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Role of microRNAs in leukemia stem cells

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1. ABSTRACT

Leukemia cells can carry a small subset of poorly differentiated cells, which are considered to be precursors of lymphoblasts, myeloblasts, or monoblasts. Thus these cells are also called leukemia stem cells (LSCs) because they are capable of instigating, maintaining and serially propagating leukemia *in vivo*, while retaining the ability to differentiate into committed progeny that lack these properties. Like hematopoietic stem cells (HSCs), LSCs possess the ability of self-renewal under a complex regulatory system. The recent discovery of microRNAs may shed new light on regulation of LSCs and leukemogenesis. As master gene regulators, microRNAs participate in these processes through coordinated work with key transcription factors required for hematopoiesis. Therefore, microRNAs could play a critical role in normal HSCs as well as LSCs. The purpose of this review is to provide updates on the role of microRNAs in HSCs and LSCs and to highlight their potential in differentiation therapy of leukemias.

2. INTRODUCTION

It has been suggested that tumors are derived from mutated stem cells, the so-called cancer stem cells because stem cells and cancer cells share self-renewal and differentiation capacities. Although this hypothesis was postulated in early reports, definite proof of existence of cancer stem cells came from recent studies in leukemia where among the tumor cell population only a small subset of cells could initiate, regenerate and maintain the leukemia after transplantation into immunocompromised mice. Using similar functional approaches, researchers have identified a variety of cancer stem cells from an increasing number of epithelial tumors, including breast, prostate, pancreatic, and head and neck carcinomas, all of which are distinguished by the expression of the cell-surface glycoprotein CD44. Another cell surface marker, the CD133 glycoprotein, is highly expressed in the tumor-initiating cells of brain and colon carcinomas. The concept of cancer stem cells not only changes our current understanding of cancer biology, but may also have profound consequences on cancer

diagnostics and therapeutics. For example, gene expression profiles associated with the stemness/differentiation states of tumors might be used as molecular predictors of therapeutic outcome. Especially in chronic myeloid leukemia (CML), the tyrosine kinase inhibitor imatinib has replaced IFNα as the standard therapy (1).

Hematopoiesis is under delicate and complex control; similarly, regulation of HSCs and LSCs is also complex, involving numerous factors among which are microRNAs. The focus of this review is to provide current understanding of how microRNAs impact these processes. In particular, we will discuss possible links between normal hematopoiesis and regulation of LSCs through microRNA-mediated gene regulations, as well as potential differentiation therapy by modulation of microRNAs.

3. HEMATOPOIETIC STEM CELLS (HSCS)

3.1. HSCS DIFFERENTIATION

It is well known that blood cells derive from pluripotent progenitor cells with the capacity for self-renewal and differentiation. More differentiated cells are generally considered to be more committed to a certain lineage with concomitant loss of self-renewal capacity. Although cell morphology provides good information about the developmental stages of bone marrow cells, immunophenotyping of blood cells is of great help in identifying lineage determination, especially in early progenitor cell compartments. Differentiated cells display distinct patterns of antigen expression, such as CD11b, CD13, CD33, CD14, CD15 and CD66 antigens on myelomonocytic cells. These lineage markers (lin^+) are not expressed on early hematopoietic cells. Cells at the intermediate differentiation level can express both the progenitor cell antigen CD34 and lineage markers, and they are capable of forming colonies in clonogenic assays. In the case of myeloid differentiation, they typically carry the CD33 and CD13 antigen.

Lineage-specific transcription factors are key regulators of gene expression in multiple cell-fate decisions that govern hematopoietic differentiation (Fig.1). Given the important role of microRNAs in development and differentiation, it is not surprising that these regulatory RNAs also play crucial roles in hematopoiesis. In this regard, transcription factors and microRNAs act in concert to regulate gene expression during hematopoietic differentiation. For instance, transcription factors may regulate the expression of microRNA genes, whereas transcription factors themselves are also subject to microRNA regulation, forming a feedback regulatory system. Overall microRNA expression generally increases with cellular differentiation, suggesting that these microRNAs are capable of shutting down genetic programs that are required for maintaining alternate cell fates or preventing differentiation more generally. For instance, mouse embryonic stem (ES) cells deficient in the Dicer and microprocessor (Drosha-DGCR8-Ddx5) components of the microRNA processing apparatus exhibit defects in ES cell differentiation and development (2). On the other hand, the promoter regions of microRNAs that function in stem cells are typically occupied by key members of the stem cell transcription factors, including Nanog, Oct4, and Sox2. Interestingly, Nanog, Oct4, and Sox2 are themselves subject to microRNA and Polycomb group (PcG) modulation during ES cell differentiation. Indeed, a number of microRNAs have been implicated in HSC differentiation at various stages (see below).

