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MicroRNAs as a New Molecular Biomarker for Diagnosis and Prognosis of T-cell Acute Lymphoblastic Leukemia (T-ALL): A Systematic Review

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Abstract
MicroRNAs (miRNAs, miRs) are small endogenous non-coding RNAs that regulate the expression of protein-encoding genes at the post-transcriptional level. Several studies have described the role of miRNAs in T-cell acute lymphoblastic leukemia (T-ALL), including tumor suppressor and oncogenic miRNAs. Down-regulation of miRNA expression is a prominent feature of human malignancy. This down-regulation can be triggered by certain chromosomal rearrangements, such as somatic deletions or translocations. New functional studies showed that dysregulation of microRNAs plays significant roles in cellular proliferation, differentiation, apoptosis, in human cancers such as leukemia. In this review, we focused on the major recent findings in the microRNA signatures in ALL pathogenesis and we discussed the potential use of cellular and circulating miRNAs as new molecular biomarkers for diagnosis and prognosis of acute lymphoblastic leukemia. In this systematic review article, 76 articles were collected from 2004 to February 2019. We chose research and review articles from open access journals which include MeSH terms such as ALL, T-ALL, microRNA, oncogenic miRNA, Tumor Suppressor miRNA, microRNA Expression and signaling pathway. Investigation of data showed that alterations in oncogenic and tumor suppressor miRNAs expression changed the expression of genes related to accurate cell functions and consequently the pathogenicity of T-ALL.
Keywords: T-cell Acute Lymphoblastic Leukemia, microRNAs, Oncogenic miRNAs, Tumor Suppressor miRNAs, Biomarkers

Introduction

A malignant neoplasm found in both adults and children is acute lymphoblastic leukemia (ALL) (1). While ALL is less common in adults, it represents a devastating disease in adults (2). The first symptoms of ALL occur in children with a peak prevalence at 1–4 years of age (3) and a second peak occurs around the age of 50. The World Health Organization classified ALL in two types, namely B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia (T-ALL). Fifteen percent of pediatric and 25% of adult ALL cases are T-ALL with a clinically aggressive hematologic malignancy (4). Oncogenic transformation in immature thymocytes caused by cooperative genetic changes was seen in T-ALL development (5). These genetic changes include constitutive activating mutations in the *NOTCH1* gene, (6) inactivating mutations in the *FBXW7* gene (the most prominent oncogenic pathway in T-ALL pathogenesis) (5), gene amplification (in *NUP214-ABL1*, *MYB* genes), inactivation of tumor suppressor genes (*PTEN*, *NF1*, and *PHF6*), and inappropriate expression of transcription factors (*TAL1*, *LMO1*, *LMO2*, *LYL1*, *TLX1*, and *TLX3*) (6). These genetic variations affect different biological processes, e.g. proliferation, survival, and the differentiation of precursor T cells (Table I) (7).

Table I: Comparison of mutation frequencies between pediatric and adult T-ALL

Gene	Type of genetic disorders	Frequency (%)	
		Pediatric	Adult
NOTCH1 signaling pathway and Cell cycle genes			
FBXW7	Loss of function mutations (5)	14	14
NOTCH1	Chromosomal rearrangements/Gain of function mutations (6)	50	57
CDKN2A	9p21 deletion (7)	61	55
CDKN2B	9p21 deletion (7)	58	46
RB1	Gene deletions (2)	12	12
Transcription factor genes			
BCL11B	Loss of function mutations/deletions (7)	10	9
ETV6	Loss of function mutations/deletions (7)	8	14
GATA3	Loss of function mutations/deletions (10)	5	3
HOXA	Chromosomal rearrangements/inversions/Over-expression (11)	5	8
LEF1	Loss of function mutations/deletions (7)	10	2
LMO2	Chromosomal rearrangements/deletions (6)	13	21
MYB	Chromosomal rearrangements/duplications (5)	7	17
NKX2.1	Chromosomal rearrangements (7)	8	8
RUNX1	Loss of function mutations/deletions (12)	8	10
TAL1	Chromosomal rearrangements/enhancer suppressor mutations/deletions (6)	30	34
TLX1	Chromosomal rearrangements/deletions (6)	8	20
TLX3	Chromosomal rearrangements (6)	19	9
WT1	Loss of function mutation/deletions (7)	19	11
Signaling pathway genes			
AKT	Gain of function mutations (4)	2	2
DNM2	Loss of function mutations (7)	13	13
FLT3	Gain of function mutations (7)	6	4
JAK1	Gain of function mutations (7)	5	7
JAK3	Gain of function mutations (7)	8	12
IL7R	Gain of function mutations (7)	10	12
NF1	Deletions (6)	4	4
KRAS	Gain of function mutations (7)	6	0
NRAS	Gain of function mutations (7)	14	9
NUP214-ABL1/ ABL1	Chromosomal rearrangement/duplications (6)	8	8
PI3KCA	Gain of function mutations (7)	1	5
PTEN	Loss of function mutations/deletion (11)	19	11
PTPN2	Loss of function mutations/deletion (7)	3	7
STAT5B	Gain of function mutations (7)	6	6
Epigenetic factor genes			
DNMT3A	Loss of function mutations (13)	1	14
EED	Loss of function mutations/deletions (7)	5	5
EZH2	Loss of function mutations/deletions (7)	12	12
KDM6A/UTX	Loss of function mutations/deletions (7)	6	7
PHF6	Loss of function mutations/deletions (14)	19	30
SUZ12	Loss of function mutations/deletions (7)	11	5
Translation and RNA stability			
CNOT3	Missense mutations (7)	3	8
mTOR	Gain of function mutations (15)	5	5
RPL5	Loss of function mutations (7)	2	2
RPL10	Non-synonymous mutations (7)	8	1
RPL22	Loss of function mutations/deletions (7)	4	0

Strong experimental results support critical role of microRNAs in the pathogenesis of non-Hodgkin's lymphomas (NHL) originating from lymphatic hematopoietic tissues. T-cell lymphomas account for approximately 10% of non-Hodgkin's lymphomas (3). The most significant miRNAs in malignant T-ALL includ tumor suppressor (miR-150, miR-193b-3p, miR-155 and miR-200) and oncogenic (miR-19b, mir-20a, miR-26a, miR-92, and miR-223) miRNAs (8, 9).

