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The Epigenomic Viewpoint on Cellular Differentiation of Myeloid Progenitor Cells as it Pertains to Leukemogenesis

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Abstract

The new millennium has brought with it a surge of research in the field of epigenetics. This has included advances in our understanding of stem cell characteristics and mechanisms of commitment to cell lineages prior to differentiation. The nature of stem cells is similar to that of malignant cells in that they have unlimited self-renewal and protection from apoptosis, leading researchers to suspect that stem cells are the target of oncogenesis. This review will explore the idea of how epigenetic control of gene expression may contribute to mechanisms controlling differentiation of myeloid progenitor cells and its importance to our understanding of myelogenous leukemias. Recent developments in epigenetic research pertaining to differentiation of myeloid progenitor cells and hematopoietic stem cells are presented including aspects of cellular memory, general myelopoiesis, change in gene expression patterns, signal transduction, and the influence of the microenvironment.

Abbreviations

AML	=	acute myelogenous leukemia
CLP	=	common lymphoid progenitor
CML	=	chronic myelogenous leukemia
CMP	=	common myeloid progenitor
DNMT	=	DNA methyltransferase
EMP	=	erythroid-megakaryocyte progenitor
GMP	=	granulocyte-monocyte progenitor
HSC	=	hematopoietic stem cell
MDS	=	myelodysplastic syndrome
MP-B	=	myeloid progenitor for basophils
MP-E	=	myeloid progenitor for erythrocytes
MP-Es	=	myeloid progenitor for eosinophils
MP-M	=	myeloid progenitor for monocytes
MP-Meg	=	myeloid progenitor for megakaryocytes
MP-N	=	myeloid progenitor for neutrophils
MPD	=	myeloproliferative disease
MPC	=	myeloid progenitor cell compartment
Pc-G	=	polycomb group
SFRPs	=	secreted frizzled-related proteins
Trx-G	=	trithorax group

Myelopoiesis is a system of differentiation starting with the pluripotent hematopoietic stem cell (HSC) and proceeding to progressively determined progenitor cell types until terminating in the mature cell types of the myeloid lineages. This review will examine the role of epigenetic gene regulation on myelopoiesis and its relevance to our understanding of leukemogenesis. Epigenetics is defined as changes in gene expression that are not sequence based, which can be propagated through mitosis or meiosis. The most extensively studied forms of epigenetic gene control are histone modification and DNA methylation. Many aspects of epigenetic control of differentiation have been characterized in *Drosophila*. The connection of epigenetics to cancer has lately become one of the most extensively studied aspects of oncogenesis. The concept of stem cells being the target of oncogenesis in leukemias has been reviewed recently[1], as well as the general concepts of epigenetics and cancer[2]. This review will explore the idea of how epigenetic control of gene expression may contribute to mechanisms controlling differentiation in this milieu of progenitor cells, known as the myeloid progenitor cell compartment (MPC), and its importance to our comprehension of myelogenous leukemias (Fig. (1)).

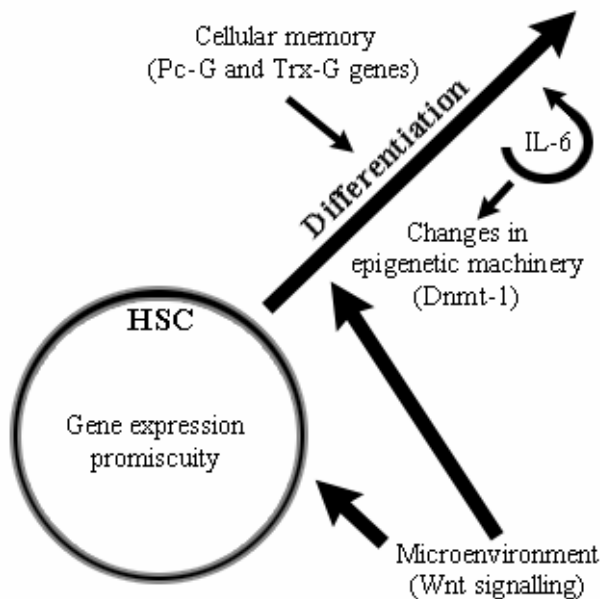


Figure 1: Epigenetics in Myelopoiesis. The processes that are the targets of epigenetic regulation are summarized. The HSC exhibits gene expression promiscuity that may be the result of the loss of gene silencing. Differentiation of the HSC into the myeloid lineages is influenced largely by the microenvironment through signal transduction pathways such as Wnt, as well as autocrine signaling through the IL-6. During the process of differentiation, Hox gene expression patterns must be maintained in the form of cellular memory by the Pc-G and Trx-G genes.