3.2. MicroRNAs in HSC differentiation

The link between microRNA expression and hematopoiesis was first demonstrated in 2004 by Chen *et al* (3). In this study, 150 microRNAs were cloned from murine bone marrow and three of them (miR-181, miR-223 and miR-142) were specifically expressed in hematopoietic cells, and dynamically regulated during early hematopoiesis and lineage commitment. In particular, miR-181 was preferentially expressed in the B-lymphoid cells of mouse bone marrow; ectopic expression of miR-181 in hematopoietic stem/progenitor cells increased fraction of B-lineage cells in both tissue-culture differentiation assays and adult mice, suggesting that microRNAs are components of the molecular circuitry that controls mouse hematopoiesis. In fact, expression of microRNAs in normal hematopoiesis has also been studied in human hematopoietic tissue by several groups (4-6); these non-coding RNAs play critical roles in almost every stage of hematopoiesis (Figure 1). Therefore, we will provide a few examples of microRNA-mediated regulation of HSC differentiation at various stages and their dysregulation that may lead to LSCs.

3.2.1. MicroRNAs in granulocyte differentiation

Work by Johnnidis et al indicates that miR-223 is an intrinsic modulator of neutrophil differentiation (7). This has to do with the ability of miR-223 to negatively regulate granulocyte differentiation and activation through suppressing expression of Mef2c, a transcription factor that promotes myeloid progenitor proliferation. In addition, two transcriptional factors, Nuclear Factor I/A (NFI-A) and CCAAT Enhancer Binding Protein alpha (C/EBP alpha), compete for binding to the miR-223 promoter: NFI-A keeps miR-223 at low levels, whereas its replacement by C/EBP alpha, following retinoic acid (RA)-induced differentiation, upregulates miR-223 expression. Moreover, RNAi against NFI-A or ectopic expression of miR-223 in acute promyelocytic leukemia (APL) cells enhance differentiation (8, 9). C/EBP alpha is crucial for granulopoiesis and is deregulated by various mechanisms in acute myeloid leukemia (AML). Mutations in the C/EBPα gene are reported in 10% of human patients with AML (10). Even though the C/EBPα-mutants are known to display distinct biological function during leukemogenesis, the molecular basis for this subtype of AML remains elusive. On the other hand, miR-34a blocks myeloid cell proliferation through targeting E2F3 (11). A lower expression of miR-34a is often associated with high levels of E2F3 as well as E2F1, a transcriptional target of E2F3in AML samples with C/EBPα mutations. Thus, manipulation of miR-34a can re-program granulocytic differentiation of AML blast cells with C/EBPα mutations.

Runx1is a known transcription factor for the differentiation of myeloblasts into granulocytes. Using the

