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Brief Reviews

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Small RNAs Guide Hematopoietic Cell Differentiation and Function

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MicroRNAs (miRNAs) are key regulators of gene expression that help direct normal differentiation and malignant transformation of hematopoietic cells. This review summarizes our current knowledge of how miRNAs function in normal and malignant hematopoiesis and how miRNAs might be applied for disease treatment. ***The Journal of Immunology*****, 2010, 184: 5939–5947.**

MicroRNAs (miRNAs) are small noncoding RNAs that suppress gene expression by binding to partially complementary sequences mostly in the 3′UTR of mRNAs and inhibiting their translation into protein or accelerating their degradation (1). miRNAs regulate all aspects of a cell, including its differentiation, function, proliferation, survival, metabolism, and response to changes in its environment. Although initial studies suggested that miRNAs might act as rheostats to fine tune the functional state of a cell, some miRNAs appear to act as master regulators of cell fate decisions in response to environmental or developmental cues by altering the expression of key regulatory genes and/or by regulating many genes in a pathway. Since their discovery in *Caenorhabditis elegans* in 1993, great progress has been made in understanding miRNA function and mechanisms of action. The contribution of miRNAs to regulating gene expression in many different cellular processes and contexts is just beginning to be understood. We will review what is known about miRNA function during normal and malignant hematopoietic cell differentiation.

miRNA biogenesis, mechanisms of action, and target recognition

miRNA genes are encoded in intergenic chromosomal regions or within introns of protein coding genes. They are transcribed by RNA polymerase II or III to generate a primary (pri-miRNA) transcript that contains a characteristic stem loop structure. The same transcription factors (TFs) that control transcription of coding genes also regulate transcription of miRNA primary transcripts. The primary transcript is processed in the nucleus by the Drosha-DGCR8 microprocessor complex to generate a shorter, ~70 nt pre-miRNA, which is exported to the cytoplasm by exportin 5 (1). Once in the cytoplasm, the pre-miRNA is further processed by the RNase Dicer to an imperfectly paired, ~22-bp double-stranded mature miRNA. One strand, the guide strand, is loaded into the RNA-induced silencing complex (RISC), the cellular machine for post-transcriptional gene regulation.

miRNA biogenesis is regulated at multiple levels, which are just beginning to be described. The SMAD transcription factors, activated by TGF-β and bone morphogenic protein signaling, enhance processing of pri-miRNAs, such as miR-21, that regulate cell differentiation mediated by these growth factors (2). The tumor suppressor p53 enhances the activity of the microprocessor complex to facilitate the posttranscriptional maturation of several miRNAs that suppress cell growth (3). Both of these mechanisms involve binding to an RNA helicase (p68) that boosts microprocessor function. The turnover of pre-miRNAs of the let-7 family, which facilitates stem cell differentiation, is accelerated in many kinds of stem cells by the lin28 and lin28B proteins that promote addition of polyU to the 3′ end of the pre-miRNA, a signal for RNA degradation (4). In addition, control of mature miRNA turnover by specific exonucleases has been described in plants and worms (5, 6). Although this latter layer of regulation of miRNA function has not yet been described in mammals, RNA editing of the pri-miRNA precursor can modulate biogenesis of the mature miRNA and affect target recognition by altering the mature sequence (7). In addition, alternative processing by Drosha can generate different forms of a mature miRNA (8). However, the functional consequences of these variants remain unclear.

miRNAs repress gene expression by binding to partially complementary sequences mostly in the 3′UTR of their target mRNAs. Inhibition of gene expression is achieved by blocking protein translation and/or enhancing mRNA degradation. The degree of sequence complementarity between the miRNA and its target sequence may determine the relative role of these distinct, but coordinated, mechanisms of inhibiting gene expression. A high degree of complementarity may favor mRNA degradation over translational repression. Regardless of the mechanism, the final outcome is reduced protein. A single miRNA can potentially modulate the expression of hundreds or thousands of proteins in a cell, both by direct and indirect effects.

Immune Disease Institute and Program in Cellular and Molecular Medicine, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115

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Address correspondence and reprint requests to Judy Lieberman, Immune Disease Institute, Warren Alpert Building, Room 255, 200 Longwood Avenue, Boston, MA 02115. E-mail address: lieberman@idi.harvard.edu

Abbreviations used in this paper: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; B-ALL, B-lineage ALL; B-CLL, B cell chronic lymphocytic leukemia; BL, Burkitt's lymphoma; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; DLBCL, diffuse large B cell lymphomas; E, erythrocyte; HSC, hemopoietic stem cell; IGH, Ig H chain; miRNA, microRNA; MK, megakaryocyte; pre-miRNA, precursor microRNA; pri-miRNA, primary miRNA; RA, retinoic acid; TF, transcription factor; Treg, T regulatory.

