Trends in Cancer Research
Vol. 15, 2020

Review

Regulatory mechanisms of microRNAs in childhood acute lymphoblastic leukemia

Tania S. de Liz, Michele P. Rode and Tânia B. Creczynski-Pasa*

Department of Pharmaceutical Sciences, Federal University of Santa Catarina, Florianópolis, SC 88040-900, Brazil.

ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, occurring mainly in children three to five years of age. ALL is a complex disease that involves both B-lineage and T-lineage precursor cells. Several studies have shown that microRNAs (miRNAs) can be used as a new class of biomarkers for ALL. These small, single-stranded, non-coding RNA molecules regulate gene expression through direct interaction with specific messenger RNAs. This review aims to summarize data from studies on miRNA and childhood ALL and presents the results of a gene ontology enrichment analysis performed on gene sets regulated by miRNAs. Most studies reported that different miRNAs are involved in childhood ALL; miR-196b-5p, miR-128-3p, miR-223-3p, miR-181a-5p, miR-27a-3p, and miR-708-5p were the most frequent. These miRNAs regulate genes and biological functions related to hematopoiesis, cell death, and proliferation. Although some miRNAs are promising biomarkers for ALL diagnosis, their use in clinical practice is still a challenge. This review reveals the need for further investigations on the role of miRNAs in disease development, diagnosis, and prognosis.

KEYWORDS: biomarkers, hematopoiesis, cell death, cell proliferation, apoptosis, miRNA.

*Corresponding author: tania.pasa@ufsc.br

INTRODUCTION

Leukemia accounts for 29% of all childhood cancers, 76% of which are lymphoid leukemia [1]. According to data from the Global Cancer Observatory (2018) [2], leukemia has the highest annual incidence: 3.4 per 100,000 children and adolescents aged 0 to 14 years, followed by tumors of the brain and central nervous system, with 1.2 per 100,000 and other cancers [3]. The incidence of acute lymphoblastic leukemia (ALL) peaks between 2 and 5 years of age, representing 70% of cases [4] and is higher in men than in women [5]. In the United States of America (USA), ALL is more common in whites than in blacks, with an incidence of 1.5 per 100,000 white population and 0.8 per 100,000 black population [6].

ALL is part of a family of genetically heterogeneous lymphoid neoplasms derived from B- and T-lymphoid progenitors [7]. The disease is characterized by the accumulation of immature lymphoid cells within the bone marrow. Generally, patients present with typical manifestations, such as anemia, neutropenia, thrombocytopenia, and leukemia cell infiltration [5]. The World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues states that a multiple approach is necessary to diagnose diseases, including analysis of clinical features, morphology, immunophenotype, and genetic data [8]. However, the difficulty in classifying some cases and the need for more accurate diagnoses has led to the search for other diagnostic parameters. Recently,

studies revealed altered microRNA (miRNA) expression profiles in acute leukemia, showing the important role of miRNAs in leukemia development. miRNAs are small, single-stranded, non-coding RNA molecules with 18 to 25 nucleotides capable of hybridizing target messenger RNAs (mRNAs) and post-transcriptionally controlling mRNA expression. miRNAs are expressed differentially in distinct stages of normal lymphopoiesis and have been shown to contribute to the maturation of lymphoid precursors [9]. In hematologic cancers, miRNA expression is altered. This review aims to discuss the differences in miRNA expression profiles and the regulatory mechanisms of miRNAs in childhood ALL.

METHODOLOGY

A web-based search of the scientific literature was performed using Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/) and Mendeley v. 1.19.2. The terms "miRNA," "childhood," and "acute lymphoblastic leukemia" were used separately and combined. Studies published from 2009 to 2019 were included in the search. Only articles on childhood ALL and miRNA using human samples or cell lines were included. Reference lists of all included articles were examined to find other relevant publications that might not have been identified by the computer search. miRNAs cited in more than three articles were further evaluated to find predicted targets using the StarBase v. 2.0 (assembly hg19) database. Enrichment analysis of genes regulated by miRNAs in ALL was performed using Gene Ontology (2019-02-01 release) database.

RESULTS AND DISCUSSION

miRNAs as biomarkers of ALL

Changes in miRNA expression profile in ALL can be evaluated through well-established methods, such as quantitative polymerase chain reaction (qPCR) and microarray analysis. Alterations in miRNA expression in peripheral blood and bone marrow samples from leukemia patients are important for the diagnosis, prognosis, and treatment of this disease and provide a better understanding of the biological processes involved in carcinogenesis.

Oliveira *et al*. [10] evaluated the expression profile of seven miRNAs in 128 bone marrow samples from ALL pediatric patients. The authors reported that miR-128a and miR-181b were upregulated and miR-100, let-7e, and miR-196b were downregulated compared with healthy subjects [10]. In contrast, an investigation carried out by Schotte *et al*. [11] showed that miR-196b is upregulated in *MLL*-rearranged ALL patients. The authors analyzed the expression profile of 397 miRNAs in 81 cases of childhood leukemia and 17 control cases using mononuclear cells isolated from peripheral blood or bone marrow. ALL subtypes and drug-resistant leukemic cells displayed unique miRNA signatures. *MLL*-rearranged, *TEL-AML1*-positive, *E2A-PBX1*-positive, and hyperdiploid ALL miRNA expression profiles differed from each other and from those of healthy hematopoietic cells. Precursor B-cell ALL samples and healthy bone marrow samples were clustered separately. miR-143 expression was 70-fold ($P = 0.0007$) lower in precursor B-cell ALL than in healthy bone marrow. Many hyperdiploid samples were clustered together and showed distinct patterns of miRNA expression compared with those of non-hyperdiploid, highly expressing miR-223, miR-222/222*, miR-98, and miR-511 [11]. Furthermore, *TEL-AML1*-positive ALL was distinguished from non-*TEL-AML1* ALL subtypes by a 5- to 1700-fold upregulation of miR-99a, miR-100, miR-125b, miR-383, and let-7c ($P < 0.001$). Among *TEL-AML*-positive patients, two distinct groups were identified based on miRNA expression. One group had an expression profile similar to that of hyperdiploid patients, characterized by upregulation of miR-126/126*, miR-151, and miR-545. The second group showed miRNA expression profiles different from those of hyperdiploid patients, with miR-383, miR-125b, miR-100, miR-99a, and let-7c expression increased by 5 to 1670-fold [11].

Analyzing samples from children with newly diagnosed ALL, Schotte *et al*. [12] found that the expression level of miR-196b was upregulated by 500 to 800 fold in most cases of *MLL*-rearranged B-cell acute lymphocytic leukemia (B-ALL) compared to B-ALL without *MLL* translocation and normal bone marrow cells. However, high miR-196b expression is not unique to *MLL*-rearranged ALL; it also occurs in patients with T-cell ALL carrying other alterations, such as *CALM-AF10*, *SET-NUP214*, and inversion of

chromosome 7. Similar to *MLL*-rearrangements, these abnormalities have been functionally linked with upregulation of *HOXA*.

Nemes *et al*. [13] investigated the expression profile of eight important miRNAs (miR-16, -21, -24, -29b, -128b, -142-3p, -155, and -223) in ALL pediatric patients and observed the overexpression of miR-128b. miR-128b expression decreased significantly during treatment until reaching the normal expression level of cells in remission. The authors reported a positive correlation between prognosis and miR-128b expression.