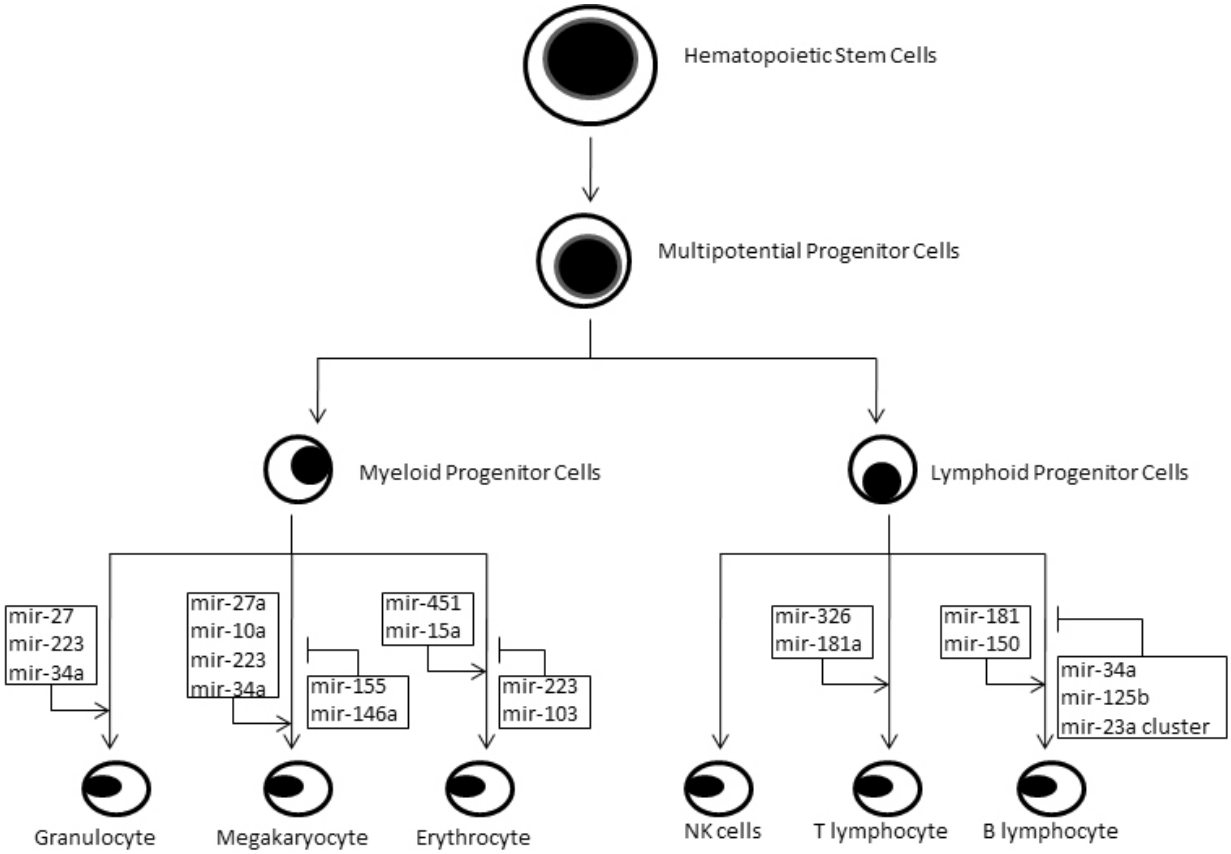

Figure 1. Implication of microRNAs in hematopoietic differentiation. While certain microRNAs promote linage specific differentiation, the others block this process. See detail explanations in the text.

32D.cl3 cell differentiation model, Feng *et al* showed that during differentiation induced by granulocyte colony stimulating factor (CSF3), Runx1 is blocked (12). At the same time, levels of miR-27 and its precursor increases substantially. While ectopic expression of miR-27 suppresses, anti-miR-27 enhances Runx1 protein levels. Of interest, the CSF3-induced transcription factor C/EBPα also enhances transcription of a host gene of miR-27, C9orf3, via activation of its promoter. Thus, miR-27 enhances differentiation of myeloblasts into granulocytes via post-transcriptional downregulation of Runx1.

3.2.2. MicroRNAs in megakaryocyte differentiation

The role of microRNAs in regulating megakaryocyte differentiation has been examined in bipotent K562 human leukemia cells (13) (14). Genome-wide expression profiling identified 21 microRNAs that are induced by Phorbol 12-myristate 13-acetate (PMA). Particularly, three microRNAs (miR-34a, miR-221, and miR-222) are induced in the early stages and maintained throughout the late stages of differentiation. Cell signaling analysis also showed that the activation of extracellular signal-regulated protein kinase (ERK) in response to PMA strongly induces miR-34a expression through the activator protein-1(AP-1) binding site in the upstream region of the miR-34a gene. On the other hand, the mitogen-activated protein kinase 1 (MEK1) serves as a direct target of miR-34a, and c-fos as a direct target of miR-221/222. Thus, miR-34a represses the proliferation of K562 cells through suppression MEK1; it also induces cell-cycle arrest in G1 phase, and promotes megakaryocyte differentiation (13). In contrast, a locked nucleic acid (LNA) antisense oligo against miR-34a (anti-miR-34a) enhances the cell proliferation. miR-34a can also directly regulate expression of MYB, facilitating megakaryocyte differentiation, and of CDK4 and CDK6, to inhibit the G1/S transition. In addition, induction of megakaryocytic differentiation in K562 cells by 12-o-tetradecanoylphorbol-13-acetate induces miR-27a expression, and facilitates Runx1 bind to the miR-27a regulatory region (15).

Also in K562 cells, expression of miR-223 is down-regulated during hemin-induced erythroid differentiation, but up-regulated during PMA-induced megakaryocytic differentiation (16). Peaks corresponding to 4N cells in stable transfectants overexpressing miR-223 are higher than that in control K562 cells during megakaryocytic differentiation, suggesting that miR-223 may stimulate megakaryocytic differentiation. This is in part through regulation of expression of LIM domain only 2 (LMO2). For example, ectopic expression of miR-223 suppresses both LMO2 mRNA and protein levels in K562 cells.

miR-155 is abundantly expressed in early CD34+ haematopoietic progenitor cells (HPCs), but decreases sharply during megakaryocytic differentiation (17). Functional studies showed that enforced expression of miR-155 impairs proliferation and differentiation of megakaryocyts. Furthermore, HPCs transfected with miR-155 showed a significant reduction of their clonogenic capacity. Consistent with this observation, miR-155 also directly targets Ets-1 and Meis 1, two transcription factors with well-known functions in megakaryocyts. Thus, the decline of miR-155 is required for megakaryocyts proliferation and differentiation at progenitors and precursors level, implying that sustained expression of miR-155 inhibits megakaryopoiesis.

