REVIEW ARTICLE

The Importance of miRNAs and Epigenetics in Acute Lymphoblastic Leukemia Prognosis†

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Abstract

Acute lymphoblastic leukemia (ALL), one of the most common malignant human disorders, originates in different important genetic lesions in T-cell or B-cell progenitors. ALL is a malignant lymphoid progenitor with peak prevalence in children (2–5 years). The rate of survival when one is suffering from ALL depends on various agents including the age of the patient, responses to anti-leukemic therapy, and cell biology. miRNAs and epigenetics are important regulatory factors in the expression of genes. miRNAs are noncoding RNA with inhibitory effectors on specific mRNA. Patterns of DNA methylation are profoundly changed in ALL by epigenetic mechanisms. The deciphering of miRNA and the epigenetic pathogenesis in ALL could revolutionize response to the therapy and outcome, and create an enormous promise for novel approaches to reduce the toxic side-effects of intensive leukemia. Hence, pathogenetic miRNAs and epigenetics leading to the initiation and the progression of ALL are summarized in this review.

Keywords: epigenetics; leukemia; non coding RNA.

Abbreviation:

ABCB1; ATP-binding cassette sub-family

DCC; Deleted in Colorectal Cancer

DDX51; DEAD (Asp-Glu-Ala-Asp) box polypeptide 51

DLC-1; deleted in liver cancer 1

KCNK2; Potassium channel subfamily K member 2

LRP1B; eted protein 1B

NKX1-6 ; NK1 Homo box 1-6

PCDHGA12 ; Proto cadherin gamma-A12

RPIB9 **;** Rap 2-interacting protein 9

SLC2A19 ; Solute carrier family 2, facilitated glucose transporter member 19

NOPE ; neighbor of punc E11

TRBP; (The HIV transactivating response RNA-binding protein)

RISC; RNA-induced silencing complex (RISC)

TRIM71;(tripartite motif71).

LMP1;(latent membrane protein1)

HMGA2;(high mobility group A2)

MYC;(myelocytomatosis)

KRAS(kirsten rat sarcoma viral oncogene homologe)

NRAS;(neuroblastoma RAS viral oncogene homologe)

Introduction

Acute lymphoblastic leukemia (ALL) consists of 12% of hematopoietic malignancy with worldwide incidence of 75 cases per 100 000 population. This disease has a high incidence rate among children and involves 80% of children leukemia. The risk of ALL is higher in modern and developed countries. The USA, Switzerland, Costa Rica, and Italy are among the countries with high incidence rates of ALL, while Asian and African countries have lower rates. Japan has the highest rate of incidence among Asian countries (1, 2). In the USA about 3000 to 4000 new cases of ALL are diagnosed annually, two-third of them being children of 2–5 years (3). A study in 2000 had shown that the incidence ratio of male to female populations was 1.5:1.3 and the mortality rate was higher among the blacks (4)

According to recent analyses of the detected genetic and epigenetic abnormalities, the effective prognosis and molecular therapy methods have been developed that has been increased 80% and 40% of 5 years life span in children and adults, respectively. However, the treatment of adults and infants of less than 12 months is still not optimal (5–7). The presence of genetic abnormalities in lymphoblastic leukemia result in poor response to treatments in adults (8).

Moreover, as in some other cancers, congenital and environmental history could be involved in ALL development. However, only 5% of ALL cases are associated with congenital agents such as Down syndrome, bomes, ataxia telangiectasia, nijeman or exposure to ionizing and chemotherapy drugs. The nuclear exposure, pesticides, benzene, smoking, ionizing radiations, drug consumption during gestation, nitrate-rich diets, drinking water containing tricolor ethylene and synthesized inhibitor of topoisomerase II are risk factors that could be associated with ALL.

However, the detection of related risk factors and identification of their roles in the pathophysiology of the different stages of ALL is a challenge and could be an effective parameter in developing new therapeutic methods (9) (table 1).

The symptoms of ALL include fever, anemia, hemostatic abnormalities, pitches, joint and bone pains, and, occasionally, central nervous system (CNS) complications (29, 30). Genetic mutations in lymphoid hematopoietic cells are a cause of ALL involving T and B cells lineages in all possible stages of their maturation. These mutations lead to aberrant cellular proliferation and disrupted differentiation of lymphoid cells. Furthermore, the presence of a large number of lymphoid blast cells found in bone marrow leads to bone pain and dysfunction of erythroid, megakaryocytes and granulocytes through competition among these malignant cells and other normal cells and their replacement(31, 32). In advanced stages of the disease, other tissues might be affected by malignant lymphoid cells (33).

