

Review Article

B-cell acute lymphoblastic leukemia-related microRNAs: uncovering their diverse and special roles

Mengqi Lv^{1,2}, Shicong Zhu³, Hongling Peng^{1,2}, Zhao Cheng^{1,2}, Guangsen Zhang^{1,2}, Zhihua Wang^{1,2}

¹Department of Hematology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China;

²Institute of Molecular Hematology, Central South University, Changsha, Hunan, China; ³Department of Geriatrics, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China

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Abstract: B-cell acute lymphoblastic leukemia (B-ALL) is a common type of hematologic malignancy characterized by the uncontrolled growth of immature B lymphocytes. Genomics, transcriptomics, and proteomics at different levels contribute to early diagnosis and can thereby provide better treatment for cancer. MicroRNAs (miRNAs) are conducive to the diagnosis and treatment of patients with B-ALL. Moreover, evidence suggests that runaway miRNAs and exosomes containing miRNA may be involved in the occurrence of B-ALL, which can then be used as potential biomarkers. This review summarizes the role of miRNAs in the pathogenesis, diagnosis, prognosis, and treatment of B-ALL.

Keywords: MicroRNA, exosome, biomarker, diagnosis, treatment, B-cell acute lymphoblastic leukemia

Introduction

B-ALL is an aggressive malignant tumor of the blood system, with B-lymphocyte precursor cells reproducing uncontrollably in the bone marrow. Although advances in chemotherapy have significantly improved disease outcomes in children, chemotherapy has a poor outcome in adults [1]. The recurrence of B-ALL is the leading cause of death, and the disease-related mortality rate is approximately 60%. B-ALL is a common subtype of ALL, and identification of new biomarkers for the diagnosis and classification of B-ALL may lead to improved patient prognosis and treatment [2].

miRNAs can attach to the 3'-untranslated region (UTR) of mRNA targets and inhibit their translation, thus, functioning in proliferation, differentiation, and apoptosis [3]. miRNA has been used as tumor suppressor or oncogene in leukemia [4]. Nanocarriers, a type of exosome, can carry proteins, miRNAs, and mRNAs, which can then be released to multiple cells in the body, such as immune cells, nerve cells, and tumor cells, thus participating in RNA transport and tumor metastasis [5]. Exosomes containing miRNAs (exo-miRNAs) result in the sur-

vival and progression of B-ALL cells because of their abundance and convenient access of these molecules in the body. exo-miRNAs can be used for both the diagnosis and treatment of B-ALL patients.

In this review, we describe the comprehensive relationship between miRNA and B-ALL: explaining the function of miRNAs in B-ALL as well as the role of exosomes in delivery.

miRNA and the pathogenesis of B-ALL

miRNA regulates gene expression

The treatment for B-ALL has improved over the past 50 years, especially in children. However, recurrence remains a threat to these patients. miRNAs act as tumor suppressors and carcinogen. Here, we summarize the latest studies that attempt to link aberrantly expressed miRNAs with their targets and describe the complex regulatory networks in B-ALL (**Table 1; Figure 1**).

miRNA as a carcinogen in B-ALL: As carcinogens, miRNAs can inhibit tumor suppressor genes. Many hematological tumors generally

The function of miRNAs in B-ALL

Table 1. Selected miRNAs involved in B-ALL pathogenesis

MicroRNA	Expression in B-ALL	Target	Mechanism of Dysregulation	Function	Material	Sample	Ref
miR-21	Upregulation	<i>PDCD4</i> , <i>PTEN</i> and <i>TPM1</i>		Promoted cell growth, invasion, angiogenesis, and metastasis	PB or BM	75 pediatric B-ALL and 50 controls	[6]
miR-339-5p	Upregulation	<i>BCL2L11</i> ↓		Avoided FGFR1 inactivation	Cell line	-	[9]
miR-509	Upregulation	<i>RAB5C</i> ↓	-	Promoted cell growth	Nalm-6 cells	-	[11]
miR-3173	Downregulation	<i>PTK2</i> ↑	-	Promoted proliferation, migration and invasion.	PB	135 pediatric B-ALL and 97 controls	[12]
miR-206	-	<i>NET1</i> ↑	-	Enhanced proliferation and chemoresistance.	PB and BM	20 pediatric B-ALL and 20 controls	[13]
miR-181a	Upregulation	<i>TLR4</i> , <i>TLR8</i> , <i>IRF8</i> and <i>IL6R</i>	-	An inhibition of innate immunity and inflammation.	PB and BM smears	9 T-ALL patients	[15]
miR-181a	Downregulation	<i>Smad7</i> ↑	TGF-β1↓	Increase in proliferation and decrease in apoptosis; Increased diagnostic sensitivity of ALL to 100%.	PB	23 pre-B-ALL, 5 B-ALL, 2 T-ALL	[18]
miR-181a	Downregulation	<i>ETV6/RUNX1</i> ↑	-	-	PB	PALL carrying t(12;21) translocation	[19]
exo-miR-181a	Upregulation	<i>PCNA</i> , <i>Ki-67</i> , <i>MCL-1</i> and <i>bcl2</i> ↑, <i>BAD</i> , <i>BAX</i> ↓	-	Upregulated proliferation, cell survival and an inhibition of apoptosis.	Cell line and serum	-	[56]
miR-125b, miR-17 and miR-181b	Upregulation	<i>PPP1CA</i> , <i>BTG2</i> , and <i>PTEN</i> ↓	Methylation of the gene promoter region	Increase in proliferation and decrease in apoptosis; Decitabine downregulates these miRNAs.	PB	5 PALL and 7 healthy controls	[20]
miR-125b	Upregulation	<i>P53</i> , <i>Bak1</i> and <i>Bmf</i> transcript↓	Enhanced carcinogenicity of <i>BCR-ABL</i> fusion protein	Increase in proliferation and an inhibition of apoptosis.	Cell line	B-ALL with t(11;14) (q24;q32) translocation	[23]
miR-17~92	Upregulation	<i>BCL2</i> ↑	-	An inhibition of apoptosis; An inhibition of <i>BCL2</i> (e.g., <i>ABT-737</i>) in <i>BCR-ABL</i> -positive ALL cells promoted apoptosis.	Blasts and CD34 cells	13 <i>BCR-ABL</i> -positive B-ALL and controls	[25]
sol-miR-23	Downregulation	<i>BCL2</i> ↑	May promote <i>BCR-ABL1</i> -dependent leukemogenesis	May affect differentiation and resist to cytotoxic drugs.	BM and PB	Pre-B-ALL patients	[26]
miR-142-3p	Downregulation	<i>MLL-AF4</i> ↑	Down-regulated <i>HOXA7</i> , <i>HOXA9</i> and <i>HOXA10</i>	Promoting cell proliferation.	Cell line	B-ALL with <i>MLL-AF4</i> fusion	[27]

