

SPOTLIGHT REVIEW

Unraveling the complex regulation of stem cells: implications for aging and cancer

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Substantial progress in embryonic and adult stem cell research in the past several years has yielded a wealth of information regarding the mechanisms regulating self-renewal and differentiation, two processes often used to define stem cells. Recent evidence suggests that epigenetic as well as genetic processes maintain stem cells in a pluripotent state as well as dictate their transition to more restricted stages of development. In this review, we discuss two emerging themes in stem cell biology, epigenetic control of gene expression and post-transcriptional regulation via microRNAs. We summarize how these regulatory mechanisms facilitate various aspects of normal stem cell biology and extend the discussion to their involvement in aging and tumorigenesis, two biological phenomena intimately tied to stem cells. We speculate that aberrant epigenetic events and altered miRNA expression profiles in aged stem cell populations play important roles in carcinogenesis.

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Introduction

Stem cells are indispensable entities in most multicellular organisms in that they are responsible for forming tissues during early development and maintaining them in the adult. This property places stem cells at the forefront of both developmental biology and regenerative medicine. But what defines a stem cell and provides it with its unique properties? Because all somatic cells have the same genetic material, every cell type has the potential to express a stem cell phenotype under specific conditions that ultimately contribute to the stemness state, including extrinsic microenvironmental signals needed to maintain stem cells in their tissue-specific niches. Gurdon^{1,2} first introduced this concept when he transferred nuclei from intestinal epithelial cells of feeding *Xenopus* tadpoles to activated enucleated eggs and achieved successful generation of all cell lineages of a living tadpole. Although met with some controversy, these initial experiments displayed the pluripotent potential of somatic cells. Thus, it is not necessarily the DNA sequence that distinguishes stem cells from their more mature counterparts, but rather how the DNA is organized, regulated and/or modified. Identification of the genetic and epigenetic mechanisms that regulate stem cells is necessary to understand how these unique cells transition from a pluripotent to a more restricted state.

The blood-forming stem cells of the hematopoietic system are perhaps the best characterized adult stem cell population and was the first stem cell population to be successfully used therapeutically.³ Like all stem cells, hematopoietic stem cells (HSCs) possess the ability to self-renew and differentiate into multiple mature cell types. HSCs must balance self-renewal and differentiation to ensure adequate numbers of cells are provided to sustain hematopoiesis throughout the lifespan of an organism. Over the past several years much progress has been made in determining the transcriptional profiles of HSCs and their more differentiated progeny in an effort to define a stemness profile that distinguishes stem cells from more mature cells.^{4–7} Akashi *et al.* compared the gene expression profiles of HSCs, multipotent progenitor cells, and lineage-restricted common lymphoid and myeloid progenitor cells and found that the self-renewing stem cells expressed transcripts from multiple differentiated hematopoietic lineages, whereas progenitor cell populations were more limited in their expression profiles. This suggests that the stem cell state is characterized by an open chromatin structure with accessibility to numerous differentiation programs, whereas lineage commitment is associated with a restricted expression profile. More recently, several groups are beginning to define the chromatin organization state and epigenetic modifications specific to stem cells.^{8–10} It seems that epigenetic programs, including chromatin remodeling and modification, provide additional layers of complexity that influence the genetic repertoires of specific cell types. These modifications likely maintain the stem cell state and regulate differentiation into various lineages by altering the transcription profiles of each developmental stage.

Yet another layer of complexity that influences the stemness state can be ascribed to microRNAs (miRNAs), small non-protein-coding RNAs that negatively regulate gene expression at the post-transcriptional level.^{11,12} Although a multitude of transcription factors have been identified as important regulators of stem cell self-renewal and differentiation,^{13–16} post-transcriptional modifications, mediated mainly by miRNAs, are emerging as important determinants of stem cell biology. Several studies indicate that stem cells express specific profiles of miRNAs that are thought to regulate self-renewal and differentiation by negatively regulating expression of specific genes. Recent studies have analyzed the miRNA expression profiles in murine hematopoietic stem and progenitor cells.^{17–19} Results indicate that miRNAs are differentially expressed in hematopoietic lineages and that expression of specific miRNAs can alter differentiation potential. Because undifferentiated HSCs display an open chromatin profile and express many lineage-specific genes, it seems that selective gene silencing by miRNAs may be a key factor regulating hematopoietic differentiation events.

But what of these regulatory processes when stem cells age, and more importantly, do these processes contribute to the aging process? In the hematopoietic system, we and others have

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shown that both quantitative and qualitative changes do occur in the stem cell population with age.^{20–24} Further, in mice, the extent of the aging of HSCs is strain dependent, highlighting the importance of genetic influences on the aging process.^{25–28} In addition to the genetic factors regulating stem cell aging, it is likely that epigenetic changes in chromatin structure play important roles in regulating gene transcription and thus influence the aging process.

Several studies have shown that epigenetic changes accompany the normal aging process^{29–31} and alterations in DNA methylation and chromatin structure have been studied extensively in cancer development.³² Yet, very little progress has been made in evaluating the temporal epigenetic changes in stem cell populations. Recent data also suggest that epigenetic disruption of tissue-specific stem and progenitor cells may play a role in cancer development.³³ The correlation between aging and loss of epigenetic control is consistent with the established connection between aging and cancer. Thus, it seems that the age-associated epigenetic changes that occur in stem cells may overlap with those that facilitate tumorigenesis. Likewise, miRNAs play essential roles in normal stem cell division and differentiation^{34,35} and have been reported to regulate these processes in cancer cells.^{36,37} Few studies have assessed the age-associated changes in miRNA profiles, but it seems likely that these changes may coincide with those that contribute to malignancy – especially in stem cell populations. The culmination of aberrant epigenetic events in the form of chromatin modifications and hypo- and hypermethylation of DNA, in addition to erroneous miRNA expression profiles in aged stem cell populations, likely play important roles in cancer development.

