REVIEW

The functional role of microRNA in acute lymphoblastic leukemia: relevance for diagnosis, differential diagnosis, prognosis, and therapy

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Abstract: MicroRNAs (miRNAs), a new class of noncoding RNAs, which can hybridize to target messenger RNAs and regulate their expression posttranscriptionally, express differentially in distinct stages of lymphopoiesis and influence the direction of lymphoid precursor maturation. Hence, there is aberrant expression of miRNAs involved in malignant lymphopoiesis, and these aberrations can be used as signatures of acute lymphoblastic leukemia (ALL) with different subtypes. In addition, changes in the expression of several miRNAs may have functional relevance with leukemogenesis or drug resistance. As a result, the reversal of the expression of these miRNAs may alleviate the disease to some extent and improve clinical outcomes. However, among the studies of miRNAs, there are still some problems that need to be solved to understand the function of miRNAs in ALL more thoroughly.

Keywords: ALL, microRNA, lymphopoiesis, molecular diagnosis, lymphoid malignant, molecular therapy

Introduction

MicroRNAs (miRNAs) are small RNA sequences of approximately 18 to 25 nucleotides. They come from 70 to 100 nucleotide hairpin precursors cleaved by a complex protein system including the RNase III Drosha and Dicer[1–4] shown in Figure 1. miRNAs sequences distribute throughout the whole genome and are classified as intergenic or intronic miRNA.[5] Mature miRNAs repress and/or degenerate the protein-coding messenger RNAs (mRNAs) posttranscriptionally through interaction with 3′-untranslated region of mRNAs,[1–3,6] and this is outlined in Figure 2. Lin-4 is the first discovered miRNA during a genetic screen of the nematode *Caenorhabditis elegans* by Lee et al in 1993.[7] Currently, more than 24,000 miRNAs have been discovered, spanning more than 200 species including flora, fauna, and some microorganisms,[8] and more than 2,000 human mature miRNAs have been identified and reported in miRBase and miRBase Tracker.[9–13] The regulation of mRNA by miRNA is a common biological phenomenon.[14] Friedman et al[15] reported that approximately 60% of human mRNA could be regulated by miRNAs.

Dysregulation of miRNAs has been discovered in different solid tumors and leukemia.[16] The study illustrating the aforementioned phenomenon demonstrated that miRNAs were frequently localized in common breakpoint regions related to tumors or in fragile sites, minimal regions of heterozygosity lost, and minimal amplification regions.[17] The first report published in 2004 showed that during murine hematopoiesis, miRNAs were expressed specially and regulated dynamically.[18] Several groups described the miRNAs expression profile and/or function during the normal and malignant hematopoiesis in murine and humans.[18–24]
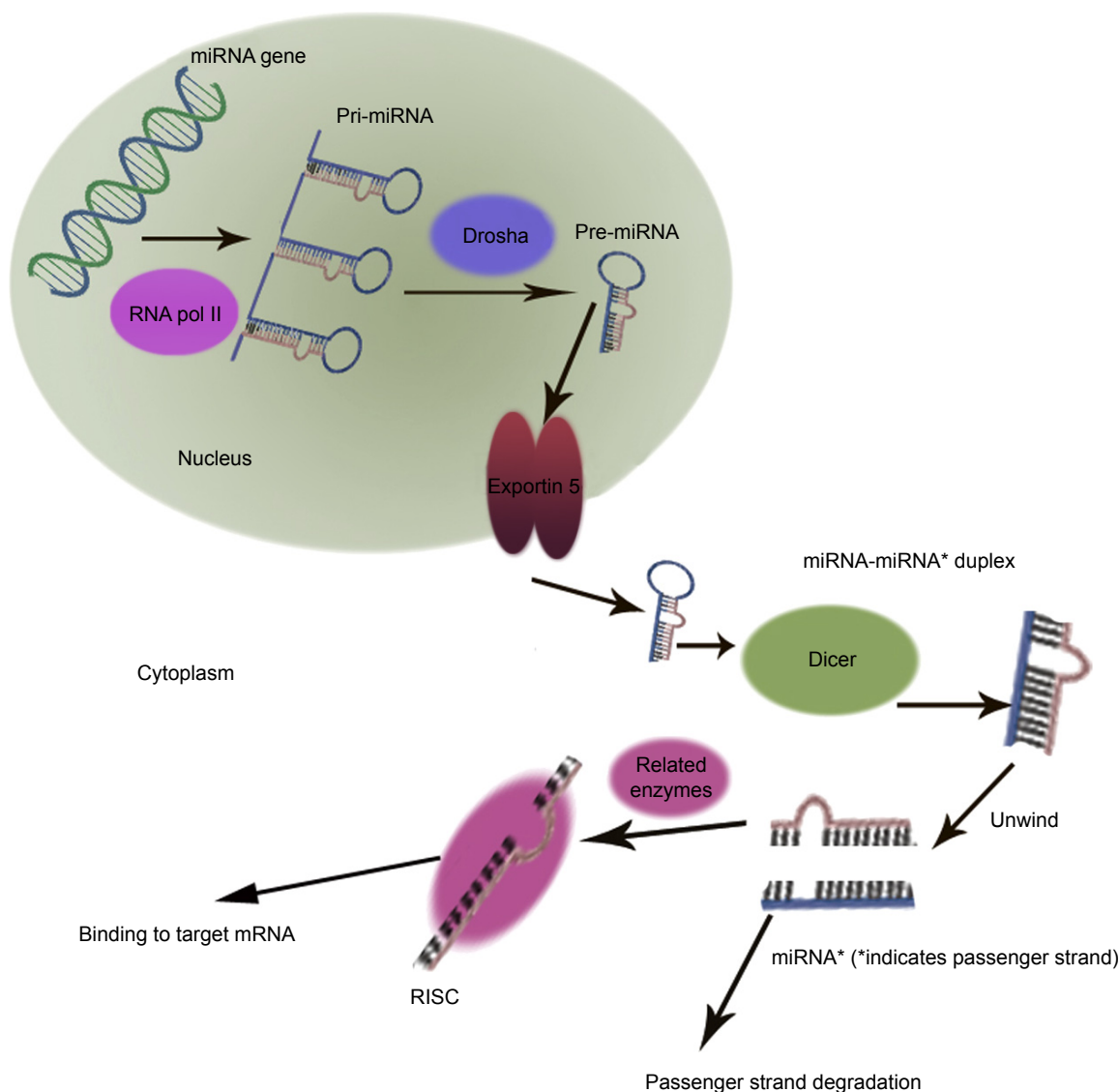
Figure 1 Biogenesis of miRNA.
Abbreviations: miRNA, MicroRNA; mRNA, messenger RNA; RNA pol II, RNA polymerase II; Pri-miRNA, primary miRNA; Pre-miRNA, precursor miRNA; RISC, RNA induced silencing complex.