Epigenetics and differentiation

The trithorax group (Trx-G) and polycomb group (Pc-G) genes were initially characterized in *Drosophila* and yeast as regulators of transcription through chromatin remodeling, an epigenetic mechanism. The Trx-G genes maintain stable transcription of genes, while the Pc-G genes are responsible for gene silencing. These two gene families act antagonistically to control the expression of the homeobox transcription factor gene family by providing cellular memory of cell fate decisions. The homeobox gene family (or Hox gene family in mammalian systems) is responsible for making cell fate decisions. Since the homeobox genes are under control of the Trx-G and Pc-G genes, mutations in these two gene families result in loss of regulated expression of the

homeobox genes and aberrations in cell lineage decisions. Identification of direct targets of Pc-G gene complexes has been thwarted by the lack of sequence specific DNA elements in mammalian systems, though they have been identified in *Drosophila*[3]. A recent discovery in this field through the use of clever microarray experiments is several direct targets of the histone modifying Pc-G complexes[4].

Hox genes have been implicated in leukemic transformation since 1988, when the WEHI-3B leukemic cell line was found to contain proviral integrations resulting in transcriptional activation of *Hoxb8* and *Interleukin-3*[5]. Evidence has continued to accumulate implicating other Hox genes in leukemia, including *myeloid ecotropic integration site 1 (Meis1)*[6,7]. This gene has recently been found to be inactivated in acute myelogenous leukemia (AML) by hypermethylation[8] and its cofactors in leukemic transformation, HoxA7 and HoxA9, have been found to be expressed in HSCs[9].

Myelodysplastic syndrome (MDS), myeloproliferative disease (MPD), and AML are related hematopoietic malignancies in that MDS and MPD often progress to AML. AML is characterized by loss of regulation of cell lineage decisions, resulting in the over-proliferation of immature myeloid cells. The impact of epigenetics in leukemia is seen in multiple significant examples. These epigenetic effects include increased methylation of the Pa promoter of *Abi* seen in advanced phases of chronic myelogenous leukemia (CML)[10,11], in addition to methylation of *Bcr* in lymphoid blast crisis[12]. Hypermethylation of *p15*, an inhibitor of cyclin-dependent kinase 4 (CDK4) and CDK6, is also associated with CML transformation[13]. The importance of *p15* is seen in disease progression from MDS to AML, where *p15* is targeted for hypermethylation in 78% of samples at the time of leukemic transformation[14]. Also, increased expression levels of the DNA methyltransferase (DNMT) genes *DNMT1*, *DNMT3A*, and *DNMT3B* correlate with blast phase CML[15]. Epigenetics may be a significantly more important mechanism in AML progression than in other cancers, because chromosomal instability does not seem to be predominant (57.6% *de novo* AML patients have normal cytogenetics)[16]. Hypermethylation as a mechanism of gene regulation is seen frequently in AML, and may be more frequent in young adults where AML is the most common form of leukemia[17].

An overview of myelopoiesis

Hematopoiesis is a robust system of cell production capable of replacing about five hundred billion blood cells each day in the human body[18]. The production of these cells occurs largely in the bone marrow. The HSC is a rare cell type, with a frequency of 10^{-3} to 10^{-4} in bone marrow. In order to provide the large number of cells needed on a regular basis, an exponential amplification system is employed. In this system, the largely quiescent HSC[19-21] produces progenitor cells with increased proliferative capacity, but reduced differentiation potential (Fig. (2)). Maintenance of the HSC pool and production of progenitors occurs through asymmetric cell division[22], where one daughter cell retains its stem cell nature and the other enters a transient amplifying population. This is a conserved mechanism in adult tissues with a high turnover rate such as blood, intestinal epithelium, and skin. These progenitor cells are highly prolific and are responsible for providing for the daily need of new blood cells. The frequency of these progenitor cells in bone marrow is 5 to 10 fold greater than that of the HSC.