This review discusses the genetic and epigenetic mechanisms that regulate stem cell function and addresses how these regulatory processes contribute to both aging and cancer.

Mechanisms of epigenetic regulation

The transition of multipotent stem cells to a more differentiated state is associated with simultaneous activation and inactivation of specific genes. The promiscuous expression of many lineage-specific genes in primitive stem cells gradually decreases as cells reach a more mature state. Recent studies suggest that the establishment of cellular identity (making the decision to self-renew or differentiate) is dependent not only on transcription factors that mediate cellular transcriptional profiles but also on the epigenetic status of the DNA, including chromatin structure and its modification in addition to DNA methylation of CpG dinucleotides.³⁸

In the nucleus, the DNA helix is wrapped around core histones (H2A, H2B, H3 and H4) to form the ‘beads on a string’ structure that is then folded into chromatin. Chromatin is further organized into euchromatin and heterochromatin, architectures associated with transcriptional activation and inactivation, respectively. Nucleosomes are considered the core units of DNA and consist of two copies of each of the core histones wrapped by 146 base pairs of DNA.³⁸ The covalent epigenetic modification of DNA itself and of the core histones determines whether DNA is in an active or inactive state, and thus mediates the repertoire of genes expressed in a cell at any given time. The proteins that regulate the modification and remodeling of chromatin interact with each other and often form large protein complexes (Table 1).

Covalent modification of N-terminal tails of histones plays an important role in regulation of gene expression. Modifications include acetylation, methylation, phosphorylation and

ubiquitination,³⁹ and the transcriptional status of genes correlates with specific modifications to core histones.⁴⁰ Transcriptionally active genes are hyperacetylated, whereas hypoacetylation is associated with gene silencing. Acetylation of the amino-terminal histone tails is thought to loosen the interactions between neighboring nucleosomes and result in a more open chromatin structure.⁴¹ In contrast to histone acetylation, which is associated with active gene transcription, direct alterations to the DNA sequence, in the form of methylation at CpG dinucleotides, are associated with stable gene silencing and the formation of heterochromatin structures.⁴² DNA methylation can block transcription factors from binding to their target sequences and repress gene expression by interacting with MeCP2 and other methyl-CpG-binding proteins that bind methylated DNA and form complexes with histone deacetylases (HDACs) that in turn repress transcription (Figure 1).^{43–45} Thus, it seems that the gene expression program of stem cells must maintain a critical balance between transcriptional activation and inactivation mechanisms to allow cells to maintain a pluripotent state but also differentiate into more specialized states.

Epigenetic regulation of stem cells by polycomb group proteins

Notably, in stem cells, a particular class of transcriptional repressors, the polycomb group (PcG) proteins have been identified as important regulators of cell self-renewal and cell fate determination decisions.^{9,10,46–48} PcG regulators were first described in *Drosophila*, where they repress homeotic genes controlling segment identity in the developing embryo.^{49–51} Mechanisms of PcG-mediated stable gene repression are highly conserved from fruit fly to human⁵² and include the establishment of a repressive chromatin structure, inhibition of chromatin remodeling machinery, inhibition of transcriptional initiation machinery, and inhibition of enhancer/promoter interactions to facilitate transcription.⁴⁸ There are two distinct polycomb repressive complexes (PRCs), PRC1 and PRC2, that associate at the initial site of repression and act through epigenetic modifications of chromatin to initiate gene silencing.^{51,52} PRC1 contains Cbx, Mph, Ring, Bmi-1, and Me118. PRC2, which initiates silencing by catalyzing histone H3 Lysine-27 (H3K27) methylation, contains Ezh2, Suz12, and Eed.^{53,54} H3K27 methylation is thought to recruit PRC1 binding to chromatin to maintain stable gene silencing.^{55,56}

Two recent studies have assessed the role of PcG proteins in mammalian development by mapping the binding sites of PRCs in murine and human embryonic stem (ES) cells.^{9,10} Reports indicate that PcG proteins bind to regions of the genome associated with transcription and development, indicating that PcG target genes are transcription factors with important roles in developmental processes. The most prominent observation is that PcG proteins silence transcriptional regulators that promote differentiation in ES cells; the subsequent activation of such genes coincides with differentiation.^{9,10} Thus, Polycomb complexes are required for maintaining pluripotency and differentiation potential in ES cells.