MicroRNAs (miRNAs, miRs) are non-coding RNA molecules. They are single strand short evolutionary conserved RNAs (approximately 22 nucleotides) which bind to the target mRNAs to prevent protein translation by a distinct mechanism (16). The human genome has over 2,500 miRNAs (17). Negative regulation of miRNAs is applied by binding miRNA response elements (MRE) at the 5′ end with a length of approximately 6-8 nucleotides of complementary sequence (seed sequence) to 3′UTR regions of their target mRNAs (11).

RNA polymerases, which transcribe pri-miRNA transcript, are pol II and pol III. Pol II produces the mRNAs and some noncoding RNAs including four of the small nuclear RNAs (snRNAs) and the small nucleolar RNAs (snoRNAs). However, pol III produces noncoding RNAs, including tRNAs, the U6 snRNA, and 5S ribosomal RNA (18). Following transcription, Drosha cleaves the stem-loop of pri-miRNAs and creates approximately 70 nucleotides (nt) precursors called pre-miRNAs. It is one of the members of the RNase III family. Subsequently, Exportin-5 exports pre-miRNAs into the cytoplasm, (19) where they cleave by Dicer ribonuclease and then change to small double-stranded RNA molecules. In the next stage, pre-miRNA molecules are guided into the RNA-induced silencing complexes (RISC), which comprise Argonaut (Ago) proteins and Dicer. In the RISC complex, one of the miRNA strands is released (passenger strand), while the second strand acts as a gene expression regulator (guide strand) (11). Guide strand converts into 'mature miRNA' which targets mRNAs (19). A complementarity level between mature miRNA and its mRNA target controls silencing mechanism, target degradation (deadenylation and decapping), or in inhibition of initiation and elongation levels of translation (16).

MiRNA genes are located in the introns of protein-coding genes or in the situation of non-coding transcription elements. Interestingly, many miRNAs are in the "clusters" of the poly-cistronic miRNA, where several miRNA genes are produced from a single primary transcript (20).

As one miRNA can have several mRNA targets and an mRNA can have numerous signals for miRNA recognition, it has been considered that at least 10-40% of human mRNAs are targeted by miRNAs. Therefore, it is very important to identify the validated targets of miRNAs (21).

MiRNAs are regulators of almost all processes in the cell, including cell division and proliferation, differentiation, viral defense, exocytosis, apoptosis, phenotype modulation in response to intra- and extracellular factors (11), tumorigenesis (22), developmental timing, and hematopoiesis (23). They are crucial time- and tissue-specific (11) or developmental stage-specific in cancers (22).

Dysregulation of miRNAs occurs in many types of cancer, including (4), breast cancer, colorectal cancer, hepatocellular cancer, lung cancer, ovarian cancer, and hematological malignancies (11) such as T-ALL (4).

The results of systematic investigations for finding an association between the genomic locus of miRNAs and the position of cancer-related regions have suggested that more than half miRNAs are located at fragile chromosomal regions which are complicated in the most of human cancers (22).

There are numerous reasons for miRNAs dysregulation expression in cancer cells. The first reason is an irregular expression of regulatory elements, such as transcription factors. Briefly, the overexpressed regulatory genes can result in several miRNAs expression for transcription activators or repression of expression for transcription repressor. Down-expression of the regulatory genes can cause deficient activation or even repression of miRNA biogenesis (11).

Epigenetic dysregulation also lead to abnormal miRNA expression (11). Methylation process in ALL causes the inactivation of three significant cellular pathways, namely the cell-cell adhesion, growth-deregulating events, including those that target the checkpoints of the principal G1 phase of cell-cycle and those that regulate the G2-M transition, and the apoptotic cascade process (24). In fact, hypermethylation of miRNA-coding regions results in their silencing, while miRNAs overexpression is because of hypomethylation of their promoters (11). Interestingly, members of the miR-124a family are the most frequently methylated genes in ALL cases. In many patients, one of the members of this family shows methylation at least.

CDK6 is a direct target of miR-124a. Recently, it has been shown that the loss of epigenetic of miR-124a increases the activation of *CDK6* and phosphorylation of retinoblastoma and causes abnormal ALL cell proliferation both in vitro and in vivo (24). MiRNA expression profiles represent lower levels of expression in most of the miRNAs in tumors as compared with normal tissues (22).

Another reason for aberrant miRNA expression is dysregulation of miRNA processing, deficiency of the main miRNA processing enzymes, Drosha or Dicer, and other constituents of the complex miRNA biogenesis and processing machinery. The presence of a mutation in miRNA precursors is the next reason (11). Chromosomal rearrangements, such as translocations (for example, *TCRβ*-miR-17-92 translocation in T-ALL) and somatic deletions (for example, miR-15a/miR-16-1 deletions in chronic lymphoblastic leukemia), can also be an important cause (5).

Although with less frequency, loss of function alterations in either miRNA or mRNA can result in miRNA-mediated gene silencing dysregulation. Nucleotide substitutions and polymorphisms within the seed sequence of miRNA or in MRE sequence of a target mRNA lead to ineffective miRNA-mRNA binding (11). In this systematic review, an overview of the effects of diverse microRNAs in T Cell Acute Lymphoblastic Leukemia was evaluated.

Materials and Methods

To address the objectives of this systematic review, we listed all genetic articles which had published in the area of microRNAs in T-ALL since 2004. Articles prior to 2004 were not considered due to being outdated.

Search policy for identification of studies

Databanks searched for potentially acceptable studies for this systematic review encompassed Nature, PLOS ONE, ELSEVIER, NIH public PMC, Research Gate, Bio Med Central, PNAS, Dove Press, Karger, Science Direct, MDPI, HINDAWI, Cell Press, and OXFORD. In this systematic review, no searches about gray literature were made.