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The number of human miRNAs annotated in the miRBase database surpasses 700 and will most likely increase as powerful deep-sequencing techniques identify new miRNAs. As many as 90% of human genes may be regulated by miRNAs. Because miRNAs bind to their targets by partial complementarity over a short sequence, and the factors that influence miRNA target recognition are not completely understood, identification of the critical genes regulated by a particular miRNA is still a challenge, especially in the context of the large number of genes whose expression changes after overexpressing or inhibiting an individual miRNA. mRNA complementarity to nucleotides 2–8 of the guide strand of the miRNA, called the "seed region," is an important determinant of mRNA recognition (9). Common strategies for identifying miRNA targets include the use of bioinformatics predictive tools, sometimes combined with mRNA microarray analysis of cells in which a particular miRNA is overexpressed or knocked down. Although these methods have been successfully applied to study miRNA function and have helped to identify important miRNA targets, they have important limitations. Bioinformatics algorithms typically predict hundreds or even thousands of targets of a single miRNA and have high false-positive rates. Algorithms that rely on 3′UTR miRNA seed sequences, especially those that are conserved during evolution, seem to perform better, but they still fail to predict biologically relevant targets, such as RAS for let-7 and E2F2 and MYC for miR-24 (10, 11). Seedless miRNA binding sites or sites in the coding region or 5′UTR of genes can mediate recognition of biologically relevant miRNA targets (11, 12). Additional factors, including RNA secondary structure, sequence context and binding of RNA-binding proteins also influence miRNA target binding, adding more complexity. A major caveat for the use of gene microarrays to study miRNA function is that often miRNA-mediated changes in mRNA levels are modest compared with the variations in microarray hybridization signals; moreover, this method does not distinguish direct mRNA targets from miRNA-mediated secondary effects and also misses targets that are regulated primarily through translational inhibition. Alternative biochemical approaches based on the pull-down of tagged components of the RISC complex or biotin-conjugated miRNAs have been recently developed (13). Interestingly, the miRNA targets identified in pull-downs of the RISC protein Ago1 differ in the molecular, structural, and functional properties of their binding sites compared with the targets upregulated after Ago1 depletion (14). Although this finding suggests that biochemical methods might be useful to identify targets that might otherwise be overlooked by other approaches, their efficacy in identifying biologically relevant targets still requires further experimental assessment. Proteomics analysis of proteins differentially labeled with stable isotopes (SILAC) in cells with manipulated miRNA expression has also been used to identify miRNA targets (15, 16). However, this method does not distinguish between direct and indirect miRNA effects, and its technical complexity and high cost limit its use.

miRNAs and normal hematopoiesis

Tight control of gene expression is required for hematopoiesis. Traditionally, the major focus of research has been to study the role of TFs in regulating hematopoiesis. Lineage-specific TFs are key regulators of gene expression in multiple cell-fate decisions that govern hematopoietic differentiation. Given the important role of miRNAs in development and differentiation, it is not surprising that these regulatory RNAs also play crucial roles in hematopoiesis. TFs and miRNAs act in concert to regulate gene expression during hematopoietic differentiation; TFs regulate the expression of miRNA genes, whereas TFs are key miRNA targets. Overall miRNA expression generally increases with cellular differentiation. This finding suggests that miRNAs might play a role in shutting down genetic programs that are required for maintaining alternate cell fates or that prevent differentiation more generally. In this section, we will review examples of miRNAs that regulate hematopoiesis (Table I).

miRNA profiling in mice and humans has identified changes in miRNA expression during hematopoietic cell differentiation. Some examples follow. miR-223, miR-181, and miR-142 are preferentially expressed in mouse hematopoietic tissues (18). miR-150 is upregulated during B and T cell maturation (26). miR-146 is upregulated during differentiation of naive T cells to Th1 effectors (26). Specific miRNAs are expressed during T cell development in the thymus; moreover, their stage-specific expression correlates with the depletion of transcripts containing seed matches for the enriched miRNAs. In particular, miR-181a is highly enriched at the double-positive $CD4^+CD8^+$ stage of thymocyte development, and its expression inversely correlates with the expression of its putative targets Bcl-2, CD69, and TCRalpha, indicating a potential role for miR-181a in regulating positive selection (see below and Fig. 1*A*) (27). miRNA expression analysis of human $CD34^+$ hematopoietic stem cell precursors from bone marrow and mobilized peripheral blood identified a common signature of 33 miRNAs (28). Bioinformatics analysis of combined miRNA and mRNA expression data and miRNA target predictions suggested a potential role for miRNAs in suppressing genetic programs that specify hematopoietic differentiation. However, although an interesting hypothesis, it requires further experimental proof.

