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The microRNAs involved in human myeloid differentiation and myelogenous/myeloblastic leukemia

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Abstract

MicroRNAs (miRNAs) are endogenously expressed, functional RNAs that interact with native coding mRNAs to cleave mRNA or repress translation. Several miRNAs contribute to normal haematopoietic processes and some miRNAs act both as tumour suppressors and oncogenes in the pathology of haematological malignancies. While most effort is engaged in identifying and investigating the target genes of miRNAs, miRNA gene promoter methylation or transcriptional regulation is another important field of investigation, since these two main mechanisms can form a regulatory circuit. This review focuses on recent researches on miRNAs with important roles in myeloid cells.

Keywords: microRNAs • myeloid haematopoiesis • myeloid leukemia

Introduction

MicroRNAs (miRNAs) are non-coding RNAs 19–24 nucleotides (nt) long that down-regulate gene expression by regulating the translation and degradation of target mRNAs, through base pairing to complementary sites. This binding can be perfect in plants and partial in mammals [1, 2]. The discovery of miRNAs casts doubt on the 'central dogma' that genetic information flows directly from DNA to protein, where RNAs only serve as mediators. The first miRNA, discovered in the nematode *Caenorhabditis elegans* in 1993, is lin-4, which contains antisense sequences complementary to a repeated sequence element in the 3' untranslated region (UTR) of the lin-14 mRNA [3]. Further studies demonstrated that the lin-4 gene product was a noncoding RNA that regulated several critical genes during development at the post-transcriptional level [4]. Several years later, let-7 was discovered as another small regulatory RNA in *C. elegans*, which regulates the expression of many genes that control developmental timing and cellular differentiation [5].

The biogenesis of miRNAs is divided between the nucleus and the cytoplasm. MiRNAs are initially transcribed by RNA polymerase II to produce long primary miRNAs (pri-miRNAs) [6]. Like protein-coding mRNAs, pri-miRNAs can be spliced, capped by 7-methyl guanosine at the 5' end, and have a polyadenylated tail added to the 3' end [7]. The human RNase III Drosha cleaves the pri-miRNA hairpin-shaped stem-loop structure to produce the precursor of miRNA (pre-miRNA) about 70–100 nt long with an extended stem-loop structure [8]. The pre-miRNAs are then transported to the cytoplasm by exportin-5 (Exp5)/RanGTP [9]. Once in

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the cytoplasm, pre-miRNAs are processed further by Dicer, a second RNase III endonuclease to produce the 19–24 nt miRNA duplexes [10]. To mediate the degradation or translation inhibition of target mRNAs, one strand of the duplex is incorporated into the protein effector complex miRISC (miRNA-containing RNA induced silencing complex) [11].

Almost 50% of mammalian miRNA genes are located in introns of protein-encoding genes or long noncoding RNA (ncRNA) genes, while the remaining miRNA genes are independent transcription units with specific promoter core elements and polyadenylation signals [2, 12]. Approximately half of known miRNAs are found in clusters, and are transcribed as polycistronic primary transcripts [13].

MiRNAs can be predicted in different species by computational methods based on the following criteria. First, miRNAs usually are highly conserved between the genomes of related species. Second, miRNAs display a characteristic pattern of evolutionary divergence. Large numbers of miRNAs are induced in specific temporal and spatial patterns during mid- to late embryonic development. The induction of miRNAs peaks in adult tissues, where a large fraction of the known miRNA genes are expressed [14, 15]. Early studies revealed most miRNA genes are down-regulated in cancer, perhaps reflecting a loss of cellular differentiation during tumorigenesis [16]. However, as more miRNAs involved in cancer are identified, miRNA expression during tumorigenesis is known now to be either up-regulated or down-regulated, depending on their specific function.

Identification of signature miRNAs and their role in lymphocytic leukemia has provided significant evidence that miRNAs are components of the molecular circuitry controlling leukemogenesis. MiR-15 and miR-16 are frequently deleted and/or down-regulated in B-cell chronic lymphocytic leukemia [17], the first evidence connecting the expression of miRNAs to a specific cancer-related abnormality. The miR-142 gene is located 50 nucleotides from the t(8;17) break between chromosome 17 and *c-myc*, a common chromosomal translocation occurring in leukemia cells. The t(8;17) translocation positions the coding region of the oncogene *c-myc* under the control of the regulatory elements of the B-cell lymphoma 3(*Bcl3)* gene located on chromosome 17, leading to aggressive acute prolymphocytic leukemia. Thus, miR-142 may be associated with overexpression of MYC [18]. However, the role of miRNAs in myeloid leukemia has been reported in only a few papers.