The function of miRNAs in B-ALL

miR-222, miR-339, miR-142-3p	Upregulation	miR-142-3p targets <i>BCLAF1</i> , <i>LIFR</i> , <i>BCL2L1</i> ↓; miR-222 targets <i>c-kit</i> ↑; miR-339 targets <i>BCL-6</i>	Affected hematopoietic development	Activated cell differentiation and growth; MiR-142 was low in T-ALL and the erythroid lineages.	BM	40 children with pre-B-ALL	[28]
hsa-miR-451 and hsa-miR-373*	Downregulation	<i>AKTIP</i> and calcium binding protein; <i>BCL11A</i> ↓; <i>BTG1</i> ↓	-	Affected cell differentiation; Promoted proliferation.	BM	40 children with pre-B-ALL	[28]
miR-143	Downregulation	<i>ERK5</i> and <i>MLL-AF4</i> ↑	Formed a methylation/miR-143/ <i>MLL-AF4</i> network	Promoted cell proliferation and induced apoptosis; 5-azacytidine can be used in B-ALL.	Cell line	B-ALL with <i>MLL-AF4</i> fusion	[29]
sol-miR-6, let-7 miRNAs, miR-125b, miR-126* and miR-383	Upregulation	-	-	-	-	<i>TEL-AML1</i> -positive ALL cases	[26]
miRNA-100/99a	Downregulation	<i>FKBP51</i> ↑; the anti-apoptotic gene <i>MCL1</i> ↑	Activated IFG1R/mTOR signaling pathways; May downregulate the GR signaling pathway	Increase in proliferation and decrease in apoptosis; Mediated response to Dex in ALL cells.	BM	ALL patients with <i>MLL</i> -rearrangement and <i>BCR-ABL</i> fusion genes	[51]
miR-126/126*, miR-151 and miR-545	Upregulation	-	Different miRNA expression patterns	-	-	<i>TEL-AML1</i> and hyperdiploid ALL	[31]
miR-383, miR-125b, miR-100, miR-99a, let-7c	Upregulation	-	Higher levels of miR-125b causing vincristine-resistant express	-	-	ALL patients with <i>TEL-AML1</i> fusion genes	[31]
miR-222/222*, miR-223, miR-511, miR-660	Upregulation	-	-	-	-	Hyperdiploid ALL	[31]
miR-10a, miR-134 and miR-214	Downregulation	<i>SOX2</i> ↑	DNA hypermethylation of miR-10a	Decreased apoptosis, increased proliferation; the level of miR-10a can be reversed by DNA hypermethylation in ALL patients with <i>MLL</i> -rearrangement.	-	P-ALL	[31]
miR-196b, miR-151, miR-148a	Downregulation	<i>c-Myc</i> ↑; <i>Bcl-2</i> , <i>hTERT</i> and <i>AATF</i> ↑	-	Increased proliferation and decreased apoptosis.	Cell line	-	[37]
miR-196b	Downregulation	-	-	-	PB or BM	Non- <i>MLL</i> pre-B-ALL	[32]
miR-196b	Upregulation	<i>HOXA</i> ↑	DNA hypomethylation of <i>HOXA</i>	Increase in proliferation.	Cells from BM or PB	<i>MLL</i> -rearranged pre-B ALL	[33]
miR-708	Upregulation	-	-	Expressed highly in <i>TEL-AML1</i> , <i>E2A-PBX1</i> , <i>BCR-ABL</i> , hyperdiploid and B-other cases.	PB or BM	ALL	[32]
291 differentially expressed miRNAs	miR-1246 (the most upregulated); miR-106b-5p (the most downregulated)	19 hub TFs such as MYC, STAT5A, STAT5B, FOXO1 and HLF	Formation of feedback loops or feed-forward loops regulates genes	Regulation of gene expression and hematopoiesis; Association with 25 significantly enriched pathways.	BM	15 patients and 10 controls	[34]
miR-15a-5p	Downregulation	<i>FLT3</i>	Formed MYC/miR-15a-5p/ <i>FLT3</i> feed-forward loop	Increased proliferation.	BM	15 patients and 10 controls	[34]