The first experimental evidence of the functional importance of miRNAs in mammals was provided by studying the differentiation of hematopoietic precursor cells in transplanted mice. Transduction of mouse Lin^- hematopoietic precursors with miR-181a, which was examined because it is selectively highly expressed in the thymus and mature B cells, alters lineage differentiation in vitro (18). Overexpression of miR-181a in these cells doubles B cell numbers, but does not significantly change the number of T cells. On the contrary, enforced expression of miR-223 and miR-142 modestly, but significantly, increases T cell numbers, but does not affect B cell numbers. Consistent with the in vitro data, transplantation of miR-181a–transduced Lin^- hematopoietic precursors into irradiated mice increases $CD19^+$ B cells and substantially decreases T cells, especially $CD8^+$ T cells. This study placed miRNAs on the central stage of hematopoiesis research. The requirement for miRNAs during hematopoietic differentiation was further demonstrated by conditionally deleting Dicer in specific hematopoietic compartments. Targeted deletion of Dicer in the thymus blocks peripheral $CD8^+$ T cell development and reduces the $CD4^+$ T cell compartment (29). Dicer-deficient $CD4^+$ T cells have reduced cell proliferation, have increased apoptosis, and are skewed toward the Th1 lineage. Targeted deletion of Dicer at early stages of B cell differentiation blocks

Table I. *Expression of some of the most biologically relevant miRNAs in normal and malignant hematopoiesis*

miRNA	Hematopoietic Lineage	Hematopoietic Malignancy	Relevant Targets	In Vivo Studies and Phenotype	References
miR-223	Myelocytes	ALL, CLL	*NFI-A, E2F1, MEF2c*	Knockout mouse; neutrophilia, granulocyte hyperactivation, inflammation	(17)
miR-181	Lymphocytes	B-CLL	*Bcl2, CD69, TCRα AID, SHP-2, PTPN22, Tcl1, PLAG1*	Transplantation of miR-181 transduced Lin^- precursors; increase in B cell numbers and decrease in $CD8^+$ T cells	(18)
miR-150	Lymphocytes MKs	CLL	*MYB*	B1 cell expansion in miR-150 knockout mice; defective B cell development in miR-150 transgenic mice	(19)
miR-155	Lymphocytes Myelocytes	CLL, AML, Lymphoma	*SHIP1, C/EBPβ, AID, SOCS1, PU.1, Cepbb, Csfr1, HIFα, TAB2*	Eμ-miR-155 transgenic mice have aberrant B cell proliferation and leukemia; miR-155 knockout mice have defective immune response and inflammation and reduced Treg cells	(20, 21, 22)
miR-17∼92	Lymphocytes	CML, Lymphoma	*Bim, E2F1, PTEN*	Targeted deletion of miR-17∼92 results in defective B cell development; miR-17∼92 transgenic mice develop lymphoproliferative disease and autoimmunity; increased tumorigenesis in a mouse model of B cell lymphoma	(23, 24, 25)

miRNAs were chosen based on evidence from in vivo models.

B cell development at the pro- to pre-B cell transition, with a pronounced increase in pre-B cell apoptosis (30). Global analysis of gene expression in Dicer-deficient pro-B cells revealed a significant enrichment of mRNAs that contain 3′UTR heptamer sequences complementary to the seed motif of several members of the miR-17∼92 cluster. Ectopic expression of this oncogenic miRNA cluster in Eμ-myc mice accelerates B cell lymphomagenesis and cell death (23). More recently, this effect has been shown to be due to a single miRNA family in the cluster, miR-19, which suppresses expression of the tumor suppressor phosphatase Pten. Homozygous deletion of Bim in the Dicer knockout background partially restores the block in B cell development (30). The crucial role for the miR-17∼92 cluster in regulating the expression of the proapoptotic protein Bim during B cell development has also been independently demonstrated by studies in mice with targeted deletion of the miR-17∼92 miRNA family (24). Another miRNA that plays a crucial role in B cell differentiation is miR-150 (Fig. 1*B*). miR-150 is expressed only in hematopoietic cells and is highly expressed in mature lymphocytes. Mice deficient in miR-150 have an expansion of B1 cells, whereas mice expressing an miR-150 transgene have impaired B cell development owing to a block at the pro-B to pre-B transition (19). Similarly, premature expression of miR-150 in hematopoietic precursors blocks B cell differentiation at the same stage (31). The key TF c-myb is directly regulated by miR-150 (19). In mice, even small changes in c-myb levels have a profound effect on B cell development. Therefore, the moderate decrease in c-myb levels observed in miR-150 transgenic mice might be sufficient to block B cell development.