In a study of bone marrow samples from 43 patients with B- and T-ALL, Duyu *et al*. [14] observed that 12 miRNAs were upregulated (miR-548i, miR-708, miR-181b, miR-449a, miR-146a, miR-155, miR-181a, miR-3121, miR-128, miR-1323, miR-195, and miR-587) and two miRNAs were downregulated (miR-640 and miR-145) compared to control patients. Confirmation analysis of dysregulated miRNAs by real-time RT-PCR revealed five upregulated miRNAs (miR-128, miR-146a, miR-155, miR-181a, and miR-195). After six months of treatment, the levels of miR-146a, miR-155, miR-181a, and miR-195 decreased significantly, indicating that analysis of miRNA expression during treatment can be used for disease monitoring.

miR-708-5p has been widely studied for its oncogenic role. Higher levels of miR-708-5p expression were observed in B-cell ALL compared with normal $CD34^+$ progenitor cells [15]. In two other studies, miR-708-5p was also overexpressed in childhood B-cell ALL, but this overexpression was not associated with clinical prognostic markers [14, 16].

Another study indicated that miR-708-5p expression was increased in patients with pre-B cell ALL, unchanged in patients with pro-B cell ALL, and significantly lower in patients with T-cell ALL compared to normal bone marrow [17]. A study investigating patients with ALL during treatment found that miR-708-5p was overexpressed at diagnosis, decreased in patients who presented complete response, and significantly increased in patients with relapse [18]. However, contradictory results were obtained in this study. Low miR-708-5p expression increased the risk of relapse and high expression at diagnosis was correlated with increased relapse-free survival. To better understand these divergent data, the authors examined other factors. High miR-708-5p expression was observed in patients who responded well to glucocorticoid and chemotherapy, whereas poor responders had low expression of miR-708-5p [18]. These results show the complexity of miR-708-5p in ALL and the need to determine whether miR-708-5p is a marker of response, relapse, or survival.

Some studies make use of cell lines to investigate the expression profile of miRNAs. Scheibner *et al*. [19] showed that miR-27a expression is downregulated in different acute leukemia cell lines. In 86% of precursor B-cell acute lymphocytic leukemia cell lines, miR-27a was expressed at levels $\geq$2 fold lower than in healthy human $CD34^+$ hematopoietic stem/progenitor cells. Enforced expression of miR-27a resulted in decreased cell growth and increased cell death, attributed, in part, to an increase in apoptosis.

Adopting a sequencing-by-synthesis strategy to generate small RNA libraries, Zhang *et al*. [20] identified 40 miRNAs significantly and differentially expressed in ALL patients and healthy donors (counts > 200, fold change > 2.0, $P < 0.001$). Of these, miR-9*, miR-9, miR-181a, and miR-128 exhibited a significantly high abundance, while miR-582-5p, miR-223, miR-143, and miR-126 displayed the most significant reduction in the patient group.

Many miRNA genes are reportedly downregulated in association with DNA hypermethylation in cancer. miR-196b is often overexpressed in tumors, and its overexpression is associated with hypomethylation [21]. Tsai *et al*. [21] performed methylation and expression analyses using different human cell lines and showed that the transcriptional activity of miR-196b was suppressed in different cells *via* methylation of cell/tissue-specific DNA. Popovic *et al*. [22] attributed the increased proliferation and survival capacity of bone marrow progenitor cells to miR-196b overexpression. Thus, deregulation of this microRNA may contribute to the treatment of hematopoietic malignancies [23].

Table 1 summarizes the data presented in this review, including up/downregulated miRNAs,

Table 1. Characteristics of studies showing up/down-regulated miRNAs, leukemia subtypes, samples and methods of analysis.

miRNA	Up ↑/Down↓ regulated	Leukemia subtype	Sample	Method of analysis	Ref.
miR-100-5p, miR-196b-5p, let-7e-5p	↓	ALL	BM	qRT-PCR	[10]
miR-181b-5p, miR-128-3p	↑				
miR-9-5p, miR-181a-5p, miR-128-3p,	↑	ALL	BM	Sequencing confirmed by qRT-PCR	[20]
miR-126-3p, miR-143-3p, miR-223-3p, miR-582-5p	↓				
miR-196b-5p	↑	MLL-rearranged and T-ALL	BM or PB	qRT-PCR	[11]
miR-126-3p, miR-126-5p, miR-151a-3p, miR-545-3p	↑	TEL-AML1-positive and hyperdiploid ALL			
miR-10a-5p, miR-214-3p and miR-134-5p	↓	precursor B-ALL			
miR-33a-5p	↑	T-ALL			
miR-128-2-5p, miR-146a-5p, miR-155-5p, miR-181a-5p, miR-195-5p	↑	ALL	BM	Microarray confirmed by qRT-PCR	[14]
miR-150-5p	↑	ALL compared with AML	BM or PB	Microarray confirmed by qRT-PCR	[35]
miR-199b-5p, miR-199a-3p, miR-223-3p, miR-27a-3p, miR-27b-3p, miR-23a-3p, miR-340-5p, miR-340-3p, miR-221-3p	↑	AML compared with ALL			
miR-128-2-5p	↑	ALL	PB and BM	qRT-PCR	[13]
miR-223-3p	↓				
miR-708-5p	↑	T-ALL and B-ALL compared with healthy control	BM	Microarray and qRT-PCR	[48]
miR-223, miR-27a	↓				
miR-125b-1	↑	Pre-B-ALL, T-All and Biphenotypic ALL	Serum from peripheral blood	qRT-PCR	[75]
miR- 203	↓				

Table 1 continued..

miRNA	Up ↑/Down↓ regulated	Leukemia subtype	Sample	Method of analysis	Ref.
miR-100, miR-146a	↑	Pre-B-ALL, T-All and Biphenotypic ALL healthy control	PB	qRT-PCR	[76]
miR-196a	↓				
miR-143, miR-182	↓	Pre-B-ALL and T-ALL compared with healthy control	BM	qRT-PCR	[77]
miR-361-3p, miR-196b, miR-708-5p	↑	T-ALL and B-ALL	BM and PB	qRT-PCR and sequencing technology	[78]
miR-708-5p, miR-497-5p, miR-151a-5p, miR-151b, miR-371b-5p, miR-455-5p, miR-195-5p, miR-1266-5p, miR-574-5p, miR-425-5p	↓	T- compared with B-ALL	BM or PB (used only in qRT-PCR)	sequencing by synthesis and qRT-PCR	[25]
miR-450b-5p, miR-450a-5p, miR-542-5p, miR-424-5p, miR-629-5p and miR-29c-5p	↑				
miR-128a, miR-128b, miR-151*, j-miR-5, miR-130b, miR-210	↑	ALL compared with AML	BM or PB	Bead-based miRNA expression profiling and stem-loop, TaqMan real-time PCR	[38]
let-7b, miR-223, let-7e, miR-125a, miR-130a, miR-221, miR-222, miR-23a, miR-23b, miR-24, miR-27a, miR-27b, let-7a, let-7c, miR-199b, miR-26a, miR-335, miR-21, miR-22, miR-424, miR-451	↓	AML compared with ALL			
miR-326 and miR-200c	↓	ALL (T-cell; Pre-B; early Pre-B lineage and Burkitt type) compared with non-cancer control	BM	qRT-PCR	[79]

Table 1 continued..

miRNA	Up ↑/Down↓ regulated	Leukemia subtype	Sample	Method of analysis	Ref.
miR-155a, miR-181a-5p	↑	ALL group (Pre-B-ALL; T-ALL; Biphenotypic) compared with control group	BM	qRT-PCR	[80]
miR-708-5p, miR- 210, miR-181b	↑	common precursor B-cell ALL compared with healthy control	BM and PB	Microarray and qRT-PCR	[64]
miR-345, miR-27a	↓				
miR-17, miR-18, miR19a, miR-19b, miR-20a, miR-92	↑	BCR-ABL+ ALL compared with BCR-ABL- ALL B-lineage	BM and PB	qRT-PCR	[81]
miR-149	↑	T-ALL compared with healthy control, AML, and B-ALL	BM	qRT-PCR	[82]
miR-124	↑	PPR-ALL compared with PGR-ALL	BM	qRT-PCR	[83]
miR-139	↓	T-ALL compared with health control	BM	qRT-PCR	[84]
miR-128	↑	ALL compared with healthy control	BM	qRT-PCR	[85]