The function of miRNAs in B-ALL

miR-187-5p	Upregulation	<i>DKK2</i>	Activated Wnt/ β -catenin signaling	Promoted cellular proliferation and inhibited apoptosis.	BM	20 children with B-ALL and Nalm-6 cells	[38]
hsa-miR-124a	Downregulation	<i>CDK6</i> †; phosphorylated retinoblastoma protein†	Cooperation with cyclinD; Promoter methylation	Increased proliferation; Predicted DFS and OS.	Cell lines	Cell lines and 353 patients	[39]
miR-128b	Upregulation	-	Methylation of CpG islands in miR-128b	Accurate diagnosis of ALL and AML.	BM	18 ALL cases	[40]
miR-126	Upregulation	<i>Cdkn2aip</i> ↓	Reduced <i>P53</i> activity in HSPC; Prevented pre-BCR signaling	Evasion of senescence, cell-cycle arrest and apoptosis, and maintaining blasts.	Cell line	17 adult patients and an experimental murine model	[44]
miR-148a-3p, miR-27a-3p, miR-375	Upregulation	-	Regulating the crosstalk between transcription factors and histones	-	BM	B-ALL in CD19-CAR-T therapy	[47]
miR-124-3p	Upregulation	<i>IFI44L</i> ↓	-	Affecting inflammatory response.	Analysis of database	PALL patients	[52]
hsa-miR-103a-3p, hsa-miR486-3p	Downregulation	<i>HOXA7</i> †, <i>S100A10</i> †	Regulating cell growth, motility, cell cycle progression and differentiation	Poor outcomes and chemoresistance.	Analysis of database	PALL patients	[52]

miRNA, microRNA; B-ALL, B-cell acute lymphoblastic leukemia; TF, Transcription factors; HSPC, Hematopoietic stem and progenitor cells; PB, peripheral blood; BM, bone marrow; PALL, Pediatric ALL. T-ALL, T-cell acute lymphoblastic leukemia; AML, acute myeloid leukemia; DFS, disease-free survival; OS, overall survival; *refers to the star strand of the miRNA duplex that is (partly) complementary to the mature miRNA.

The function of miRNAs in B-ALL

Figure 1. Description of microRNAs implicated in the pathogenesis of B-cell acute lymphoblastic leukemia (B-ALL). The miRNAs studied in B-ALL can act as carcinogens or tumor suppressors. The targets, including genes, transcription factors, proteins, and signaling pathways, linked to the miRNAs are noted in the geometric figures. Increased and decreased expression of microRNAs are noted with a gray and green background, respectively. Increased and decreased expression of genes are noted with red and blue backgrounds, respectively. Transcription factors and proteins are annotated with orangey-red and pink background, respectively. Purple arrows and green arrows indicate promotion and inhibition roles, respectively.

overexpress the same miRNAs, such as miR-21 and miR-155. Overexpression of miR-21 results in the acceleration of precursor B (pre-B) cells developing into malignant lymphoid phenotypes. The study indicates that miR-21 is upregulated in children with B-ALL, targeting tumor suppressor genes (*PDCD4*, *PTEN*, and *TPM1*) and promoting invasion, angiogenesis, and metastasis [6, 7]. Medina et al. found that the overexpression of miR-155 promotes B cell proliferation, and de Yébenes et al. showed that miR-155 can serve as a key miRNA for therapeutic targets in children with hepatitis virus leukemia [7, 8]. miR-339-5p is also important for B-cell precursor acute lymphoblastic leukemia (BCP-ALL) where it promotes cell cycle progression and reduces apoptosis [9]. Additionally, the upregulation of miR-708 can suppress *CNTFR*, *NNAT*, and *GNG12*, causing loss of control of hematopoietic differentiation in BCP-ALL [10].

miRNA as a tumor suppressor in B-ALL: miRNAs can act as tumor suppressors and negatively regulate proto-oncogenes. Overexpression of miR-509 in Nalm-6 cells increased the number of apoptotic cells. They did this by targeting *RAB5C*, which is important for cell growth [11]. miR-3173 inhibits *PTK2* expression at the protein level and is downregulated in B-ALL. miR-3173 mimics inhibit the proliferation, migration, and invasion of B-ALL cells, while miR-3173 inhibitors do the opposite [12]. miR-206 can inhibit these effects by targeting neuroepithelial cell-transformation 1 (*NET1*), thus reducing the proliferation of B-ALL cells [13].

Special miRNAs in B-ALL: The same type of miRNA can have different roles in various parts of the body. For example, miR-181 regulates the differentiation of B cells, T cells, and NK cells during normal hematopoiesis [14]. However, miR-181a is one of the most controversial miRNAs in ALL with some researchers regarding miR-181a as an oncogenic factor [15], while other studies have shown miR-181a

as a tumor suppressing factor. However, these may depend on the different cell backgrounds and post-target expressions [16, 17]. miRNA-181a expression is significantly increased in patients with acute myeloid leukemia and T-cell acute lymphoblastic leukemia (T-ALL) through the downregulation of *EGR*, which produces carcinogenic effects [16]. By targeting *SMAD7* and regulating TGF- β 1 signaling pathways, miR-181a can suppress ALL in children. In BCP-ALL, miR-181a can effectively target the fusion protein *ETV6/RUNX1*, reducing its protein level, and inducing significant anti-leukemia effects [18, 19]. Hence, the role of miR-181a has not been fully determined. Moreover, miR-181b tends to be a carcinogenic factor in B-ALL, which can be downregulated by decitabine through the reduced expression levels of miR-125b and miR-17 [10, 20]. miR-125b can determine hematopoietic differentiation and selectively induce myeloid or lymphocytic leukemia according to expression levels and duration. Furthermore, it may regulate certain factors, such as interferon regulatory factor 4 (*IRF4*). In *IRF4* homozygous mutant mice, miR-125b-induced B cell leukemia is significantly accelerated [21, 22]. Another study showed that high levels of miR-125b shortened the incubation period of *BCR-ABL*-induced leukemia. Ectopic expression of miR-125b in hematopoietic embryonic hepatocytes could also induce the development of a B-ALL, a myeloproliferative neoplasm, or T-ALL mouse model [23].

miRNAs and different subtypes of B-ALL: B-ALL can be subdivided into haploid fusions, including *ETV6-RUNX1* fusion, *BCR-ABL1* fusion, *TCF-PBX1* fusion, and *MLL* fusion. The first two subtypes have a better disease prognosis. The appearance of *BCR-ABL* fusion proteins in 3% of children with B-ALL indicates a possible poor prognosis [24]. Some miRNAs in B-ALL are dependent on *BCR-ABL* expression. After deleting miR-17~92 in normal lymphocyte production, accompanied by higher levels of