Different stages and subgroups of ALL usually have specific morphological, immune-phenotyping and genetic disorders. The differentiation of the subgroup is considered the most important step to select the optimal therapeutic approach suited to the type of disease (34). On the basis of the French American British (FAB) classification, ALL is divided to three groups, including L1, L2, and L3, with specific morphological features (35). L1 is marked by small uniform cells with few cytoplasm and without any apparent nucleolus within nucleus; L2 is marked by large varied cells with more cytoplasm and apparent nucleus, and L3 shows large and homogeneous lymphoblastic cells with multi nuclei, and more cytoplasm, containing vacuole. L3 is the most common ALL type and known as Burkit lymphoma. Furthermore, WHO classified ALLs into genetic and prognostic groups, according to systemic immune-phenotyping and cytogenetic analysis (35, 36). For instance, in the subtypes of B-ALL found t(9;22)[*BCR-ABL*], t(1;19)[*E2A-PBX1*], t(12;21)[*TEL-AML1*], rearrangements in the *MLL* gene on chromosome 11 band q23, or a hyperdiploid karyotype

(i.e., >50 chromosomes). Hyperdiploidy and t(9:21) have a desirable prognosis but hypodiploidy and MLL rearrangement are considered with poor prognosis (37, 38). T(1:19)[E2A-PBX1] with expression of E2A-PX1 fusion protein poorly responding to the base therapy of anti-metabolite, while responding better to more effective chemotherapy, so that the successful therapy rate reaches 80% (38, 39). Owing to the evaluation of the prognostic and clinical backgrounds, the WHO classification is more suitable than FAB. There are some cases of diseases that cannot be detected without immune-phenotyping. As a result, today, with immune-phenotyping of superficial markers, 90% of these cases can be diagnosed with high specificity and sensitivity(40).

About 15% and 25% of acute lymphoblastic leukemia cases in children and adults are T-ALL, respectively(39). Contrary to B-ALL cases, T-ALL is often regarded as a single malignancy in clinical and therapeutic processes and has not divided into subtypes. Recent studies have shown that in T-ALL there are many blast cells with different biological characteristic that play their roles in disease progression, response to treatment, and assessment of the malignancy(41). T-ALL ought to be considered more heterogenic than a single malignancy; so different findings of T-ALL treatment indicate that, for acquiring optimized therapeutic strategies and desirable rate of treatment, it is mandatory to classify T-ALL cases into prognostic subgroups to dedicate specialized therapeutic procedures for each. In T-ALL, the rearrangement of the T-cell Antigen Receptor gene with TAL1, 1TLX, TLX3, LYL1 genes are often seen(42, 43).

As per definition, a cancer cell has characteristics such as spontaneous growth signaling, preventing cell-growth inhibitory signaling, evading apoptosis, showing extreme replication potential, escaping the immune system, making alterations in cell metabolism pathways, and promoting angiogenesis. Interaction between cell signaling and transcription factors, proteins involving proliferation, differentiation, homeostasis and apoptosis are mandatory for normal

cells. Like other tumor cells, in ALL, such mechanisms are disrupted in the malignant lymphoblastic cells. The important regulators of cells that deserve mention are miRNA and epigenetic. Both play notable roles in the prognosis, diagnosis, and even treatment of different malignancies. However, the status and the precise mechanism that lead to the development of ALL are being investigated. Therefore, in this review, the various aspects of prognosis and factors related to miRNA and the epigenetics of ALL are discussed as novel factors.

miRNAs

In 1993, for the first time, Ambros and Ruvkun discovered miRNA through complementary base pairing with mRNA and inhibiting its expression in Caenorhabditis elegans worm(44). This miRNA was called Lin-4(Lineage 4). The increased attention to the function of miRNA was due to their major role in many biological processes such as growth, differentiation, cell cycle regulation, apoptosis, metabolism, and in developmental processes(45, 46).

miRNAs are a novel class of non-coding small 60–110 nucleotides. The enzyme complex includes a protein known as Pasha that binds double-strand RNA and Drosha, an RNase III, which plays the role of splitting(47). miRNAs process transport to the cytoplasm via exportin-5 and Ran-GTP. In cytoplasm, As shown in figure 1, miRNAs undergo the final processing by another RNase III called dicer and is transformed into 19–22 nucleotides double strand RNA(48). Human argonat protein(EIF2C2) binds to the dicer complex by another trans-activating response RNA-binding RNAs that are strictly conserved(49). They have a length about 22 nucleotides, as intracellular regulator RNAs. miRNAs regulate post-transcriptional expression of genes in animals and plants through cleaving and inhibiting the

translation of mRNA. miRNA genes are dispersed as clusters and singles in genome and can be found in intra-gene and in transcriptionable regions such as introns and exons(50).