BM, bone marrow; PB, peripheral blood; qRT-PCR, real-time quantitative polymerase chain reaction.

leukemia subtypes, samples and methods of analysis.

miRNA expression profile in B- and T-ALL

In B-ALL and T-ALL, lymphoid maturation arrest occurs in distinct stages, and each disease exhibits specific miRNA signatures [24, 25]. B-ALL and T-ALL can be discriminated by analysis of miRNA expression profiles. For instance, a study reported that the expression profile of 135 miRNAs varied among ALL samples. T-ALL and *E2A/PBX1*-, *BCR/ABL*-, and *MLL/AF4*-positive cases were aggregated based on miRNA expression. miR-425-5p, miR-191, and miR-128 were preferentially expressed in *E2A/PBX1*-positive cases, miR-629 was highly expressed in *MLL/AF4*-positive cases, and miR-146b and miR-126 were highly expressed in *BCR/ABL*-positive cases. Four out of the six B-ALL cases without major molecular aberrations were clustered together. A significant downmodulation of miR-151 and high expression of miR-148a and miR-424 were observed in T-ALL patients as compared with B-ALL patients [26]. Duyu *et al*. [14] observed that the most significantly distinctive miRNAs were miR-548i, miR-3140, and miR-181b in T-ALL and miR-708, miR-181b, and miR-369-3p in B-ALL.

miRNA polymorphism

Single nucleotide polymorphisms (SNPs) occurring in the miRNA gene, target genes, or genes involved with miRNA biogenesis and processing may impair miRNA regulatory activity, possibly affecting cancer risk, disease prognosis, and treatment response [27]. Several polymorphisms in genes involved in biogenesis pathways and miRNA processing were identified in hematological malignancies, such as in *DROSHA*, *XPO5*, *TNRC6A*, *CNOT1*, *DDX20*, *GEMIN4*, and *EIF2C1* [28]. Gutierrez-Camino *et al*. [29] found that 11 SNPs in miRNA genes (*mir-612*, *mir-499*, and *mir-449b*) and in miRNA biogenesis pathway genes (*TNRC6B*, *DROSHA*, *DGCR8*, *EIF2C1*, *CNOT1*, and *CNOT6*) were significantly associated with ALL susceptibility. Two SNPs (rs12803915 in *mir-499* and rs3746444 in *mir-612*) showed a more significant ($P < 0.01$) relationship with ALL.

Studies suggest an association between some SNPs in miRNA genes and B-ALL susceptibility. An Iranian study [30] genotyped SNPs located at the miRNA hairpin region of miR-146a (rs2910164 G>C) and evaluated their relationship with childhood ALL risk. The authors found a significant association, at genotypic and allelic levels, between the hsa-mirRNA146a rs2910164 G>C variant and ALL risk. The rs2910164 GC and CC genotypes were more prevalent in cases than in controls and were associated with an elevated risk of ALL. Tong *et al*. [31] analyzed blood samples from 570 children with ALL and 673 control patients to investigate the association between hsa-miR-196a2 T>C polymorphism and ALL risk in a Chinese population. The authors found that the hsa-miR-196a-2 variant TC heterozygote and CC/TC genotypes were associated with a significantly increased risk of childhood ALL compared with the TT wild-type homozygote.

A case-control study of 75 ALL patients and 115 healthy Iranian children showed the influence of rs16917496 polymorphism within the miR-502 miRNA seed region at the 3′-untranslated region (3′-UTR) of SEDT8 on childhood ALL [32]. The product of this gene is a histone H4 methyltransferase, which plays an essential role in various biological processes, including transcription regulation, mitotic regulation, DNA repair, and cell-cycle progression [33]. The results showed that CT, as well as CT+TT, decreased the risk of ALL compared with the CC genotype. Single nucleotide polymorphism in mRNAs 3′-UTR affects the binding of miRNA, changes the expression of target genes, and may increase the risk of cancer [32].

These findings show that miRNA and mRNA polymorphisms are important in cancer pathogenesis and might be correlated not only with the onset of the disease but also with progression and response to treatment.

miRNAs in acute leukemia of ambiguous lineage

Some acute leukemia patients co-express lymphoid and myeloid markers. These cases are classified as acute leukemias of ambiguous lineage (ALAL) and have worse prognosis than acute myeloid leukemia (AML) and ALL [34]. De Leeuw *et al*. [35] investigated and compared miRNA expression

among ALAL, ALL, and AML cases. Generally, ALAL cases cannot be distinguished as a separate entity, as they have miRNA expression profiles similar to those of AML, B-ALL, or T-ALL. However, the authors identified five miRNAs (miR-199b, miR-221, miR-223, miR-23a, and miR-27a) that could assign ALAL cases to either AML or ALL, a finding that might allow reclassification of ALAL as AML or ALL and thus assist in treatment decisions.

The expression of some miRNAs differs significantly between ALL and AML. Zhu *et al*. [36] studying 147 newly diagnosed acute leukemia patients found that the expression of miRNA-128 was significantly higher in ALL than AML ($P < 0.001$). Several factors may influence miR-128 expression, for example, loss of heterozygosis, amplification of the *miR-128* gene, point mutation or SNPs in *miR-128*, and DNA methylation in promoter regions [37].

By comparing samples from patients with ALL and AML, Mi *et al*. [38] demonstrated that the expression of some microRNAs differed significantly between the two hematological cancers. Whereas the expression levels of miR-128a, miR-128b, miR-151*, j-miR-5, miR-130b, and miR-210 were higher in ALL, the expression of let-7b, miR-223, let-7e, miR-125a, miR-130a, miR-221, miR-222, miR-23a, miR-23b, miR-24, miR-27a, miR-27b, let-7a, let-7c, miR-199b, miR-26a, miR-335, miR-21, miR-22, miR-424, and miR-451 was higher in AML. In this same study, the authors estimated the minimum number of miRNAs required for ALL diagnosis and discrimination between ALL and AML. They found four miRNAs with high diagnostic power. miR-128a, miR-128b, let-7b, and miR-223 showed a >5-fold difference in expression between ALL and AML, and a combination of any two of these miRNAs could distinguish ALL from AML cases with a diagnostic accuracy of 97-99%. These findings show that miR-128a, miR-128b, let-7b, and miR-223 are potential discriminatory markers [38].

Functional pathways associated with miRNAs in ALL

As data found in the literature do not allow a clear distinction among leukemia subtypes based on miRNA profiles, we decided to investigate functional and genetic pathways regulated by some of the miRNAs mentioned in this review by conducting a gene ontology (GO) enrichment analysis and analyze their role in the leukemic process. The miRNAs reported in three or more articles were included in this investigation: miR-196b-5p, miR-128-3p, miR-223-3p, miR-181a-5p, miR-27a-3p, and miR-708-5p. The genes regulated by each miRNA were obtained from StarBase v. 2.0 (assembly hg19) using medium stringency (≥ 2). StarBase compiled the results from five databases (TargetScan, PicTar, RNA22, PITA, and miRanda). Then, GO was used to assign a biological process for selected genes regulated by the six most reported miRNAs (false discovery rate < 0.05). Figure 1 represents some genes regulated by the highlighted miRNAs, as well as the major pathways involved.