Bim (*BCL2L11*), apoptosis is induced, thereby preventing the conversion of pro-B cells. miR-17~92 targeted the overexpression of BCL2 to induce apoptosis, which provides application potential for using ABT-737, a BCL2 inhibitor to cure B-ALL [25]. The decrease in sol-miR-23 levels is related to the increase in BCL2 [26]. The study found that miR-142-3p was down-regulated and hence promoted B-ALL cell proliferation with *MLL-AF4* fusion by targeting the *HOXA7*, *HOXA9*, and *HOXA10* genes [27, 28]. Various other miRNAs (miR-143, miR-223, miR-222/222*, miR-98, hsa-miR-101, miR-511, Let-7 miRNA, miR-100, miR-99a, miR-196b, and miR-708) were differentially expressed in the different types of ALL [26, 29-33]. These results indicate that miRNA expression is specific to different ALL subtypes.

miRNA and the network of B-ALL gene expression: At present, the regulatory network of B-ALL gene expression is unclear. miRNAs play an important role in various physiological and pathological processes by targeting and regulating gene expression and regulating signaling pathway processes. The JAK-STAT signaling pathway, PI3K-AKT signaling pathway, cell cycle pathway, and RAS signaling pathway are associated with B-ALL pathogenesis [34]. Transcription factors (TFs) are important in biological processes. As one of the most common TFs. MYC is important in cell differentiation, proliferation, survival, and metabolism [35], and it can activate miR-17~92 clusters, thereby controlling *BIM*, *PTEN*, and p21 (*CDKN1A*). The rearrangement of MYC or ABL proto-oncogenes relieves the expression restriction of these key regulators, and members of the BCL-2 family transfer downstream oncogenes. Double transgenes of MYC and BCL-2 lead to excessive proliferation of immature cells and thereby promote B-ALL [36]. miR-196b functions by targeting the transcription factor MYC, thus inducing apoptosis in B-ALL [37], however, this is different for T-ALL expression. Using TF array technology and Illumina deep-sequencing, a study of microRNAs and TFs has shown that there is a MYC/miR-15a-5p/*FLT3* feed-forward loop in B-ALL, which may regulate the JAK-STAT signaling pathway. This has been regarded as a vital motif in the miRNA-TF network of B-ALL. Other hub miRNAs and hub TFs were also predicted to be related to B-ALL. Among them, STAT5A, STAT5B, and MYC were

associated with the JAK-STAT signaling pathway [34]. Moreover, Lou et al. first proved that uncontrolled WNT signaling can lead to B-ALL, which is regulated by the miR-187-5p-DKK2 pathway [38].

miRNAs and epigenetic regulation

The formation of acute leukemia is closely related to epigenetic regulation. Promoter methylation regulates hsa-miR-124a and can cooperate with cyclinD by targeting *CDK6*, thus, participating in the regulation of the cell cycle and helping to develop glucocorticoid resistance in ALL [39]. Methylation of CpG islands in miR-128b promoters leads to upregulation of miR-128b, thereby resulting in the pathogenesis of high-risk pre-B-ALL [40]. In addition, miRNAs can regulate epigenetic mechanisms by affecting DNA methylation and histone changes. Researchers have found that *BTG2* and *PTEN* are targets of miR-181b and miR-125b, this was confirmed when their expression decreased after demethylation therapy. These results suggest that demethylation drugs can be used as a first-line therapy for patients with B-ALL [20].

Effects of miRNA on hematopoietic function

Different tissues express different miRNA patterns at different stages. Various miRNAs, including miR-15a, miR-146, miR-150, miR-10a, miR-221, and miR-222 are important for normal hematopoiesis [41]. miR-155 and miR-150 affect B and T lymphocyte functions [42]. As mentioned before, miR-125b, miR-17~92, and miR-181 are important in the regulation of hematopoietic differentiation, however, their abnormal expression can lead to hematological tumors. Moreover, premature expression of miR-150 prevented the migration of pro-B cells to pre-B cells [43]. miRNA-126 regulates hematopoietic stem cell differentiation. Nucera et al. designed a mouse model with hematopoietic engineering to express miRNA-126 at all stages of differentiation. miRNA-126 expression in human progenitors and mice reduces p53 transcriptional activity by regulating p53-related targets, thus, 30% of mice developed monoclonal B-cell leukemia. The development of this type of leukemia is controlled by inhibiting miRNA-126 expression [44]. Leukemia cells can interact with the bone marrow microenvironment (BMM). BMM can reduce miR-221 expression in leukemia cells,

leading to an increase in CDKN1B protein and thereby promoting cell cycle progression, increasing ALL survival, and inducing chemoresistance. By targeting the miR-221/222-p27 pathway, the residue of quiescent leukemia cells is reduced [45].

Immunomodulatory effects of miRNA

miRNAs can promote the release of mediators that activate cancer and anticancer immune activities. miRNA analysis was conducted in 19 adult and 79 pediatric patients with ALL. Immune-associated miRNAs in children and adults were significantly similar. However, researchers have observed that the level of miR-18a is low in children with ALL. This is appropriate for future study because miR-18a may play a role in the better medical response in pediatric patients with ALL [46]. Chimeric antigen receptor (CAR) T-cell therapy is an important immunotherapy [47]. Zhang et al. revealed that many miRNAs, such as members of the let-7 family, are involved in the immune process during CD19-specific CAR-T therapy. miRNAs including miR-148a-3p, miR-375, and miR-27a-3p regulate the interaction between TFs and histones. The combination of histone genes with lncRNA and miRNA-TF-gene regulatory networks is necessary for these patients [48]. These findings help us to understand the mechanisms of CAR-T immunotherapy, and the effects of miRNAs on B-ALL, in cancer. Additionally, genes targeting PD-1 can also activate anticancer immunity and initiate miRNAs (including miR-424 and miR-34a) that regulate apoptosis and block the expression of PD-1 [49]. Moreover, miR-142-5p was found to suppress PD-L1 by directly binding to PD-L1 mRNA [50].