miR-146a is another microRNA that may be involved in megakaryopoiesis. In this case, a regulatory pathway in normal megakaryopoiesis involves the PLZF transcription factor; miR-146a and the SDF-1 receptor CXCR4 serve as either miR-146a targets or as a regulator of miR-146a (18). In leukemic cell lines, PLZF represses miR-146a and enhances CXCR4 protein, whereas PLZF knockdown induces the opposite effects. *In vitro* assays showed that PLZF interacts with the miR-146a promoter and inhibits its expression. In contrast, miR-146a targets CXCR4 mRNA. In megakaryopoietic cultures of CD34+ progenitors, PLZF is upregulated, whereas miR-146a expression decreases and CXCR4 protein increases.

Garzon *et al* (19) performed microRNA expression profiling of *in vitro*-differentiated megakaryocytes derived from CD34+ hematopoietic progenitors to identify novel regulatory pathways during megakaryocytic differentiation. Their finding suggests that several microRNAs including miR-10a, miR-126, miR-106, miR-10b, miR-17 and miR-20 are downregulated. In particular, miR-10a expression in differentiated megakaryocytes inversely correlates with expression of HOXA1, and HOXA1 is a direct target of miR-10a.

3.3.3. MicroRNAs in erythrocyte differentiation

Gene complementation combined with microarray screening has identified two conserved microRNAs, miR-144 and miR-451, required for the formation of erythroid cells (20). Of interest, these two microRNAs are direct targets of the critical hematopoietic transcription factor GATA-1. *In vivo*, GATA-1 binds a distal upstream regulatory element to activate RNA polymerase II-mediated transcription of a single common primary microRNA (pri-microRNA) encoding both mature microRNAs (20). Furthermore, expression of the locus encoding miR-144 and miR-451 is strictly dependent on Argonaute 2 and is required for erythroid homeostasis (21). Mice deficient for the miR-144/451 cluster display a cell autonomous impairment of late erythroblast maturation, resulting in erythroid hyperplasia, splenomegaly, and a mild anemia. Analysis of gene expression profiles from wild-type and miR-144/451-deficient erythroblasts revealed that the miR-144/451 cluster acts as a "tuner" of gene expression, leading to different levels of expression for many genes.

The role of miR-451 in erythrocyte differentiation is further supported by a recent study with the knockout mouse model. The mice lacking miR-451 display a reduction in hematocrit, an erythroid differentiation defect, and ineffective erythropoiesis in response to oxidative stress, (22). Specifically, 14-3-3 δ is an intracellular regulator of cytokine signaling and is subject to negative regulation by miR-451. While 14-3-3 δ is up-regulated in miR-451 (-/-) erythroblasts, inhibition of 14-3-3 δ rescues their differentiation defect. These findings reveal an essential role of 14-3-3 δ as a mediator of the proerythroid differentiation actions of miR-451, and highlight the therapeutic potential of miR-451 inhibitors.

Separately, Fu *et al* (23) showed that miR-144, which is expressed at specific developmental stages during zebrafish embryogenesis, negatively regulates the embryonic α-globin, but not embryonic β-globin, through physiologically targeting Klfd, an erythroid specific kruppel-like transcription factor. This is because Klfd selectively binds to the CACCC boxes in the promoters of both α-globin and miR-144 genes to activate their transcriptions, thus forming a negative feedback circuitry to fine-tune the expression of embryonic α-globin gene. Microarray analysis further reveals miR-210 as a highly expressed microRNA in the erythroid precursor cells from the hereditary persistence of fetal hemoglobin (HPFH) patient and miR-210 can be induced by mithramycin in K562 cells in time-dependent and dose-dependent fashion, along with increased expression of the fetal gamma-globin genes (24). Furthermore, miR-223 reversibly regulates erythroid and megakaryocytic differentiation of K562 cells (16) where expression of miR-223 is downregulated during hemin-induced erythroid differentiation, but up-regulated during PMA-induced megakaryocytic differentiation.

Hemin can also induce miR-126, but suppress miR-103, miR-130a, miR-210, and miR-18b in K562 cells (25). The same expression tendency of the five microRNAs was observed following erythroid induction of CD34+ cells derived from human cord blood (25). Furthermore, ectopic expression of miR-103 in K562 could inhibit hemin-induced K562 erythroid differentiation, which is likely through direct silencing forkhead box J2 (FOXJ2), a transcription factor that is involved in the development of many tissues.