Genes regulated by the six miRNAs participate in some of the same biological processes. For example, miR-128-3p and miR-27a-3p participate in the regulation of hematopoietic progenitor and stem cell differentiation; miR-196b-5p, miR-128-3p, miR-223-3p, miR-181a-5p, and miR-27a-3p participate in the regulation of cell cycle processes and mitotic cell cycle phase transition; miR-181a-5p, miR-27a-3p, and miR-128-3p are involved in hematopoietic or lymphoid organ development and in the negative regulation of cell death. miR-27a-3p, miR-128-3p, and miR-181a-5p had the highest number of regulated genes in common (n = 279, 8.37%) (Figure 2). These miRNAs also had some biological processes in common, including positive regulation of gene expression, hematopoiesis, negative regulation of cell death, DNA damage response and signal transduction by the p53 pathway.

miR-196b-5p

mir-196b is located between *HOXA9* and *HOXA10* [39], which belong to the *HOXA* group of transcription factor genes [40]. *miR-196b* is encoded by the *HOXA* cluster, and, therefore, *miR-196b* and *HOXA* are co-activated in ALL [12], resulting in a block of differentiation of bone marrow hematopoietic progenitor cells [21]. Coskun *et al*. [41, 42] observed that miR-196b induces downregulation of *ERG*, a transcription factor required for hematopoiesis. Some studies

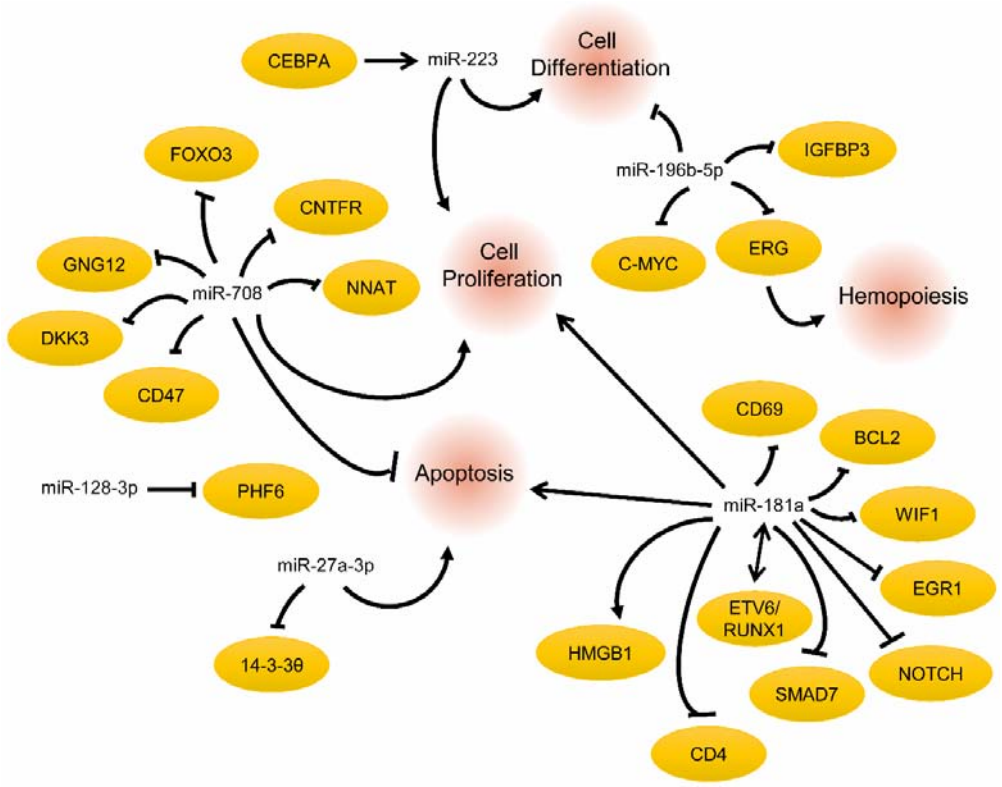

Figure 1. miRNAs and some related genes involved in acute lymphoblastic leukemia, including apoptotic and proliferative pathways. References can be found in Table 1 or in the main text.

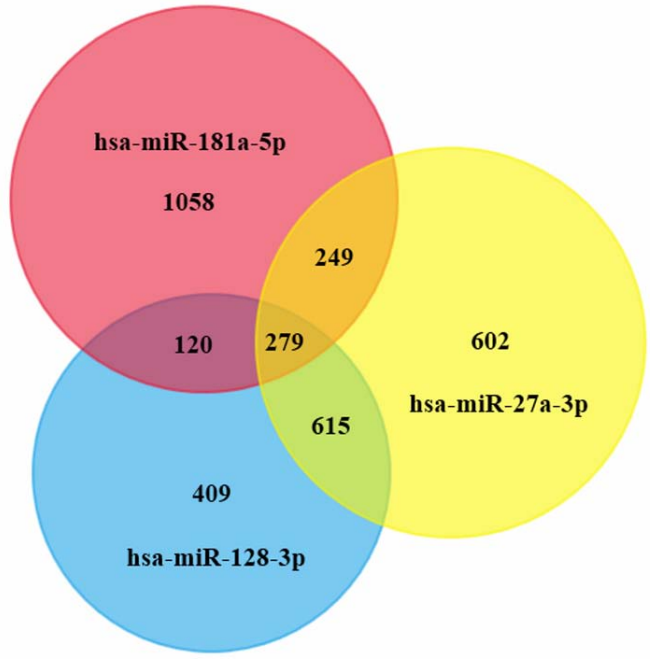

Figure 2. Venn diagram presenting absolute number of genes co-regulated by miRNA-128-3p, miRNA-181a-5p and miRNA-27a-3p.

showed that miR-196b-5p has tumor suppressor activity. Bhatia *et al*. [39] investigated the EB-3 cell line, B-ALL patient samples, and negative controls and found that miR-196b can downregulate the *C-MYC* gene, which is amplified in some types of cancer, particularly in B-ALL. Another study showed that miR-196b loses the ability to downregulate *C-MYC* in T-cell ALL cells with mutations in the 3'-UTR of the *C-MYC* gene [43]. Zhou *et al*. [44] reported that miR-196b downregulates *IGFBP3* expression in ALL cells.

miR-128-3p

Mets *et al*. [45] investigated new microRNAs with an oncogenic role in T-ALL and found that miR-128-3p suppresses the *PHF6* tumor suppressor gene.

miR-223-3p

miR-223 is an important regulator of myeloid differentiation and proliferation and is upregulated by the transcription factor CEBPA, which binds to the miR-223 promoter during granulocytic differentiation [46, 47].

Han *et al*. [48] performed a genome-wide miRNA microarray analysis and validation by qRT-PCR to evaluate miRNAs differentially expressed in childhood ALL. The qRT-PCR results showed that miR-708 and miR-223 expression differed significantly between relapse and complete remission patients.

miR-181a-5p

miR-181a has been reported to have a role in the development and differentiation of B cells and cytotoxic T cells [49]. Expression of genes that participate in thymocyte maturation, such as *BCL2*, *CD69*, and T-cell receptor genes, were repressed by miR-181a [14]. Furthermore, it was found that members of the miR-181 family are significantly overexpressed in T-cell leukemia [14]. A prediction analysis showed that miR-181a-5p has many target genes, including *BCL2*, *KRAS*, *GATA6*, and *CDX2*, suggesting that this miRNA is involved in proliferation and apoptosis pathways [50]. An *in vitro* study using the luciferase assay, REH cells, and Western blotting demonstrated that miR-181a alters the expression of the fusion gene *ETV6/RUNX1* [51], an indication that *miR-181a1* may be associated with ALL.