Several genetic keywords and Medical Subject Headings (MeSH) terms were combined and used in PubMed, includng “Acute Lymphoblastic Leukemia” OR “T Cell Acute Lymphoblastic Leukemia” OR “T-ALL”, “MicroRNA” OR “miRNAs” OR “miRs” OR “miRNA expression” AND “Signaling Pathways”, “Onco microRNAs” OR “Oncogenic miRNAs” AND “Tumor Suppressor microRNAs” OR “TSmiRs”. Time period between January 2004 and February 2019 was considered during search for articles.

Standards for selecting studies

Annexation and rejection criteria were itemized prior to inaugurating the search strategy. These criteria were related to

Types of participants

Patients with T-ALL were our priority, but in some cases, we also reported general ALL. Sex and age were not considered in the election of acute lymphoblastic leukemia.

The population in the reference articles was from different countries, namely Brazil, China, Japan, Poland, the United States of America, and the United Kingdom.

types of studies (research or review), types of participants (T-ALL patients), and article publication year (2004 to 2019).

Results

Published articles selection

In figure 1, PRISMA diagram for article's selection was shown. After deletion of repetitive articles, 282 articles was acquired. From these articles and after excluding the studies in the field of microRNA signaling pathways in other types of leukemia, 76 articles were fully reviewed for eligibility.

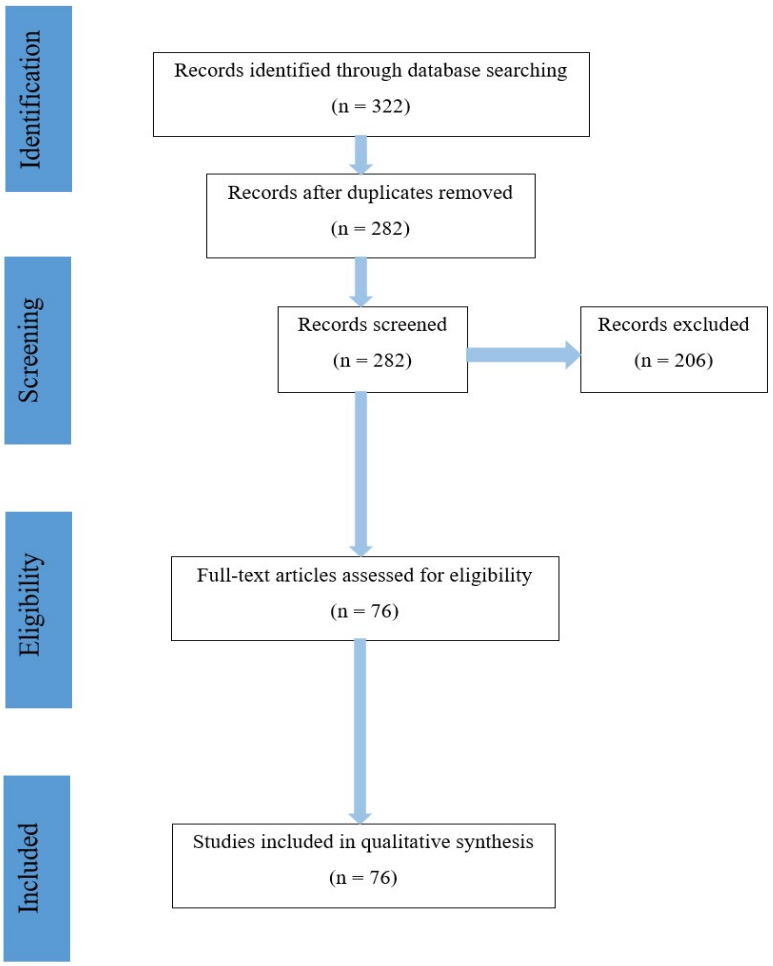

Figure 1. Flow Diagram of PRISMA instructions detailing the article selection steps to guide the systematic review

MiRNAs roles in leukemia

MiRNAs play a significant regulatory role in normal and malignant hematopoiesis (11) and leukemogenesis (3). In patients with hematologic malignancies (chronic leukemias, AML, and ALL), several miRNAs are up-or down-regulated (11). Depending on the cellular context, including the hematopoietic lineage, the type of tissue, and the presence of chromosomal translocations, miRNA expression levels and their functions are very different (25).

As each miRNA regulates numerous genes and cell-signaling pathways, the effects of the miRNAs are thought to be complex and have a combinational output. Consequently, it is essential to define the acute signaling pathways, which are important in leukemia and to recognize possible therapeutic treatments subsequently (26).

Oncogenic miRNAs

Oncogenic miRNAs target tumor suppressor genes. They overexpressed abnormally in cancer cells in comparison with healthy cells. In the cancer cells, this process can cause dysregulation of cell proliferation, cell differentiation, apoptosis, and other crucial pathways in cells (11). In the following, there is a list of oncogenic miRNAs and their targets (Table II).

Table II: Oncogenic miRNAs

MicroRNA Name	Expression	Role in T-ALL	Target Gene
miR-16	Over	Oncogenic	*PTEN, BIM, IKZF1, NF1, FBXW7, PHF6*
miR-17	Over	Oncogenic	*PTEN, CDKN1A, BIM*
miR-18a	Over	Oncogenic	*PTEN, CDKN1A, BIM*
miR-19(a & b1)	Over	Oncogenic	*PTEN, BIM, PRKAA1, PP2A*
miR-20a	Over	Oncogenic	*PTEN, PHF6, BIM*
miR-21	Over	Oncogenic	*PDCD4*
miR-26a	Over	Oncogenic	*PTEN,PHF6*
miR-92a	Over	Oncogenic	*PTEN, CDKN1A, BIM, FBXW7, IKZF1, NF1*
miR-93	Over	Oncogenic	*PTEN, BIM, IKZF1, NF1, FBXW7, PHF6*
miR-128 (a & b)	Over	Oncogenic	*PHF6,FADD,MLL,AF4*
miR-142-3p	Over	Oncogenic	*ADCY9, GRα, cAMP/ PKA*
miR-149	Over	Oncogenic	*JunB*
miR-153	Over	Oncogenic	*PTEN, BIM, IKZF1, NF1, FBXW7, PHF6*
miR-181(a & b)	Over	Oncogenic	*EGR1, NRARP*
miR-223	Over	Oncogenic	*FBXW7*
miR-342	Over	Oncogenic	*PTEN, BIM, IKZF1, NF1, FBXW7, PHF6*
miR-590	Over	Oncogenic	*RB1*
miR-664	Over	Oncogenic	*PLP2*