MiRNAs involved in myeloid differentiation may be involved in myeloid leukemia

Haematopoiesis is a highly regulated process in which the self-renewal and differentiation of pluripotent haematopoietic stem cells (HSC) into specific cell lineages is controlled by the coordinated regulation of gene expression. Leukemias result when either the self-renewal pathways become uncontrollable or when cells lose their ability to differentiate [19]. MiRNAs participate in the regulatory network, where their expression level is associated with different stages of cell differentiation. Chen and colleagues [20] first found that miR-181a, miR-142 and miR-223 are expressed preferentially in murine haematopoietic tissue. These miRNAs are expressed in HSCs, both B and T lymphocytes, monocytes, granulocytes and erythroid cells; ectopic expression of these miRNAs in murine HSCs *in vitro* and *in vivo* dramatically altered the proportion of differentiated cells [20]. Ramkissoon and colleagues [21] found that normal human cells expressed miR-223 and miR-142 in a manner very similar to the mouse, but that, in great contrast to the mouse, expression of miR-181a was detected only in normal human B cells, T cells, monocytes and granulocytes. Multiple miRNAs, including miR-17, -24, -146, -155, -128 and -181, may hold early haematopoietic cells at an early stem-progenitor stage, blocking their differentiation to more mature cells. MiR-16, -103 and -107 may block differentiation of later progenitor cells; miR-221, -222 and -223 most likely control the terminal stages of haematopoietic differentiation [22].

Myeloid cells (erythrocytes, granulocytes, monocytes and megakaryocytes/platelets) are the dominant cell population in bone marrow. Several miRNAs are involved in the control of myeloid gene expression, acting in cooperation with other regulatory molecules to modulate gene expression at post-transcriptional level. Since they have essential roles in myeloid differentiation, it is reasonable that abnormal expression of these miRNAs would contribute to aberrant myeloid development such as acute myeloid leukemia (AML) and chronic myeloid leukemia (CML).

MiR-221 and -222, clustered on the x chromosome, are markedly down-regulated in erythropoietic culture of cord blood (CB) CD34+ haematopoietic stem-progenitor cells (HSPCs). These miRNAs target c-KIT receptor mRNA to block expression of this key functional protein in CD34+ cells. The decline in miR-221 and -222 expression unblocks kit protein production and leads to early expansion of erythroid cells in normal HSPCs and the erythroleukemic cell line TF-1 [23]. As activation of c-KIT occurs in diverse neoplasias [24], and constitutive activation of c-KIT receptor tyrosine kinase prevents AML blasts from developing into normal blasts [25], studies on miR-221 and -222 may contribute to improving therapy in AML patients.

MiR-223, specifically expressed in myeloid cells, is up-regulated during granulocytic differentiation of acute promyeloid leukemia (APL) cells mediated by retinoic acid (RA) *in vivo* or *in vitro* [17, 26]. The primary action of miR-223 is to influence granulopoiesis. However, a recent study in mice reported quite opposite result that miR-223 negatively regulates progenitor proliferation, as well as granulocyte differentiation and activation. A transcription factor that promotes myeloid progenitor proliferation, myocyte enhancer factor 2C (Mef2C) is a target of miR-223. Genetic ablation of Mef2c suppresses progenitor expansion and corrects the neutrophilic phenotype in miR-223 null mice [27]. Moreover, expression of miR-223 is not limited to granulocytic differentiation but has been reported in erythroid cells [28].

MiR-196 participates in myeloid differentiation of HL-60 cells, a cell line that was classified as AML-M2 by the French–American–British (FAB) system [29], *via* regulation of homeobox B8 (HOXB8) expression. HOXB8 is a member of the mammalian HOX complex, a group of 39 transcription factors best known for their roles providing positional information during early development [30]. HOXB8 is activated transcriptionally in AML cells to prevent differentiation of factor-dependent myeloid progenitors [31]. MiR-196 has perfect complementarity with the target sequence in the 3'-UTR of HOXB8 mRNA. Exogenous miR-196 repressed HOXB8 expression by cleaving the mRNAs, and thus enhanced myeloid differentiation of HL-60 cells [32].