Nabhan *et al.* [52] observed that miR-181a expression was lower in serum samples of pediatric ALL patients than in samples of healthy subjects, leading to higher expression of the target gene *SMAD7*, which regulates and mediates the interaction between *TGFB1* and other signaling pathways. The authors concluded that SMAD7 overexpression may have therapeutic implications and that miR-181a may act as a tumor suppressor in ALL.

It is known that dysregulation of Wnt signaling is related to leukemogenesis. Lyu *et al*. [53] showed that miR-181a-5p was overexpressed in leukemia cell lines and ALL samples compared to control peripheral blood mononuclear cells. In a mechanistic study, the authors showed that miR-181a-5p directly targeted *WIF1*, downregulated its expression, and activated Wnt/β-catenin signaling, suggesting that the activation of signaling pathways involving miR-181a-5p and Wnt may be implicated in the pathogenesis of ALL. Using Jurkat cells, Verduci *et al*. [54] demonstrated that the expression of *EGR1* can be reduced by increasing miR-181a expression, leading to increased cell proliferation and progression from G1 to S phase. These data suggest an onco-miRNA function for miR-181a and show that *EGR1* is a direct target of this miRNA.

HMGB1 and *CD4*, two genes involved in immunoreactivity, are potential targets of miR-181a. To confirm the effects of miR-181a on HMGB1 and CD4 expression, Dahlhaus *et al*. [55] performed an inhibition assay using the ALL cell lines REH and MOLT-4, which naturally have high miR-181a levels. Inhibition of miR-181a induced a strong reduction in HMGB1 levels in both cell lines and a significant increase in the relative mean fluorescence intensity of CD4 in MOLT-4 cells. Inhibition of miR-181a expression in the AML cell line HL60 led to reduced cell growth and metabolic activity, effects that were not observed in ALL cells.

In another study, Fragoso *et al*. [49] reported that *NOTCH* oncogene activity can be selectively inhibited by targeting the molecular components controlled by mir-181a-1/b-1.

Zeng *et al*. [56] performed bioinformatics analyses using microarray from B-ALL data downloaded from the Gene Expression Omnibus database to

screen differentially expressed miRNA and predict target genes, long non-coding RNAs, and transcription factors. The author found that hsa-miR-181a-2 and hsa-miR-181b-2 regulate a large number of transcription factors, including CDX2 and YY1.

miR-27a-3p

According to TargetScan prediction analyses (www.targetscan.org), miR-27a-3p targets numerous genes related to hematological malignancies, for instance, B-cell translocation gene 1 anti-proliferative (*BTG1*), early B-cell Factor 3 (*EBF3*), cell death-inducing p53 target 1 (*CDIP1*), and B-cell CLL/lymphoma 7A (*BCL7A*). BTG1 acts as a transcriptional cofactor by recruiting effector molecules to specific transcription factors, affecting cell proliferation and differentiation. Deletions in *BTG1* are present in 20% of all *ETV6/RUNX1*-positive B-cell precursor ALL cases [57]. *BTG1* expression is highly regulated during cell growth and proliferation; it is strongly expressed in the G0/G1 phases and downregulated in the G1 phase. A negative correlation between BTG1 mRNA expression and cell proliferation was observed in T lymphocytes and macrophages [58]. *EBF3* gene encodes a member of the early B-cell factor (EBF) family of DNA binding transcription factors that is involved in B-cell differentiation, bone development, and tumor suppression [59]. CDIP1 acts as an important apoptotic effector, regulating TNF-alpha-mediated apoptosis in a p53-dependent manner. CDIP can also induce apoptosis through the cleavage of caspase-8, leading to the extrinsic pathway of cell death [60].

miR-27a-induced apoptosis occurs through downregulation of the 14-3-3θ gene. Thus, miR-27a plays a tumor suppressor-like role in acute leukemia by regulating apoptosis, and miR-27a and 14-3-3θ are potential targets for the development of leukemia therapeutics [19].

Han *et al*. [48] showed that miR-27a was differentially expressed in patients in relapse or at diagnosis compared with patients in complete remission ($P < 0.01$). In the analysis of predicted functions of target genes based on gene ontology, the authors found that genes associated with stem-cell development and differentiation might be correlated with miR-27a.

miR-708-5p

miR-708-5p has been shown to have important roles either in the promotion or suppression of oncogenesis in solid and hematological tumors. Located on chromosome 11 (11q14.1) as a mirtron, *MIR708* is found in intron 1 of the *ODZ4* gene, which encodes a transmembrane protein called teneurin 4 (Tenm4). Teneurins are a family of highly conserved type II transmembrane proteins, which can be released from the plasma membrane and act as transcriptional regulators [61]. Two transcriptional activators with binding sites within the *ODZ4* promoter regulate miR-708-5p expression, E2F transcription factor 1 (E2F1) and c-Myc. E2F1 influences cell-cycle progression or induction of apoptosis in response to DNA damage [62] and c-Myc affects the transcription of genes participating in cell growth, metabolism, and apoptosis [63].

Li *et al*. [64] showed that miR-708 was upregulated in the bone marrow and peripheral blood from common precursor B-cell ALL patients. Using REK-293 cells, the authors demonstrated that miR-708 binds to the 3′-UTR of CNTFR, NNAT, and GNG12 mRNAs. In Jurkat cells, miR-708 upregulation was associated with reduced CNTFR, NNAT, and GNG12 levels.

Han *et al*. [18] found that miR-708 was upregulated in samples from relapsed patients compared to complete remission patients. In Jurkat cells transfected with miRNA mimics, miR-708 significantly decreased the expression levels of *FOXO3*, an important transcription factor for stem cell self-renewal and apoptosis. Response to glucocorticoid therapy, as well as disease risk, was also related to miR-708 expression.

Huang *et al*. [65] showed that miR-708 downregulates CD47, a transmembrane inhibitor of phagocytosis in acute T-cell lymphoblastic leukemia. MiR-708 targets CD47 binding to 3'-UTR and is inversely correlated with CD47 expression. These findings suggest that miR-708 may be an attractive candidate for T-ALL immunotherapy. Zhang *et al*. [66] showed that miR-708 promotes cell proliferation through cell cycle promotion and apoptosis inhibition by downregulating *DKK3*, a tumor suppressor gene. A summary of the main functions associated with these six miRNAs is presented in Table 2.

Table 2. miRNAs that play an important role in childhood ALL and their mainly functions.

miRNA	**Related functions**
miR-27a-3p	B-cell differentiation and a tumor suppressor-like role by regulating apoptosis
miR-128-3p	Regulatory function on cell proliferation, hematopoietic or lymphoid organ development and regulation of hematopoietic stem cell differentiation
miR-181a-5p	Tumor suppressor and up-regulating B-cell apoptosis
miR-196b-5p	Mitotic cell cycle phase transition and regulation of cell proliferation
miR-223-3p	Regulation of Pro-B differentiation, sister chromatid segregation and T-cell proliferation
miR-708-5p	Promoting cell proliferation and apoptosis inhibition

Challenges of using miRNAs as biomarkers

miRNA profiling is a potential complementary tool for clinical routine and has diagnostic and prognostic value in various types of cancer. Because circulating miRNAs show relative stability in biological fluids, tumor-derived miRNAs can be detected through less invasive methods, such as serum or blood plasma sampling [67-69].