Acute lymphoblastic leukemia (ALL) is the most common hematologic malignancy in children, and its incidence peaks from 2 to 5 years of age, while it is relatively rare in adults.[25–28] The classification of ALL by French–American–British cooperative group based on morphology had been abandoned because it failed to meet clinical relevance. The current classification system based on morphology, immunology, cytogenetics, and molecular biology was introduced by World Health Organization, while immunophenotyping based on cell surface and cytoplasmic proteins is more widely applied. According to immunophenotyping, ALL could be classified into two types, T-ALL and B-ALL. The main markers of T-ALL include the terminal deoxynucleotidyl transferase (TdT), CD2+, CD3+, CD4+, CD5+, CD7+, and CD8+. B-ALL mainly includes three subtypes: early pre-B-cell, pre-B-cell, and mature B-cell. The main markers of early pre-B-cell include TdT+, HLA-DR+, CD19+, D34+, and CD10+/−. The main markers of pre-B-cell include TdT+, HLA-DR+, CD19+, CD10+, and CD20+, and the main markers of mature B-cell include HLA-DR+, CD19+, CD10+/−, CD20+, and surface Ig (sIg)+.[29] Recently, besides the immunophenotyping of ALL, an increasing number of studies showed that the miRNA expression profiles in acute leukemia have cooperative interactions in the development of leukemia. Therefore, the miRNA expression profile can be used as biomarkers in diagnosis, differential diagnosis, prognosis, and therapy of hematologic cancers.[30–32] In this review, the role of miRNA expression profiles as biomarkers in diagnosis, differential diagnosis, prognosis, and therapy of ALL is summarized.
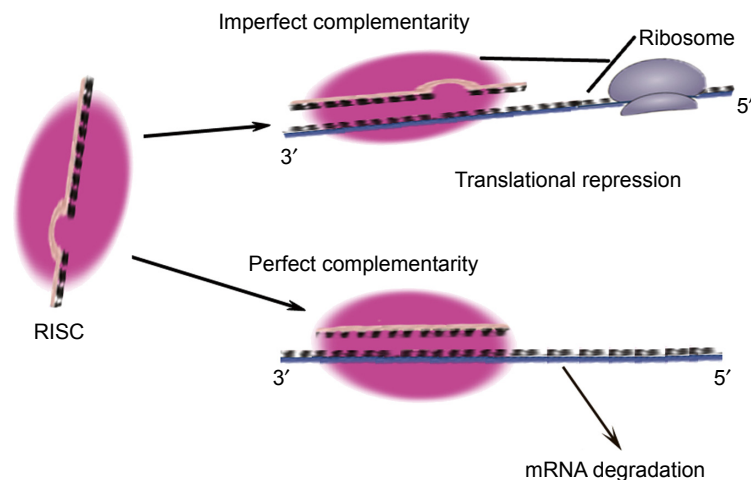
Figure 2 The functional mechanism of miRNA.
Abbreviations: mRNA, messenger RNA; miRNA, microRNA; RISC, RNA-induced silencing complex.