Human monocyte/macrophage differentiation is regulated by miR-424, miR-17-5p, miR-20a and miR-106a. The expression of miR-424 is up-regulated by PU.1, a transcriptional factor specific to the monocytic lineage. Elevated miR-424 targets nuclear factor-A (NFI-A) mRNA to repress its translation. Since decreased NFI-A expression is required for the activation of differentiation-specific genes, these three components coordinate to designate monocytic differentiation of precursor cells [33]. Another mechanism regulating monocytopoiesis includes miR-17-5p, -20a and -106a, the multipotent transcriptional factor AML1 and macrophage colony-stimulating factor (M-CSF) receptor (M-CSFR). The three miRNAs target AML1 and down-regulated its expression, which promotes M-CSFR gene transcription, resulting in enhanced blast proliferation and inhibition of monocytic differentiation and maturation [34].

Some miRNAs, although not directly involve in leukemogenesis, function in the development of normal blood cells by a mechanism that suggests a possible pathological function. MiR-155 and miR-451 are key regulators of normal erythroid differentiation. Functional studies using both gain-of-function and loss-of-function approaches in murine erythroleukemia (MEL) cells showed that miR-451 is associated with erythroid maturation [25]. MiR-155 and its non-coding RNA host gene *BIC* are up-regulated 100-fold in Burkitt's lymphoma patients [35]. Analyses of miRNA expression using the quantitative real-time polymerase chain reaction showed that expression of miR-155 decreased by 95%, while expression of miR-451 increased about 270-fold during a 12-day culture of erythroid progenitor cells. Moreover, in a microarray study of both mouse spleen erythroblasts and human CB CD34+ stem-progenitor cells, miR-451 was up-regulated most significantly during erythroid maturation, and miR-451 expression was restricted to red blood cells. A moderate down-regulation of miR-221 and miR-223 in this research is in accordance with their expression pattern in AML [36]. Haematopoietic transcription factor GATA-1 that is essential for the formation of platelets, eosinophils, mast cells and erythrocytes, binds to the miR 144/451 locus in erythroid cells. This transcription factor results in the increase of miR-144/451 level by activating RNA polymerase II-mediated transcription of a common primary RNA including both miRNAs [37]. MiR-24 is another important inhibitor in erythropoiesis. Studies on CD34+ stem-progenitor cells showed that miR-24 decreased erythroid colony-forming and burst-forming units by base pairing with the 3'-UTR of the human activin type I receptor ALK4 mRNA [38]. These findings suggest that altered levels of these miRNAs, either through naturally occurring genetic changes or through pharmacologic manipulation, could affect red blood cell production in various diseases, including myeloid leukemia and hypercythemia.

Correlation analysis on umbilical CB (UCB)-derived CD34+ cells showed that miR-15b, miR-16, miR-22 and miR-185 have specific, strong, positive correlation to the appearance of erythroid surface antigens (CD71, CD36 and CD235a) and haemoglobin synthesis, while miR-28 has an inverse correlation to the expression of these markers. The restriction of these correlations to the erythroid lineage indicates the specific role of these miRNAs in erythroid differentiation, especially since they are predicted to target mRNAs involved in cell development and differentiation [39].

During human megakaryocytopoiesis, the mRNAs of two megakaryocytic transcription factors, v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) and HOXA1, are targeted by miR-130 and miR-10a, respectively. The downregulation of these miRNAs can unblock the expression of MAFB and HOXA1 and thus activate genes involved in megakaryocyte/platelet differentiation [40].

MiRNAs directly involved in myeloid leukemia

MiRNAs in acute myeloid leukemia

AMLs represent the clonal expansion of haematopoietic precursors blocked at different stages of erythroid, granulocytic, monocytic or megakaryocytic differentiation, thus altering the developmental programs of normal haematopoiesis [41]. AML is a heterogeneous disease. The genetic reprogramming of AML blasts renders them ineffective at generating mature red cells, neutrophils, monocytes and platelets; the most common cause of death in AML patient is bone-marrow failure. AML blasts also inhibit normal blasts from differentiating into mature progeny (for review see [42]). Prognosis is poor for the majority of AML patients, and, after 25 years of research, the standard therapy for AML is highly toxic and poorly tolerated (for review see [43]).