However, the use of miRNAs as biomarkers for cancer diagnosis in clinical practice is still a challenge because of the high implementation costs and difficulties in the standardization of methods used to validate miRNA expression profiles for each disease. The potential of miRNAs for clinical diagnosis needs to be further investigated. To increase data reliability and ensure the correct identification of miRNA signatures, some precautions must be taken. Studies and approaches using miRNAs as diagnostic biomarkers need to consider patient age, sex, and treatment history. Methods should be standardized considering sample type, storage, and preparation. Furthermore, data normalization should be carried out before statistical analysis [67, 70]. In terms of new detection methods, a highly innovative device is currently being developed to detect miRNAs without sample laborious handling. This approach allows rapid detection of circulating microRNA with advantage in terms of result consistency, time and cost [71].

Although the limitations of miRNAs as biomarkers are very meaningful currently, miRNAs could be important secondary biomarkers for the diagnosis and prognosis of some medical conditions. For instance, our research group recently reported that high miR-21 levels were associated with reduced transplant-free survival in outpatients with stable cirrhosis [72] or ALAL [35]. The expression profiles of five miRNAs (miR-199b, miR-221, miR-223, miR-23a, and miR-27a) allowed reclassification of ALAL as AML or ALL, contributing to appropriate treatment choices [35]. Luna-Aguirre *et al*. [73] investigated the diagnostic ability of miR-511 by receiver operating characteristic curve analysis (ROC) and observed that this miRNA was the most valuable biomarker for differentiating B-ALL from normal controls (AUC = 1, sensitivity and specificity of 100%). In the serum of lung cancer patients, the levels of miR-10b, miR-141, and miR-155 were significantly higher compared with a control [74]. These results suggest that miRNAs will be important predictive biomarkers in the near future.

CONCLUSION

The findings discussed here highlight the importance of miRNA expression profiling for childhood ALL diagnosis and prognosis. miR-27a-3p, miR-128-3p, miR-181a-5p, miR-196b-5p, miR-223-3p, and miR-708-5p were reported by three or more articles to play an important role in childhood ALL. Bioinformatics analysis revealed that miR-27a-3p is involved in B-cell differentiation and plays a tumor suppressor-like role by regulating apoptosis. miR-196b-5p is involved in mitotic cell cycle phase transition and regulation of cell proliferation, and miR-708 promotes cell proliferation and apoptosis inhibition. miR-128-3p also modulates cell proliferation and is involved in hematopoietic or lymphoid organ development and regulation of hematopoietic stem cell

differentiation. miR-223-3p regulates pro-B cell differentiation, sister chromatid segregation, and T-cell proliferation. miR-223-3p and miR-181a-5p participate in cellular responses to leukemia inhibitory factor, a cytokine that inhibits the growth of leukemic cells. miR-181a-5p also downregulates T-cell apoptosis while upregulating B-cell apoptosis. The selected miRNAs might be important candidates for ALL markers because they are involved in the regulation of genes and biological functions essential to the development of the disease. However, the role of miRNAs in the pathophysiology of cancer has not been completely elucidated, and the search for new markers is of utmost importance to improve current diagnostic approaches, therapies, and the life quality of childhood ALL patients.

ACKNOWLEDGMENTS

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC).

CONFLICT OF INTEREST STATEMENT

Nothing to declare.

ABBREVIATIONS

ALAL	:	acute leukemias of ambiguous lineage
B-ALL	:	B-cell acute lymphocytic leukemia
miRNA	:	microRNA
mRNA	:	messenger RNA
pre-B-ALL	:	precursor B-cell acute lymphocytic leukemia
SNP	:	single nucleotide polymorphism
UTR	:	untranslated region
WHO	:	World Health Organization

REFERENCES

1. Siegel, R. L., Miller, K. D. and Jemal, A. 2017, CA Cancer J. Clin., 67(1), 7-30.
2. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A. and Jemal, A. 2018, CA Cancer J. Clin., 68(6), 394-424.
3. GLOBOCAN. Estimated age-standardized incidence rates (World) in 2018, Worldwide, both sexes, ages 0-14.
4. Farias, M. G. and Castro, S. M. 2004, J. Bras. Patol. e Med. Lab., 40(2), 91-98.
5. Hunger, S. P. and Mullighan, C. G. 2015, N. Engl. J. Med., 373(16), 1541-1552.
6. Kebriaei, P., Anastasi, J. and Larson, R. A. 2003, Best. Pr. Res. Clin. Haematol., 15(4), 597-621.
7. Onciu, M. 2009, Hematol. Oncol. Clin. North Am., 23(4), 655-674.
8. Cazzola, M. 2016, Blood, 127(20), 2361-2364.
9. Luan, C., Yang, Z. and Chen, B. 2015, Onco. Targets Ther., 8, 2903-2914.
10. De Oliveira, J. C., Scrideli, C. A., Brassesco, M. S. Morales, A. G., Pezuk, J. A., Queiroz, R. P., Yunes, J. A. Brandalise, S. R. and Tone, L. G. 2012, Leuk. Res., 36(3), 293-298.
11. Schotte, D., De Menezes, R. X., Akbari Moqadam, F., Khankahdani, L. M., Lange-Turenhout, E., Chen, C., Pieters, R. and Den Boer, M L. 2011, Haematologica., 96(5), 703-711.
12. Schotte, D., Lange-Turenhout, E. A. M., Stumpel, D. J. P. M., Stam, R. W., Buijs-Gladdines, J. G. C. A. M., Meijerink, J. P. P., Pieters, R. d. B. and Monique L. 2010, Haematologica., 95(10), 1675-1682.
13. Nemes, K., Csóka, M., Nagy, N., Márk, Á., Váradi, Z., Dankó, T., Kovács, G., Kopper, L. and Sebestyén, A. 2015, Pathol. Oncol. Res., 21(3), 597-604.
14. Duyu, M., Durmaz, B., Gunduz, C., Vergin, C., Yilmaz Karapinar, D., Aksoylar, S., Kavakli, K., Cetingul, N., Irken, G., Yaman, Y., Ozkinay, F. and Cogulu, O. 2014, Biomed. Res. Int., 2014, 1-7.
15. Schotte, D., Chau, J., C. K., Sylvester, G., Liu, G., Chen, C., van der Velden, V. H. J., Broekhuis, M. J. C., Peters, T. C. J. M., Pieters, R. and den Boer, M. L. 2009, Leuk. Off. J. Leuk. Soc. Am. Leuk. Res. Fund, UK., 23(2), 313-322.
16. Candia, J., Banavar, J. R., Civin, C. I., Losert, W., Cherukuri, S., Doshi, K. A. and Guo, Y. 2015, Converg. Sci. Phys. Oncol., 1, 025002.