The interplay between TFs that regulate myelopoiesis and miRNAs may be complex. TFs and miRNAs work together to regulate this important transition. miR-223, another miRNA whose expression is largely restricted to hematopoietic cells, has emerged as an important regulator of myelopoiesis. Mice knocked out for miR-223 have an expanded granulocytic compartment owing to enhanced proliferation and differentiation (17). This phenotype can be reverted by genetic ablation of the TF MEF2c, which promotes myeloid differentiation and is a direct target of miR-223. Another recently identified target of miR-223 is the E2F1 TF, which regulates cell cycle progression through the G1-S transition. miR-223 expression can be dysregulated in leukemia cells. Retinoic acid (RA)-induced granulocyte differentiation of acute promyelocytic leukemia (APL) cells strongly upregulates miR-223 expression. Enforced miR-223 expression in APL cells enhances granulocyte differentiation, whereas miR-223 knockdown blocks differentiation in response to RA. Decreased miR-223 expression is linked to poor prognosis in chronic lymphocytic leukemia (CLL). In humans, miR-223 can be expressed from more than one promoter. A longer transcript that is conserved in mice and humans and specific to myeloid cells is regulated by binding of the TFs PU.1 and C/EBPβ (32). Another promoter within the 1-Kb region upstream of the pre-miR-223 sequence is regulated by TFs C/EBPα and NFI-A (33). NFI-A inhibits miR-223 transcription from this promoter in APL cells. After treatment with RA, C/EBPα replaces NFI-A at the promoter region and induces miR-223 expression, which in turn, in a positive feedback loop, inhibits translation of NFI-A. The transcriptional repressor Gfi1, which is required for myelocytic differentiation at the granulocyte-monocyte progenitor (GMP) stage, coordinately inhibits the expression of miR-21 and miR-196b during normal myelopoiesis. Additional examples of functionally relevant interactions between miRNAs and TFs during myelopoiesis are illustrated by the transcriptional activation of miR-424 by PU.1 and the translational inhibition of AML1 by miRNA-17-5p/20a/106a to regulate NFI-A and M-CSF, respectively, during monocytic differentiation.

miRNA expression is also dynamically regulated during megakaryocyte (MK) and erythrocyte (E) differentiation. A discrete subset of miRNAs is downregulated during in vitro MK differentiation of human $CD34^+$ hemopoietic stem cell (HSC) progenitors from bone marrow (34). Expression of the downregulated miRNAs, miR-10a, and miR-130a, correlates inversely with expression of their predicted targets, *HOXA1* and *MAFB*, respectively. Both of these genes have been experimentally validated as miRNA targets, but the functional impact of knocking them down during MK differentiation of HSC progenitors has not been experimentally assessed. miR-150 is highly expressed in MKs as compared with Es or MK-E progenitor cells (35). miR-150 promotes MK differentiation at

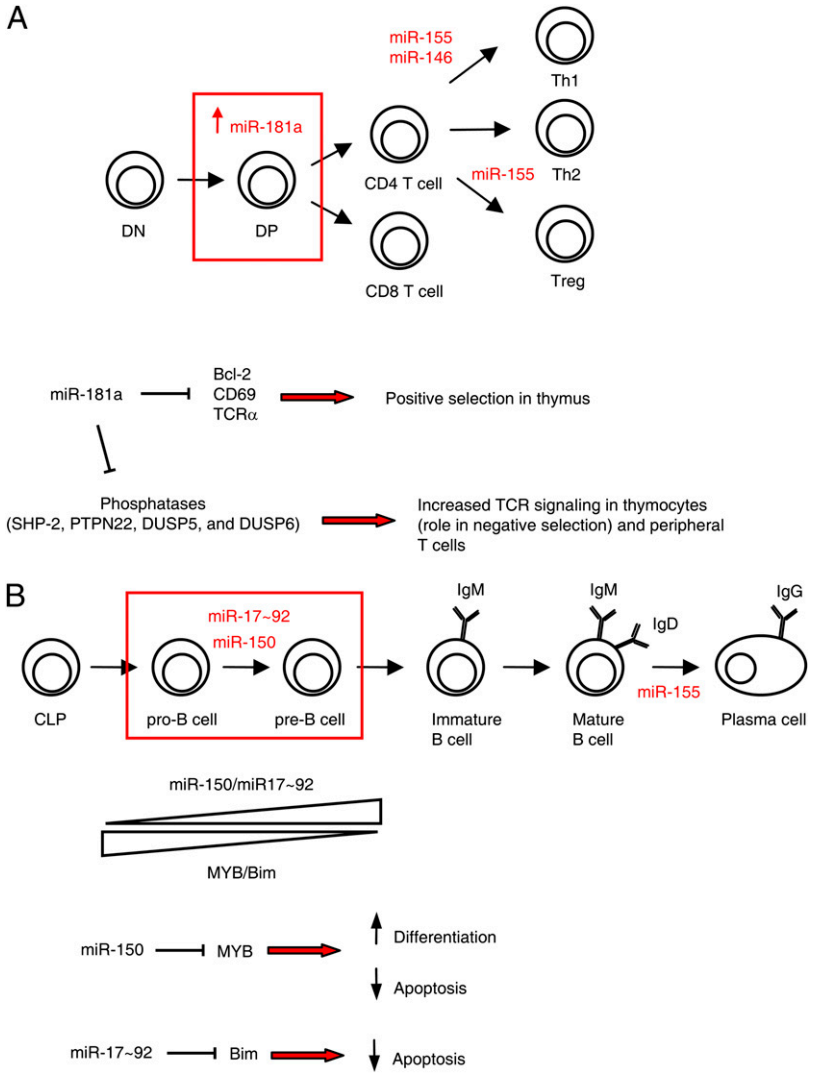

FIGURE 1. Role of miRNAs in lymphocyte differentiation. *A*, T cells. miR-181a is a key regulator of TCR Ag sensitivity during T cell differentiation in the thymus. In the periphery, miR-146 and miR-155 favor Th1 differentiation. miR-155 is also important for Treg cell differentiation. *B*, B cells. miR-150 and the miR-17~92 cluster are crucial regulators of B cell differentiation at the pro-B to pre-B transition. miR-155 is important for Ag-driven B cell maturation and production of high-affinity IgG Abs.