In an earlier study Shakti and colleagues [44] showed high miR-181 expression in HL-60 cells, compared to the low expression in normal myeloid cells. In samples with AML-M1 or AML-M2 morphology, miR-181a expression was elevated, while in the samples with AML-M4 or AML-M5 morphology miR-181a was repressed. By contrast, in normal human bone marrow, miR-181a is expressed preferentially in B cells, T cells, monocytes and granulocytes [21], which are more closely allied to the M4 and M5 morphological leukemic subtypes. The elevated expression of miR-181a observed in myeloblastic leukemias implies its involvement in leukemogenesis. Studies on the expression of the three miRNAs located in intergenic regions in the HOX gene clusters, miR-10a, miR-10b and miR-196a-1 [45–47]

also revealed their potential connection to leukemia [48]. A distinct correlation of these miRNAs with the HOX genes was reported, which exhibited an elevated expression in leukemogenesis [49]. Nearly 30% of the genes showing a high correlation with the HOX genes have oncogenic potential.

More than one-third of AMLs were characterized by aberrant cytoplasmic localization of nucleophosmin (NPMc + AML), a consequence of mutations in the putative nucleolar localization signal NPM. These AMLs have a unique gene expression profile [50, 51]. Recent studies have found that relapse-free and overall survival are better in patients with *NPM1* mutations who lack internal tandem duplications (ITDs) in FMS-like tyrosine kinase 3 (FLT3; FLT3-ITD+) than in the other three subgroups (for review see [42]). Microarray analysis of AML patients with the normal karyotype (AML-NK) revealed a strong miRNA signature that distinguishes NPMc+ from the cytoplasmic-negative (unmutated NPM1) cases, including the up-regulation of miR-10a, miR-10b, as well as several let-7 and miR-29 family members. Many of the down-regulated miRNAs including miR-204 and miR-128a are predicted to target several HOX mRNAs. In fact, miR-204 targets HOXA10 and myeloid ectopic viral integration site 1 (MEIS1) mRNAs, suggesting that the HOX up-regulation observed in NPMc+ AML may be due, at least in part, to loss of HOX-regulating miRNAs. Overexpression of HOX genes or formation of fusion genes is one of the two lesions essential to AML [32]. FLT3-ITD+ samples are characterized by up-regulation of miR-155. Further experiments demonstrated that the up-regulation of miR-155 was independent of FLT3 signaling [52]. In addition, miR-155 is overexpressed in undifferentiated CD34+/CD38- AML stem cells, and ectopic overexpression of miRNA-155 in AML cells blocks differentiation [22]. Combining the human HSC mRNA expression data with miRNA-mRNA target predictions, Robert and colleagues [22] predicted that miRNA-155 could target multiple haematopoietic differentiation-associated factors, including CCAATT-enhancer binding protein α (C/EBPα), cyclic AMP response element binding protein (CREBBP), MEIS1, PU.1, angiotensin II type 1 receptor (AGTR1), AGTR2 and FOS, and determined that miR-155 acts to regulate normal myelopoiesis negatively.

At the single miRNA level, miR-23B was repressed in AML specimens compared to normal bone marrow and purified CD34+ HSPCs. In contrast, the miR-221/miR-222 cluster and miR-34a were expressed at significantly higher levels in AML blasts. Patients with high miR-221/miR-222 expression had low levels of c-KIT mRNA and protein expression but the correlation between c-KIT protein and c-KIT mRNA was significantly stronger than the correlation of either one with miR-221/miR-222. A global analysis comparing miRNA expression to the mRNA expression of the predicted target genes revealed only weak associations for the majority of miRNA species. Nonetheless, the presence of two or more miRNA binding sites within c-KIT mRNA usually is associated with a decrease in mRNA levels [53].

Comparison of miRNA expression in megakaryoblastic leukemic cell lines and differentiated megakaryocytes and CD34+ stem-progenitor cells *in vitro* revealed up-regulation of 10 miRNAs, including miR-101, miR-126, miR-106, miR-135 and miR-20, that are involved in the megakaryocytic differentiation signature. Moreover, miR-106, miR-135 and miR-20 are predicted to target AML1 [35], suggesting potential role in acute megakaryoblastic leukemia.

Northern blot experiments to detect miR-223, miR-181a, miR-142 and miR-155 in leukemia and lymphoma cell lines failed to detect obvious miR-142 expression in all cells, although miRNA was present in normal human myeloid and T cells [21]. This finding is in contrast to miR-155 expression, which is undetectable in normal human haematopoietic cells but found in Raji Burkitt lymphoma cells and erythroid-colony forming cells before day 3 of the induction [25, 36]. Therefore, miR-142 is a promising regulator in the pathogenesis of myelogenous malignancies.