Studies show that miR-16 can down-regulate the progression of the cell cycle, inhibit cell proliferation, induce cell apoptosis and, have a suppress tumorigenicity effect in both in vitro and in vivo conditions. Thus, in various types of cancers, such as chronic lymphocytic leukemia, lung cancer, and prostate cancer, it is recurrently deleted and/or down-regulated (27). Identification of a genomic deletion in 13q14 locus in patients with chronic lymphocytic leukemia led to the discovery of two tumor suppressor miRNAs called miR-15a and miR-16-1. In patients with T-cell lymphoblastic lymphoma (T-LBL) and T-ALL, overexpression of cellular miR-16 was linked with longer life span (11).

MiR-17, miR-18a, miR-19a, miR-19b-1, miR-20a, and miR-92a are six miRNAs from the prototypic oncogenic miR-17-92 cluster (11, 20). This cluster targets significant T-ALL tumor suppressor genes, including *PTEN*, *CDKN1A*, and *BCL2L11* (*BIM*). In adult patients with T-ALL, overexpression of miR-17-92 cluster is the result of t(13;14)(q32;q11) arrangement which puts them under the control of a strong enhancer of T-cell receptor alpha/delta locus (*TCRD/A*) (11). Other mechanisms which lead to overexpression of this cluster include coding amplification of these miRNAs, overexpression or activation of *MYC* oncogene, or down-regulation and repression *TP53* as a tumor suppressor gene (11).

One of the core miRNAs of the miR-17-92 cluster is miR-19. This miRNA with oncogenic properties is overexpressed in T-ALL both in vitro and in vivo conditions. By using bioinformatics tools and luciferase reporter assay, four tumor suppressor genes are identified as miR-19 targets. *BIM*, *PRKAA1*, *PP2A,* and *PTEN*, which are involved in phosphatidylinositol-3-OH kinase (*PI3K*) pathway, are down-regulated by miR-19 (11).

As an oncogenic miRNA, miR-21 is overexpressed in different malignancies, including prostatic carcinoma, cholangiocarcinoma, lung cancer, colon carcinoma, and breast cancer. miR-21 has important roles in several processes, including cell proliferation, apoptosis, invasion, and metastasis. MiR-21 down-regulate the expression of defined target genes, such as tropomyosin 1 (*TPM1*), phosphatase and tensin homolog (*PTEN*), programmed cell death 4 (*PDCD4*), and B-cell lymphoma 2 (*Bcl-2*) (28). Bioinformatics analysis has demonstrated that *PDCD4* contains a miR-21 binding site (29). MiR-21 has also an oncogenic role in NOTCH signaling in T-ALL cell lines. It is up-regulated in these cells, stimulats cell proliferation, invasion, and with inhibition of expression in signal transducer and activator of transcription 3 (*STAT3*) reduces the apoptosis frequency (17).

MiR-26b is another oncogenic microRNA in T-ALL cell lines. Decreased expression levels of miR-26b in human T-ALL cells were confirmed. *PTEN* and *PHF6* are their targets. *PTEN* as a tumor suppressor gene has either a key negative regulatory role in the *PI3K* signaling pathway or a *PI3K*-independent manner. MiR-26b inhibited the *PI3K/AKT* pathway in human T-ALL cell lines by directly targeting *PIK3CD*. *PIK3CD* gene encodes *PI3Kδ*. *CAL-101* is a *PIK3CD* inhibitor. shRNA for *CAL-101* and *PIK3CD* increases apoptosis and decreases the growth rate in T-ALL cells (4).

MiR-93 is a prologue of the miR-17-92 cluster and is up-regulated in many types of cancers (30), including breast cancer, nasopharyngeal carcinoma (31), and gastric cancer (32). It is one of the ten highly expressed miRNAs in T-ALL (11). The recognized targets of miR-93 include *AICDA*, *LATS2, PTEN*, *VEGFA*, *TP53INP1*, *DAB2*, and *ITGB8*. MiR-93 activates PI3K/Akt signaling pathway and down-regulates *PTEN*, *FOXO3* and

PHLPP2 expression through targeting their 3′UTRs. MiR-93 plays several oncogenic roles but its gene targets in cancers have not been effusively clear (30).

MiR-128-3p is a new candidate oncogenic microRNA in T-cell acute lymphoblastic leukemia. Further studies have been shown that miR-128-3p acts as an oncogene in malignancies such as breast cancer, lung cancer, and acute leukemia (14). In vitro studies have shown that miR-128 prevents the expression of *FADD* gene, a responsible gene in FAS-mediated apoptosis, and *PHF6* gene, which is a recognized tumor suppressor in T-ALL (11). The results of studies in mutagenesis of miRNA binding sites and 3'UTR luciferase assay showed that miR-128-3p directly interact with the 3'UTR of *PHF6*. Also, it is found that over-expression of miR-128-3p can lead to epigenetic regulations (by hypo-methylation of the miR-128 promoter) in T-ALL cell lines (14). The role of miR-128 in cancer mechanisms is unclear because it displays both oncogenic and tumor suppressor activity in numerous types of cancer (11).