Aside from their role in regulating repressive chromatin states during development, PcG proteins play a role in cellular self-renewal and proliferation. Specifically, several studies indicate that PcG genes are involved in regulating both normal and leukemic hematopoiesis by controlling stem and progenitor self-renewal and proliferation. The PRC1 member Bmi-1, whose expression is restricted to primitive mouse and human bone marrow cells, is necessary for self-renewal and optimal

Table 1 Examples of common epigenetic modifying complexes and their mechanisms of action

Modifying agents	Mechanism of action and/or affect on gene expression	Examples
DNA cytosine methyltransferase	Catalyze methylation of genomic DNA in mammals, commonly at the 5-position of the cytosine residue within cytosine–guanine dinucleotides (CpGs); Dnmt1 is a maintenance methyltransferase that restores methylation patterns after replication; Dnmt3a and Dnmt3b initiate <i>de novo</i> methylation and establish new DNA methylation patterns ¹¹⁵	Dnmt1, Dnmt3a, Dnmt3b, Dnmt2, Dnmt3l
Histone methyltransferase	Catalyze the addition of methyl groups to histones; mediate both gene activation (via methylation of H3 K4) and repression (via methylation of H3 K9) ¹¹⁶	H3K4 MTases, Suv39h1/Suv39h2, G9a, Eu-HMTase1, ESET/SETDB1
Histone acetyltransferase (HAT)	Catalyze histone acetylation, which is thought to increase DNA accessibility through neutralization of the positive charge of lysine residues; can also modify factors other than histones to mediate transcriptional activation ¹¹⁶	Gcn5 family proteins, MYST proteins, p300/CBP, TAF250, ACTR/SRC1 nuclear receptor cofactors
Histone deacetyltransferase (HDAC)	Catalyze the removal of acetyl groups from histones, which acts to silence gene expression; can also modify factors other than histones to mediate transcriptional repression ¹¹⁶	HDAC I family, HDAC II family, Sirtuin family (Sir2)
ATP-dependent remodeling complexes	Use the energy of ATP hydrolysis to locally disrupt or alter the association of histones with DNA; introduce superhelical torsion into DNA which can alter chromatin accessibility to proteins and restriction endonucleases ^{38,117}	SWI2/SNF2 proteins, ISWI enzymes, Mi-2/NuRD proteins
Polycomb group proteins	Form multimeric complexes; bind to DNA and block access of DNA to transcription factors; interact with HDACs and/or methyltransferases to mediate gene silencing; can also directly mediate histone methylation ^{10,41}	Bmi-1, Eed, Ezh2, Suz12, Mel-18, Rae28
Methyl-CpG-binding proteins	Most bind preferentially to methyl CpG DNA and mediate methylation-dependent repression of transcription ¹¹⁸	MECP2, MBD1–4, Kaiso

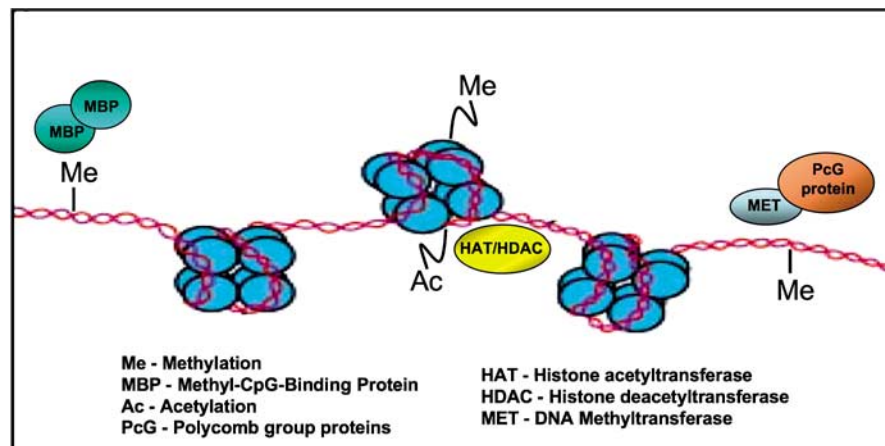


Figure 1 Epigenetic modifications that regulate gene expression. CpG dinucleotides are often methylated by DNA METs. MBPs and PcG proteins can bind to regions of DNA methylation to facilitate gene silencing. Histones are also modified by the addition of methyl and/or acetyl groups. Specific modifications can have differential effects on gene expression. Advancing age and tumorigenesis are both associated with an overall loss of repressive complexes (i.e. loss of methylation at CpG dinucleotides), ultimately resulting in dysregulation of normal gene expression patterns. Additionally, in aged stem cell populations, epigenetic modifications in the form of activation of oncogenes and silencing of tumor suppressors may contribute to cancer development.

proliferative capacity of adult HSCs.^{57,58} In competitive repopulation experiments, bone marrow and fetal liver derived *Bmi-1*^{-/-} cells were unable to contribute to long-term hematopoiesis, suggesting cellular intrinsic impairment of self-renewal capacity. *Bmi-1* was first identified as an oncogene that interacts with c-myc in the formation of mouse B- and T-cell lymphomas.⁵⁹ *Bmi-1* is an inhibitor of the INK4a-ARF tumor suppressor locus, which encodes regulators (p16^{INK4a} and p19^{ARF}) of the primary senescence checkpoint in human and rodent primary cells.⁶⁰ Over-expression of *Bmi-1* in mouse embryonic fibroblasts was shown to down-regulate p16^{INK4a} and p19^{ARF}, delay replicative senescence, and promote immortalization.^{61,62} Thus, it is not surprising that over-expression of *Bmi-1* in the lymphocyte compartment of mice leads to the development of B- and T-cell lymphoma and that *Bmi-1* is necessary for the proliferative activity of leukemic stem and progenitor cells.⁴⁷ These data provide evidence that the machinery regulating normal hematopoiesis is also critical for maintaining the leukemic phenotype and thus emphasize the point that cancer stem cells may arise from dysregulation of normal stem cell self-renewal pathways.^{63–65} *Bmi-1* protein levels decline during the replicative senescence of human fibroblasts⁶⁶ and levels of p16^{INK4a} and p19^{ARF} accumulate. It has been hypothesized that increased cellular surveillance and tumor suppressor activity account, at least in part, for the age-related decline in stem cell function.⁶⁷ Thus, mechanisms of cellular proliferation and senescence must remain balanced throughout the lifetime of an organism to prevent tumor formation and maintain stem cell populations, the decline of which may result in premature aging.