MiRNAs in chronic myeloid leukemia

Philadelphia (Ph) chromosome was first reported in 1960 as a chromosomal abnormality associated with a specific type of leukemia (CML) [54]. The Ph chromosome is the result of a t(9;22) reciprocal chromosomal translocation that involves the *Abl* proto-oncogene normally on chromosome 9 and *Bcr* on chromosome 22 [55, 56]. The deregulated ABL tyrosine kinase activity is the pathogenetic principle [57]. Clinically, chronic-phase CML is not a severe problem until the disease progresses to acceleration and blast crisis, often within 5 years of diagnosis. The fusion protein BCR-ABL participates in diverse signal transduction pathways to decrease adhesion of CML cells to bone marrow stromal cells as well as the extracellular matrix [58], activate mitogenic signaling, degrade inhibitory proteins [59] and prevent or compensate for apoptosis (for review see [60]).

The expression of miRNAs is regulated by a variety of proteins, especially the proteins involved in tumorigenesis. The miR-17–92 cluster, comprised of miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1, is located in chromosome 13, a region that is amplified in human B-cell lymphomas, and is a known oncogene in mouse Burkitt's lymphoma [61]. The miR-17–92 cluster is an important CML-associated oncogene. The polycistronic pri-miRNA transcript is up-regulated in CML but increased mature miRNAs are only detected in early chronic-phase but not in blast-crisis CML [62]. Overexpression of the miR-17–92 cluster in K562, a CML cell line, promotes cell proliferation. In addition, the overexpression also compensates for the cell cycle arrest induced by *c-MYC* silencing. SL3–3 provirus integration near the gene that encodes the miR-17–92 cistron can induce T-cell lymphoma. Insertion of the retroviral enhancers probably caused an increase in expression of the primary transcript, consequently increasing the concentration of the oncogenic miRNA [63].

The expression of the miR-17–92 cluster can be regulated by three members of the E2 transcription factor family (E2F1–3) [64]. In turn, miR-17 and miR-20 target E2F1 in a negative feedback loop [65]. Nevertheless, the function of each E2F transcriptional factor is different. When E2F3 binding is predominant, miR-17–92 will promote cell proliferation in normal tissue; in contrast, in neoplastic tissue E2F1 binding induces apoptosis. As a transcription

activator, c-MYC activates miR-17–92 cluster transcription [65]. To trace upward, the expression of *c-myc* is induced by oncogenic ABL-variants. c-MYC is required, in cooperation with BCR-ABL, for transformation of haematopoiesis arrest in CML [66]. A recent study showed significant down-regulation of the miR-17–92 cluster members, excluding miR-17–3p and miR-92, after both imatinib treatment and *bcr-abl* knock out, among three CML cell lines. These results indicate that the onco-protein BCR-ABL up-regulates the expression of miRNAs through the BCR-ABL-cMYC-miR-17–92 pathway. Moreover, *c-myc* is one of the many genes that can be transactivated by E2F1. As a result, E2F and MYC transactivate each other, providing a complex regulatory signal for miR-17–92 cluster expression. However, the exact mechanism by which overexpression of the miR-17–92 cluster might promote oncogenesis is unclear.

Effective ATRA treatment of AML involves miRNA regulation

All-trans retinoic acid (ATRA) is regularly used for the treatment of APL. The dramatic therapeutic activity of ATRA is due to induction of terminal granulocytic differentiation in the malignant promyelocytes [67–69]. Promyelocytic leukemia (PML)/RA receptor α (RARα), a characteristic fusion protein of APL chromosomal translocation t(15;17) [70], blocks the differentiation and neoplastic transformation of APL blasts by disrupting the function of PML and repressing transcription of genes regulated by RARα at the physiological level [71]. Increased doses of RA can overcome this block and induce terminal differentiation of PML/RARα-positive APL blasts both *in vitro* and *in vivo* [72]. While human myeloid leukemia cells with normal RA receptors have virtually no response to ATRA, myeloid leukemia cells with dominant negative RARs have a therapeutic response to this agent [73]. The active RARs serve as transcription factors, regulating the expression of a growing number of genes, such as *c-myc*, *p21* and *Hox* family members [74–77].