Application of comprehensive bioinformatics analysis

The identification of miRNA targets is difficult because it is challenging to locate complementary miRNA sites. Recently, many studies have used interdisciplinary methods, such as comprehensive bioinformatics analysis and open databases, to identify the correlation between potential miRNAs and disease. These studies are beneficial because they do not have the high costs and long cell experiments, thus they guide the future research direction and subsequent wet-lab experiments. One study linked

miRNAs to mRNA target genes; relying on the use of publicly available data, Ramani et al. found that miRNA-100/99a inhibits B-ALL cell proliferation by targeting FKBP51 and regulating the IFG1R/mTOR signaling pathway. Furthermore, the P53 pathway is targeted by miRNAs (miR-19, miR-221, miR-515, and miR-103), suggesting that these miRNAs may have carcinogenic effects in B-ALL [51]. During early relapse of B-ALL, upregulated miR-124-3p was predicted to target the *IFI44L* gene. Using two predictive websites and Cytoscape data to build an mRNA-miRNA network, Huang et al. found that hsa-miR-103a-3p and hsa-miR-486-3p and their target genes change during relapse [52]. Developments in the field of bioinformatics has helped us to understand the relationship between miRNAs and their targets. Although public data and bioinformatics are useful, we are more aware of its limitations, including faults in experiments, processing data, and clinical information. Therefore, further wet-lab experiments and clinical studies involving larger number of samples are necessary. Most computer methods focus on whether the association between miRNAs and disease exists. However, new computing methods are used to predict the association between various miRNAs and diseases, and these are particularly useful for clinicians.

Role of exo-miRNA in B-ALL pathogenesis

Extracellular vesicles (EVs), including microvesicles and exosomes, are released by cells. Patel et al. confirmed that exosomes secreted from pre-B ALL cells in Ph⁺ ALL patients can stimulate non-growing pre-B ALL cell growth without the dependence on direct intercellular contact [53]. Exosomes containing miRNAs (exo-miRNAs) interact with the surrounding microenvironment; assisting in the development of B-ALL cells by stimulating cell proliferation and migration and altering the microenvironment, thus, inhibiting tumor immune monitoring and anti-tumor responses (**Figure 2**) [54]. ALL central recurrence is an important reason for the high mortality rate of patients with ALL. Only a limited number of studies have explored the role of exosomes or miRNAs in the metastasis to the central nervous system (CNS) of patients with ALL (**Figure 2**). Egyed et al. found that miR-181a and its specific EV subtypes in cerebrospinal fluid can predict ALL central nervous sys-

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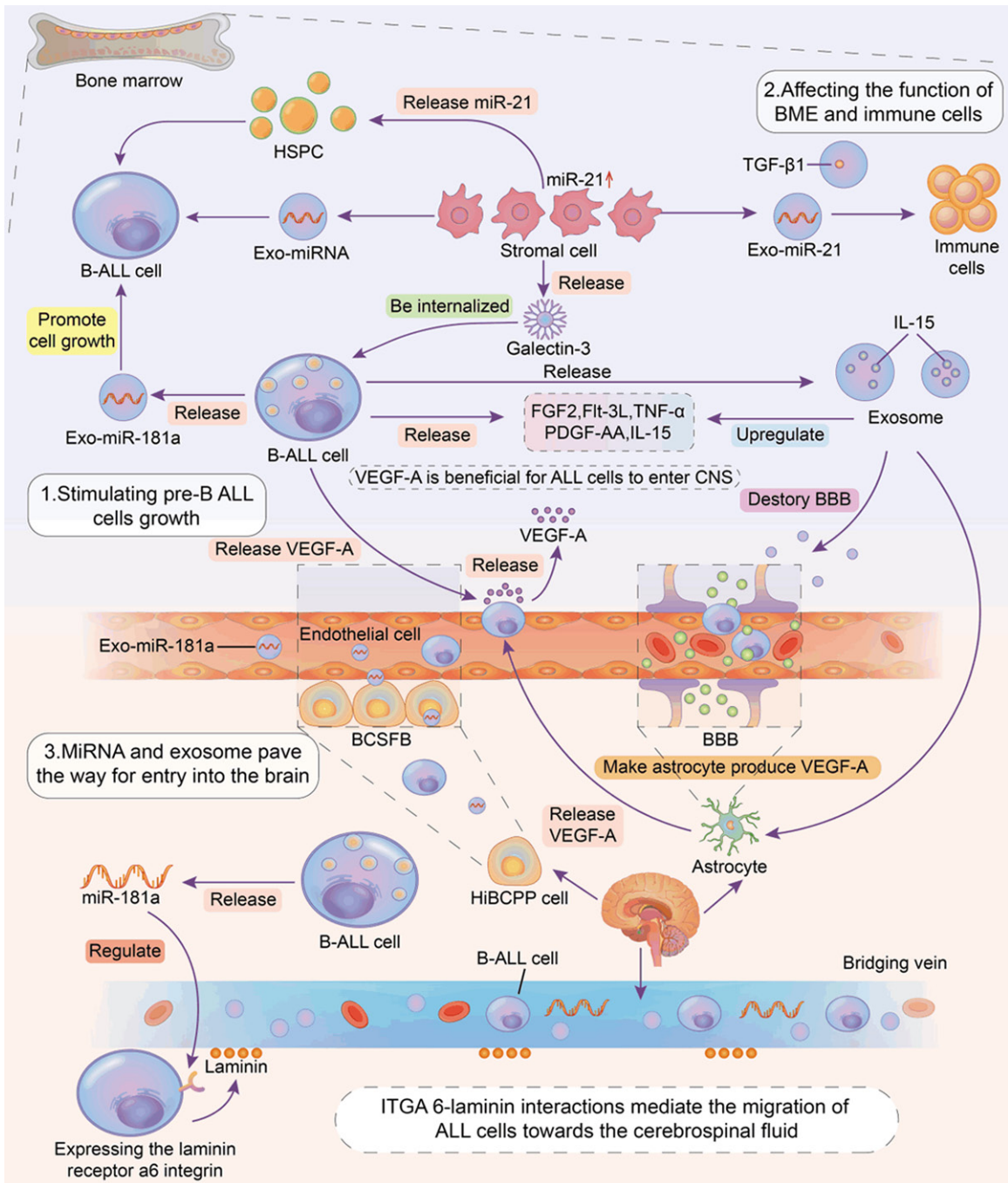