the expense of the erythroid pathway, mostly by controlling the levels of the transcriptional regulator MYB. MYB expression during MK differentiation is also suppressed by miR-34a (36). Functional studies of miRNAs differentially expressed during MK or E differentiation of purified human $CD34^+$ hematopoietic progenitors are helping delineate how these miRNAs modulate signaling pathways to influence differentiation. miR-155 and miR-146a are highly expressed in $CD34^+$ HSCs, where they repress genetic programs that promote MK differentiation. miR-155 suppresses MK differentiation by repressing the TFs Meis1 and Est-1, whereas miR-146a, which is transcriptionally inhibited by PLZF, suppresses the chemokine receptor gene CXCR4. These miRNA targets promote MK differentiation. Similarly, downregulation of miR-221/222 and miR-24 is required for E differentiation (37). miR-221/222 inhibits E differentiation at least in part by translational inhibition of c-kit, whereas miR-24 inhibits erythropoiesis by targeting the activin type I receptor ALK4. miR-15a might also inhibit erythropoiesis, possibly through regulation of MYB. In vivo studies in zebrafish identified the miRNA cluster miR-144/451 as part of the GATA-1 gene network and as an essential positive regulator of erythropoiesis (38). miR-451 regulates expression of the TF GATA2 and is important for E maturation in vivo, whereas miR-144 inhibits expression of klfd and regulates embryonic α-globin synthesis during embryonic erythropoiesis.

miRNAs and hematologic malignancies

Given the essential role of miRNAs in regulating development and differentiation, it is not surprising that miRNAs are emerging as important regulators of tumorigenesis. Dysregulation of miRNA expression is associated with oncogenic transformation. A high proportion of miRNA genes are encoded in cancer-associated genomic regions, fragile sites and regions associated with loss of heterozygosity, gene amplification, and common breakpoint regions (39). miRNA signatures can define the cell of origin, developmental stage, prognosis or response to therapeutic intervention for many types of cancer (40). Some miRNAs function as tumor suppressor genes or oncogenes; these are collectively termed "oncomirs".

One of the first clues for a role of miRNAs in cancer came from studies of B cell CLL (B-CLL). Hemizygous and/or

homozygous deletions at the 13q14 locus occur in more than half of B-CLL cases. These breakpoints do not disrupt a common coding gene. Seminal work by Calin et al. (41) identified two clustered miRNAs, miR-15 and miR-16, at this locus, whose expression is downregulated in most CLL samples. The tumor suppressor activity of miR-15/16 is mediated in part by inhibition of the antiapoptotic protein BCL2 (42). BCL2 is overexpressed in most B-CLL cells, but is rarely the site of chromosomal translocations. Enforced expression of miR-15/16 decreases BCL2 protein and induces apoptosis. However, miR-15/16 expression and BCL2 expression do not always inversely correlate in B-CLL samples, suggesting that other target genes are important. Leukemia MEG-01 cells overexpressing miR-15/16 show significantly altered expression of genes involved in cell cycle regulation and apoptosis (43). miRNA profiling of a large number of B-CLL samples identified distinct miRNA signatures associated with two known CLL prognostic factors, ZAP70 expression and mutational status of IgV_H genes (44). These miRNA signatures can be used independently to predict CLL prognosis. These studies have also identified other miRNAs with potentially relevant functions in B-CLL pathogenesis. miR-181 and miR-29, whose expression is reduced in CLL as compared with normal B cells, can act as tumor suppressors by targeting the oncogene *Tcl1*. Epigenetic silencing of the miR-181 promoter in B-CLL cells is associated with upregulation of the miR-181 oncogenic target PLAG1 (pleomorphic adenoma gene 1). miR-92a, which is upregulated in B-CLL, directly suppresses the von Hippel-Lindau protein tumor suppressor gene. In addition, apoptosis in B-CLL cells induced by treatment with deacetylase inhibitors may be mediated by miR-106b induction, which inhibits expression of the ubiquitin E3 ligase Itch to activate the proapoptotic molecule p73. Decreased expression of let-7a and miR-30d and increased levels of miR-155, miR-150 and miR-21 have also been reported in CLL, but their functional significance remains to be elucidated (45–47). Low expression of miR-34a, an important tumor suppressor miRNA, is associated with p53 inactivation in B-CLL cells.

