4-fold higher rate compared to loss of these domains, which were dominated by loss of H3K4me3 (Sun et al., 2014) (Figure 4). Similarly, in muscle satellite cells, aging-associated gains of novel bivalent domains were attributed to gains of H3K27me3 repressive marks on loci marked only for activation in young satellite cells (Liu et al., 2013) (Table 1, Figure 4). Though not fully explored in these studies, the potential restriction of genes that were in a permissive state in young stem cells, together with loss of the permissive H3K4me3 mark on bivalent domains present in young stem cells, may serve to functionally restrict the potential of the aged stem cells. Further examination of how these novel age-associated bivalent domains resolve or are maintained in differentiated progeny upon vertical transmission from the stem cell compartment will provide insights into how aging-associated alterations of these marks are fully manifest.

Furthermore, the complex nature of the histone code suggests that H3K4me3 and H3K27me3 bivalent domains are not the only histone marks used to prime loci for expression or repression during aging. As technology improves to allow more robust, high quality histone analysis on small cell numbers, it will be interesting to address how these other heritable histone modifications affect the functional potential of stem cells and their progeny during aging.

Epigenetic Crosstalk between DNA Methylation and Histone Modifications in Aged Stem Cells

Emerging evidence suggests the existence of significant crosstalk among multiple types of epigenetic modifications. As one example, DNA methylation can be restricted by the histone modification H3K4me, which has been demonstrated to inhibit recruitment of DNMT3A and DNMT3B to active promoter regions (Rose and Klose, 2014). This interaction between histone and DNA methylation is further highlighted with the loss of *Kdm1b* and *Kdm1a*, demethylases of mono- and di- methylated H3K4, which leads to disruption of DNA methylation, suggesting that removal of histone methylation is necessary for DNA methylation to occur at certain loci. In HSCs, the inability to remove the H3K4me1/2 methylation due to loss of KDM1A/LSD1 may prevent DNA methylation from repressing the HSC self-renewal program, thus contributing to the loss of differentiation potential observed in LSD1-deficient animals (Kerenyi et al., 2013).

Similar to the antagonistic relationship of DNA methylation with H3K4me, DNA methylation also appears to be mutually exclusive with repressive H3K27me marks. H3K27me3 is readily found at CpG island promoters that are hypo-methylated in ESCs (Brinkman et al., 2012; Bartke et al., 2010). However, during differentiation many of these regions acquire DNA methylation that replaces H3K27me3, perhaps as a more permanent mark of lineage repression. Interestingly, this mechanism may also be involved in restricting the potential of old stem cells because regions marked by H3K27me in ESCs preferentially become hyper-methylated in aged HSCs (Beerman et al., 2013; Sun et al., 2014) and in aged mesenchymal stem cells (Fernández et al., 2015). It has recently been suggested that the PRC2 complex directly interacts with DNMT3L (Neri et al., 2013b) and TET1 (Neri et al., 2013a) to inhibit DNA methylation, and because PRC2 core factors are significantly downregulated in aged HSCs (Beerman et al., 2013; Sun et al., 2014), loss of this direct PRC2 mediation of repression may allow DNA methylation to accumulate.

In contrast to the largely antagonistic relationship of H3K27 and H3K4 methylation with DNA methylation, H3K36me3, a hallmark of transcriptional elongation, appears to directly recruit and interact with de novo DNA methyltransferases (Dhayalan et al., 2010; Morselli et al., 2015). Consistent with this hypothesis, regions in which H3K36me3 decreased in aged HSCs also displayed DNA hypo-methylation (Sun et al., 2014). Low levels of H3K36me3 inversely correspond to expression variation associated with aging in both worms and flies (Pu et al., 2015), which might suggest that H3K36me3 recruits DNA methylation to stabilize gene expression patterns and that dysregulation or loss of H3K36me could destabilize the aged transcription networks through loss of DNA methylation. Together, these data suggest that DNA methylation and histone modifications play a complex, interactive role in maintaining and safeguarding stem cell potential during aging.