Figure 2. In patients with B-cell acute lymphoblastic leukemia (B-ALL), miRNAs and exosomes containing miRNAs play a key role in promoting the occurrence, progression and the central nervous system (CNS) metastasis of B-ALL. Pre-B lymphocytes, bone marrow microenvironment cells, and nervous system astrocytes can produce miRNAs and exosomes, which can mediate the bidirectional action between cells. Besides this, important cytokines and special humoral pathways also mediate the development of B-ALL cells. B-ALL cells enter the CNS through three main routes including the blood-cerebrospinal fluid barrier, blood brain barrier and bridging vein. miRNA, exo-miRNA and VEGF-A regulate these routes, thus they affect the metastasis of B-ALL cells. miRNA, exo-miRNA and VEGF-A regulate these ways, affecting the metastasis of B-ALL cells. miRNA, microRNA; Exo-miRNA, exosome containing miRNA; B-ALL, B-cell acute B-lymphoblastic leukemia; HSPC, hematopoietic stem progenitor cells; BME, bone marrow microenvironment; VEGF-A, vascular endothelial growth factor A; CNS, central nervous system; BBB, blood-brain barrier; BCSFB, Blood-cerebrospinal Fluid Barrier; HiBCPP cells, the human choroid plexus papilloma epithelial cells; ITGA 6-laminin, integrin alpha 6-laminin; EVs, extracellular vesicles.

tem infiltration, moreover, the sensitivity of cerebrospinal fluid miR-181a detection in the early diagnosis of CNS leukemia (90%) was significantly higher than that of conventional cytology (54.5%) [55]. Exo-miR-181a was first reported to specifically induce B lymphocyte proliferation in pediatric ALL (PALL). Moreover, miR-181a has unique and significant exosome amplification in PALL sera and leukemia cell lines. miR-181a inhibitors were successfully transfected into exosomes. Inhibiting exo-miR-181a, with the downregulation of pro-survival genes (*MCL-1* and *BCL2*) and proliferative genes (*PCNA* and *KI-67*) as well as the inhibition of pro-apoptotic genes (*BAD*, *BAX*), can inhibit exosome-induced cell proliferation. However, inhibitors based on exosome transport-targeting miRNAs have higher safety [56].

miRNA as a biomarker in B-ALL

Mortality in ALL is increasing, this is due to a large proportion of patients dying because of a lack of biomarkers to guide treatment, assess drug resistance, and tack prognosis. **Table 2** shows that miRNAs have great potential in the early diagnosis of malignancy, as well as being useful biomarkers in prognosis and drug resistance. Exosomes, a membrane-bound vesicle approximately 30-100 nm in diameter, are widely distributed in various biological body fluids [57]. Circulating miRNAs are packaged in microbubbles (e.g., exosomes), and their distribution in patients typically reflects patterns observed in tumor tissues. Their properties support their use as minimally invasive and robust biomarkers [2].

miRNAs function as diagnostic biomarkers

Some patients with ALL cannot be diagnosed using traditional approaches. Expression levels of certain miRNAs and overall miRNA expression profiles have shown that certain cancer types can be distinguished from each other. Mi et al. showed that ALL could be distinguished from AML based on four miRNAs (miR-128a, miR-128b, let-7b, and miR-223), with an accuracy of more than 95% [40]. The sensitivity of ALL diagnosis can be improved by 100% through miR-181a and TGF- β 1 [18, 31]. Other miRNAs, including miR-127 and miR-143, also have applied value in this setting [31]. Almeida et al. have used the HiSeq 2500 platform and found that miR-29c-5p is the best differentia-

tor for children with either B-ALL or T-ALL, because its participation in calcium signaling is crucial for the fate of B cells [58]. Malik et al. found a new diagnostic/prognostic marker that can distinguish between pediatric B cells and T cells: a miR-2909-KLF4 molecular axis [59].

miRNAs function as prognostic biomarkers

Expression of miRNAs and their association with prognosis can be used as biomarkers for screening leukemia progression. Nemes et al. suggested that miR-223 and miR-128b levels could predict ALL recurrence. At diagnosis, high levels of miR-128b expression predict a good prognosis [60]. El-Khazragy et al. showed that miRNA-155a and miRNA-181a are associated with poor prognosis, with the critical value of miRNA-155a being significantly different between patients with different risk stratification, however this was not the case for miRNA-181a ($P > 0.05$). Low expression of miR-335 predicts poor five-year patient survival, moreover, the level of miR-335 at diagnosis was an independent prognostic indicator for treatment outcomes of PALL [61]. Labib et al. reported that upregulated miR-21 expression was associated with reduced disease-free survival (DFS) and overall survival (OS), as well as being correlated with a poor response to induction therapy in children with B-ALL [6]. Avigad et al. found that miR-451, miR-151-5p, and miR-1290 were novel biomarkers and prognostic indicators in children with BCP-ALL regardless of the treatment options. Furthermore, they detected a significant correlation between at least one of the three indicators of abnormal expression and poor prognosis ($P < 0.0001$). Their use may improve risk stratification and allow early treatment interventions to improve survival in at-risk patients [62]. In addition, Amankwah et al. found six miRNAs related to early relapse in children with B-ALL [63].