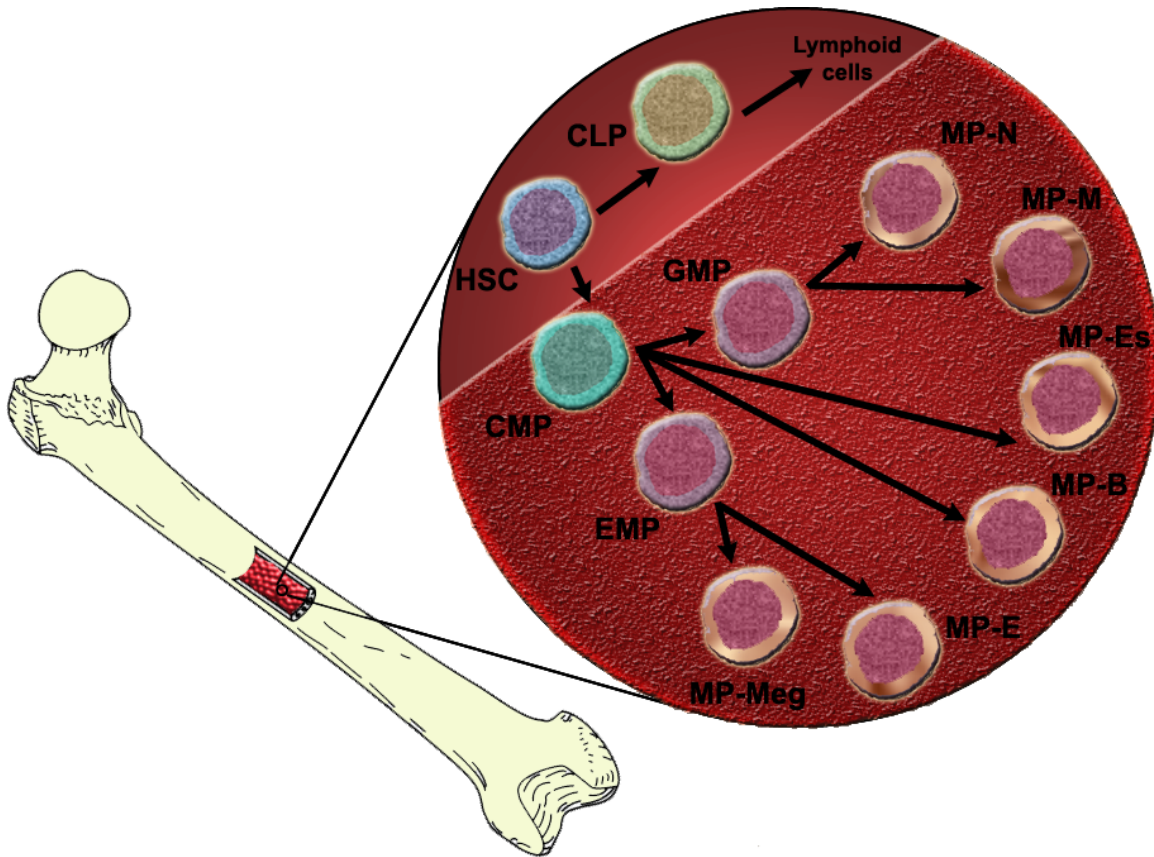


Figure 2: Schematic of Myelopoiesis. The hematopoietic stem cell (HSC) is the most pluripotent stem cell in hematopoiesis, from which all other hematopoietic cells are derived. HSCs can be grouped into short-term and long-term HSCs (not shown), which differ in that short-term HSCs no longer have unlimited self-renewal. Once self-renewal is lost it is not regained in subsequent progenitor differentiation. The HSC can differentiate into the common lymphoid progenitor (CLP) or common myeloid progenitor (CMP) that are responsible for generating their respective parts of the hematopoietic system. The myeloid progenitor cell compartment (shown as the textured area in the blow up) consists of the CMP; oligopotent progenitors, the erythroid-megakaryocyte progenitor (EMP) and granulocyte-monocyte progenitor (GMP); and committed myeloid progenitors. There are six major lineages of myeloid cells produced during myelopoiesis. Each lineage is produced from a corresponding committed myeloid progenitor shown in the figure above: megakaryocyte (MP-Meg), erythrocyte (MP-E), basophil (MP-B), eosinophil (MP-Es), monocyte (MP-M), and neutrophil (MP-N).

The current understanding of this system indicates that several classes of progenitor cells are present that vary in differentiation potential. The common progenitor cells of the lymphoid (CLP) and myeloid lineages (CMP) are produced by the HSC. These common progenitors are capable of producing all of the cells of their respective lineages. These common progenitors lose differentiation potential as they mature. This eventually results in highly prolific progenitor cells that have a differentiation potential restricted to a single cell type. These committed myeloid progenitors (committed MPs, Fig. (2)) have lost the self-renewal capacity stem cells possess. The committed MPs will proliferate, mobilize from the marrow, and differentiate to mount the cellular immune response of terminally differentiated myeloid cells in the periphery[23]. The myeloid differentiation

specific cytokine IL-6[24,25] has been found to induce the expression of *DNA methyltransferase 1 (DNMT-1)*[26], which has been found to occur through the transcriptional activation of FLI-1, a transcription factor that up-regulates DNMT-1[27]. Lately, cDNA microarray analysis of the KAS-6/1 multiple myeloma cell line treated with the demethylating drug, zebularine, showed methylation of several genes contributing to growth and survival[28]. IL-6 treatment allowed cells to recover their viability and methylation status of apoptosis and proliferation related genes after exposure to zebularine.