The relationship between ATRA treatment and miRNA expression was first reported by Nadia and colleagues [23]. After the APL cell line is treated with ATRA, C/EBPα, instead of NFI-A, binds to the promoter of the miR-223 gene and up-regulates miR-223 expression. MiR-223 mediates translational repression of the NFI-A mRNA, thereby decreasing the NFI-A protein expression, forming a regulatory circuit that controls granulocyte differentiation. While C/EBPα has critical roles in the differentiation of bipotential myeloid progenitors to granulocytes [78–80], it can down-regulate *c-myc* expression [81] and can be down-regulated by the formation of AML1-ETO fusion protein [82]. Recently, more miRNAs that are up-regulated during ATRA-induced granulocytic differentiation in APL patients and cell lines were found [83], among which are miR-15a, miR-16-1, let-7a, let-7d and miR-107. As most of these miRNAs target oncogenes such as *bcl-2* and *ras*, they can be viewed as tumour suppressors. Interestingly, it is proved that miR-107 targets NFI-A and down-regulates its expression, in a manner similar to that of miR-223. ATRA most likely functions through ATRA-modulated transcription factors like NF-κB, at least to some extent.

Regulation of miRNA expression

Regulation of miRNA expression by transcription factors

The transcription of certain miRNAs can be regulated by transcription factors in haematopoiesis and leukemia, as mentioned above. However, the exact mechanisms by which transcription factors regulate these miRNA genes are not clear. For example, GATA-1 up-regulates miR-144/451 expression by activating RNA polymerase II-mediated transcription of the miRNA gene cluster [37]. Sometimes, several transcription factors bind to the same regulatory site, and their relationship can be either competitive, such as binding of C/EBPα or NFI-A to the promoter of miR-223 gene [23], or cooperative, such as c-MYC and E2F family members regulating the expression of the miR-17–92 gene cluster [64, 66]. In contrast to the role of miR-221 and miR-222 in myeloid differentiation, studies in cutaneous melanoma identified the promyelocytic leukemia zinc finger (PLZF) transcription factor as a repressor of miR-221 and miR-222, by binding directly to their putative regulatory region. This repression down-regulates miR-221 and miR-222 expression, thereby leading to enhanced proliferation and differentiation blockade of the melanoma cells [84]. Recently, a transcription factor encoded by the tumour suppressor gene *p53*, was found to regulate the miR-34a and miR-34b/c genes. This regulation results in induction of apoptosis, cell cycle arrest and senescence by *p53* [for review see ref. 85].

Epigenetic mechanisms silence miRNA gene expression

In normal eukaryotic cells, DNA methylation and histone acetylation interplay for maintaining the equilibrium, allowing temporal expression of some genes [86, 87]. In neoplastic cells, this balance is frequently disrupted. In leukemic cells, CpG islands in the promoter region of genes critical for cell cycle progression and maturation are frequently hypermethylated [88], and DNA methyltransferases (DNMTs) are often overexpressed. These findings are paralleled by transcriptional repression of downstream genes [89], including miRNA genes. A putative AML1 binding site at the 5' end of the predicted core promoting sequence on the pre-miR-223 upstream region has been identified using computational search methods [90]. The AML1-ETO oncoprotein may bind at this site to regulate transcription [91]. The AML1-ETO fusion protein is found in t(8;21)+ AML. Expression of AML1-ETO in human HSCs maintains them as stem cells, as opposed to their differentiation

Table 1 Summary of microRNAs involvement in myeloid leukemia

Cancer type	miRNA involvement	Targets	Regulators	Reference
AML-M2	miR-221/222 ↑	KIT receptor ↓		[30, 53]
AML-M2/M3	miR-181a ↑			[48, 20]
AML	miR-10a/10b ↑	HOXA1 ↓		[48]
AML	miR-196 ↑	HOXB8 ↓		[28]
AML	miR-204 ↓	HOXA10 and MEIS1 ↑		[52]
AML-M3	miR-223 ↓	NFI-A ↑	C/EBPα, NFI-A, AML1-ETO	[23, 88]
AML	miR-155 ↑	multiple haematopoietic differentiation-associated molecules ↓		[22]
APL	miR-15a, 16-1 ↑	BCL2 ↓		[83]
CML	miR-17–92 cluster ↓	E2F1 ↑	cMYC, E2F1--3	[64, 65]

↑ represents the increase of miRNA or target gene in this type of cancer; ↓ represents the decline of miRNA or target gene in this type of cancer. See details in text.