Finally, miR-15a has also been implicated in erythroid differentiation, which is likely through suppression of c-Myb (26). Suppression of c-Myb by miR-15a blocks the cells in the G1 phase of cell cycle. On the other hand, the miR-15a promoter contains several potential c-Myb binding sites. Binding of c-Myb to the c-Myb binding sites in the miR-15a promoter is required for miR-15a expression in K562 cells. In normal human CD34+ cells, expression of c-Myb and miR-15a is inversely correlated in cells undergoing erythroid differentiation, and that ectopic expression of miR-15a blocks both erythroid and myeloid colony formation *in vitro*. These findings suggest a c-Myb-miR-15a is part of autoregulatory feedback loop of potential importance in human hematopoiesis.

3.3.4. MicroRNAs in T lymphocyte differentiation

During an immune response, T cells enter memory fate determination, a program that divides them into two main populations: effector memory and central memory T cells. Since in many systems protection appears to be preferentially mediated by T cells of the central memory it is important to understand when and how fate determination takes place. However, little is known about cellular intrinsic molecular events that determine their differentiation. Using an *in vitro* system where activated CD8 T cells driven by IL-2 or IL-15 become either effector memory or central memory cells, Almanza *et al* showed that fate determination to central memory T cells is under the balancing effects of a discrete number of microRNAs including miR-150, miR-155 and the let-7 family (27). Apparently, miR-150 targets KChIP.1 (K+ channel interacting protein 1) which is specifically upregulated in developing central memory CD8 T cells. Thus, cell fate determination such as surface phenotype and self-renewal may be decided at the pre-effector stage on the basis of the balancing effects of a discrete number of microRNAs.

While sets of transcription factors and cytokines are known to regulate T(H)-17 differentiation, miR-326 is a T(H)-17 cell-associated microRNA, and is highly correlated with disease severity in patients with multiple sclerosis and mice with experimental autoimmune encephalomyelitis (EAE) (28). *In vivo* silencing of miR-326 results in fewer T(H)-17 cells and mild EAE, and its overexpression causes more T(H)-17 differentiation. In addition to miR-326, miR-181a has been implicated in T-cell differentiation because it is highly expressed in developing thymocytes and markedly downregulated in post-thymic T cells. Papapetrou *et al* tagged lentiviral-encoded antigen receptors with a miR-181a-specific MRE and transduced into mouse BM cells that were used to generate hematopoietic chimeras (29). Their study showed that expression of a chimeric antigen receptor (CAR) specific for human CD19 (hCD19) is selectively suppressed in late double-negative and double-positive thymocytes, and at the same time, the endogenous miR-181a expression reaches the highest point. Of interest, miR-181a-1, but not miR-181c, can promote CD4 and CD8 double-positive (DP) T cell development when ectopically expressed in thymic progenitor cells (30). The distinct activities of miR-181a-1 and miR-181c are largely determined by their unique pre-microRNA loop nucleotides-not by the one-nucleotide difference in their mature microRNA sequences.

3.3.5. MicroRNAs in B lymphocyte differentiation

miR-150 is specifically expressed in mature lymphocytes, but not in their progenitors, suggesting its role in lymphocyte differentiation. This is likely attributed to its ability to specifically silence c-Myb (31), a transcription factor controlling multiple steps of lymphocyte development. Of interest, miR-150 suppresses c-Myb *in vivo* in a dose-dependent manner over a narrow range of microRNA and c-Myb concentrations (31). Evidently, miR-34a is an important microRNA that impacts HSC differentiation at multiple levels. In addition to its role in granulocyte differentiation, miR-34a is able to block B cell development at the pro-B-cell-to pre-B-cell transition, leading to a reduction in mature B cells (32). This block appears to be mediated primarily by suppression of the transcription factor Foxp1, a direct target of miR-34a. Knockdown of Foxp1 by siRNA recapitulates the B cell developmental phenotype induced by miR-34a, whereas cotransduction of Foxp1 lacking its 3'-UTR with miR-34a is able to rescue B cell maturation (32).

The B lymphocyte-induced maturation protein-1 (BLIMP-1) and IFN regulatory protein-4 (IRF-4) transcription factors are essential for plasma cell differentiation. miR-125a and miR-125b are members of a multigene family located in paralogous clusters. The miR-125a cluster on chromosome 19 in humans includes miR-99b and let-7e, whereas the miR-125b cluster on chromosome 21 includes miR-99a and let-7c. These four microRNAs are preferentially expressed by the actively dividing centroblasts in germinal centers (GC) (33). Among them, miR-125b is able to directly target BLIMP-1 and IRF-4. Furthermore, miR-125b is able to inhibit the differentiation of primary B cells and compromise the survival of cultured myeloma cells. These findings suggest that miR-125b promotes B lymphocyte diversification in GC by inhibiting premature utilization of essential transcription factors for plasma cell differentiation.