Several other PcG genes have been implicated as regulators of HSC self-renewal and proliferation. Heterozygosity for an *eed* null allele in mice resulted in extensive hyperproliferation of bone marrow myeloid and lymphoid progenitor cells.⁶⁸ This indicates that *eed* has important anti-proliferative activity in bone marrow progenitors and that its absence may lead to tumor formation. The finding that *eed* and *Bmi-1* have opposite effects on cell proliferation suggests hematopoietic cell division is regulated by a balance between enhancing and repressing PcG proteins.⁴⁸ Similarly, the knock-down or loss of *mel-18* promotes self-renewal of HSCs, thus indicating its involvement in the negative regulation of hematopoietic cell proliferation.⁶⁹ Further, *Ezh2*, which is highly expressed in purified HSCs and is down-regulated upon differentiation, was found to prevent stem cell exhaustion after multiple rounds of serial transplantation.⁴⁶ The authors hypothesize that *Ezh2*, which acts as a transcriptional repressor, stabilizes chromatin structure (which is altered during replicative stress and/or the natural aging process) and thus maintains the quality of the HSC population. Another PcG protein, *rae28/mph*, was shown to perform functions similar to *Bmi-1* and *Ezh2* in that it is involved in the positive regulation of HSC proliferation.⁷⁰ Fetal liver cells from *rae28/mph* deficient mice were impaired in their ability to form hematopoietic progenitors and displayed progressive reduction of colony-forming units in the spleen during development.⁷⁰ Together these data provide compelling evidence that Polycomb genes play important roles in hematopoiesis through epigenetic regulation of HSC self-renewal, proliferation, and lineage commitment.

Epigenetic alterations in aged stem cells – implications for cancer

The most notable epigenetic change that accompanies aging is the progressive loss of 5-methylcytosine content within gene

regulatory sites and repetitive sequences.^{71–73} Manel Esteller's group studied the epigenetic profiles of 80 pairs of monozygotic twins ranging in age from 3 to 74 years old and found that older twins exhibited large differences in their overall content and distribution of 5-methylcytosine DNA and histone acetylation, compared to pairs of young twins which were largely epigenetically indistinguishable.³⁰ The epigenetic changes that accumulated with age had a dramatic effect on gene expression; thus, the authors propose that the so-called 'epigenetic drift' that accompanies the aging process may have important effects on the health of older individuals. Perhaps not surprisingly, this same loss of DNA methylation has been noted extensively in tumor development.⁷⁴ It has been hypothesized that the epigenetic loss of gene repression may be a contributing factor to the progressive dysfunction observed during mammalian aging and in malignant transformation.²⁹ Global DNA hypomethylation can result in gene-specific oncogene activation, thus contributing to chromosomal instability and increased tumor frequency. In addition to widespread hypomethylation, the promoters of many tumor suppressor genes including RB1, p16, and Wnt associated factors show increased DNA methylation³³ during tumor formation. Given their intrinsic ability to self-renew and because stem cells are inherently long-lived and have likely been exposed to subtle genomic and environmental insults, aged stem cells are prime targets for malignant transformation. A well accepted hypothesis is that aging promotes conditions permissive to cancer development.^{75,76} In addition to direct genetic and epigenetic alterations to chromatin, the aging microenvironment may promote tumorigenesis.⁷⁶ Over time, the tissue microenvironment accumulates cells that have undergone cellular senescence and these cells secrete factors that can disrupt tissue architecture and stimulate nearby cells to proliferate, thus creating a pro-oncogenic microenvironment.⁷⁷ Conversely, tissue-specific stem cells may themselves undergo senescence. The tissue specific depletion of stem and progenitor cell pools (a phenomenon often observed in accelerated aging phenotypes) can impact both aging and tumor development. Perhaps not surprisingly, accelerated aging phenotypes often display high incidence of malignancy.^{78,79} Recently the involvement of epigenetic silencing in regulating expression of the Werner syndrome gene (WRN), a DNA helicase that belongs to the RecQ family was investigated.⁸⁰ Werner syndrome is an autosomal recessive disease characterized by premature aging and a high incidence of malignancy.^{81,82} The authors report that the WRN gene undergoes CpG-island promoter methylation-associated gene silencing in human cancer cells, a phenomenon often associated with tumor suppressor genes.⁸³ They propose that WRN acts as a tumor suppressor, the inactivation of which contributes both to accelerated aging and cancer development. Thus, the epigenetic mechanisms associated with aging and cancer do in fact overlap. It would be interesting to know the rate at which these silencing mechanisms are at work in aged stem cell populations. It is likely that epigenetic changes in the form of hypomethylation of oncogenes and specific hypermethylation of tumor suppressors in aged stem cell populations along with local microenvironmental perturbations may be important early events in the multistep progression from a normal stem cell to a cancer stem cell.