Gene expression promiscuity of the HSC

The HSC and various progenitors can be isolated by flow cytometry using appropriate cell surface markers[1], allowing for the molecular characterization of these cell types. This approach has had some success in determining the nature of the genomic events associated with stem cell differentiation and the genes responsible. Genomic technologies have helped characterize the nature of stem cell differentiation. HSCs are promiscuous in their expression profiles in that they exhibit expression of a large number of genes when compared to progenitor cells of the lymphoid and myeloid lineages. When comparing the HSC gene expression profile with that of the CMP and CLP it was found that the HSC had 8 fold more differentially expressed transcripts than the CLP and 21 fold more than the CMP[9]. As the progenitors continue to differentiate into more committed cell types, the levels of differentially expressed transcripts rise but still remain only one-quarter that of the HSC. Thus, the HSC maintains a promiscuous transcription profile that is reduced significantly when it moves from the quiescent HSC to the prolific progenitor cell pool. Experimental data suggests that this promiscuous gene expression profile includes low level transcription of many genes that are lineage specific[29,30]. This may prime the HSC for differentiation into many lineages, one of which is selected in response to environmental stimuli.

Examining this promiscuity from an epigenetic viewpoint suggests that large scale genomic silencing events occur after a lineage decision by the HSC. Gene silencing is known to be an important aspect of cellular identity and lineage choice[31-33]. The concept that genome-wide increases in methylation during early differentiation and organogenesis may restrain the lineage choices of maturing cells during late development has been postulated[34]. Recently, investigators have provided evidence that chromatin remodeling events are preliminary to relatively late occurring enhancer complex assembly and high-level gene expression in hematopoietic cell differentiation in mouse bone marrow[35]. These investigations concentrated on the *c-fms (Csf1r)*, the macrophage colony-stimulating factor receptor, and *chicken lysozyme locus (clys)* genes in macrophage development. Other experiments investigating *c-fms* found it to be selectively silenced in all hematopoietic cells except the HSC and those of the macrophage lineage[36]. This silencing may be triggered by factors asymmetrically allocated during cell division or by the signal transduction events that trigger the differentiation. Once restrictive chromatin is established through histone modification or DNA methylation, active measures must be invoked to remove these modifications since they are relatively stable, even through cell division[37].

It remains to be seen whether the transcription promiscuity of the genome as a whole is the result of an overall reduced level of chromatin silencing, or maintained transcriptional activity in the presence of chromatin silencing. A recent report provides evidence that reduced levels of DNA methylation block differentiation and are associated with

hyperacetylation of histones, supporting the idea that stem cells maintain open chromatin conformations on a genomic scale[38]. If chromatin silencing is still largely in effect, low level transcription may be necessary to prime the HSC for differentiation down multiple lineages by preventing restrictive chromatin conformations that will inhibit transcription of genes necessary for differentiation. It may be that stem cells prevent these closed conformations in chromatin through maintenance of transcription or through a general inhibition of factors involved in silencing. It will be interesting to determine if one of the defining characteristics of stem cells is a unique condition in the epigenetic regulatory machinery.

Signal transduction in myelopoiesis

In addition to lineage specific genes, the HSC and progenitor cells express many genes involved in signal transduction pathways[9]. Four signal transduction pathways are implicated in general stem cell biology including the Wnt, Notch, Bmp, and Hedgehog pathways. Evidence now exists showing that epigenetic control, of at least some of these pathways, is an important aspect of their regulation in oncogenesis. There is a substantial body of data linking the hedgehog pathway to stem cells in fish, chickens and *Drosophila* [39-42]. Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-beta super-family. The BMP signaling pathway has been found to be epigenetically activated by DNA demethylation in prostate cancer[43]. The Wnt and Notch signal transduction families have been shown to be extremely important in HSC and hematopoietic progenitor cell fate decisions[44-48]. Evidence exists that shows both of these signaling pathways are important in promoting self-renewal in HSCs and progenitors[48-51]. However, the involvement of the Wnt pathway in hematopoiesis is still under debate as inactivation of β -catenin, a downstream cytosolic/nuclear factor in the Wnt pathway, in mice was recently found to have no effect on hematopoiesis[52]. There also is evidence suggesting epigenetic control in the Notch pathway but no direct links have been found. The prospect of cellular memory in the case of blood vessel formation suggests a role for epigenetic control mechanisms in cell fate decisions mediated by the Notch pathway[53]. The Wnt pathway is an interesting story, and so far has shown the most direct connection with epigenetic modification, stem cells, and cancer.