On the other hand, the miR-23a cluster (miR-23a, miR-27a, and miR-24-2) is regulated by PU.1(34). Each microRNA of this cluster is more abundant in myeloid cells than in lymphoid cells. Expression of this cluster in hematopoietic progenitors under B-cell-promoting conditions causes a dramatic decrease in B lymphopoiesis and an increase in myelopoiesis. Although several microRNAs have been identified downstream of PU.1 in mediating development of monocytes and granulocytes, the miR-23a cluster is the first such a downstream target implicated in regulating development of myeloid vs lymphoid cells.

4. LSCs

Although it is generally accepted that there is a subset of cells, i.e., LSCs in leukemias, its origin is still controversial. For example, LSCs are not necessarily derived from their corresponding tissue stem cells such as HSCs although they may share functional characteristics and components of the self-renewal gene expression program seen in HSCs. Evidence for leukemia-initiating cells was first reported in 1994 when John Dick's laboratory isolated a sub-population (CD34+CD38-) from patients with acute myeloid leukemia (AML), demonstrating the existence of LSCs (35). These CD34+CD38- AML cells are not directly analogous to normal HSCs. Additional markers such as IL-3Rα (CD123) has been identified as a unique cell surface receptor on AML-initiating cells in contrast to normal HSCs (36).

When transduced with the leukemogenic MLL fusion gene, prospectively isolated stem cells and myeloid progenitor populations with granulocyte/macrophage differentiation potential are efficiently immortalized *in*

vitro and result in the rapid onset of acute myeloid leukemia with similar latencies following transplantation *in vivo* (37). Using a mouse model of human AML induced by the MLL-AF9 oncogene, Tim S *et al* demonstrated that colony-forming cells (CFCs) in the bone marrow and spleen of leukemic mice are also LSCs (38). These self-renewing cells are frequent, accounting for 25%-30% of myeloid lineage cells at late-stage disease with LSC features such as phenotypic, morphologic, and functional leukemia cell hierarchy. Therefore, the LSCs responsible for sustaining, expanding, and regenerating MLL-AF9 AML are downstream myeloid lineage cells.

However, in acute lymphoblastic leukemia (ALL) harboring different cytogenetic abnormalities, conflicting observations have been reported. For example, in marrow samples from TEL/AML 1-positive ALL patients, the marrow CD34+CD19- fraction contains little-to-no TEL-AML1 (39). Since the sample size is small in this study, and sorter errors may account for the little TEL-AML1 in the primitive CD34+CD19- subset. Nevertheless, it appears that the ALL-initiating cells could not be enriched through the CD34+CD19- sorting (39). Follow-up studies employing NOD/SCID xenotransplant experiments confirmed this finding in TEL-AML1 ALL, a finding that this leukemia likely arises in a lymphoid progenitor rather than a malignant multipotent hematopoietic cells (40).

In chronic myeloid leukemia (CML) the malignancy is stem cell derived, but driven in myeloid progenitors (41). CML is a proliferative disease in the bone marrow, originating in a multipotent HSC with an acquired translocation of chromosomes 9 and 22. This mutational event leads to the generation of a novel fusion gene, BCR-ABL. The gene product of this translocation is a constitutively expressed tyrosine kinase that alters signal transduction pathways, leading to abnormalities in cell cycling, decreased apoptosis and increased cell proliferation. CML is clinically manifested in the granulocytic lineage, despite the fact that it originates in the HSC and its pathogenetic chromosome translocation can be found in all blood elements except T lymphocytes.

Interestingly, the LSC maintenance program is more akin to embryonic rather than adult stem cells, which is supported by gene expression profiling for populations enriched or depleted of self-renewing LSCs (42). This approach reveals that gene sets previously shown to be characteristic of embryonic stem cells (ESCs), as opposed to adult tissue stem cells, are highly expressed in the MLL LSC-enriched cell fraction. Furthermore, genes that predict for poor prognosis in various cancers are also enriched in the high LSC data set. Therefore, there may be shared programs between different lineage cancers and different species (43).

5. MICRORNAS: FROM NORMAL TO MALIGNANT HEMATOPOIESIS

5.1. A link between microRNAs and hematological malignancies

There are an increasing number of studies trying to correlate microRNA expression in normal hematopoiesis to that in hematological malignancies. Unfortunately, there is still not sufficient evidence to support a direct role of microRNAs in hematological malignancies. This is likely due to the fact that microRNAs often target multiple genes and single gene can be targeted by different microRNAs. Moreover, most of the studies have focused on multiple aberrantly expressed microRNAs in hematological malignancies, which complicate the attempt to associate oncogenesis with a unique microRNA.