The function of miRNAs in normal lymphopoiesis

Hematopoiesis is a process by which multipotent hematopoietic stem cells (HSCs) self-renew and differentiate into different lineages cells continuously. Lymphopoiesis is a part of the hematopoiesis process by which HSCs differentiate into lymphoid progenitors and finally into B- or T-lymphocytes. The development of T-cells occurs in the thymus and the development of B-cells has two stages inside and outside the bone marrow (BM) separately. Nonetheless, their development and activation at the periphery are controlled by complex protein signaling pathways, which are regulated by the miRNAs.[33–35]

miRNA-150 is expressed in both mature B- and T-cells. The lymphoid progenitors express the miRNA-150 to give rise to the mature B-cells and assist in the transition from progenitor B-cell (pro-B) to the precursor B-cell (pre-B) stage. And premature expression of miRNA-150 results in blocked transition from the pro-B-cell stage to the pre-B-cell stage.[20,36–38] In the thymus, the expression of miRNA-150 may enhance T-cell development and its mechanisms, including enhancing key pathways of T-cell development (like the Notch Pathway) and suppressing alternative lineage differentiation (like B-cell differentiation) in progenitor cells.[39] C-Myb is a confirmed target of miRNA-150, and is an essential transcription factor involved in early lymphoid development. Its targeted loss in B-cells leads to the maturation arrest from pro-B to pre-B-cell stage, and, simultaneously, miRNA-150 is found to be overexpressed.[36,40]

B-cell differentiation is regulated by the miR-155-PU.1 axis, and the mechanism is that miR-155 inhibits PU.1 expression, which leads to Pax5 downregulation and the initiation of the plasma cell differentiation pathway.[41] Recent data show the role of miRNA-155 in the differentiation of T-cells into different effectors T-helper (Th) cell subsets. miRNA-155 regulates the differentiation of T-cells into Th type 1 cells, and its absence results in the direct differentiation from T-cells to Th type 2 cells.[33,34,42–44]

miRNA-181 is composed of three clusters located in different chromosomes.[20,32,45] miRNA-181 expression is high in the early B-cell differentiation stage and subsequently decreases.[18] In addition, miRNA-181 plays an important role in T-cell development and is expressed highly in double-positive T-cells. Its targets are BCL-2, CD69, EGR1, and T-cell receptor, all involved in positive T-cell selection.[46–48]

miRNA-17-92 cluster consists of six miRNAs: miRNA-17, miRNA-18a, miRNA-19a, miRNA-20a, miRNA-19b-1, and miRNA-92-1[49] and is highly expressed in the B- and T-lymphoid precursors and is decreased after maturation. As well as miRNA-150, absence of the cluster leads to the development disorders of B-cells from pro-B to pre-B-cell stage, due to the increased levels of the proapoptotic protein BIM that is the target of the cluster.[20,50] Another study has also showed consistency with the aforementioned conclusion.[51] It demonstrated that mice with targeted overexpression of the miRNA-17-92 cluster during lymphopoiesis develop severe lymphoproliferative disorders and autoimmunity.[20,51] miRNAs relevant to normal lymphopoiesis are shown in Figure 3.[33,52]

The function of miRNAs in ALL
The function of miRNAs in diagnosis of ALL

ALL is a lymphoid malignancy, and around three quarters of childhood ALL cases contain one or more total alterations of chromosome, and it may involve B- or T- lineages and have
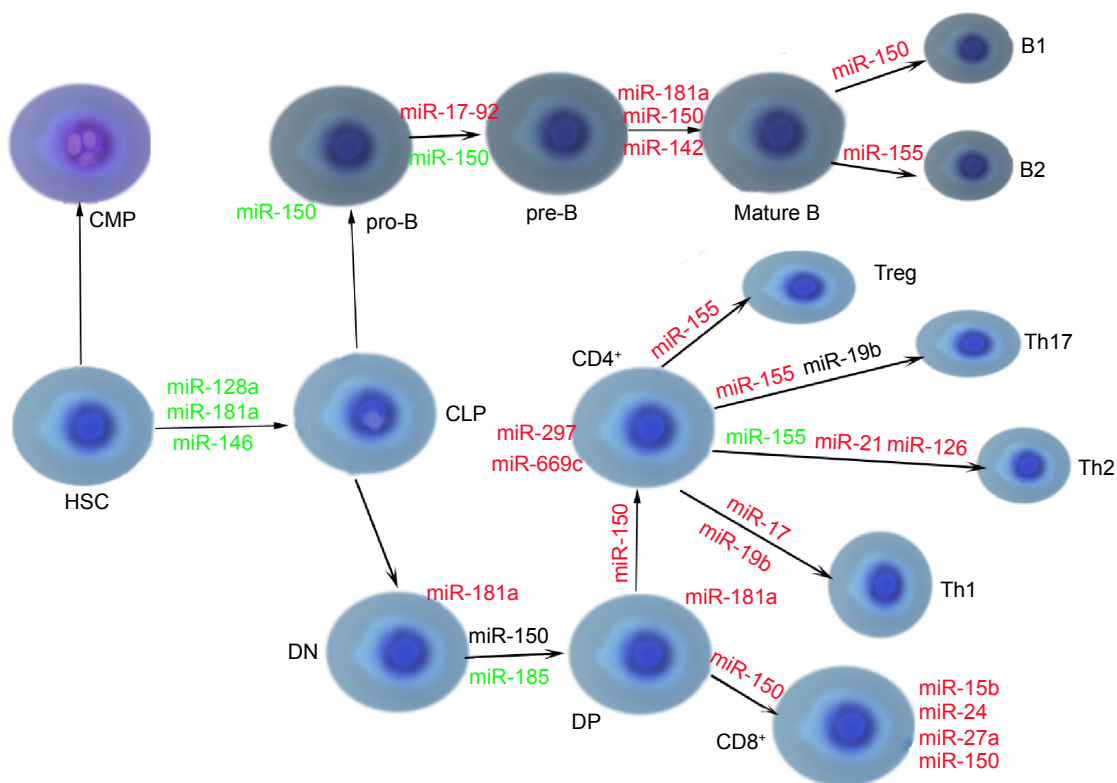
Figure 3 miRNAs relevant to normal lymphopoiesis.
Notes: Green text indicates microRNAs that are underexpressed, red text indicates microRNAs that are overexpressed, and black text indicates microRNAs that are unclear. Where miRNA is shown next to the cell, it indicates miRNA expression level at the cell stage. Where miRNA is shown below or above arrows, it indicates a level of the miRNA is needed for the cells to differentiate to next stage.
Abbreviations: miR, miRNA; miRNA, microRNA; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; DN, double-negative; DP, double-positive; HSC, hematopoietic stem cell; Th1, T-helper 1 cell; Th2, T-helper 2 cell; Th17, T-helper 17 cell; Treg, regulatory T-cell; pro-B, progenitor B-cell; pre-B, precursor B-cell.