MiR-142-3p is initially recognized as a hematopoietic-specific miRNA and expresses in numerous hematopoietic cell lineages. Some signs determine that it is a significant regulator in T-cell differentiation and function. This miRNA down-regulate of miR-142-3p in effector T cells, but not in naive or memory T cells, over-expressed miR-142-3p in conventional T cells, and reduce of expression of miR-142-3p in T-leukemia cells (33). MiR-142-3p targets mRNA coding adenylyl cyclase 9, and consequently, down-regulates cAMP/PKA pathway, which is critical for the reduction of T-cells proliferation (11). It has been recognized that dysregulation of miR-142-3p may inhibit leukemogenesis by decreasing cAMP levels and growth of T-leukemic cell (11, 33).

MiR-149, as an oncogenic miRNA, (17) is up-regulated in adult and childhood T-ALL patients (11).

JunB gene was known as a direct target of miR-149 (34). JunB is a transcription factor and leads to gene activation following the primary growth responses (17). It involves in cell cycle regulation and apoptosis initiation (11, 34).

MiR-153 has been shown to be involved in cancer cell proliferation, invasion, and migration (35). Its dysregulation is reported in some human cancers (36). MiR-153 in glioblastoma is meaningfully lower as compared with brain normal tissues. In breast cancer, miR-153 increases cell proliferation and inhibits apoptosis through targeting HECT domain E3 ubiquitin protein ligase 3 or myeloid cell leukemia 1 (37, 38). Moreover, miR-153 decreases the migration and invasion of non-small cell lung cancer cells by targeting ADAM metallopeptidase domain 19 (37). Zou et al. study recognized an important role of miR-153 in regulating autophagy and apoptosis (35). miR-153 directly targets and suppresses *HECTD3* expression in apoptosis (39). A direct downstream target for miR-153 is *MTDH* which involved in the miR-153-induced suppression of the migration and invasion of breast cancer cells (40). By using qRT-PCR, Nianxi Shan et al. confirmed miR-153 is decreased in clinical NSCLC tissues and cell lines, and miR-153 down-regulation was meaningfully connected with the status of lymph nodes. Their results highlight the importance of miR-153 and *ADAM19* in the growth and progression of NSCLC (36). Furthermore, miR-7 and miR-153 regulate intracellular signaling by targeting upstream components of the AKT pathway (41).

Another putative oncogenic miRNA (11), mir-181 has an important role in T-cell maturation (1, 8). It is recognized that miR-181a targets *EGR1* gene. *EGR1* has tumor suppressive activity in hematologic malignancies. Comparative assays of miRNA and mRNA expression in a groups

of B and T-lineage ALL patients present a negative correlation between the miR-181a expression and *EGR1* gene (11). Transcription of *TGFβ1*, *BCL2*, *TP53,* and *P73* is promoted by *EGR1*. They show a significant reduction of expression in cells with miR-181a overexpressed. *BCL2* involves in positive selection and T-cell maturation (1).

MiR-223 locus is located at the X chromosome and has an evolutionarily conserved putative *NF-kB* and *RBPjk* overlapping binding site in upstream of the transcription start site of the pri-miRNA (42). miR-223 has been reported to play a significant role in normal granulopoiesis (43). However, Over-expression of miR-223 has been informed in T-ALL (44). Most recently it has determined that miR-223 increases Notch-mediated T-cell leukemogenesis (43). *FBXW7* is the key facilitator of miR-223 pro-oncogenic activity in T cells (44). Notch signaling and *NF-kB* increase miR-223 gene expression, which then down-regulates the expression of the onco-suppressor *FBXW7*. This onco-suppressor regulates Notch signaling, negatively. Consequently, the Notch/miR-223/*FBXW7* complex effects on Notch signaling in T-ALL. Inhibition of The *Notch1, Notch3* and *NF-kB* in T-ALL cells results in reduced expression of endogenous miR-223. It is understood that miR-223 decreased expression results in cell cycle arrest, and apoptosis of T-ALL cells (42).

There are ten highly expressed miRNAs in T-ALL, including miR-16, miR-19b, miR-20a, miR-26a, miR-92, miR-93, miR-142-3p, miR-150, miR-223, and miR-342 (11). MiR-342-3p is a significant cancer-related miRNA in some types of cancers (45), including, leukemia, osteosarcoma, gallbladder, liver (46), breast, colorectal and cervical cancers (47). *AEG-1* mRNAs is one of the important targets of miR-342-3p in osteosarcoma cells. This microRNA also controls the Wnt and NF-κb signaling pathways through targeting *AEG-1*. Therefore, miR-342-3p with down-regulation of *AEG-1* inhibits the proliferation, migration, and invasion of cancerous cells (45, 46). Direct repression of *E2F1* by miR-342-3p is accomplished in the mechanism underlying the regulation of *MYC* activity. In adenocarcinoma, direct suppression of the *MYC* collaborating transcription factor *E2F1* is responsible for the *MYC* activity by regulating the effect of miR-342-3p (47). Hongju Yang et al. showed that miRNA-342-5p and miRNA-608, targeted the 3'-UTR of *NAA10* mRNA for degradation and inhibited the cell proliferation and migration, and stimulated apoptosis by down-regulating *NAA10* levels (48). Recently, it is evidenced that expression of this microRNAs which encode in an intron of the gene *EVL*, is usually suppressed in human colorectal cancer (CRC) (49).

Miao et al recognized miR-590 is overexpressed in pediatric and adult T-ALL patients' blood samples as compared with healthy controls (11). In T-ALL patients the relationship between miR-590 and *RB1* was more established(2). There is a negative relationship between miR-590 expression and *RB1* mRNA, which recommends *RB1* as a target of this miRNA (11). MiR-590 locus is located at 7q11.23 and expressed in heart, breast, nerve, stomach, and other tissues. MiR-590 plays a central role in cell proliferation, differentiation and tumor incidence (50). Like other microRNAs, it has many different targets. As predicted by TargetScan and MiRanda databases, there is an interaction between miR-590 and the 3'UTR of *RB1*. *RB1* is a negative regulator of the cell cycle. Reduction of expression of *RB1* is indicated in various types of cancers, including lung adenocarcinoma, osteosarcoma, and Retinoblastoma. Also, miR-590 has a role in the carcinogenesis of T-ALL by down-regulating of *RB1* (2).