17. de Oliveira, J. C., Scrideli, C. A., Brassesco, M. S., Yunes, J. A., Brandalise, S. R. and Tone, L. G. 2015, Pediatr. Blood Cancer, 62(1), 177-178.
18. Han, B. W., Feng D. D., Li, Z. G., Luo, X. Q., Zhang, H., Li, X. J., Zhang, X. J., Zheng, L. L., Zeng, C. W., Lin, K. Y., Zhang, P. Y., Xu, L. C. and Yue, Q. 2011, Hum. Mol. Genet., 20(24), 4903-4915.
19. Scheibner, K. A., Teaboldt, B., Hauer, M. C., Chen, X., Cherukuri, S., Guo, Y., Kelley, S. M., Liu, Z., Baer, M. R., Heimfeld, S. and Civin, C. I. 2012, PLoS One, 7(12), e50895.
20. Zhang, H., Yang, J. H., Zheng, Y. S., Zhang, P., Chen, X., Wu, J., Xu, L., Luo, X.Q., Ke, Z. Y., Zhou, H., Qu, L. H. and Chen, Y. Q. 2009, PLoS One, 4(9), e6849.
21. Tsai, K. W., Hu, L. Y., Wu, C. W., Li, S. C., Lai, C. H., Kao, H. W., Fang, W. L. and Lin, W. C. 2010, Genes Chromosom Cancer, 49(11), 969-980.
22. Popovic, R., Riesbeck, L. E., Velu, C. S., Chaubey, A., Zhang, J., Achille, N. J., Erfurth, F. E., Eaton, K., Lu, J. L., Grimes, H., Chen, J., Rowley, J. D. and Zeleznik-Le, N. J. 2009, Blood, 113(14), 3314-3322.
23. Rashed, W. M., Saad, A. M., Hammad, A. M. and Shohdy, K. S. 2019, Crit. Rev. Oncol/Hematol., 136, 70-78.
24. Mullighan, C. G. 2013, Semin. Hematol., 50(4), 314-324.
25. Almeida, R. S., Costa e Silva, M., Coutinho, L. L., Garcia Gomes, R., Pedrosa, F., Massaro, J. D., Donadi, E. A. and Lucena-Silva, N. 2018, Hematol. Oncol., 37(1), 103-112.
26. Fulci, V., Colombo, T., Chiaretti, S., Messina, M., Citarella, F., Tavolaro, S., Guarini, A., Foà, R. and Macino, G. 2009, Genes Chromosomes Cancer, 48(12), 1069-1082.
27. Dzikiewicz-Krawczyk, A. 2015, Crit. Rev. Oncol. Hematol., 93(1), 1-17.
28. López-López, E., Gutiérrez-Camino, Á., Piñán, M. Á., Sánchez-Toledo, J., Uriz, J. J., Ballesteros, J., García-Miguel, P., Navajas, A. and García-Orad, Á. 2014, PLoS One, 9(3), e91261.
29. Gutierrez-Camino, A., Lopez-Lopez, E., Martin-Guerrero, I., Piñan, M. A., Garcia-Miguel, P., Sanchez-Toledo, J. C., Bañeres, A., Uriz, J., Navajas, A. and Garcia-Orad, A. 2014, Pediatr Res., 75(6), 767-773.
30. Hasani, S. S., Hashemi, M., Eskandari-Nasab, E., Naderi, M., Omrani, M. and Sheybani-Nasab, M. 2014, Tumor Biol., 35(1), 219-225.
31. Tong, N., Xu, B., Shi, D., Du, M., Li, X., Sheng, X., Wang, M., Chu, H., Fang, Y., Li, J., Wu, D. and Zhang, Z., 2014, Mutat. Res. – Fundam. Mol. Mech. Mutagen., 759, 16-21.
32. Hashemi, M., Sheybani-Nasab, M., Naderi, M., Roodbari, F. and Taheri, M. 2014, Tumor Biol., 35(10), 10375-10379.
33. Qin, Y., Ouyang, H., Liu, J. and Xie, Y. 2013, Acta Biochim. Biophys. Sin. (Shanghai), 45(4), 303-308.
34. Weir, E. G., Ali Ansari-Lari, M., Batista, D. A. S., Griffin, C. A., Fuller, S., Smith, B. D. and Borowitz, M. J. 2007, Leuk. Off. J. Leuk. Soc. Am. Leuk. Res. Fund., UK., 21(11), 2264-2270.
35. De Leeuw, D. C., van Den Ancker, W., Denkers, F., De Menezes, R. X., Westers, T. M., Ossenkoppele, G. J., van De Loosdrecht, A. A. and Smit, L. 2013, Clin. Cancer Res., 19(8), 2187-2196.
36. Zhu, Y. D., Wang, L., Sun, C., Fan, L., Zhu, D. X., Fang, C., Wang, Y. H., Zou, Z. J., Zhang, S. J., Li, J. Y. and Xu, W. 2012, Med. Oncol., 29, 2323-2331.
37. Li, M., Fu, W., Wo, L., Shu, X., Liu, F. and Li, C. 2013, Exp. Cell Res., 319(20), 3059-3064.
38. Mi, S., Lu, J., Sun, M., Li, Z., Zhang, H., Neilly, M. B., Wang, Y., Qian, Z., Jin, J., Zhang, Y., Bohlander, S. K., Le Beau, M. M., Larson, R. A., Golub, T. R., Rowley, J. D. and Chen, J. 2007, Proc. Natl. Acad. Sci. USA, 104(50), 19971-19976.
39. Bhatia, S., Kaul, D. and Varma, N. 2010, Mol. Cell Biochem., 340(1-2), 97-106.
40. Yekta, S., Shih, I. H. and Bartel, D. P. 2004, Science, 304(5670), 594-596.
41. Coskun, E., von der Heide, E. K., Schlee, C., Kühnl, A., Gökbuget, N., Hoelzer, D., Hofmann, W. K., Thiel, E. and Baldus, C. D. 2011, Leuk. Res., 35(2), 208-213.