lymphoid maturation arrest in distinct stages, leaving different immunophenotypes with different miRNA signatures.[53–57] So, these signatures can help the diagnosis and classification diagnosis of ALL. Compared with normal pediatric BM samples, miRNA-100, miRNA-196b, and let-7e were expressed at a lower level in BM samples of pediatric ALL, while miRNA-128a and miRNA-181b were overexpressed. miRNA-100 was related to t(12; 21) positive ALL.[58] A case-control study with 570 Chinese childhood ALL cases and 673 cancer-free controls suggested that miRNA-196a2 T>C polymorphism might increase the risk of ALL of children.[59] B- and T-lineage ALL can be discriminated by the expression of miRNA-148, miRNA-151, and miRNA-424. Furthermore, B-lineage ALL subsets with special molecular lesions can be differentiated by a set of six miRNAs – miRNA-425-5p, miRNA-191, miRNA-146b, miRNA-128, miRNA-629, and miRNA-126 – which was highlighted by one-way analysis of variance.[60] Zhang et al[61] observed differential expression patterns of ALL which were composed of several known miRNAs and 20 newly identified miRNAs that had first been discovered at the genomic level in human ALL. These

patterns constituted an ALL-specific miRNA signature for diagnosis. Gutierrez-Camino et al[62] analyzed 118 single nucleotide polymorphisms (SNPs) presenting in pre-miRNAs and miRNA-processing genes. Eleven SNPs, including three SNPs presenting in three miRNA genes (*miRNA-612*, *miRNA-499*, and *miRNA-449b*) and eight SNPs presenting in six miRNA biogenesis pathway genes (*TNRC6B*, *DROSHA*, *DGCR8*, *EIF2C1*, *CNOT1*, and *CNOT6*) were significantly associated with ALL susceptibility. And, of those eleven SNPs, two SNPs presenting in miRNA-612 and miRNA-499 had a more significant association with ALL susceptibility.

Furthermore, different subtypes of ALL can be distinguished. Schotte et al[63,64] compared the miRNA expression levels of seven major subtypes of pediatric ALL, which were T-cell, MLL-rearranged, TEL-AML1-positive, E2A-PBX1-positive, hyperdiploid ALL, BCR-ABL-positive, and "B-other" ALLs. They obtained the differential expression of the special miRNAs, such as miRNA-708, which were expressed 250- to 6,500-fold higher in the 57 TEL-AML1, BCR-ABL, E2A-PBX1, hyperdiploid, and B-other cases than in the 20 MLL-rearranged and 15 T-ALL cases

$(0.0001 < P < 0.01)$. Then, they analyzed expression levels of 397 miRNAs in 81 cases of pediatric ALL and 17 normal hematopoietic control cases, demonstrating the unique miRNA signatures of each subtype. Additionally, the miRNA signature of TEL-AML1-positive and hyperdiploid cases overlapped partly, which may suggest a common underlying biology. Mavrakis et al[65] reported that five miRNAs – miRNA-19b, miRNA-20a, miRNA-26a, miRNA-92, and miRNA-223, were identified as being capable of promoting T-ALL development in a mouse model and accounting for the majority of miRNA expression in human T-ALL, which could be used to reveal the pattern of gene interactions of T-ALL.