miRNA expression and mechanism of action

Complex regulatory mechanisms are required to keep stem cells in an undifferentiated, self-renewing state and mediate their

differentiation and proliferation. Recently, miRNAs have been implicated as important regulators of a wide variety of cellular processes including developmental timing,^{84–86} carcinogenesis,^{36,87,88} and cellular fate determination.^{17,89} miRNAs are a group of small non-coding RNAs, ~22 nucleotides in length, that play important regulatory roles in animals and plants by regulating the stability or translational efficiency of target mRNAs.^{11,90} In 1993, Ambros and co-workers^{84,85} discovered the founding member of this abundant class of small RNAs, *lin-4*, while studying developmental timing in *Caenorhabditis elegans* (*C. elegans*). They found that mutations in the *lin-4* gene resulted in significant developmental defects, and went on to demonstrate that this gene encoded a small RNA that regulated the translation of a target message, *lin-14*, by binding to its 3' untranslated region (3'UTR). Seven years later, another *C. elegans* gene, *let-7*, was found to encode a small 21 nucleotide RNA that was also involved in directing developmental timing.⁸⁶ These initial experiments set the stage for subsequent investigation of this diverse group of genes, which

more recently have been implicated in the complex regulation of stem cell self-renewal and differentiation.

miRNAs are initially transcribed as long precursor transcripts in the nucleus and then subsequently processed into hairpin RNAs of ~60–80 nucleotides in length.⁹¹ Drosha, the RNase-III endonuclease, and DGCR8 (a product of the DiGeorge syndrome critical region 8) form a processing complex that recognizes the precursor transcript and excises the hairpin structure that contains the complete miRNA sequence.^{11,92} The hairpin structures are then actively transported to the cytoplasm where a second enzyme, Dicer, performs a final cleavage to generate the mature double-stranded ~22 nucleotide RNA molecule. One strand of the miRNA is then incorporated into a large RNA-induced silencing complex that binds to the target mRNA.⁹³ The degree of complementarity between the miRNA and its target mRNA determine degradation or translational inhibition of the message, with the latter being the outcome of imperfect base-pairing (Figure 2).⁹⁴