In pediatric T-ALL cases, miR-664 is overexpressed. It is an inhibitor of *PLP2* gene. PLP2 protein negatively regulates CD99 cell surface protein, which

complicated in cell adhesion and migration of T-cells (11).

MiR-19b, miR-20a, and miR-92 which belonging to the miR-17-92 cluster, and also, miR-26a and miR-223, were predicted by Bioinformatics assay that targets the main tumor suppressors in T-ALL (11).

Tumor suppressor miRNAs

On the other hands, miRNAs can target and silence oncogenes. They are down-regulated in many cancers, and therefore, act as Tumor suppressor miRNAs (11). Here is a list of Tumor suppressor miRNAs and their targets (Table III).

Table III: Tumor suppressor miRNAs.

MicroRNA Name	Expression	Role in T-ALL	Target Gene
miR-29	Under	Tumor Suppressive	*DNMT3, CDK6, TET1 & 3, HBP1*
miR-30	Under	Tumor Suppressive	*NOTCH1 & 2*
miR-31	Under	Tumor Suppressive	*HBP1*
miR-99a/100	Under	Tumor Suppressive	*IGF1R*/mTOR Pathway, *FKBP51*
miR-101	Under	Tumor Suppressive	*TAL1, NOTCH1*
miR-124a	Under	Tumor Suppressive	*CDK6*
miR-140-5p	Under	Tumor Suppressive	*TAL1*
miR-146b-(3p & 5p)	Under	Tumor Suppressive	-
miR-150	Under	Tumor Suppressive	*MYB*
miR-155	Under	Tumor Suppressive	*MYB, HBP1*
miR-193b-3p	Under	Tumor Suppressive	*MYB*
miR-196 (a & b)	Under	Tumor Suppressive	*MYC, ERG*
miR-200	Under	Tumor Suppressive	*MYB, HBP1*
miR-204	Under	Tumor Suppressive	*SOX4*
miR-448	Under	Tumor Suppressive	*TAL1*
miR-451	Under	Tumor Suppressive	*MYC*
miR-485-5p	Under	Tumor Suppressive	*TAL1*
miR-709	Under	Tumor Suppressive	*MYC, AKT, Ras-GRF1*

Reduction of expression Levels of miR-29 family members (miR-29a, miR-29b, and miR-29c) involve in several types of human cancer. Some studies define the role of miR-29 family members in hematological malignancies, as its levels are reduced in the T-ALL lineage (13). Oliveira et al. investigated on miR-29 in T-ALL, and presented that miR-29 targets are including *CXXC6*, *CDK6, DNMT3a, DNMT3b*, *MCL1*, *PXDN*, and the *p53* upstream inhibitors *p85a* (or *PIK3R1*) (11, 13). MiR-29a leads to decrease of DNA methylation (by targeting DNMTs), or increase of DNA methylation (by targeting *TET*s and *TDG*) (13).

Five highly conserved members of the mir-30 family are miR-30a, miR-30b, miR-30c-1, miR-30c-2, miR-30d, and miR-30e (51). Their loci are located at different chromosomal positions (52). This family is encoded by six genes located on human chromosomes 1, 6, and 8 (51). Several studies suggest that the miR-30 family play different roles as oncogenes or tumor suppressor genes that are depending on the type of cancer. This family has many different targets depending on the tissue and biological functions (53). Between the members, miR-30a is positioned at 6q13 and is originated from an intron transcriptional unit (52). miR-30a down-

regulated in renal cell carcinoma, colorectal cancer, gastric cancer, breast cancer, giant cell tumor, lung cancer, hepatocellular carcinoma, ovary cancer, chondrosarcoma, pancreatic cancer, urothelial carcinoma of the bladder, Ewing tumor, nasopharyngeal carcinoma, cervical cancer, and prostate cancer. It also plays an important role in the progression and development in cancer by modifying target genes, containing inducing apoptosis and inhibiting proliferation, invasion, and migration (53). This tumor suppressor miRNA negatively regulated by an overexpressed crucial T-ALL oncogene. Studies have recognized a regulatory loop which is shared between miR-30a, *NOTCH1, NOTCH2,* and *MYC* (54). *MYC* suppressed the expression of miR-30, which is targeting and inhibiting *NOTCH1* and *NOTCH2* genes. Remarkably, *NOTCH1* directly targets *MYC* and triggers its transcription (11). The association between these transcriptional factors is bidirectional, i.e., *MYC*, by suppressing miR-30a, also redacts *NOTCH* expression (54).

Inhibition miR-31 encouraged leukemogenesis in in-vivo. The main target of miR-31 is a gene which is encoding high mobility group box transcription factor (*HBP1*). *HBP1* is a suppressor of the cell cycle regulator *p21* as well as an initiator of *CD2* expression in T cells. Nevertheless, the analysis of target genes from tumor suppressor miRNAs has been shown to play important leukemogenic roles for *HBP1* (55).

MiR-100 and miR-99a are-members of the same family and have similar roles in the progression of cancers. These two miRNAs have various roles in lymphocyte pathogenesis and myeloid cell lines. miR-100 and miR-99a expression levels are very low in ALL patients in comparison with AML. The molecular basis of how miR-100 and miR-99a are associated with lymphoblastic leukemogenesis has not yet been understated. The results of Bioinformatics analysis show that FK506-binding protein 51 (*FKBP51*) is a target of miR-100 and miR-99a. *FKBP51* is an immunophilin that is expressed in lymphocytes and has the main roles in cell proliferation, malignancy, and resistance to treatments. On the other hand, *IGF1R* and mTOR are common targets of miR-100 and miR-99a in several cancer cells and they have been complicated in the initiation and progression of numerous malignant neoplasms, including ALL. MiR-100 and miR-99a are down-regulated in pediatric ALL patients and their expression levels are linked with the prognosis of the ALL patients. The supplementary studies had confirmed that miR-100 and miR-99a participate in the regulation of cell proliferation and induction of apoptosis in ALL cell lines. Finally, miR-100 and miR-99a overexpression suppressed *IGF1R* and mTOR and down-regulated *MCL1* (15).