miRNA is transcribed by RNA pol II and its 3'-end polyadenilates. Owing to base-pairing interactions throughout the transcript, stem-loop structures appear. In the nucleus, this structure is processed by an enzyme complex and forms a hairpin with protein (TRBP) and organizes an RNA-induced silencing complex (RISC) that has the potential of controlling the miRNA. A leader strand of mature double strand miRNA incorporates miRNP and forms miRISC. The sequence of this leader strand detects the mRNA binding site. Only this strand can bind to its complementary messenger RNA and suppress the translation of targeted miRNA(51). The passenger strand is believed to be non-functional and degrades after release(52, 53).

miRNAs could be oncogenes and/or tumor suppressor. It should be noted that most of miRNAs are able to control many cellular pathways such as cellular responses to infectious diseases, control stem cell signaling, cell differentiation, etc (54–57).

Profiling miRNAs called miRNome differ in normal and pathogenic conditions (58). miRNAs have broad functions and could regulate some mRNA and/or several miRNA target one mRNA(59). The major alteration of miRNome in tumor cells is inappropriate gene expression that leads to an abnormal rate of mature miRNA. Besides, the mutations in pri-miRNA sequences, abnormal transcriptions and single nucleotide polymorphism(SNP) are major alterations of miRNome in cancers (60). It has been demonstrated that alteration in the expression level of miRNA leads to marked changes in profiling mRNA expression. Therefore, oncomiRs are used as key biomarkers in prognosis, diagnosis and even in the treatment of hematological malignancy such as ALL. Further, miRNAs can be used as special markers for the type and stage of ALL (13).

miRNAs and ALL

Most studies on miRNome have shown that almost in all cancer types such as solid tumors and hematological malignancies some alterations occur in miRNAs (61–63). The significant role of miRNAs in the regulation of gene expression shows that the detection of these non-coding RNA profiling is required in the evaluation of malignancy cells. Today, miRNAs are used in the classification and differentiation of molecular and cytogenetic subtypes of acute leukemia. However, the question that arises is: what type of factor leads to miRNome alteration? Genetic abnormalities such as deletions, translocations, variety of mutations, and epigenetic modification(DNA methylations and histone modifications) could disrupt miRNA functions such as epigenetic modification-decreased miR-124a, CpG-Island hypermethylation down-regulated miR-152, and miR-9 that are tumor suppressor miRNAs (64). Hence, a decrease in the methylation of these miRNAs can be a therapeutic approach. Moreover, miRNA profiling differs among the types of leukemia. For example, the expression of miRNA is different in ALL and CLL(65). The five most highly expressed miRNAs are miR-128b, miR-204, miR-218, miR-331, and miR- 181b-1 in ALL, and miR-331, miR-29a, miR-195, miR-34a, and miR- 29c in CLL. The most represented in ALL is miR 128b with a fold difference of 436.5 compared to CD 19+ Bcell and miR-331 being the highest represented miRNA in CLL. Indeed, in ALL, most of aberrantly over expressed or down-regulated miRNAs cause more cellular proliferation and prevent maturing cells, but in CLL, which is characterized by a gradual accumulation of small mature B cells in patients, mostly the apoptosis mechanisms disrupted by dysregulation of miRNAs expression (66). Furthermore, the leukemic cells in ALL patients and normal hematopoietic cells have different profiles of miRNA expression (67–70). A quantitative analysis of 397 miRNAs in leukemic progenitor cells of pediatric B-ALL demonstrated a different miRNA expression profile compared to natural bone marrow samples and CD34+ cells sorted as miR-92a, miR-100, miR-125a-5p,

miR-128a, miR-181b, miR-196b and let-7e. Furthermore, miRNA is differently expressed among T-ALL subjects and normal thymusocytes (71); a significant association was observed between higher expression levels of miR-196b and T-ALL (72). The most important miRNAs are as follows:

Mir 128b

One of the most important altered mir in ALL is mir128b that is up-regulated. The high expression of mir128a and mir128b can be even used in differentiating between ALL and AML. Hence, both the miRNAs can serve as diagnosis biomarkers between acute leukemia with 98% precision (13). Mir128b and mir221 are reduced in ALL with the translocation of MLL-AF4. The mutations were found on mir128b gene in RS4:11 cells (the first cell line established from t[4,11]-associated acute leukemia) that is remarkably associated to resist glucocorticoid through failure in down regulation of oncogenic fusion with impaired miRNA processing and decreased expression of mature mir128b. Hence, normalizing the rate of mir128b can be an inspired therapeutic approach for MLL-AF4 ALL patients (73).