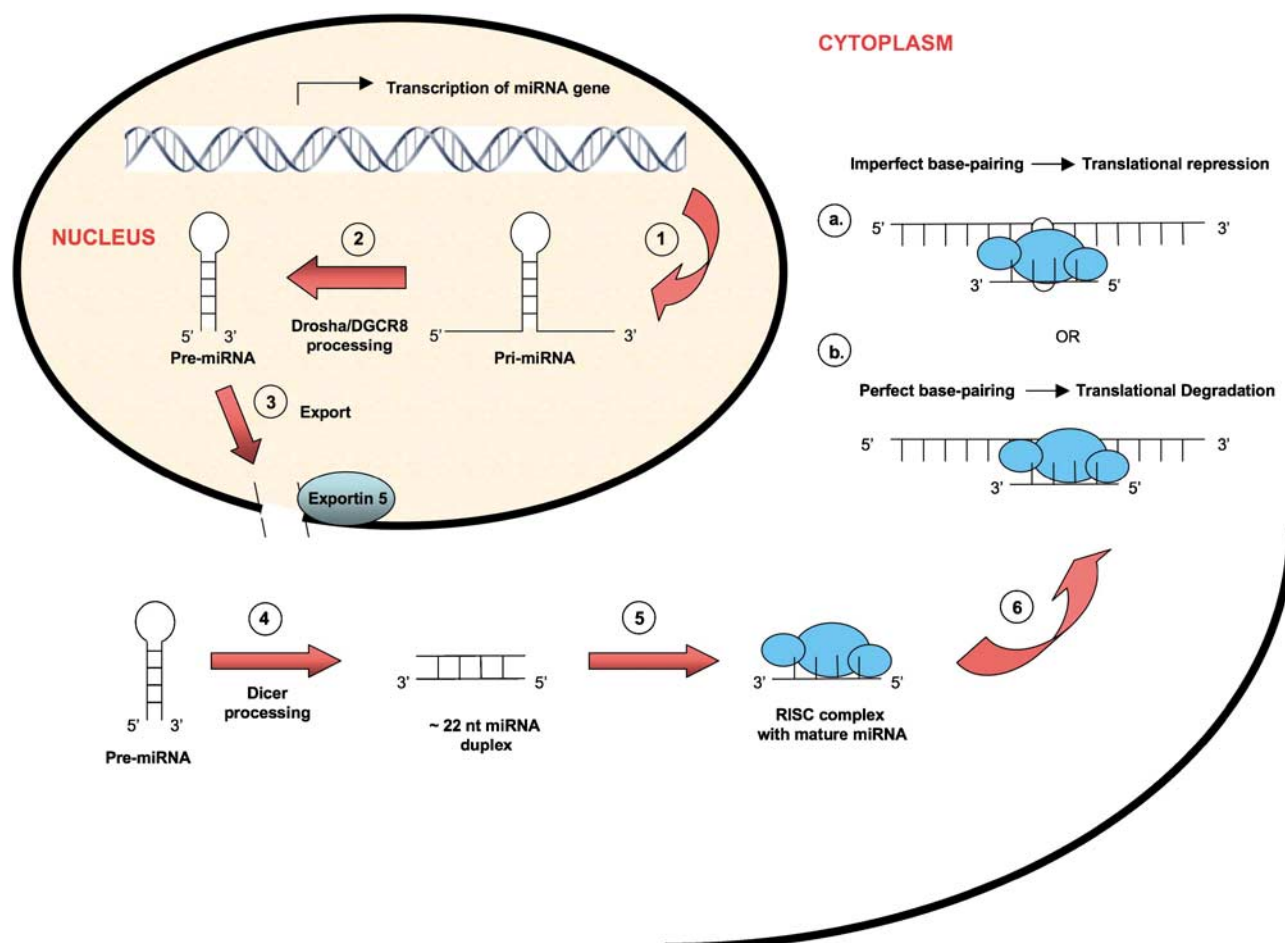


Figure 2 miRNA processing and mechanism of action. (1) In the nucleus, miRNAs are transcribed by RNA polymerase II to generate primary transcripts (pri-miRNAs). (2) These transcripts are processed by the Drosha/DGCR8 complex resulting in the formation of ~70 nucleotide pre-miRNAs. (3) The Ran-GTP-dependent-factor Exportin 5 then transports the pre-miRNAs into the cytoplasm, where (4) Dicer processes them into ~22 nucleotide duplexes that contain the mature miRNA product. (5) One strand of the miRNA duplex incorporates with the RNA-induced silencing complex (RISC), which directs the miRNA to target mRNAs. (6) Partial sequence complementarity between the miRNA and the target results in (a) translational repression, whereas complete sequence complementarity is thought to result in (b) translational degradation. The repertoire of expressed miRNAs in immature undifferentiated stem cells and tumor cells is reduced compared to mature differentiated cells. A similar miRNA expression profile likely exists in aged stem cell populations, thus providing an impetus for tumorigenic transformation in aged tissues.

miRNAs and stem cells

To date, miRNAs have only been identified in multicellular organisms. This suggests that miRNAs may be essential for organisms to differentiate into multiple cell or tissue types, or may be involved in regulating maintenance of a specific differentiation (or undifferentiated) state.⁹⁵ miRNAs are therefore attractive targets when investigating the regulatory mechanisms of stem cells. Numerous studies have begun to investigate the role of miRNAs in mammalian development and differentiation, many of which have used Dicer-deficient cells to study the functional roles of miRNAs.^{34,96–100} Mice harboring a chromosomal lesion in the *Dicer1* gene were found to die at embryonic day 7.5 and these mutant mice displayed a complete loss of multipotent stem cells.¹⁰⁰ Subsequent studies by this group demonstrated that ES cells from Dicer-deficient mice had profound proliferation defects resulting from improper maturation of mature miRNAs.⁹⁸ The authors proposed that the proliferation defect may be attributed to the loss of a miRNA that is normally required to repress a cell cycle inhibitor. Another study conducted in *Drosophila* by Hatfield *et al.*³⁴ shows that Dacapo (Dap; a homolog of the p21/p27 family of cyclin-dependent kinase inhibitors) levels are elevated in Dicer-1 (*dcr-1*) deficient germline stem cells (GSCs) that display a cell division defect. They hypothesize that miRNAs are required for GSCs to transit the G1/S checkpoint by repressing the G1/S inhibitor Dap. Together, these studies emphasize the importance of miRNAs in regulating pathways of stem cell division. Another study indicated that Dicer is also required for *in vitro* and *in vivo* differentiation of mouse ES cells, demonstrated by the failure of Dicer-deficient ES cells to form embryoid bodies in culture, as well as their inability to contribute to the generation of chimeric mice when injected into blastocysts.³⁵ The authors suggested that the differentiation defect may be attributed to the fact that the cells cannot produce mature miRNAs, the expression of which may be involved in regulating transcription factors important in cellular differentiation. Thus, it seems that miRNAs play pivotal roles in regulating multiple stem cell-specific biological processes, and that at least some of the mechanisms of regulation may be conserved across multiple species. Indeed, several ES-specific miRNA homologs have been identified in mouse and human cells.^{101,102} The homologs, which share similar genomic organization and expression patterns, are downregulated upon differentiation and thus are thought to play important roles in maintenance of the mammalian pluripotent cell state. However, all of the cloned miRNAs from the above studies are mammalian cell specific, which suggests that complete conservation of miRNA function does not exist.

HSC-specific miRNA expression patterns

Given that the expression pattern of specific miRNAs is often developmentally regulated and/or tissue specific, one approach to study these small RNAs is to examine their differential expression at various stages of development in one particular tissue or organ system. Conducting this type of analysis in the hematopoietic system revealed that miRNAs modulate lineage differentiation, and thus are key components of the molecular machinery that control mouse hematopoiesis.^{17,103} Chen *et al.* cloned ~100 miRNAs from mouse bone marrow and extensively studied three (miR-181, miR-142s and miR-223) in detail because of their differential or preferential expression in hematopoietic tissues. They showed that miR-181 is preferen-

tially expressed in B-lymphocytes (B220+); miR-142s is expressed mainly in myeloid (Gr-1+ and Mac+) and B-lymphoid (B220+) lineages; and miR-223 expression is confined to myeloid lineages (Gr-1+ and Mac+). To test the possibility that expression of these miRNAs does in fact influence lineage differentiation, they ectopically expressed each miRNA in lineage negative (Lin-) hematopoietic progenitor cells. The expression experiments supported roles for miR-181 in directing B-cell development and revealed that miR-142s and miR-223 are involved in modulating T-cell lineages. They further showed that transplantation of miR-181 expressing Lin- bone marrow cells into lethally irradiated mice resulted in a substantial increase (32 to 80%) in the percentage of B-lymphoid cells in peripheral blood. This study provides strong evidence that miRNAs play important roles in modulating hematopoietic lineage differentiation, and demonstrates the importance of post-translational regulatory mechanisms in mediating hematopoietic cell fate decisions. A recent study by Monticelli *et al.*¹⁹ reiterates and expands on these findings, providing further evidence that miRNAs influence hematopoietic lineage commitment and cellular identity. Using oligonucleotide arrays to analyze miRNA expression in murine hematopoietic cells, they found that cells at similar stages of differentiation share similar miRNA expression profiles. For example, expression patterns of early B and T precursor cells are more closely related to each other than to their more differentiated progeny, mature splenic B cells and naïve T cells.¹⁹ The challenge now is to identify targets of the miRNAs that influence hematopoiesis. Chen *et al.*¹⁷ observed that miR-181 affected the differentiation of both B and T lineages and that its over expression did not completely block myeloid development. This suggests that miR-181 may act through repression of several targets and may function more as a lineage modulator rather than an on/off switch. A similar scenario likely exists for other miRNAs at work in the hematopoietic system.