However, there is at least one study suggesting a link between microRNAs and hematological malignancies (44). Most chromosomal translocations in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) involve oncogenes that are either up-regulated or form part of new chimeric genes. The t(2;11)(p21:q23) translocation has been cloned in 19 cases of MDS and AML. In addition, this translocation is associated with a strong up-regulation of miR-125b (from 6- to 90-fold) (44). Apparently, miR-125b is able to interfere with primary human CD34+ cell differentiation, and to inhibit terminal (monocytic and granulocytic) differentiation in HL60 and NB4 leukemic cell lines. Thus, upregulation of miR-126b may represent a new mechanism of myeloid cell transformation, and myeloid neoplasms carrying the t(2;11) translocation define a new clinicopathological entity.

Deletions of the chromosomal region 13q14 are commonly associated with CLL, with monoclonal B cell lymphocytosis (MBL), which occasionally precedes CLL, and with aggressive lymphoma, suggesting that this region contains a tumor-suppressor gene. Deletion in mice of the 13q14-minimal deleted region (MDR), which encodes the DLEU2/miR-15a/16-1 cluster, causes development of indolent B cell-autonomous, clonal lymphoproliferative disorders, recapitulating the spectrum of CLL-associated phenotypes observed in humans (45). In this regard, deletion of miR-15a/16-1 accelerates the proliferation of both human and mouse B cells by modulating the expression of genes controlling cell-cycle progression and apoptosis.

It is known that all Burkitt lymphomas (BL) have a B-cell phenotype and a Myc translocation, but a variable association with the Epstein-Barr virus (EBV). A recent study suggests that EBV-positive and EBV-negative BL have different cells of origin (46). According to immunoglobulin gene mutation analysis, EBV-negative BLs may originate from early centroblasts, whereas EBV-positive BLs seem to arise from postgerminal center B cells or memory B cells. In this regard, Leucci *et al* identified that miR-127 is differentially expressed between EBV-positive and EBV-negative BLs (46). miR-127 is upregulated only in EBV-positive BL samples and plays a role in B-cell differentiation process through posttranscriptional regulation of BLIMP1 and XBP1. Thus, upregulation of miR-127 may represent a key event in the lymphomagenesis of EBV positive BL by blocking the B-cell differentiation process.

5.2. MicroRNAs involved in differentiation of LSCs

Given that the genetic lesions in leukemia may result in a block of differentiation such that myeloid

Table 1. MicroRNAs implicated in LSCs differentiation

AML-acute myeloid leukemia	miR-17 family (48), miR-29a (49), miR-155 (50), miR-34a (11), miR-29b (51), miR-126 (52)
APL-acute promyelocytic leukemia	miR-342 (47)
CML-chronic myeloid leukemia	miR-34a (62)

leukemic cells continue to proliferate and/or prevents the terminal differentiation and apoptosis seen in normal white blood cells, differentiation therapy has been used successfully for treatment of human myeloid leukemias. A well-known model for such treatment is acute promyelocytic leukemia (APL), where retinoic acids can target the retinoic acid receptor (RARα) component of the fusion product derived from the PML-RAR alpha (t15:17) translocation, causing degradation of the fusion protein by ubiquitinization. Of interest, ATRA treatment induces granulocytic maturation and complete remission of leukemia which involves induction of miR-342 (47). Furthermore, miR-342 is regulated by several critical hematopoietic transcription factors including PU.1 and interferon regulatory factor (IRF)-1 and IRF-9. For instance, IRF-1 keeps miR-342 at low levels, whereas the binding of PU.1 and IRF-9 in the promoter region following retinoic ATRA-mediated differentiation upregulates miR-342 expression (47). As listed in Table 1, there are several other microRNAs implicated in LSC differentiation.

For example, microRNAs are differentially expressed in LSC-enriched cell fractions (c-kit$^+$) in a mouse model of MLL leukemia (48). In particular, members of the miR-17 family are highly expressed in LSCs, compared with their normal counterpart granulocyte-macrophage progenitors and myeloblast precursors. Once differentiation, expression of the miR-17 family is substantially reduced; on the other hand, ectopic expression of this cluster significantly shortens the latency for MLL leukemia development. Importantly, leukemias expressing high levels of the miR-17 family reveal a higher frequency of LSCs, more stringent block of differentiation, and enhance proliferation associated with reduced expression of p21. Expression of c-Myc, a crucial upstream regulator of the miR-17 cluster, correlates with miR-17~92 levels, enhanced self-renewal, and LSC potential.