The BCR-ABL1 translocation, which aberrantly activates the tyrosine kinase ABL1, is a hallmark of chronic myelogenous leukemia (CML) that drives the pathogenesis of this disease. Comparison of miRNA expression between CML cells with intact BCR-ABL1 protein and cells with impaired BCR-ABL1 activity indicated that the miRNA cluster miR-17∼92 is activated by BCR-ABL1 in CML (48). This cluster is regulated by MYC in these cells. Upregulation of the miR-17∼92 cluster, which correlates with increased proliferation of CML cells, was observed in $CD34^+$ cells from chronic-phase, but not blast-crisis, patients (48). miR-10a is downregulated, independently of BCR-ABL1 activity, in CML samples. miR-10a downregulation enhances cell proliferation, possibly through derepression of its oncogenic target, upstream stimulatory factor 2. Inactivation of miR-203, which is frequently deleted in some hematopoietic malignancies and is also silenced by promoter hypermethylation in CML, may also play an important role in CML pathogenesis (49). miR-203 suppresses the expression of ABL1 and BCR-ABL1.

Acute leukemias, which originate from genetic alterations in poorly differentiated blood cells, can be subtyped based on their miRNA expression. miRNA expression not only distinguishes chronic versus acute lymphoblastic leukemias (ALLs), but also accurately identifies the lineage of acute leukemias. Differential expression of four miRNAs (miR-128a, miR-128b, let-7b, miR-223) distinguishes ALL from acute myeloid leukemia (AML) (50), whereas a signature of three miRNAs (miR-148, miR-151, miR-424) differentiates B-lineage (B-ALLs) from T-lineage ALLs (51). In addition, B-ALL subgroups with different genetic abnormalities can also be distinguished based on their miRNA profiles. The first indication of a potential role for miRNAs in ALL pathogenesis was the identification of the insertion of miR-125b into the Ig H chain (IGH) locus in a B-ALL patient (52). More recently, high expression of miR-125b has been described in a subtype of childhood ALL bearing the ETV6/RUNX1 translocation, potentially conferring increased resistance to apoptosis. Epigenetic silencing of the tumor suppressor miR-124a by hypermethylation of its promoter and downregulation of miR-124a is also observed in a significant proportion of patients with ALL and is associated with poor prognosis. Enforced expression of miR-124a in ALL cells inhibits cell proliferation in vitro and tumorigenesis in vivo, effects that can be ascribed at least in part to repression of CDK6 by miR-124a.

AMLs are heterogeneous because of a wide range of cytogenetic and molecular genetic alterations. Distinct miRNA signatures are associated with specific cytogenetic and molecular AML subgroups (53), highlighting the potential relevance of miRNAs as diagnostic markers. Studies of AML-associated miRNAs have helped define novel molecular pathways that govern AML leukemogenesis. Enforced miR-155 expression in hematopoietic precursors in mice induces myeloid dysplasia (54). miR-155 is overexpressed in bone marrow from AML patients and $FLT3^-ITD^+$ AML samples (53). A possible role for miR-125b in myeloid transformation is supported by the observation of higher miR-125b levels in AML samples harboring the t(2;11)(p21;q23) translocation and by in vitro experiments indicating that miR-125b overexpression inhibits myeloid differentiation (55). Epigenetic silencing of miR-34b might be responsible for aberrant upregulation of the TF CREB in AML, which could contribute to uncontrolled leukemia cell proliferation. miR-29b, which is downregulated in certain types of AML, can act as a tumor suppressor because its ectopic expression in AML cells induces their apoptosis (56). miR-29b exerts its tumor suppressor activity by inhibiting DNA methyltransferases, which results in global hypomethylation, and by coordinated repression of genes that regulate the cell cycle and apoptosis, such as CDK6 and MCL-1 (56). Loss of miR-145 and miR-146a, owing to an interstitial deletion of chromosome 5q in the 5q-myelodysplastic syndrome, explains some of its features. Knockdown of these miRNAs in mouse HSCs results in thrombocytosis, megakaryocytic dysplasia, and mild neutropenia—phenocopying the 5q- syndrome. TIRAP and TRAF6, which participate in TLR signaling, are targets of miR-145 and miR-146a, respectively, and mediate the effects of these miRNAs in the 5q- syndrome.

miR-155 and the miR-17∼92 cluster are well validated oncomirs in lymphoma pathogenesis. miR-155 is overexpressed in diffuse large B cell lymphomas (DLBCLs), Hodgkin's lymphoma, primary mediastinal B lymphomas and pediatric Burkitt's lymphoma (BL) (57). Eμ-miR-155 transgenic mice with targeted overexpression of miR-155 in the B cell compartment develop aberrant pre-B cell proliferation