miRNAs, cancer and stem cells – implications for aging

Many human miRNA genes are located at sites known to be involved in cancers such as regions of loss of heterozygosity, regions of amplification, and genomic breakpoint regions thus supporting their involvement in tumorigenesis.^{95,104,105} The first study linking miRNAs and cancer involved a deletion at chromosome 13q14, the most frequent chromosomal abnormality observed in chronic lymphoid leukemia (CLL).³⁶ Two miRNA genes, *miR15* and *miR16*, were identified to lie in the 30 kilobase minimal region of loss in CLL and deletion of this region correlated with down-regulation of both genes. More recent data from this group provided evidence that *miR15* and *miR16* potentiate the normal apoptotic response by repressing the anti-apoptotic gene *BCL-2*.¹⁰⁶ Thus, these miRNAs act as tumor suppressors and their re-expression in lymphoma cells harboring the 13q14 deletion was shown to promote apoptosis in leukemic cells. Another miRNA cluster, denoted *miR-17-92*, is located in a region commonly amplified in B cell lymphoma.¹⁰⁷ This genomic cluster contains six miRNAs, all of which display elevated expression levels in primary lymphomas.^{88,108} A study conducted by He *et al.*⁸⁸ provided the first solid evidence that miRNAs can act as oncogenes by demonstrating that enforced expression of the miR-17-92 cluster accelerated tumor development in a mouse model of B cell lymphoma. In sum, data from these studies suggest that miRNA-mediated tumorigenesis results from either down-regulation of tumor suppressors and/or upregulation of oncogenes.¹⁰⁹

Surprisingly, a study conducted by Golub and co-workers¹¹⁰ revealed that tumors from both mouse and human displayed a general down-regulation of miRNAs compared to normal tissues. They used an innovative bead-based flow cytometric miRNA expression profiling method to determine the expression pattern of all known mammalian miRNAs in a diverse panel of cancer samples. They determined that miRNA expression profiles could be used to group cancers of different developmental origin and found that miRNAs could also be used to distinguish tumors from normal cell types. Their observation that miRNA expression was higher in normal tissues compared to tumors led them to hypothesize that miRNA expression profiles reflect states of differentiation. This hypothesis is in agreement with Strauss *et al.*,¹¹¹ who demonstrated that immature samples (ES cells, embryoid bodies, and day 11 mouse embryos)

exhibited a less complex miRNA profile than did more mature somatic tissues. They too suggested that the degree of cellular or tissue differentiation can be characterized by a particular miRNA signature. The obvious parallel that can be drawn is that undifferentiated stem cells display miRNA expression profiles reminiscent of cancer cells, thus further facilitating the potential transition from a normal cell to a cancer cell.

Despite the observation that most miRNAs display reduced expression in cancer cells, there are exceptions. For example, the miR-17-92 cluster, as mentioned above, is commonly over-expression in B cell lymphoma and a causal role for these genes in cancer has been conclusively demonstrated.⁸⁸ Perhaps not surprising, although having reduced expression of many miRNAs, stem cells retain the expression of those that are important to maintain the stemness state, including