[92]. Intensive chromatin immunoprecipitation (ChIP) in AML cell lines HL-60 and U937 revealed that the chromatin regions containing miR-223 gene occupied by AML1-ETO are minimally acetylated, but have hypermethylated CpG dinucleotides. However, another conserved promoter region located at about 3400-bp upstream of the miR-223 gene that contains three CpG dinucleotides is not methylated constitutively [93], which indicates that the remodeling complex aberrantly formed by AML1-ETO, and its methylation mechanism is the key event in the miR-223 gene silencing. Thus, like other genes that regulate important cell functions, miRNA genes can be epigenetically transcriptional-silenced by increased DNA methylation at the promoter regions of genes. The AML1-ETO subtype of AML, in which the leukemogenic mechanism involves corepressor protein complexes containing histone deacetylases (HDAC) and DNMT can be treated by HDAC inhibitors and DNMT inhibitors, which inhibit cell proliferation and induce apoptosis by increasing histone acetylation as well as decreasing DNA methylation [94, 95]. These studies suggest improved prognosis of patients treated with such enzyme inhibitors.

Post-transcriptional regulation of miRNA expression

The expression of a large fraction of miRNAs can also be regulated during the Drosha processing step. This regulation has a major impact on mature miRNA production during embryonic development and in cancer [96]. In cancer samples, the correlation between mature miRNAs and the abundance of their primary transcripts is unknown, which suggests that the Drosha processing step is disrupted in cancer.

Discussion and perspectives

Until now, many miRNAs have been reported to play critical roles in myeloid leukemogenesis (Table 1). It is clear that miRNAs have dual functions as tumour suppressors and oncogenes in tumour formation. On one hand, some miRNAs identified as tumour suppressors, such as miR-223, -15a, -16-1 and let-7a, target oncogene mRNAs, and can be down-regulated in leukemia. On the other hand, miRNAs highly expressed in leukemia silence tumour suppressor genes, and therefore are considered oncogenes, such as miR-17–92 cluster, miR-181a, -10a, -10b, -155 and -196a-1. The current understanding of the mechanism of these miRNAs suggests that chromosome translocations and the resulting fusion proteins are often upstream of the regulatory network involves miRNAs (Fig. 1).

Methods to identify miRNAs have improved greatly, and miRNAs are being linked now to an expanding number of physiological processes, as well as multiple diseases. Initially, the majority of miRNAs were identified by direct cloning of small RNAs [97]. Many computational procedures have been developed to predict miRNAs in the genome of different organisms according to phylogenetic conservation and the structural characteristics of miRNA precursors, such as miRNAscan (http://genes.mit.edu/mirscan) in human beings and miRNaseeker (http://www.fruitfly.org/seq_tools/miRseeker.html) in *Drosophila* [98]. Recently new methods have been developed and proven effective, using bioinformatic approaches based on conserved sequences in the stems of miRNA hairpins and 5' region of mature miRNA [99, 100]. These advances indicate that, in addition to the miRNAs registered in publicly available databases, such as miRBase (http://microrna.sanger.ac.uk/sequences/) [101], more miRNAs are likely to be discovered in the near future.