42. Loughran, S. J., Kruse, E. A., Hacking, D. F., de Graaf, C. A., Hyland, C. D., Willson, T. A., Henley, K. J., Ellis, S., Voss, A. K., Metcalf, D., Hilton, D. J., Alexander, W. S. and Kile, B. T. 2008, Nat Immunol., 9(7), 810-819.
43. Bhatia, S., Kaul, D. and Varma, N. 2011, Mol. Cell. Biochem., 346(1-2), 103-116.
44. Zhou, W., Wang, S., Ying, Y., Zhou, R. and Mao, P. 2017, Oncol. Rep., 37(2), 1075-1083.
45. Mets, E., van Peer, G., van der Meulen, J., Boice, M., Taghon, T., Goossens, S., Mestdagh, P., Benoit, Y., De Moerloose, B., van Roy, N., Poppe, B., Vandesompele, J., Wendel, H. G., van Vlierberghe, P., Speleman, F. and Rondou, P. 2014, Haematologica., 99(8), 1326-1333.
46. Leroy, H., Roumier, C., Huyghe, P., Biggio, V., Fenaux, P. and Preudhomme, C. 2005, Leukemia., 19, 329-334.
47. Fazi, F., Rosa, A., Fatica, A., Gelmetti, V., De Marchis, M. L., Nervi, C. and Bozzoni, I. 2005, Cell., 123(5), 819-831.
48. Han, B. W., Feng, D. D., Li, Z. G., Luo, X. Q., Zhang, H., Li, X. J., Zhang, X. J., Zheng, L. L., Zeng, C. W., Lin, K. Y., Zhang, P. Y., Xu, L. and Chen, Y. Q. 2011, Hum. Mol. Genet., 20(24), 4903-4915.
49. Fragoso, R., Mao, T., Wang, S., Schaffert, S.,Gong, X., Yue, S., Luong, R., Min, H., Yashiro-Ohtani, Y., Davis, M., Pear, W. and Chen, C. Z. 2012, PLoS Genet., 8(8), e1002855.
50. Chen, G., Shen, Z. L., Wang, L., Lv, C. Y., Huang, X. E. and Zhou, R. P. 2013, Asian Pacific J. Cancer Prev., 14, 3871-3875.
51. Yang, Y. L., Yen, C. T., Pai, C. H., Chen, H. Y., Yu, S. L., Lin, C. Y., Hu, C. Y., Jou, S. T., Lin, D. T., Lin, S. R. and Lin, S. W. 2015, PLoS One, 11, e0142863.
52. Nabhan, M., Louka, M. L., Khairy, E., Tash, F., Ali-Labib, R. and El-Habashy, S. 2017, Gene, 628, 253-258.
53. Lyu, X., Li, J., Yun, X., Huang, R., Deng, X., Wang, Y., Chen, Y. and Xiao, G. 2017, Oncol Rep., 3, 1469-1476.
54. Verduci, L., Azzalin, G., Gioiosa, S., Carissimi, C., Laudadio, I., Fulci, V. and Macino, G. 2015, Leuk. Res., 39(4), 479-485.
55. Dahlhaus, M., Schult, C., Lange, S., Freund, M. and Junghanss, C. 2013, Anticancer Res., 33(2), 445-452.
56. Zeng, M. N., Ma, W. L. and Zheng, W. L. 2016, Zhonghua Xue Ye Xue Za Zhi, 37(7), 585-590.
57. Tijchon, E., van Emst, L., Yuniati, L., van Ingen Schenau, D., Gerritsen, M., van der Meer, L. T., Williams, O., Hoogerbrugge, P. M., Scheijen, B. and van Leeuwen, F. N. 2018, Exp Hematol., 60, 57-62.e3.
58. Iwai, K., Hirata, K. I., Ishida, T., Takeuchi, S., Hirase, T., Rikitake, Y., Kojima, Y., Inoue, N., Kawashima, S. and Yokoyama, M. 2004, Biochem. Biophys. Res. Commun., 316(3), 628-635.
59. Tao, Y. F., Xu, L. X., Lu, J., Hu, S. Y., Fang, F., Cao, L., Xiao, P. F., Du, X. J., Sun, L. C., Li, Z. H., Wang, N. N., Su, G. H., Li, Y. H., Li, G., Zhao, H., Li, Y. P., Xu, Y. Y., Zhou, H. T., Wu, Y., Jin, M. F., Liu, L., Zhu, X. M., Ni, J., Wang, J., Xing, F., Zhao, W. L. and Pan, J. 2015, J. Exp. Clin. Cancer Res., 34(4), 1-21.
60. Brown, L., Ongusaha, P. P., Kim, H. G., Nuti, S., Mandinova, A., Lee, J. W., Khosravi-Far, R., Aaronson, S. A. and Lee, S. W. 2007, EMBO J., 26(14), 3410-3422.
61. Tucker, R. P. and Chiquet-Ehrismann, R. 2006, Dev. Biol., 290(2), 237-245.
62. Engelmann, D. and Pützer, B. M. 2012, Cancer Res., 72(3), 571-575.
63. Dang, C. V. 2015, Mol. Cell Biol., 19(1), 1-11.
64. Li, X., Li, D., Zhuang, Y., Shi, Q., Wei, W. and Ju, X. 2013, Pediatr Blood Cancer, 60(12), 2060-2067.
65. Zhang, Y., Li, H., Cao, R., Sun, L., Wang, Y., Fan, S., Zhao, Y., Kong, D., Cui, L., Lin, L., Wang, K., Li, Y. and Zhou, J. 2017, Oncotarget., 8(38), 64114-64128.
66. Huang, W., Wang, W. T., Fang, K., Chen, Z. H., Sun, Y. M., Han, C., Sun, L. Y., Luo, X. Q. and Chen, Y. Q. 2018, Mol. Cancer, 17(12), 1-6.
67. Glinge, C., Clauss, S., Boddum, K., Jabbari, R., Jabbari, J., Risgaard, B., Tomsits, P., Hildebrand, B., Kaèaèb, S., Wakili, R., Jespersen, T. and Tfelt-Hansen, J. 2017, PLoS One, 12(2), e0167969.

68. Grasedieck, S., Sorrentino, A., Langer, C., Buske, C., Döhner, H., Mertens, D. and Kuchenbauer, F. 2013, Blood, 121(25), 4977-84.
69. Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., Peterson, A., Noteboom, J., O'Briant, K. C., Allen, A., Lin, D. W., Urban, N., Drescher, C. W., Knudsen, B. S., Stirewalt, D. L., Gentleman, R., Vessella, R. L., Nelson, P. S., Martin, D. B. and Tewari, M. 2008, Proc. Natl. Acad. Sci. USA, 105(30), 10513-10518.
70. Wang, J., Chen, J. and Sen, S. 2016, J. Cell Physiol., 231(1), 25-30.
71. Pernagallo, S., Ventimiglia, G., Cavalluzzo, C., Alessi, E., Ilyine, H., Bradley, M. and Diaz-Mochon, J. J. 2012, Sensors (Switzerland)., 12(6), 8100-8111.
72. do Amaral, A. E., Cisilotto, J., Creczynski-Pasa, T. B. and de Lucca, S. L. 2018, Pharmacol Res., 128, 274-287.
73. Luna-Aguirre, C. M., Martinez-Fierro, M. D. L. L., Mar-Aguilar, F., Garza-Veloz, I., Treviño-Alvaradod, V., Rojas-Martinez, A., Jaime-Perez, J. C., Malagon-Santiago, G. I., Gutierrez-Aguirre, C. H., Gonzalez-Llano, O., Salazar-Riojas, R., Hidalgo-Miranda, A., Martinez-Rodriguez, H. G., Gomez-Almaguer, D. and Ortiz-Lopez, R. 2015, Cancer Biomarkers, 15(3), 299-310.
74. Roth, C., Kasimir-Bauer, S., Pantel, K. and Schwarzenbach, H. 2011, Mol. Oncol., 5(3), 281-291.
75. Swellam, M., Hashim, M., Mahmoud, M. S., Ramadan, A. and Hassan, N. M. 2018, Biochem. Genet., 56(4), 283-294.
76. Swellam, M. and El-Khazragy, N. 2016, Tumor Biol., 37(8), 10571-10576.
77. Piatopoulou, D., Avgeris, M., Drakaki, I., Marmarinos, A., Xagorari, M., Baka, M., Pourtsidis, A., Kossiva, L., Gourgiotis, D. and Scorilas, A. 2018, Ann. Hematol., 97(7), 1169-1182.
78. Schotte, D., Akbari Moqadam, F., Lange-Turenhout, E. A. M., Chen, C. van Ijcken, W. F. J., Pieters, R. and den Boer, M. L. 2011, Leukemia, 25(9), 1389-1399.
79. Ghodousi, E. S. and Rahgozar, S. 2018, J. Cell Biochem., 119(7), 6024-6032.
80. El-Khazragy, N., Noshi, M. A., Abdel-Malak, C., Zahran, R. F. and Swellam, M. 2018, J. Cell Biochem., 120(4), 6315-6321.
81. Scherr, M., Elder, A., Battmer, K., Barzan, D., Bomken, S., Ricke-Hoch, M., Schröder, A., Venturini, L., Blair, H. J., Vormoor, J., Ottmann, O., Ganser, A., Pich, A., Hilfiker-Kleiner, D., Heidenreich, O. and Eder, M. 2014, Leukemia, 28(3), 554-565.
82. Fan, S. J., Li, H. B., Cui, G., Kong, X. L., Sun, L. L., Zhao, Y. Q., Li, Y. H. and Zhou, J. 2016, Leuk. Res., 41, 62-70.
83. Liang, Y. N., Tang, Y. L., Ke, Z. Y., Chen, Y. Q., Luo, X. Q., Zhang, H. and Huang, L. B. 2017, J. Steroid Biochem. Mol. Biol., 172, 62-68.
84. Qin, L., Deng, H. Y., Chen, S. J., Wei, W. and Zhang, Y. T. 2017, Am. J. Transl. Res., 9(9), 4059-4070.
85. Shafik, R. E., Abd El Wahab, N., Senoun, S. A., Ebeid, E. and El Taweel, M. A. 2018, Asian Pac, J. Cancer Prev., 19(8), 2263-2267.