In addition to its role in stem cell maintenance, the Wnt pathway has been implicated in cellular proliferation, polarity, and fate decisions[54]. Not surprisingly, the WNT pathway has been implicated in colorectal and skin cancers[55-57]. Furthermore, it has been shown that deregulated WNT pathway signaling is an early progression event in 90% of colorectal cancers[58]. My colleagues and I have found that the Wnt pathway can be upregulated in *Drosophila* in response to perturbations in epigenetic regulators[59], indicating a role of epigenetic gene control in this pathway. In these studies, mutations in Trx-G genes induced up regulation of the Wnt pathway. Since Trx-G genes are responsible for maintenance of active transcription, a mutation in Trx-G genes will result in loss of transcription. These results indicate a gene under epigenetic control involved in repression of the Wnt pathway, since loss of the gene product upregulates the WNT pathway. A possible explanation of these results has surfaced recently with the discovery that the secreted frizzled-related proteins (SFRPs)[60]. These proteins have been implicated in colorectal cancers and shown to be negatively regulated by DNA methylation early in these cancers[61,62]. The SFRPs negatively regulate the Wnt pathway by sequestering the ligand. The importance of these results to

stem cell biology is that they suggest that epigenetic control may be a mechanism of Wnt pathway regulation in normal physiology.

Microenvironmental effects on myelopoiesis

It is becoming increasingly apparent that the cellular microenvironment is drastically different in cancerous tissue and normal tissue. This was elegantly shown in SAGE (serial analysis of gene expression) studies of malignant breast tissue and normal breast tissue[63]. The researchers found that even early in breast tumor progression the surrounding tissue had a dramatically different gene expression profile than normal tissue. When combined with a recent finding that mammary tumors only formed when the stromal network was treated with a carcinogen[64], regardless of whether the injected endothelial cells were treated with carcinogen *in vitro*, indicate that the surrounding cells of a tumor greatly impact its progression to full malignancy. There is corroborating evidence for this supposition in MDS, where marrow stromal layers from patients with MDS had an increased ability relative to normal marrow stroma to augment apoptosis of the GM-CSF and IL-3-dependent cell line F-36P[65].

Since SFRPs are secreted molecules, they are a potential mechanism under epigenetic control to allow the microenvironment of the bone marrow to influence HSC and progenitor cell homeostasis. The microenvironment of bone marrow is determined by stromal cells present in the bone marrow. These stromal cells are a diverse network of cell types including fibroblasts, adipocytes, endothelial cells, and macrophages. Additionally, osteoblasts may serve a role in HSC maintenance and progenitor cell maturation[66]. Both osteoblast and stromal cell lines have been found to express SFRP1[67].

The effect of microenvironmental influences of bone marrow stroma could be a factor in the idea of “field leukemogenic effect” postulated recently[68]. This idea is an adaptation of field cancerization in solid tumors developed in the 1950s[69]. Field cancerization is defined as diffuse injury to an organ resulting from long-term exposure to carcinogens. This results in a variety of mutations to different cells in the organ, which can be propagated through the tissue as the cells divide resulting in large areas of cells or “fields” that contain the same mutation. In field leukemogenic effect there are several abnormal hematopoietic cell clones that produce different types of aberrant cells as the result of a generalized insult to bone marrow. One of these clones predominates, while the others remain present but below detectable levels. It is possible that field effects may include regions of stromal cells that have been altered to be supportive for premalignant cells. This is important to treatment strategies in hematopoietic malignancies because the secondary clones can become more pronounced once the primary clone has been reduced by treatment, resulting in new malignancies. The idea of field leukemogenic effect can be extrapolated to include epigenetic modifications to the genome that can affect both the microenvironment and stem/progenitor cells of bone marrow.

Conclusion

New techniques are allowing studies of epigenetic gene regulation on the genomic scale. These will continue to provide exciting new results that will increase our understanding of this regulatory mechanism in differentiation and its impact on cellular transformation. Currently, the five-year survival rate for AML is the lowest of the

leukemias at 21%, necessitating urgency in new chemotherapy. Because epigenetic gene regulation correlates with early malignancies and progression, epigenetic etiological studies hold the promise of producing powerful diagnostic tools. Insights into new treatment and diagnostic modalities in AML, MDS, and MPS will result from our discoveries in epigenetic gene regulation of myelogenesis.

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