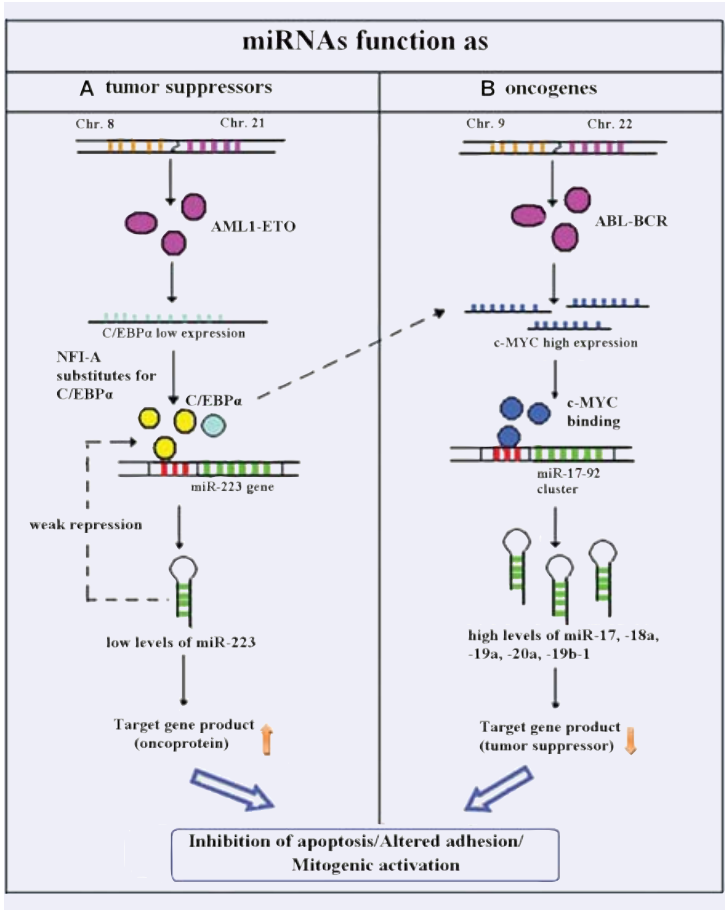

Fig. 1 MiRNAs act as tumour suppressors or oncogenes in myeloid leukemia. During leukemogenesis, (**A**) miR-223 may act as tumour suppressor [23, 88], while (**B**) the miR-17–92 gene cluster members act as typical oncogenes [64, 65]. The transcription of the miRNA-223 gene and miR-17–92 gene cluster are regulated initially by fusion protein AML1-ETO and ABL-BCR, which result from characteristic AML and CML chromosome translocations, respectively. Although their target mRNAs have not been identified, the decreased miR-223, and increased miR-17, -18a, -19a, -20a and -19b-1 may be involved in the pathogenesis of leukemia. C/EBPα can also regulate the expression of *c-myc*. The regulatory network is complex and some key molecules may participate in several subtypes of myeloid leukemia.

Quantitative real-time PCR to detect precursor and mature miRNAs [for review see 102], microarray technology and the newly developed RNA-primed array-based Klenow enzyme assay (RAKE) [103] make it possible to study the expression patterns of all miRNAs in a tissue or cell in a single time, and are better than classic Northern blotting to study the expression patterns of miRNAs. The induction of stable loss-of-function phenotypes for specific miRNAs by lentivirus-mediated antagomir expression provides an alternative way to study the function of miRNAs. A set of successful lentivirally expressed antagomirs are transcribed from an H1-promoter located within the lentiviral 3' LTR and directed against miRNAs encoded on the polycistronic miR-17–92 transcript [104]. All these methods, combined with the commonly used algorithms to predict target genes of miRNAs, facilitate research on both miRNA functions and their mechanism in normal myeloid differentiation, as well as in developing myeloleukemia.

The regulation of miRNAs and their post-transcriptional silencing of target genes are likely to be more complicated than has been stated. Many genes are under combined regulation at the transcriptional and post-transcriptional levels. A comprehensive network of combinatorial regulatory interactions spanned by miRNAs and transcription factors and conserved binding sites of transcription factors in promoters has been predicted using evolutionarily conserved potential binding sites of miRNAs in human targets [105]. Hundreds of miRNA target genes have been nominated as hubs because they are regulated by dozens of miRNAs, and they are involved in a diversity of developmental processes, as well as in transcription regulation. Moreover, the miRNA-transcription factor regulatory network features several instances in which transcription factor and miRNA partners that regulate multiple target genes often regulate one another, forming a large number of feed-forward loops. In conclusion, many genes are under combined regulation at the transcriptional and post-transcriptional silencing levels, and an miRNA that post-transcriptionally silences a set of genes also will silence the transcriptional regulator of those genes, presumably to prevent *de novo* transcription of its target genes.

Compared to the approximately 4000 existing miRNAs reported to date, the proportion of miRNAs involved in myeloid leukemia is far too low. More myeloid leukemia-related miRNAs need to be identified. Additionally, further research on their target genes, specific transcription factors and their interactions is needed to integrate miRNAs into the regulatory network in the genesis of myeloid leukemia. Future studies will also provide potential

treatment for the disease, adding to the available targeted therapies (for review see [39]) that focus on pathophysiological events critical for leukemogenesis, such as unbridled proliferation, failure to differentiate, stromal cell-mediated survival factors, and failure to undergo normal programmed cell death.

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