In addition, miRNA expression profiles may reveal new subset of ALL. A new subset of ALL with T-cell origin, which has similar a gene expression profile as acute myeloid leukemia (AML), was identified by comparing the mRNA and miRNA expression profiles with other cases. It has significantly higher levels of miRNA-223 expression than the other subsets, which suggests an unfavorable clinical course.[66] Other groups also have performed analogous studies to discover miRNAs expression signatures of ALL.[67–70]

Differential diagnosis from AML

As well as gene expression profile,[71] differential miRNAs expression can be utilized to define myeloid or lymphoid lineage leukemia and distinguish ALL from AML. De Leeuw et al[72] reported five of the most lineage-discriminative miRNAs – miRNA-23a, miRNA-27a, miRNA-199b, miRNA-221, and miRNA-223 – which could distinguish ambiguous lineage acute leukemia either as AML or ALL. With a bead-based miRNA-expression profiling assay, Mi et al[73] suggested that there were 27 differently expressed miRNAs between ALL and AML in a large-scale genome-wide miRNA expression profiling assay. Compared with AML, let-7b and miRNA-223 were downexpressed and miRNA-128a and -128b were overexpressed in ALL. Also, no less than two miRNAs of these four miRNAs could discriminate ALL from AML with an accuracy rate more than 95%. Using quantitative PCR (qPCR), Wang et al[74] separated patients with ALL from those with AML based on differential expression of 16 miRNAs, including previously reported eight miRNAs and newly identified eight miRNAs. The documented information of miRNAs in diagnosis and differential diagnosis of ALL is listed in Table 1.

Prognostic impact of miRNAs in ALL

miRNA signatures can be used not only in the diagnosis the ALL, but also in the prognosis of patients. Several miRNAs, involved in cell proliferation and apoptosis regulation,

may interfere with either oncogenic or tumor-suppressor pathways and are implicated in leukemogenesis, influencing the prognosis of patients. For instant, Ohyashiki et al[75] reported that cellular miRNA-92a expression was significantly increased in a subset of ALL cells, and ALL patients with overexpression of miRNA-92a had poor prognoses. Compared with peripheral blood mononuclear cells from healthy volunteers, the cell-to-plasma ratio of miRNA-92a expression was particularly higher in both ALL and AML cells. Nemes et al[76] suggested that expression level of miRNAs could be used as indicators of prognosis in children with ALL, such as higher expression of miR-128b at diagnosis predicted a better prognosis and prednisolon response. A study of 147 patients with acute leukemia (AL) and 100 healthy individuals showed that AL (including both ALL and AML) patients with high miRNA-24 expression tended to have shorter overall survival $(P < 0.05)$.[77]

High miRNA-16 expression was involved in hyperleukocytosis and poor cytogenetic groups. In B-cell ALLs, patients with miRNA-16 above quartile 75 had a significantly shorter disease-free survival (DFS), and in T-cell ALLs, a significant trend that was a survival shortening from the lowest to the highest miRNA-16 levels was revealed for both DFS and overall survival.[78] Another study with 38 cases of T-LBL/ALL patients and 15 cases of reactive hyperplasia of lymph nodes as controls conducted by Tong et al[79] claimed that although the overall survival rate in miRNA-16 high-expression group decreased compared with control group, the miRNA-16 expression correlated with BCL-2 protein $(r=0.51, P < 0.05)$, and the prognosis in BCL-2-positive expression group was better than that in the negative expression group, indicating that BCL-2 may be also a factor influencing prognosis. The study with 70 cases of T-LBL/ALL and 30 cases of reactive lymph node as controls conducted by Li et al[80] reported that the high-expression group of miRNA-16 had longer overall survival than the low-expression group and that the prognosis of BCL-2 negative was better than BCL-2 positive. So, further studies are required to elucidate the definite role of miRNA-16 on the prognosis of ALL and the relationship between miRNA-16 and BCL-2.

The function of miRNAs in therapy of ALL

Glucocorticoids

Glucocorticoids (GCs) induce apoptosis in lymphoid lineage cells and, therefore, are used in the therapy of ALL and related malignancies.[81] However, a proportion of patients with ALL are insensitive to prednisone. Here, eight miRNAs

Table 1 The documented information of miRNAs in diagnosis and differential diagnosis of ALL