that evolves to full-blown B cell neoplasm (20). Suppression of the protein phosphatase SHIP1 and the TF C/EBPβ, which negatively regulate IL-6 signaling, contributes to miR-155–induced lymphomagenesis in this model (58). Suppression of SHIP1 by miR-155 also plays a role in DLBCL, in which blocking TNF-α reduces miR-155 levels that is accompanied by an increase in SHIP1 and a concomitant reduction in cell proliferation and tumor growth in DLBCL xenografts. The miR-17∼92 cluster is located at human chromosome 13q31, a region frequently amplified in malignant lymphomas. miR-17∼92 enhances Eμ-myc–induced B cell lymphomas in mice (25). Moreover, mice bearing a miR-17∼92 transgene expressed in the lymphocyte compartment develop lymphoproliferative disease and autoimmunity (23). In the tumors, miR-17∼92 regulates the balance between proliferation and apoptosis. Transcriptional activation of miR-17∼92 by MYC or E2F3 inhibits the expression of its targets E2F1, PTEN, and Bim, which reduces apoptosis and favors cell proliferation. Although MYC enhances expression of the oncogenic miR-17∼92 cluster, MYC more typically represses expression of many miRNAs (59). MYC-mediated repression of global miRNA expression facilitates lymphoma cell growth in vivo. Consistent with these data, a regulatory loop involving MYC/miR-26a/EZH2 contributes to aberrant proliferation of BL cells (60). Down-regulation of miR-34b or let-7a in BL cells might further contribute to lymphomagenesis by derepressing MYC. In addition, downregulation of miR-143 and miR-145 has also been reported in BL and might contribute to its pathogenesis by increasing ERK5 levels.

miRNAs as modulators of immune function

Aside from their contributions to the development of mature blood cells that compose the immune system, miRNAs also regulate immune cell effector function. Precise and coordinated control of gene expression is essential for a proper immune response to pathogens and to control its strength and duration so that it does not turn deleterious to the host. miRNAs participate in immune cell lineage maintenance, effector function, inflammation, and autoimmunity. Consistent with this idea, functionally distinct subpopulations of B and T cells express characteristic miRNA signatures, indicating dynamic miRNA regulation upon Ag recognition.

miR-181 and miR-155 are emerging as important regulators of lymphocyte function (Fig. 1). miR-181a acts as a rheostat to modulate TCR signaling during T cell selection in the thymus (61). High expression of miR-181a in immature thymocytes contributes to increased T cell sensitivity to low-affinity self-Ags by coordinately suppressing the expression of multiple phosphatases that inhibit TCR signaling. Reduction of miR-181a in double-positive thymocytes interferes with positive and negative selection to generate self-peptide reactive T cells. Genetic ablation of miR-155, which is highly expressed in activated B and T lymphocytes and activated macrophages and dendritic cells, severely impairs immune function (21, 22). miR-155 knockout mice have reduced T regulatory (Treg) cells and develop lung fibrosis with an abnormally elevated number of leukocytes, have enteric inflammation, and fail to mount a protective immune response to bacterial pathogens after vaccination (21). The dampened immune response is caused by decreased Ab and cytokine production because of intrinsic defects in B cell function and defective activation of T cells by dendritic cells. In addition, the T cell response is skewed toward Th2 cells, and the germinal center reaction is suppressed as evidenced by a defect in T cell-dependent Ab production (22). miR-155 also regulates the levels of AID to control class switch recombination, but not somatic hypermutation (62). miR-181b can also inhibit class switch recombination by reducing AID expression.

Intact miRNA function is critical for Treg cell lineage maintenance and function. Conditional disruption of Drosha or Dicer in Treg cells leads to reduced expression of multiple genes associated with suppressor function (CTLA4, IL-10, granzyme B) and aberrant upregulation of markers characteristic of other T cell lineages (IL-4, IFN-γ) (63–65). More importantly, miRNA disruption results in loss of Treg cell suppressor function in vivo, which leads to systemic autoimmunity, phenocopying the FoxP3 knockout *scurfy* mouse. Normally, FoxP3 activates expression of miR-155, which in turn represses SOCS1, which promotes Treg proliferation in nonlymphopenic settings when the amount of growth factors is limiting (66). Although miR-155 contributes to Treg cell survival by enhancing IL-2 signaling, it does not appear to affect Treg cell suppressor function. This finding suggests that other unidentified miRNAs mediate the effect of Dicer or Drosha knockout on Treg cell function. miRNA disruption by targeted deletion of Dicer in hematopoietic precursors and endothelial cells also affects the development and maturation of invariant NKT cells and prevents α-GalCer–induced activation and cytokine production. miR-17∼92 represses BCL6, which regulates differentiation of follicular Th cells, a specialized T cell effector subset important for T cell-dependent Ab responses.