MiR-101 is well-known as another recognized tumor suppressor in numerous types of cancer, including melanoma, gastric cancer, prostate cancer, and renal cell carcinoma (56). Qian et al. showed that miR-101 with possible property in drug-resistance is down-regulated in T-ALL patients (11). However, the exact role of miR-101 in T-ALL progression and chemical resistance is still ambiguous (56). MiR-101 targets *NOTCH1* (11). *NOTCH1* is a transmembrane receptor and regulates cell proliferation, differentiation, angiogenesis, and metastasis. The activation of Notch1 signaling plays a significant role in the mainstream of hematological malignancies, including T-ALL (56). MiR-101 can increase doxorubicin-mediated apoptosis by limiting *NOTCH1*, although down-regulation of miR-101 may be associated with chemoresistance in T-ALL (11).

Hypermethylation and histone modifications of miR-124a in the promoter region (11) decrease levels of 3mk4H3 and AcH3 and increase levels of 2mK9H3, 3mK9H3, and 3mK27H3 (22). Consequently, down-regulation of this

miRNA leads to suppression of cyclin-dependent kinase 6 (*CDK6*), which is its target. CDK6 phosphorylates retinoblastoma protein (*RB1*). In this state, RB1 is more efficiently phosphorylated and is in an inactive form, which successively stimulates cell proliferation (11). This led to an abnormal proliferation of ALL cells both in vitro and in vivo (22). MiR-140-3p and miR-140-5p are both down-regulated in various cancer cells in comparison with their normal tissues. Therefore, they have important roles as tumor suppressor microRNAs in cancers. Based on findings, miR-140-3p has 637 exclusive targets and miR-140-5p has 813 distinctive targets (57). One of the miR-140-5p targets is *TAL1*. *TAL1*, as a T-cell oncogenes (e.g. *HOX11* and *LMO2*), (58) is a helix-loop-helix (bHLH) transcription factor. *TAL1* makes a heterodimer with the class I bHLH E-proteins, including *TCF3*/*E2A* and *TCF12*/*HEB*. In hematopoietic cells, *TAL1* regulates the transcription of its target genes by binding to E-box motifs and a large complex that contains the E-proteins, *GATA* family members, and numerous non-DNA-binding *LMO* proteins. *TAL1* expression is generally suppressed during early thymocyte differentiation, (10) but it is typically found that over-expressed in T-ALL patients (58).

T-ALL primary cells expressed significantly lower levels of miR-146b-5p as compared with normal hematopoietic control cells, such as bone marrow ancestors, T-cells, thymocytes and CD34+ hematopoietic stem cells (59). Upregulation of *TAL1* causes differential expression of several miRNAs, such as miR-146b-5p. MiR-146b-5p is a critical target of *TAL1*-mediated suppression. *TAL1* as a transcription factor binds directly to the promoter region of miR-146b-3p (11).

Several studies have shown that dynamic changes in expression of miR-150 in the of lymphoid and myeloid lineage development in both mice and humans (60). MiR-150 is a miRNA selectively expressed in a mature and resting B and T cells, but not their progenitors (61). MiR-150 inhibits the progression of leukemia by regulating genes in many biological pathways and signaling cascades. They are involved in the metabolic process of small molecules such as regulators of transcription (*FOXO4*, *IKZF1*, *TET3*, *NFIC*, *RUNX1*, *EIF4B*, and *CTIF*), proteoglycan synthesis in cancer (*PDCD4*, *PRKCA*, *FZD4*, and *EIF4B*), RNA metabolic process (*EIF4B*, *PRKCA*, and *PDCD4*), mTOR signaling pathway (*PRKCA* and *EIF4B*), and the Wnt signaling pathway (*FZD4* and *PRKCA*) (12). An important predicted target for miR-150 is the transcription factor *c-MYB* (62). Functional studies show miR-150 and miR-155 levels decrease in T-ALL cell lines simultaneous with *MYC* overexpression (11).

In human, miR-155, an evolutionarily well-conserved microRNA,(63) is encoded by B cell integration cluster locus and *MIR155HG* gene. This locus is located on chromosome 21 (64). Transcription of miR-155 is regulated by the nuclear factor-κB (*NF-κB*) transcription complex and the activator protein-1 (*AP-1*) complex. (63, 64) MiR-155 is mostly expressed in the spleen and the thymus. It is one of the miRs specifics for the hematopoietic system (associated with miR-142, miR-144, miR-150, and miR-223). Subsequent studies have shown that miR-155 may signify as an oncogenic miRNA, which its expression is activated in various tumors, predominantly those of the lymphoid tissue (65). Approximately 140 genes are regulated by miR-155 which contain mRNAs encode for inflammation-related proteins, tumor suppressor proteins, and immune-modulatory proteins (63). MiR-155 targets are some important regulatory proteins such as Ras homolog family member A (*RhoA*), fork-head box O3A (*FOXO3a*) and suppressor of cytokine signaling 1 (*SOCS1*) (64). miR-155 has been commonly studied in the

immune system under circumstances of normal and abnormal immune responses and hematologic malignancies (63).

MiR-193b-3p was known as a unique tumor-suppressor miRNA which targets *MYB* during malignant T-cell transformation (5). miR-193b-3p blocks the expression of *MYB* (11). The *MYB* proto-oncogene is a leucine zipper transcription factor which has a key role in cell proliferation, differentiation, and lineage commitment during normal hematopoiesis (5). The level of miR-193b-3p in T-ALL patients is lower than normal T-cells and in T-ALL patients with high *TAL1* levels than *TAL1* negative cases, (11) which relates to the earlier reported higher expression levels of *MYB* in TAL-rearranged leukemia (5).