miRNA	Gene locus	Target	Main effect of the target in hematopoiesis or oncogenesis	Value in diagnosis or differential diagnosis of ALL
miRNA-223	Xq12	FOXO[103] LMO2[104]	FOXO represses tumor suppression genes, such as BIM, Trail, and Fas L, which induce apoptosis;[103] LMO2 is a pro-oncogene in T-cells[105,106]	Higher expression in the subset of adult T-cell ALL displaying myeloid features than the other subsets;[66] downregulated in ALL compared with AML[73,74]
Let-7b	22q13	HMGA[107]	HMGA is oncogenic	Downregulated in ALL compared with AML[73,74]
miRNA-128a	2q21	BMI-1[108]	A gene regulates self-renewal of leukemic cells[109]	Upregulated in ALL compared with AML;[73,74] highly expressed in E2A/PBXI-positive cases[60]
miRNA-196a	17q21-22	HOXB8[110] ERG[111]	HOXB8 and ERG increase cell survival and proliferation of progenitors[110]	Downregulated in T-ALL compared with AML; high miRNA-196a expression is significantly associated with an early immunophenotype of T-ALL[110]
miRNA-196b	7p15, between HOXA9 and HOXA10[110]	c-myc[101] ERG[111]	ERG and c-myc increase cell survival and proliferation of progenitors[112]	Downregulated in B-cell ALL;[113] upregulated in MLL-rearranged[63] and other HOXA-activated ALLs[114]
miRNA-125b-1	11q24	May be Trp53inp1[115]	Trp53inp1 is a proapoptotic gene[116]	Upregulated in B-ALL with chromosomal translocation t(11; 14) (q24; q32)[115]
miRNA-128-3p	Not clear	PHF6[117]	Might be a tumor suppressor gene[118]	Significantly higher expressed in ALL samples than in AML or normal samples[63,73]
miRNA-148a	7p15.2	BIM, PTEN[119]	BIM and PTEN are proapoptotic genes[119]	Highly expressed in T-ALL cases[60]
miRNA-151	Chromosome: 8	ITK, ZAP-70[60]	ITK plays an important role in normal T-cell functions and in the pathophysiology of both autoimmune diseases and T-cell malignancies;[120] ZAP-70 expression is associated with the E2A/PBXI rearrangement[21]	Downmodulated in T-ALL[60]
miRNA-424	Xq26.3	NFIA,[122] VEGFR2[123]	NFIA modulates the differentiation of hematopoietic progenitors;[124] VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex[125]	Highly expressed in patients with T-ALL and down modulated in AMLs with NPM1mutA[60,122]
miRNA-23a	19p13.13	HOXB4[126] BCR/ABL[127] CXCL12[128]	HOXB4 is oncogenic;[126] BCR/ABL is oncogenic;[127] CXCL12 regulates the interaction between hematopoietic stem and progenitor cells and BM stromal cells[128]	Higher expressed in AML compared with ALL[72,129]
miRNA-27a	19p13.13	4-3-3θ[30]	4-3-3θ is antiapoptotic[130]	Higher expressed in AML compared with ALL;[72] downregulated in ALL compared with hematopoietic stem-progenitor cells[130]
miRNA-221	Xp11.3	p27[131]	p27 prevent cell-cycle progression from G1 to S phase[131]	Higher expressed in AML compared with ALL[72,132]

Abbreviations: miRNA, microRNAs; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; BM, bone marrow.

can help to distinguish the patients sensitive from those insensitive to prednisone, which are miRNA-18a, miRNA-532, miRNA 218, miRNA-625, miRNA-193a, miRNA-638, miRNA-550, and miRNA-633.[82] And, suppose the patients with MLL-rearranged ALL were insensitive to GCs, miRNA-128b and miRNA-221 may serve as GCs sensitizers potentially. Both miRNAs are downregulated in MLL-rearranged ALL. The restoration of miRNA-128b downregulates target genes including *MLL*, *AF4*, and both *MLL-AF4*, and *AF4-MLL* fusion oncogenes, and the restoration of miRNA-221 downregulates CDKN1B cooperatively. Thus, the sensitivity of two cultured lines of MLL-AF4 ALL cells to GCs is strengthened.[83] In a subsequent study, Kotani et al[84] illustrated that one novel mutation of miRNA-128b significantly reduced its processing, and the resultant downregulation of mature miRNA-128b gave rise to GCs resistance due to the failure to downregulate the fusion oncogenes. Harada et al[85] transiently overexpressed pre-miRNA-17, an miRNA precursor, in the SUP-B15 cell line by electroporation and monitored the dexamethasone-induced levels of apoptosis using annexin/propidium iodide (PI) staining. They found that overexpression of miRNA-17 reduced dexamethasone-induced cell death. By inhibition of miRNA-17 through locked nucleic acid (LNA) inhibitor, sensitivity to dexamethasone was increased. Therefore, by regulating miRNAs, therapeutic effect of GCs may be improved.

Tyrosine-kinase inhibitors (TKI)
For the ALL with *BCR-ABL* fusion gene, the application of TKI may be a promising strategy, but the prognosis remains suboptimal.[86] BCR-ABL1 and ABL1 are the direct targets of miRNA-203, which is silenced by genetic and epigenetic mechanisms in hematopoietic malignancies expressing either ABL1 or BCR-ABL1, and the restoration of miRNA-203 expression reduces ABL1 and BCR-ABL1 levels and inhibits cell proliferation.[86,87] The inhibition of DNMT3A by forced expression of miRNA-217 may benefit in preventing drug resistance to TKI treatment in Philadelphia-chromosome-positive ALL patients. Hence, it may indicate another therapeutic strategy for BCR-ABL-positive ALL.[88]

Demethylation
Demethylation may be a potential therapeutic strategy for ALL.[89] In the MLL-AF4 ALL, miRNA-143 is epigenetically repressed by promoter hypermethylation in MLL-AF4-positive primary blasts and cell lines, but not in normal BM cells and MLL-AF4-negative primary blasts. Meanwhile, miRNA-143 was identified as a regulator of MLL-AF4 expression, and its restoration could induce apoptosis, negatively contributing to leukemia cell growth. Therefore, upregulation of miRNA-143 expression has therapeutic promise for MLL-AF4 B-cell ALL.[90] Other documented information of miRNAs for prognosis and/or treatment of ALL is listed in Table 2, and miRNA-196b is presented as an example to illustrate the interaction between miRNA and its target in Figure 4.