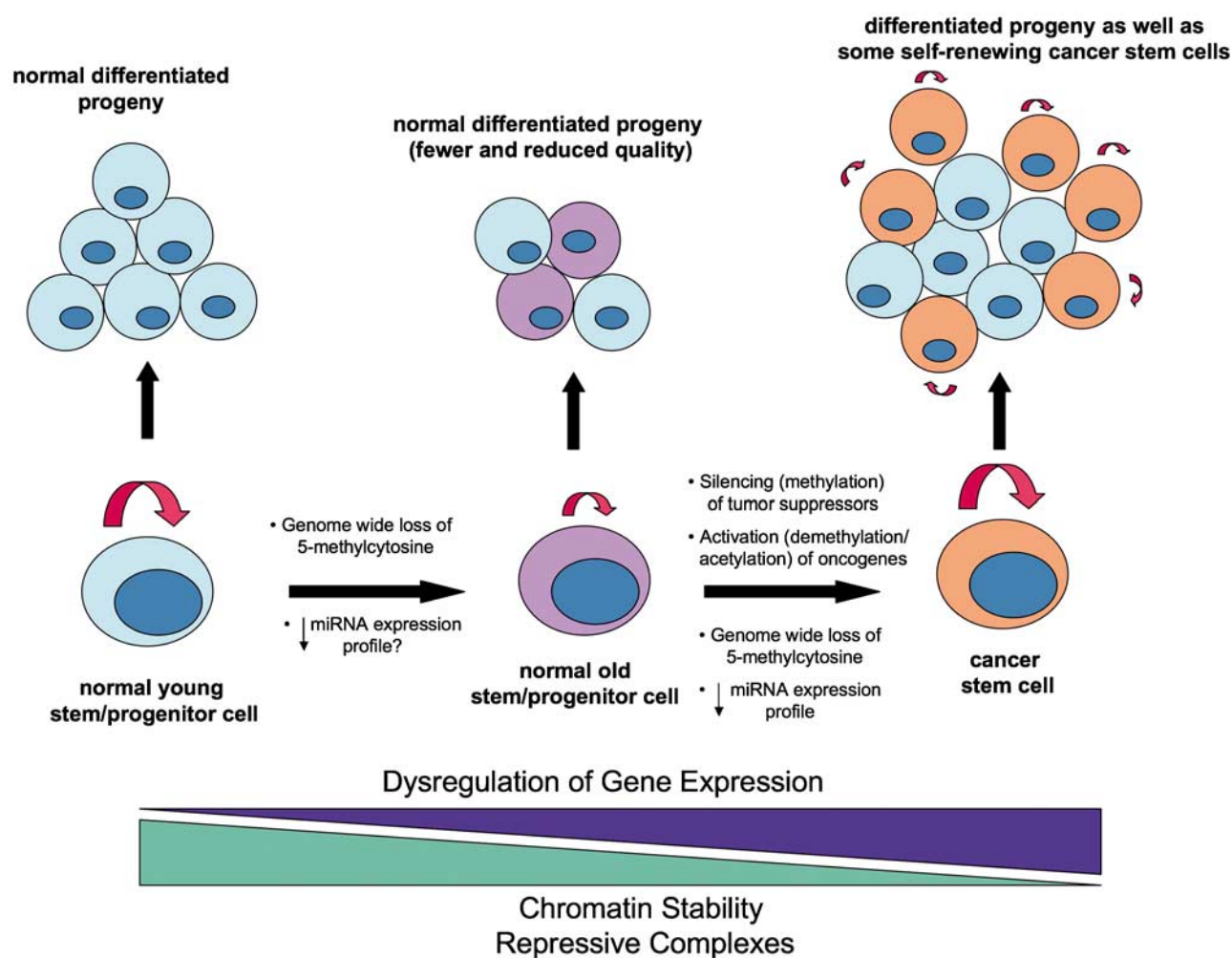


Figure 3 Epigenetic and miRNA changes that accompany aging and tumorigenesis. Normal young stem cells undergo controlled self-renewal divisions and differentiate to produce large numbers of high-quality mature cells. These well-orchestrated processes rely heavily upon regulated epigenetic modifications of gene expression and miRNA-mediated gene repression events. However, as stem cells age, their functional attributes decline. They have a reduced capacity for self-renewal and differentiate to give rise to fewer, low-quality daughter cells. A general loss of repression, resulting in dysregulation of gene expression and decreased chromatin stability, also accompany the aging process. It is hypothesized that, in addition to alterations in gene expression and reduced chromatin stability, the aging microenvironment creates conditions permissive to cancer development. Subsequent cancer-promoting epigenetic events, including silencing of tumor suppressor genes and activation of oncogenes, as well as alterations in miRNA gene expression promote the transition from a normal to a cancerous state. Changes in miRNA gene expression include amplification of miRNA oncogenes and deletion of miRNA tumor suppressors. The cancer stem cell has extensive self-renewal capacity and differentiates to give rise to self-renewing (disease sustaining) as well as terminally differentiated progeny. It is important to note, however, that the cancer stem cell does not necessarily have to arise from aged stem cell populations, but instead can arise from young stem cells whose normal developmental processes have gone awry.

miR-17–92.³⁷ Thus, miRNAs important in maintaining stem cell identity also seem to be important in cancer development.¹¹² It remains to be determined if and how miRNA expression patterns change as a function of age. Because aging provides conditions permissive to tumorigenesis, only subtle miRNA gene expression changes would be needed for normal stem cell regulatory pathways to go awry. Indeed, cancer stem cells could hijack the miRNA mechanisms that enable stem cell division.^{34,113} We speculate that aged stem cells and cancerous cells share similar miRNA expression profiles, perhaps contributing to the fact that aging is the greatest risk factor associated with cancer development.¹¹⁴

Conclusions

Current knowledge linking stem cells, aging and cancer can be distilled into three general but compelling bonds as depicted in Figure 3.

1. There is overall agreement that repressive complexes (PcG proteins and miRNAs) are important to maintain normal stem cell identity (by repressing genes that promote differentiation) and to determine differentiation down a particular lineage path (by repressing genes that promote self-renewal and proliferation). It seems that a general loss of repression, in the form of widespread hypomethylation and decreased expression of miRNA genes, accompanies both aging and cancer, and this potentially synergistic loss of repression may be a contributing factor to both states.
2. Dysregulation of gene expression in the form of repression of tumor suppressors and/or activation of oncogenes, mediated both by epigenetic chromatin remodeling and miRNA pathways, are common events in the multistep progression to cancer. An overall dysregulation of gene expression accompanies the aging process as well, resulting in decreased chromatin stability. Thus, only subtle cancer-promoting epigenetic and miRNA mediated alterations would be needed to potentially drive an aged stem cell from a normal to a cancerous state.
3. During aging, the gene expression profiles of stem cells change, likely due to epigenetic and miRNA-mediated events. This altered gene expression profile may impair stem cell function, ultimately resulting in loss of stem cells pools, which then further potentiates the aging process. Alternatively, distorted gene expression profiles may facilitate or promote tumorigenesis. Thus, the regulatory pathways mediating stem cell self-renewal and differentiation must remain optimal throughout the lifetime of an organism to ensure proper maintenance of stem cells pools and to protect against cancer development.

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