Epigenetic Dysregulation of Stem Cells as a Prelude to Disease

Aging is invariably associated with increased disease prevalence in all tissues; however, epigenetic contributions to disease are most well characterized in blood. Hematopoietic aging is associated with onset of many different malignancies including myelodysplastic syndromes (MDS), leukemias (acute myeloid leukemia [AML], CML, CLL, and CMML), myeloproliferative disorders (CMD), myeloma, and lymphomas (Hodgkins and Non-Hodgkin) (http://seer.cancer.gov). MDS encompasses a diverse group of aging-associated disorders in which aberrant DNA methylation patterns are associated with clonal expansion of HSCs with abnormal differentiation potential (Will et al., 2012). The stem cells that clonally expand in MDS patients often have mutations in key epigenetic regulators and inappropriate DNA methyl-silencing appears to be a functional driver of MDS (Issa, 2013; Itzykson and Fenaux, 2014). The importance of dysregulated DNA methylation in the pathophysiology of MDS has been clinically validated by treatment with potent inhibitors of cytosine methylation, 5-Azacytidine and 5-Aza-2′-deoxycytidine (decitabine), that act by incorporating into DNA and inhibiting DNA methyltransferases (Wijermans et al., 2000; Kantarjian et al., 2006, 2007; Fukumoto and Greenberg, 2005). Though the exact mechanism through which these agents act is unknown, global demethylation appears to de-repress inappropriately silenced genes, allowing for re-establishment of normal stem cell function (Daskalakis et al., 2002). Clonally expanded MDS HSCs are believed to serve as the reservoir through which additional mutations promote progression to AML.

More generally, clonal expansion of epigenetically dysregulated stem cells is emerging as a common mechanism underlying pre-disease states (Figure 1). Consistent with this, loss of *Dnmt3a* confers a competitive self-renewal advantage in HSCs (Challen et al., 2012, 2014), and *DNMT3A* is frequently mutated in myeloid malignancies including AML, MDS, and myeloproliferative neoplasms (MPNs) (Yang et al., 2015). Similarly, *Tet2* mutations provide a selective advantage to HSCs leading to clonal expansion (Ko et al., 2011; Moran-Crusio et al., 2011; Shide et al., 2012), and *TET2* mutations are common to MPNs, AML, MDS, and B and T cell lymphomas (Ko et al., 2015; Xie et al., 2014b). Furthermore, recent studies have begun to shed light on the clonal evolution of AML, as pre-leukemic clones have been found to almost invariably harbor mutations in critical epigenetic regulators including *DNMT3A*, *TET2*, *ASXL1,* and IDH1/2 (Corces-Zimmerman et al., 2014; Shlush et al., 2014; Jan and Majeti, 2013). Interestingly, clonal hematopoiesis indicative of clonally expanded stem cells is strikingly increased in the peripheral blood of aged, non-diseased individuals, with over-representation of mutations in the same cadre of epigenetic regulators (Busque et al., 2012; Jaiswal et al., 2014; Xie et al., 2014b). Thus, though mutations in these genes are often associated with frank disease, it is also clear that epigenetic dysregulation plays a significant role in establishing the pre-disease state.

In addition to mutation of epigenetic regulators, there is also evidence that direct alteration of epigenetic marks may also underlie disease emergence. During aging, the most frequently identified mutation in human stem and progenitors are cytosine to thymine (C-T) transitions (Welch et al., 2012), which occur when an mC is deaminated. In many cancers, C-T transitions are the most common mutation, but this transition is also highly prevalent in aged, non-diseased blood (Kandoth et al., 2013; Jaiswal et al., 2014; Xie et al., 2014b). Moreover, the direct conversion of mC to thymine permanently alters potential DNA methylation at that site. The age-associated loss of mC through deamination together with mutations in epigenetic regulators likely collaborate to underwrite the global DNA hypo-methylation associated with cancer (Watanabe and Maekawa, 2010).