Han *et al* (49) showed that miR-29a is highly expressed in HSC and down-regulated in hematopoietic progenitors. Ectopic expression of miR-29a in mouse HSC/progenitors results in acquisition of self-renewal capacity by myeloid progenitors, biased myeloid differentiation, and the development of a myeloproliferative disorder that progresses to acute myeloid leukemia (AML). It appears that miR-29a promotes the initiation of AML by converting myeloid progenitors into self-renewing LSC. On the other hand, miR-155 has been implicated in granulocyte/monocyte (GM) expansion (50). For example, miR-155 is overexpressed in the bone marrow of patients with certain subtypes of AML. Furthermore, miR-155 is able to repress a subset of genes involved in hematopoietic development and disease, thereby implicating miR-155 as a contributor to physiological GM expansion during inflammation and to certain pathological features associated with AML.

As discussed early, the C/EBPα-mutants are known to display distinct biological function during leukemogenesis. Of interest, miR-34a is a novel target of C/EBP alpha in granulopoiesis (11). During granulopoiesis miR-34a targets E2F3 and blocks myeloid cell proliferation. Analysis of AML samples with C/EBPα mutations revealed a lower expression of miR-34a and elevated levels of E2F3 as well as E2F1. MicroRNA-mediated differentiation may also involve epigenetic regulation of gene expression by suppression of genes involved in methylation. For example, enforced expression of miR-29b in AML cells can result in marked reduction of the expression of DNA methyltransferases DNMT1, DNMT3A, and DNMT3B (51). This in turn decreases global DNA methylation and re-expression of p15 (INK4b) and ESR1 via promoter DNA hypomethylation.

Finally, three microRNAs (miR-126, miR-145, and let-7) have been implicated in progenitor cell differentiation by regulation of HOXA9, because forced expression of these microRNAs in Hoxa9-immortalized bone marrow cells reduces the HOXA9 protein and causes loss of biological activity (52). For example, miR-126 and Hoxa9 mRNA are coexpressed in hematopoietic stem cells and downregulated in parallel during progenitor cell differentiation; however, miR-145 is barely detectable in hematopoietic cells, and let-7s are highly expressed in bone marrow progenitors, suggesting that miR-126 may function in normal hematopoietic cells to modulate HOXA9 protein.

6. MICRORNAS AS BIOMARKERS IN LEUKEMIAS

Overwhelming evidence indicates that alterations of microRNA expression are often associated with many types of human cancers (53-57). Thus, microRNA signatures have been well demonstrated as biomarkers in solid tumors (58). Similarly, several microRNAs including miR-142, miR-144, miR-150, miR-155, and miR-223 may serve the same role in leukemias because they are specifically expressed in the hematopoietic system. For instance, lymphoid and myeloid cells could be clearly distinguished based on their microRNA profiles, as could three diagnostic AML bone marrow (BM) samples from remission samples from the same patients (59).

Several reports support this notion. Notably, Mi *et al* showed that microRNA expression patterns are able to discriminate AML from ALL (60). They used BM or peripheral blood (PB) mononuclear cells obtained at the time of diagnosis or relapse from 11 patients with ALL and 47 patients with AML with common translocations, as well as 7 ALL cell lines, 7 AML cell lines, and mononuclear or CD15 positive cells from BM of three healthy donors. These samples were subject to bead-based microRNA expression profiling (61). Among 112 microRNAs tested, 27 microRNAs are significantly differentially expressed between AML and ALL. Bousquet *et al* (44) identified miR-125b-1 to be located in chromosome band 11q23 in

the vicinity of the break-point region involved in the rare but recurrent translocation t(2;11) (p21;q23), miR-125b-1 is highly expressed in patients with AML and myelodysplastic syndromes carrying this translocation as compared to other patients or to healthy BM. Ectopic expression of miR-125b-1 inhibits myeloid differentiation, suggesting that this microRNA is the oncogene specifically associated with, and activated by, the t(2;11). Together, these findings highlight the diagnostic potential of microRNAs in leukemias.

7. CONCLUSION

As master gene regulators, microRNAs could play a crucial role in human hematopoiesis. This is very likely through regulation of critical transcription factors required for this process. While certain microRNAs may promote, the other may suppress this process. Despite the controversy over the origin of LSCs, microRNA-mediated gene regulation is an important component of this complex regulatory system. Apparently, microRNAs do not act alone; they cooperate with key transcription factors. Of particular interest, microRNAs and transcription factors seem to form an autoregulatory circuit to determine cell fate. Therefore, a better understanding of how microRNA-mediated gene expression leads to the formation of LSCs and leukemia initiation and progression will help identify novel therapeutic targets. This is particularly valuable for differentiation therapy strategy. Unfortunately, we still know very little about this complex regulatory system, and more work is needed to illustrate the molecular mechanism of how microRNAs target different sets of genes under different cellular contents.

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