Future directions
miRNAs promote lymphoblastic leukemogenesis through different mechanisms such as enhancing the expression

Table 2 Other documented information of miRNAs for prognosis and/or treatment of ALL

miRNA	Gene locus	Target	ALL type	Value for prognosis or therapy
miRNA-210	11p15.5	May be *SAR1B* or *HINT1*[133]	Not mentioned	Lower expression level in patients prone to relapse and insensitive to chemotherapeutic drug than in other patients ($P<0.001$)[133]
miRNA-124a	8p23	*CDK6*, *Rb*[97]	Not mentioned	Low expression was associated with higher relapse rate ($P=0.001$) and mortality rate ($P<0.001$)[97]
miRNA-152	17q21	*DNMT1*[89,134]	MLL rearranged: t(4;11)-positive	Low expression was strongly correlated with a poor clinical outcome[89]
miRNA-664	Chromosome 1	*PLP2*[135]	T-ALL	Inhibition of miR-664 may be a potential therapeutic strategy for the treatment of T-ALL[135]
miRNA-100/99a	11q24 and 21q21, respectively	*FKBP51* and *IGF1R/mTOR*[136]	Not mentioned	Expression levels were related to the patient's 5-year survival;[136] approximately 20-fold upregulation predicted resistance to vincristine and daunorubicin[64]
miRNA-708	11q14	*CNTFR*, *NNAT*, and *GNG12*[137]	B-ALL	Low expression of miR-708 was correlated with resistance to GCs in pediatric B-ALL[138]
miRNA-193b-3p	Not found	*MYB*[139]	T-ALL	An entry point for efficient MYB targeting-oriented therapies for human T-ALL[139]
miRNA-27a	19p13.13	*Bax*, *Bad*, and *14-3-3θ*[130]	Not mentioned	May be a potential therapeutic target[130]

Abbreviations: miRNA, microRNAs; ALL, acute lymphoblastic leukemia; GCs, glucocorticoids.
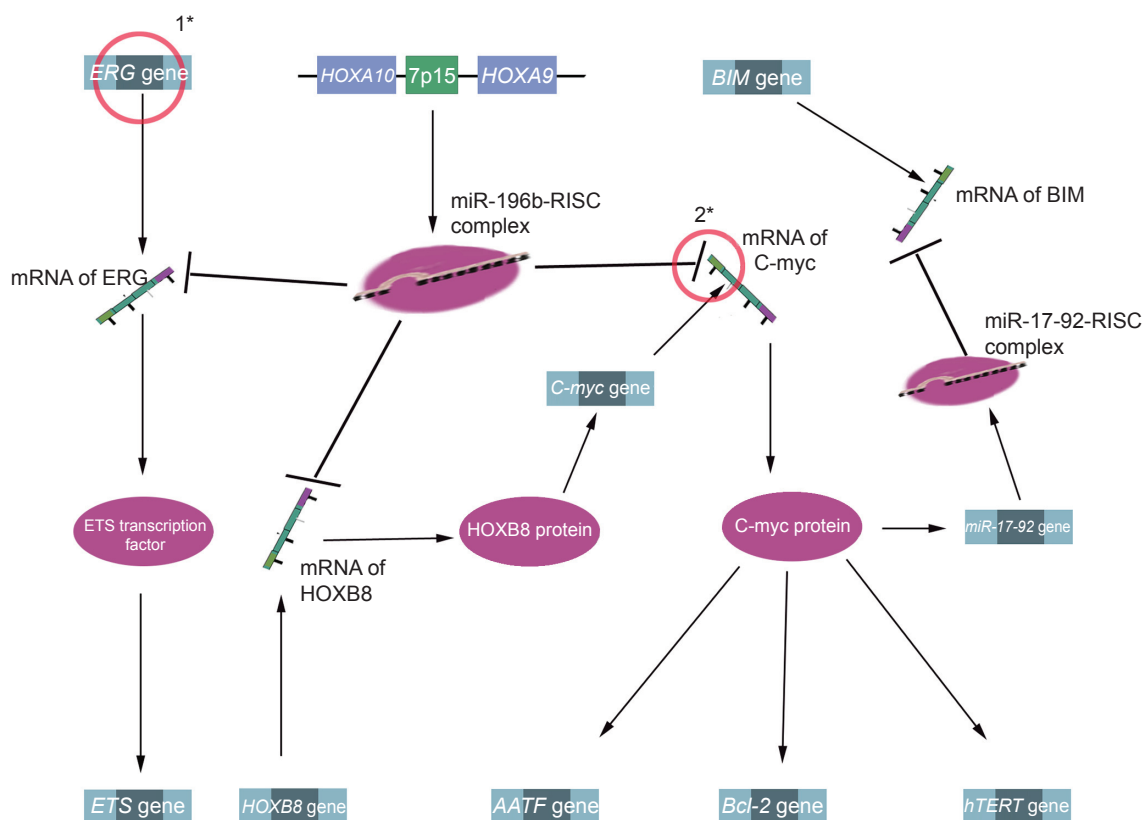
Figure 4 The mechanism between miRNA-196b and their targets.

