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 aggressive hematologic clinically 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 pathogenesis) (5),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	30	34				
	mutations/deletions (6)						
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/	Chromosomal rearrangement/duplications (6)	8	8				
ABL1	(*)						
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 primiRNA 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 stempri-miRNAs of and approximately 70 nucleotides (nt) precursors called pre-miRNAs. It is one of the members of the RNase III family. Subsequently, Exportin-5 exports premiRNAs 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 RNAinduced 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, defense, exocytosis, apoptosis, phenotype modulation in response to intraand extracellular factors tumorigenesis (22), developmental timing, and hematopoiesis (23). They are crucial and tissue-specific time-(11)developmental stage-specific in cancers

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 regulatory elements, such transcription factors. Briefly, the overexpressed regulatory genes can result in several miRNAs expression 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 expression miRNA 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 "miRNAs" 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.

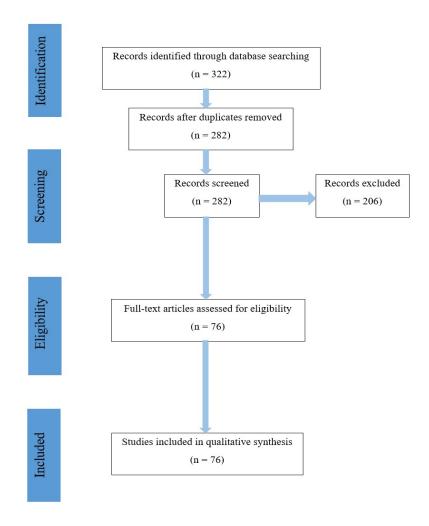


Figure 1. Flow Diagram of PRISMA instructions detailing the article selection steps to guide the systematic review

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 downregulate the progression of the cell cycle, inhibit cell proliferation, induce cell apoptosis and, have 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 downregulated (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 (T-LBL) lymphoma 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 downregulation 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, carcinoma, including prostatic 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 downregulate the expression of defined target genes, such as tropomyosin 1 (TPMI), phosphatase and tensin homolog (PTEN), programmed cell death 4 (PDCD4), and Blymphoma (Bcl-2)2 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 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 T-cell in 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 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 Tleukemic 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 downregulation 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 targeting signaling by 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 $TGF\beta1$, 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 targeting pathways through AEG-1. Therefore, miR-342-3p downwith

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 the mechanism underlying regulation of MYCactivity. 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 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. MiRplays 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 cell surface protein, CD99

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	TALI, NOTCHI
miR-124a	Under	Tumor Suppressive	CDK6
miR-140-5p	Under	Tumor Suppressive	TALI
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	TALI
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 TETs 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 transcriptional unit (52). miR-30a downregulated 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, 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 genes. and NOTCH2 Remarkably, NOTCH1 directly targets MYCand 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 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 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 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 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 proliferation, differentiation, cell angiogenesis, and metastasis. activation of Notch1 signaling plays a significant role in the mainstream of hematological malignancies, including T-MiR-101 can ALL (56).increase doxorubicin-mediated apoptosis by NOTCH1, although limiting downregulation of miR-101 may be associated with chemoresistance in T-ALL (11). Hypermethylation and 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 cyclindependent kinase 6 (CDK6), which is its CDK6 phosphorylates target. 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 TCF12/HEB. TCF3/E2A and 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 and numerous non-DNAmembers, binding LMO proteins. TAL1 expression is generally suppressed during 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 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 such regulators molecules as transcription (FOXO4, IKZF1, TET3, NFIC, RUNXI, EIF4B, and CTIF), proteoglycan synthesis in cancer (PDCD4, PRKCA, FZD4, and EIF4B), RNA metabolic process (EIF4B, PRKCA, and signaling pathway PDCD4), mTOR (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 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- $\kappa B (NF - \kappa B)$ 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 inflammationrelated 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 MYBduring 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, 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 TALrearranged 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 hematopoiesis. The normal transcription of miR-196b was 500-800 fold upregulated in the majority of MLLrearranged 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 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 expression development. SOX4 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 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 proteins is oncogenic the 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 NOTCH1 signaling. for 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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