As briefly mentioned earlier, miR-181a and its specific subtype of EV in the serial cerebrospinal fluid could also be a predictor of central nervous system involvement in ALL [55]. Human hematological malignancy-derived exosomes have special molecular membrane markers, including TGF- β 1, MHC class I polypeptide-related sequences A/B, and medulloblastoma cell markers, such as CD34, CD33, and CD117 [64]. The miRNAs contained in exosomes have

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Table 2. Application of MiRNA in Diagnosis and Treatment of B-ALL

MicroRNA	Expression in B-ALL	Target	Mechanism of Dysregulation	Function	Material	Sample	Ref
miR-29c-5p	Downregulation	AFF1; <i>KMT2A</i>	Transcriptional dysregulation in cancer; Calcium signaling may be upregulated	Distinguish childhood T-ALL from B-ALL	BM	8 B-ALL and with 8 T-ALL patients (Brazilian children)	[58]
miR-2909	Upregulation	<i>KLF4</i> ↓	Decrease in tumor suppressor	Inhibition of apoptosis; Distinguish T-ALL and B-ALL	-	30 pediatric B-ALL and 20 T-ALL cases and 50 controls	[59]
miR-21	Upregulation	<i>PDCD4</i> , <i>PTEN</i> and <i>TPM1</i>	-	Poorer DFS and OS	PB or BM	75 pediatric B-ALL and 50 healthy controls	[6]
miR-151-5p, miR-451	Downregulation	-	-	Independent prognostic markers for relapse; MiR-151 could distinguish T-ALL and B-ALL	BM	189 pediatric ALL patients	[63]
miR-1290	Upregulation	-	-	Independent prognostic markers for relapse	BM	189 pediatric ALL patients	[63]
miR-101-3p, miR-1324, miR-4774-5p, miR-631, miR-922 and miR-4699	Upregulation	-	-	-	BM	6 early relapse and 6 prolonged remission patients and an independent set of 14 early relapse and 14 prolonged remission specimens	[64]
miR-708	Upregulation	<i>CNTFR</i> , <i>NNAT</i> and <i>GNG12</i> ↓	Affecting hematopoietic differentiation; Activating JAK/STAT signal transduction	Increase in cell proliferation	BM	34 pre-B-ALL cell patients and cell line	[10]
miR-708, miR-223 and miR-27a	miR-708 was upregulated; miR-223 and miR-27a were downregulated	<i>E2F1</i> , <i>MDR1</i> , <i>FOXO3</i>	Activating cell proliferation and leukemia remission to relapse	Biomarkers for Glucocorticoid therapy response and predicting RFS	BM	B-ALL patients	[67]
miR-335	Downregulation	Activating MAPK1-mediated survival of cells	DNA methylation; PRED resistance	Poorer 5-year EFS and GC resistance	BM	56 B-ALL and 7 T-ALL patients	[68]
hsa-miR-17-5p	Upregulation	Bim protein levels↓	-	GC resistance	A computational approach	PALL	[69]
miR-99a, miR-100, miR-125b-5p	-	-	-	Resistance to daunorubicin and vincristine	-	Pediatric B-ALL patients	[70]
hsa-miR-3143, hsa-miR-744-3p, hsa-miR-6503-3p, hsa-miR-1226-3p, hsa-miR-4658, hsa-miR-493-3p, hsa-miR-10-5p	Differentially expressed between LPs and LSCs	-	Affecting leukemogenesis, clone and stemness capacities	Prediction for the presence of resistant LSCs	BM	Tree adult Ph ⁺ B-ALL patients	[71]
miR-3173	Downregulation	<i>PTK2</i> ↑	Activated proliferation, migration and invasion	Having therapeutic potential	PB	135 pediatric B-ALL and controls	[12]
miR-19b	Upregulation	-	Increase in apoptosis	miR-19b inhibitors could inhibit the proliferation	-	-	[75]

miRNA, microRNA; B-ALL, B-cell acute B-lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; Ph⁺ B-ALL, B-cell Acute lymphoblastic leukemia with chromosome positive in Philadelphia; DFS, disease-free survival; OS, overall survival; LSC, stationary leukemia stem cells; LPs, leukemia progenitor cells; GC, Glucocorticoid; EFS, event-free survival.

a higher clinical research potential. Through monitoring B-ALL exosome contents, the efficacy of treatments can be evaluated providing more accurate means of diagnosis and evaluation of the curative effects of B-ALL.

MiRNA and treatment of B-ALL

miRNA and chemotherapy resistance and adverse reactions

Currently, there is plenty of evidence that miRNAs tend to influence treatment responses by modulating a variety of transport proteins in patients with ALL, including ATP-binding cassette (ABC) transporters [65]. Ghodousi and Rahgozar found miRNA-326 and miRNA-200c target the transporter genes *ABCA3* and *ABCA2*, respectively, and all patients showed that a depression in miR-326 and miR-200 had new diagnostic functions [66].

Han et al. showed that miR-708 results in a poor reaction to glucocorticoids in pediatric B-ALL [67]. Ectopic expression of miR-335 increases sensitivity to prednisone [68]. Chen et al. cloned two microRNAs (*hsa-miR-142-3p* and *hsa-miR-17-5p*) and bioinformatically predicted their function in glucocorticoid resistance in PALL [69]. Some miRNAs (including miR-125b-5p, miR-99a, and miR-100) have been shown to result in daunorubicin and vincristine (VCR) resistance in pediatric B-ALL [70]. Thirty-nine percent of patients with VCR resistance had the *ETV6/RUNX1* subtype. These patients showed high levels of miR-125b expression; affecting miR-125b function may enhance the chemotherapeutic response in patients [31]. The survival of stationary leukemia stem cells (LSCs) after therapy is one of the causes of relapse in patients with Ph⁺ ALL. A recent study identified that *hsa-miR-6503-3p* and *hsa-miR-3143* may affect the production, cloning, and cell capacity of these subpopulations of Ph⁺ B-ALL and thus help develop drug-resistant LSCs [71].