Notes: 1*, chromosomal rearrangements lead to overexpression of ERG; 2*, mutation in the 3′UTR of C-myc leads to the lost ability of down regulation of miRNA-196b in T-ALL; *ETS* gene is a oncogene; *AATF* gene is anti-apoptotic; *Bcl-2* gene represses the mitochondrial apoptosis signals; *hTERT* gene encodes telomerase catalytic submit related to immortalization and carcinogenesis; *BIM* gene is pro-apoptotic. Data from Bhatia et al[101] and Loughran et al.[102]

Abbreviations: miR, miRNA; miRNA, microRNA; mRNA, messenger RNA; RISC, UTR, untranslated region; ALL, acute lymphoblastic leukemia.

of oncogenes or suppressing apoptosis. However, many problems need to be solved in the future.

Some miRNAs involved in the control of lymphopoiesis are deregulated in ALL, for instance, miRNA-128a, miRNA-126, and miRNA-146 are deregulated miRNAs in ALL and they also play roles in lymphopoiesis. As miRNAs are frequently localized in common breakpoint regions related to tumors or in fragile sites, one may speculate that miRNAs that play a role in lymphopoiesis are prone to be deregulated in lymphocyte original cancers like ALL. For example, a study reported that many of the miRNAs deregulated in multiple lymphoma are also intimately involved in lymphocyte biology under physiological conditions.[91] While one should keep in mind that it is a very young field, the documented miRNAs involved in lymphomagenesis or deregulated miRNAs involved in cancer like ALL are incomplete as is our knowledge about their function. Therefore, further studies are needed to verify this speculation.

In the aforementioned studies, different methods were used in detect the miRNAs in cells or plasma, including bead-based array, planar array, and qPCR.[60,73,74] Consequently, the lack of uniform methods for detecting miRNAs leads to the inability to compare results between different researches. Second, different studies may share few similar miRNA profiles when comparing the differences in origin of normal and aberrant cells. For instance, normal CD34+ cells can be obtained under different conditions, such as after growth factor mobilization versus collected directly from the BM with no mobilization.[3] Therefore, it is possible that the growth factor changed the expression profile of miRNAs in CD34+ cells as well as the change of mRNAs expression reported previously.[3,92] Additionally, some studies use unselected peripheral blood mononuclear cells or BM mononuclear cells from normal donors as controls instead of CD34+ cells.[73,75] From the aforementioned points, better and uniform methods for the detection of miRNAs will help to understand normal and aberrant lymphopoiesis more thoroughly. Also, the uniformity of collecting cells in experimental and control groups separately will help make the results more accurate, enabling comparability among different studies to be obtained. Therefore, standardization of the related studies is the most imperative problem that must be settled in the future.

Considering that miRNAs are the underlying mechanism in the development of human disease, regulation of miRNA function may have therapeutic utility. Although some miRNAs are indentified to have therapeutic effect on some disease, the strategy to interrupt the function of miRNA is limited. In vitro, transfection with miRNA mimics or miRNA inhibitors into cells is a common way to increase miRNA expression or to decrease miRNA expression, respectively, while safety concern and degradation limit their utility in vivo. Many strategies like chemical modifications, LNA, and phosphorothioate linkages have been developed to increase stability and safety.[93] Antagomirs, which are improved miRNA inhibitors, are antisense single-stranded oligonucleotides that are chemically modified, cholesterol conjugated, etc. Antagomirs could silence miRNAs after combining with them and are stable enough to be administrated by intravenous injection.[94] Besides the stability of the miRNA mimics or miRNA inhibitors themselves, delivery methods also play a significant role.[95] The advantages of miRNA mimics expressed from plasmid vectors or viral vectors have longer expression compared with transfection of lipid reagents or electroporation.[96] Some drugs were also reported to potentially modulate miRNAs expression in diseases, but further studies of their pharmacodynamics, pharmacokinetics, safety, etc are required.[97]

Off-target effects, which are brought about by interactions between the RNA interference (RNAi) molecules and nontarget genes, or other cellular components, RNAi molecules, mainly include small interfering RNA (siRNA), short hairpin RNA, and miRNA. Off-target effects could be generally classified as specific off-target effects, also known as miRNA-like off-target effects, and nonspecific off-target effects.[98] Many strategies are studied to diminish or eliminate the undesired effects, such as designing new vectors and chemical modification of RNAi molecules.[99,100] Compared with siRNAs, few studies are related to off-target effects of miRNAs, and therefore, further studies are needed in the future.

Conclusion

There are still many problems that need to be solved before the clinical application of miRNAs in ALL, such as comprehensive understanding of the role of related miRNAs both in physiology and pathology of ALL, standard detecting methods, effective and specific-targeting delivery methods, and acceptable off-target effect. miRNA-based therapeutics is an attractive research area and is a promising field to improve the treatment of cancers like ALL and other diseases.

Disclosure

The authors report no conflicts of interest in this work.

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