MiR-196b expression in pediatric T-ALL patients is meaningfully lower than normal blood T-cells. The miRNA loss of function can also the outcome of a structural change involving the region regulating or encoding specific miRNA, which exists in fragile sites related to cancer. MiR-196b is placed in the *HOXA*-cluster. *HOX* gene family plays a significant role in regulating normal hematopoiesis. The level transcription of miR-196b was 500-800 fold upregulated in the majority of *MLL*-rearranged precursor B-ALL patients in comparison with precursor B-ALL cases who had no *MLL* translocations. MiR-196b locus is located between the *HOXA9* and *HOXA10* genes on chromosome 7p15.2. miR-196b expression level may be related to *HOXA* gene transcription, nevertheless of the *HOXA* activating mechanism (either MLL fusions or other factors) (66). miR-196b is involved in *MYC* regulation (11). *c-MYC* is a transcription factor which regulates cell proliferation and tumorigenesis (67).

The miR-200 family consist of five members (miR-141, miR-200a, miR-200b, miR-200c, and miR-429), situated in two clusters on two different chromosomes in humans, chromosomes 1 and 12 (68). The first cluster in chromosome 1 carries miR-200a, miR-200b, and miR-429. The second cluster is placed on chromosome 12 which takes miR-200c and miR-141 (69). Members of the miR-200 family, directly activated by *OCT4,* and *SOX2* (68). *MYB*, *HBP1,* and *NOTCH1* are targets of this microRNA. The effect of miRNA-200b on the development and progression of T-cell acute lymphoblastic leukemia (T-ALL) remains generally mysterious. New studies have shown miR-200b may function as a potential therapeutic target for T-ALL by negatively regulating the *NOTCH1* signaling pathway (70).

Several types of research have verified that miR-204 has a twofold function as a tumor-suppressor gene and/or an oncogenic miRNA in different cancers. 30 target genes of miR-204 in different cultured cells representing 19 types of cancers. *BCL-2* is the target of this miR in intrahepatic cholangiocarcinoma, Gastric cancer, Neuroblastoma, and Colon cancer. *BDNF* is expressed differently in Breast cancer. Glioma, Gastric cancer, and Squamous cell carcinomas result from the low expression of *SOX4* gene (71). MiR-204 is down-regulated in T-ALL. MiR-204 ectopic expression inhibits T-ALL cell proliferation, migration, and invasion. MiR-204 function as a tumor suppressor is down-regulating of *SOX4*. The observations showed that miR-204 was significantly upregulated in T-leukemic cells compared to the normal T cells. *SOX4* (sex determining region Y-box 4) is a developmental transcription factor and a direct target of miR-204. New studies have recommended *SOX4* played important roles in tumor progression and development. *SOX4* expression was meaningfully increased in malignant cancers and positively correlated with leukemia (72).

MicroRNA-448 is an important miRNA and down-regulated, as a tumor suppressor, in several types of cancer, including breast cancer, gastric cancer, ovarian cancer, and hepatocellular

carcinoma (73). *Bioinformatics* analysis predicted that is a gene target for miR-448 (74). The results of Luciferase assays show that *SIRT1* expression level is suppressed by miR-101, miR-140-5p, miR-448, and miR-485-5p. In T-ALL patients these miRNAs are down-regulated in compare with normal cases which indicate their potential tumor suppressor function in T-ALL (11).

Li et al. have demonstrated that intracellular Notch1 protein has a repressive role in the down-regulation of tumor suppressor miR-451. The repression of miRs transcription by over-activation of oncogenic proteins is the major mechanism of down-regulation of miRNAs expression. Human *NOTCH1* with transcription factor activity is a transmembrane receptor. It is necessary for normal T-cell proliferation and differentiation. The intracellular *NOTCH1* decrease the expression of miR-451 by inducing degradation of *E2a* transcription factor which is an activator of the miR-451 transcription. Also, miR-451 target a key oncogene, called *MYC*. In T-ALL patients, decreased levels of miR-451 associated with increased levels of *MYC,* which is an activator for *NOTCH1* signaling. Overexpression of *MYC* leads to enhanced proliferation T-cells and leukemia progression. This is a feedback regulatory mechanism by alteration in expression levels of miRNA (11).

MiR-485-5p lies in the 14q32-31 chromosomal region and is reported as an antineoplastic gene in many human cancers, such as melanoma, oral tongue squamous cell carcinoma, gastric cancer, breast cancer, and lung adenocarcinoma (75).

MiR-709 is a surprise miRNA. It expresses in various tissues. MiR-709 locus is located in intron 8 of the *Rfx1* gene (Regulatory Factor X1). *Rfx1* is a member of the winged-helix subfamily of helix-turn-helix transcription factors. This transcription factor has both activation and repression functions. *Rfx1* is ubiquitously expressed, Like miR-709. MiR-709 has a role in response to cell proliferation and cellular stress processes. By targeting the oncogene *c-MYC*, *AKT,* and *RAS-GRF122*, miR-709 inhibits *NOTCH1*-induced T cell acute lymphoblastic leukemia (T-ALL) (76).

Conlusion

The identification of new oncogenic miRNAs and tumor suppressor miRNAs and their molecular characterization in ALL have provided new insights to the understanding of the pathogenesis of this cancer. Several number of crucial signaling pathways which play important roles in ALL pathology and progression are involved in this study. Despite many reports on microRNA subjects, there is still a necessity to find new information.

According to our knowledge, high expression levels of miR128, miR181b-1, miR204, miR218, and miR332 present in ALL samples. For example, miR128 has the highest expression in ALL. There are several microRNAs which interference in ALL but their role and targets have not determined yet. These miRs are miR-126, miR-132, miR-151, miR-190, miR-191, miR-221, miR-222, miR-342-3p, miR-363, miR-425, miR-520d-5p, miR-542-5p, miR-576-3p, miR-638, miR-708, miR-1972 to 1979, miR-2909, miR-3136, miR-3140, miR-3150b, miR-3154, miR-3190, miR-3942, miR-4474, miR-5006, miR-5187 and miR-5190. Thus, the study of alteration in MicroRNAs expression profiles as new molecular biomarkers is a good diagnostic and prognostic tool for acute lymphoblastic leukemia in human.

Conflicts of interest

There are no conflicts of interest.

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