DNA hypo-methylation is not the only aberrant methylation profile associated with cancer, as various cancers exhibit DNA hyper-methylation at specific promoter-associated regions (Esteller, 2003) that are over-represented as targets of PcG proteins (Widschwendter et al., 2007). Again, hyper-methylation at PcG targets is similar to phenotypes seen in aged stem cells, where atypical DNA methylation occurs on regions that are typically regulated by H3K27me3 (Maegawa et al., 2010; Beerman et al., 2013; Sun et al., 2014). Interestingly, ASXL1, which is frequently mutated in pre-leukemic clones, is a member of the PcG family, and mutations of *ASXL1* result in loss of PRC2-mediated tri-methylation of H3K27 (Abdel-Wahab et al., 2012). Further, complete loss of ASXL1 in mice leads to onset of MDS that is associated with global loss of H3K27me3 and H3K4me3 (Wang et al., 2014). This loss of antagonistic histone methylation could allow for accumulation of DNA methylation at these loci typically associated with histone modifications (as discussed above), and aberrant DNA methylation could contribute to the MDS phenotypes. Similarly, *EZH2* is also frequently mutated in cancers and MDS, further implicating a mechanism by which diminished PRC2 regulation could lead to aberrant DNA methylation at histone-regulated regions.

Cumulatively these studies demonstrate that epigenetic dysregulation plays a central role in cancer development in blood from a pre-disease state through to the emergence of frank disease. They also raise the possibility that targeted epigenetic therapies may be effective in treating such diseases (Neff and Armstrong, 2013).

Coda

Unlike genomic lesions that arise during aging, age-associated epigenetic alterations are not permanent. This raises the possibility that resetting the epigenetic landscape of aged stem cells, either globally or in a targeted fashion, may be sufficient to restore function to a youthful level. Indeed, this concept was recently tested in a study demonstrating that aged hematopoietic progenitors which were reprogrammed to pluripotency and then re-differentiated to blood cells no longer exhibited the characteristic functional decline of aged HSCs (Wahlestedt et al., 2013). This study provides proof of principle that resetting epigenetic landscapes via reprogramming to a pluripotent state is sufficient to rejuvenate the functional potential of aged stem cells.

Stem cell rejuvenation has also been achieved in muscle satellite cells and NSCs using the experimental paradigm of heterochronic parabiosis, in which the circulatory systems of young and old mice are surgically conjoined (Conboy et al., 2005; Sinha et al., 2014; Katsimpardi et al., 2014). The exposure to a youthful systemic environment was able to restore functional potential to the stem cell compartments of the old parabiont, and though the epigenome was not examined in these studies, it will be very

interesting to determine the extent to which restoration of stem cell function mediated by heterochronic parabiosis is dependent upon re-establishment of a youthful epigenetic profile.

Though few in numbers, the studies that have examined aging-associated epigenetic alterations in stem cell compartments have demonstrated that by and large the stem cell epigenome is relatively stable during aging, with only a small number of loci consistently altered (Beerman et al., 2013; Fernández et al., 2015; Liu et al., 2013; Sun et al., 2014). This raises the possibility that targeted restoration of relatively few aberrant epigenetic marks may be sufficient to rejuvenate the functional potential of aged stem cells. Until recently, however, this type of directed epigenetic manipulation was not possible, and the only available tools for modulating epigenomic states did so on a global scale. The advent of targeted genome editing tools such as Cas9/CRISPR (Jinek et al., 2012) or TALENs (Bedell et al., 2012), and the repurposing of these tools to interrogate locus-specific epigenetic marks, promises to reshape how epigenetic marks are manipulated and studied. Indeed, nuclease-deficient Cas9 or TALEs can be fused to the catalytic domains of different epigenetic regulators, offering the potential to direct locus-specific alteration of epigenetic marks (Kearns et al., 2015; Hilton et al., 2015; Mendenhall et al., 2013; Konermann et al., 2013). Such technologies, together with the growing number of studies establishing both the location and specific epigenetic marks altered during stem cell aging, provide a powerful means through which aging-associated epigenetic dysregulation can be studied and perhaps eventually mitigated.

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