miR-155 and miR-146 are important regulators of inflammation. Both miRNAs are upregulated in response to LPS in human and mouse monocytic cell lines, but they have opposing effects—miR-146 inhibits, whereas miR-155 enhances, inflammation. miR-146 expression is also induced by other bacterial components and proinflamatory cytokines, such as TNF-α and IL-1β, in an NF-κB dependent manner (67). miR-146 attenuates the inflammatory response by directly inhibiting TRAF6 and IRAK1. miR-146 participates in a negative feedback loop that fine tunes TLR and cytokine signaling cascades. miR-155 is also upregulated by TLR ligands other than LPS and by IFN-β in murine macrophages (68). In both cases, miR-155 activation depends on the JNK pathway. The biologic relevance of miR-155 as a key mediator of the inflammatory response is underscored by in vivo data showing that enforced miR-155 expression in mouse HSCs drives myeloid cell proliferation, which mimics the proinflammatory effect of LPS (54). Among the potential target genes in the proinflammatory effect of miR-155 are genes that activate transcription in myeloid cells (PU.1, Cepbb), cytokine receptors (Csfr1), and the stress response transcription factor HIF1α. In human monocyte-derived dendritic cells, miR-155 also suppresses inflammation by directly targeting TAB2, an adaptor in the TLR/IL-1 signaling cascade, to attenuate IL-1 receptor signaling during the response to LPS (69). Activation of the protein kinase AKT1 also affects miRNA expression to reduce endotoxin sensitivity in murine macrophages. AKT1 positively regulates expression

of let-7e, which inhibits TLR4, and negatively regulates miR-155, which represses SOCS1.

Implications for diagnosis, prognosis, and therapeutic intervention

Because miRNA profiling can identify tumor origin and prognosis with a high degree of accuracy, miRNAs might serve as useful biomarkers for the clinical diagnosis of blood malignancies and other cancers, replacing or supplementing more traditional tests that rely on cell morphology, cytochemistry, or cytogenetics. Some recent studies suggest that measuring miRNA plasma levels might be clinically useful for cancer detection or diagnosis. Serum from patients with DLBCL contains increased levels of the tumor-associated miRNAs miR-155, miR-210, and miR-21, compared with healthy controls (70). Moreover, increased miR-21 was also associated with relapse-free survival in patients with DLBCL. miRNA profiling might also be valuable for predicting clinical outcome. In patients with CLL, a 13-miRNA signature could distinguish patients with high expression of ZAP-70 and unmutated IgV_H, factors known to predict disease progression (44). This miRNA signature also correlated with the time from diagnosis to disease progression. Within this miRNA signature, miR-29c and miR-223 might be particularly valuable in predicting treatment-free survival and overall survival. In other cancers, miRNA profiles have also been linked to responsiveness to specific therapies (i.e., miR-26 expression and response to IFNs in hepatocellular carcinoma). The refined cancer subtyping that is possible using miRNA signatures should provide useful information for stratifying patients as to therapeutics (both in and beyond clinical trials), especially as more targeted cancer therapies directed at specific cancer pathways become available.

Because miRNAs play fundamental roles in the etiology of cancer, manipulating miRNA function, by delivering miRNA mimics that resemble the Dicer-processed endogenous miRNA or by antagonizing a miRNA using antisense oligonucleotides, is a promising approach for cancer therapy (71). miRNA manipulation might also be used to modulate diseases of inappropriate immune activation (i.e., in autoimmunity, transplant rejection, sepsis) or to enhance immune responses to vaccination or infection. miRNA-targeted therapies have the advantage of altering the expression of multiple genes that coordinately regulate key functional networks. Because of their multiple targets, drug resistance might be less of a problem than for conventional small molecule drugs. I.v. administration of a locked nucleic acid-modified oligonucleotide complementary to miR-122, a liver-specific miRNA required for hepatitis C virus replication, reduces viral load and improves hepatitis C virus-induced liver pathology in chronically infected chimpanzees (72). This miRNA-based drug is currently being evaluated in a phase I clinical study. However, a number of hurdles, especially efficient and specific small RNA delivery into cancer cells and/or immune cells, need to be overcome first (73). Because of their similar chemical structure, strategies that have been developed to deliver siRNA drugs in vivo can also be applied to deliver miRNAs. Systemic siRNA delivery has been achieved in animal models in vivo using liposomes, cationic polymers, and cholesterol-conjugated siRNAs. However, these methods are not efficient at transducing circulating cells, and lymphocytes are especially refractory to transduction. Alternative strategies for more specific delivery to lymphocytes and other hematopoietic cells—based on ligand or Ab-coated liposomes or nanoparticles, Ab fusion protein-RNA complexes, or aptamer-siRNA chimeras—have shown some promise in animal models (74–78).

Conclusions

miRNAs play a critical role in regulating fundamental processes within a cell. Many of the most elegant examples are studies of hematopoietic cell differentiation, where tight control of gene expression is required to coordinate genetic programs that favor differentiation toward a particular cell lineage and suppress alternate pathways. miRNAs contribute to this process by suppressing the expression of key genes that act as "master regulators," especially of lineage-specific TFs. miRNA deregulation contributes to the underlying etiology of leukemias and lymphomas. miRNAs also regulate programs of both innate and adaptive immunity. The next few years should see many studies that further unravel the role of miRNAs and the molecular basis for their action in normal and malignant hematopoiesis and immunity in addition to new efforts to harness this endogenous pathway for therapy.

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Disclosures

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