Single nucleotide polymorphisms (SNPs) in miRNAs can alter the levels or functions of miRNAs. Iparraguirre et al. detected SNPs in miR-5189, miR-595, and miR-6083, which may affect the regulation of the MTX transporter genes *SLC46A1*, *SLC19A1*, and *SLC01A2*, thus influencing the effect of MTX in children with

B-ALL [72]. In terms of drug side-effect-related miRNA expression, this study identified two SNPs that might be involved in VCR-and neurotoxicity-related miR-3117-3p [73]. Gutierrez-Camino et al. found an association between the rs2114358 variant of miR-1206 in ALL patients with MTX-induced oral mucositis [74].

miRNA and prognosis of B-ALL patients

The role of miRNAs in tumor pathogenesis provides a new research perspective on therapeutic targets (**Table 2**). miRNAs regulate protein expression by altering the mRNA transition to a lower state. Changing protein levels by manipulating miRNAs is possible; however, these techniques are still in their infancy. The transfection of miRNA mimics or miRNA inhibitors into cells is the usual way to regulate miRNA expression. miR-3173 inhibits PTK2 expression at the protein level. *In vitro* experiments demonstrated that miR-3173 mimics inhibited proliferation, migration, and invasion in B-ALL cells, while miR-3173 inhibitors had the opposite effect. Therefore, miR-3173 shows therapeutic potential [12]. miR-206 inhibits cell proliferation and chemical resistance by targeting neuroepithelial cell-transformation 1 (*NET1*); this suggests the possibility of developing therapeutic interventions with this miRNA [13]. In addition, *in vitro* studies showed that miR-19b inhibitors could inhibit the proliferation of B cells better than T cells [75].

However, safety and degradation limit the application of miRNA mimics or miRNA inhibitors *in vivo*. It is important to maintain the stability of miRNA mimics or miRNA inhibitors *in vivo*; therefore, delivery methods need to be adequately studied [76]. miRNAs in exosomes have non-degradable stability, therefore, the application prospects of exosomes in transporting miRNAs are extensive [77]. A previous study successfully transfected miR-181a inhibitors into exosomes, which could then inhibit exo-miR-181a, thereby inhibiting the proliferation of B-ALL cells. This provides the possibility for accurate and effective treatment of B-ALL [56]. The technology and miRNA utilization of edited miRNAs need to be improved, and the possible risks of miRNA non-target effects need to be further studied. Moreover, clinical studies based on transport miRNA therapy are underway and should elucidate more data [78].

Conclusion and future perspectives

Our understanding of the molecular mechanisms underlying B-ALL remains limited. miRNAs can act as carcinogens or proto-oncogenes in different cell types. The specific mechanisms of miRNA in B-ALL disease progression and therapeutic drug resistance is not fully understood. The establishment of miRNA-mRNA interactions and signaling pathways in B-ALL helps to understand the role of miRNAs in disease occurrence, progression, epigenetic regulation, immunosuppression, and drug resistance. Since an miRNA can target many gene transcripts, it usually has related functions. Moreover, several individual transcripts can be targeted by many miRNAs that are often regulated together. Combining miRNA-ome data with both transcriptome and proteome integration will be the basis for a more comprehensive analysis of the consequences of disrupting the cellular structure maintained by the microRNA. However, this requires a large number of patients and appropriate healthy tissues. Therefore, interdisciplinary methods are required. Although studies on exo-miRNAs are limited, exo-miRNAs specifically regulate the pathogenesis of B-ALL, especially during the invasion of BCP-ALL cells into the CNS.

There is good research potential for disease progression biomarkers and therapeutic targets for miRNAs. Specific miRNAs help diagnose B-ALL and determine the prognosis of patients. However, their roles also require large-scale clinical trials to confirm their clinical potential. So far, most studies on miRNAs in B-ALL have focused on children. However, there are differences in the expression patterns of miRNAs between children and adults with ALL. Therefore, it is important to analyze the differences, which may help explain the poor effect of chemotherapy in adults and provide a new perspective for the choice of treatment options available for adult patients with B-ALL. Understanding the interaction between miRNA-mediated immune cells and leukemia cells is essential for the successful development of immunotherapy.

At present, exosomes can replace liposomes in transporting chemical drugs, miRNAs, and many other components, moreover, they are highly specific to target cells. Because exosomes are small, the internalization mechanism of exosomes is not clear. In the future it

is necessary to determine how the exosomes are transferred *in vivo* and how long they maintain stability in systemic circulation, furthermore, new methods to isolate and identify exosomes must be established. EV-based clinical trials are underway, but most of them focus on solid cancer. Moreover, the Exo ReBly project (NCT03985696) is a clinical trial on Diffuse Large B-cell Lymphoma (DLBCL) aiming to explore whether there is a large number of CD20 and PD-L1 in DLBCL derivative EVs. This study will determine whether they are bait targets and whether they have strong immunosuppressive signals for rituximab antibodies, which may then lead to therapeutic resistance. As mentioned before, the killing activity of CAR-T cells in transfusion patients will still be weakened by the PD-1/PD-L1 immunosuppressive pathway. We must discover whether exosomes secreted by B-ALL leukemic cells carry PD-L1, thus decreasing CAR-T immunotherapy. This question requires further exploration, which may help to improve the effectiveness of CAR-T immunotherapy in patients with B-ALL.

In summary, studies of both miRNAs and exo-miRNAs have revealed the pathogenesis of B-ALL. Specific miRNAs and exo-miRNAs are promising biomarkers for the diagnosis and prognosis of patients with B-ALL. However, in the future it is necessary to determine how exosomes are transferred *in vivo* and how long they are maintained. Furthermore, disease progression biomarkers and therapeutic targets for miRNAs have good research potential.

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Disclosure of conflict of interest

None.

Address correspondence to: Zhihua Wang, Department of Hematology, The Second Xiangya Hospital, Central South University, No. 139 Middle Renmin Road, Changsha 410011, Hunan, China. E-mail: wangzhihuazsc@csu.edu.cn

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