Mir 125b and let-7

ALL with t(12, 21)(TEL-AML1) and other types of leukemia have shown an aberrant expression of mir-125b associated with neighboring genes, including let7c, mir99a, and mir100 (74–76). These alterations lead to an increased survival of pro-B cells and highly expressed mir125b disrupts the differentiation of CD34+ to myeloid progenitors. The important role of mir125b in enhancing the susceptibility to leukemia has been evaluated in mice recently. The mir125b over expressed in embryonic hepatocytes were transplanted into mice, resulting in B-ALL and T-ALL (77). It is assumed that mir125b plays an oncogenic role by decreasing IRF4, a BCL6 proto-oncogene transcript factor (figure 2 and 3). Furthermore, mir125b facilitates the IL3 independent growth of Ba/F3 murine cells (a murine

Pro-Bcell line). Studies have shown that mir125b has an oncogenic characteristic because this miRNA leads to drug resistance in ALL with TEL-AML1+, and the suppression of mir125a resulting in the sensitization of TEL-AML1 cells to doxorubicin(78, 79).

miRNA LET7 have been taken into consideration recently. miRNA LET7 indicated a high suppressive potential by targeting oncogenes such as tripartite motif 71(TRIM71), latent membrane protein1 (LMP1) (80), high mobility group A2 (HMGA2) (81), myelocytomatosis (MYC) (82), Kirsten rat sarcoma viral oncogene homologue (KRAS) and neuroblastoma RAS viral oncogene homologue (NRAS) (83). Decreased levels of LET7c and LET7b have been seen in different types of leukemia (51, 84). Let7b is related to increased levels of the C-MYC onco protein in children with ALL associated with rearranged MLL. This finding showed that disruption of LET7b is not just related to solid tumors but also to leukemia (71).

Mir-155

Mir-155 is another mir that is over expressed in different ALL subtypes associated with children. Interestingly, it has been found that Emicro-mir155 in transgenic mice resulted in the development of ALL/high-grade lymphoma. This mir led to clonal expansion of pre-B cells. It has been suggested that two key regulators of IL-6 signaling, called the Src homology 2 domain containing inositol -5-phosphatase (15) and CCAAT enhancer-binding protein beta(C/EBPbeta), are down regulated by direct targeting of Mir-155. Mir-155 with decreasing expression of ship and C/EBP beta) triggers some cascades that lead to large pre-B cell accumulation and incidence of ALL/high-grade lymphoma (85–89).

Mir 196b

According to studies done in 2011, the expression of mir196b is significantly decreased in Burkitt lymphoma and ALL patients. Through the re-expressing of mir196b in Burkitt lymphoma cell lines, considerable down regulation in C-MYC and its target genes occur.

These findings showed tumor suppressor activity of miR-196b in B-ALL patients (90). On the other hand, in patients with T-ALL and Human T lymphoblast, ALL cell line (MOLT-4) has been shown to be down-regulatory mir196b, due to lake in the reduction of c-myc mutated in T-ALL. MiR195b is up-expressed in rearranged-MLL and activated by MLL and fusion-MLL genes. The expression of mir196b in children with acute leukemia is not restricted to MLL but occurs in TALL as well. Such children are carriers of CALM-AF10, SET-NUP214 gene fusion and inversion on chromosome 7 (91). These disorders, like MLL rearrangements, are related to HOXA. The expression of mir196 is highly related to the members of the HOXA family since this mir196 is coded on a HOXA cluster. Over expression of mir196b is accompanied by DNA methylations in GpC islands in the prompter of mir196b. Patients with rearranged-MLL are highly resistant to prednisolone and L-asparginase (91).

Mir17-92 cluster

The Mir17-92 cluster increased significantly in patients with T-ALL and MLL-rearranged acute leukemia. This miR is relatively high in all of ALL cases. This cluster, also known as mir17HG, is located on 13q31-32 and coded miR-17/17, miR-mir19b, miR-19a, miR-18a, miR-20a and miR-92a. Mir 17HG plays a key role in the growth of lymphocytes and deletion of this cluster in mice results in an increased expression of bcl2L11 pro-apoptotic protein(also known BIM) in B cells and prevent the differentiation of pro B into pre-B. Mir17-92 acts as an oncogene in lymphoid tissues by associating with the c-myc gene to improve B lymphoma(92). Over expression of this cluster led to a decrease in p21. Mir 17-92 in MLL-rearranged leukemic cells and T-ALL has the same effect to knock down p21 gen. Mice transplanted with hematopoieticprogenitors with over expression of mir19/notch1 ALL had more rapid growth than those transplanted only with notch1. Zhang et al. detected miRNA signatures in children with CNS-recurrent ALL. The findings have demonstrated a

high level of miR-7, 198, and 663, and low levels of miR-126, 345, 222 and 551 are associated with CNS-recurrent ALL (66, 93).

Mir-124a

Recent studies have shown that DNA methylation in carcinogenesis led to less expression of mi RNA (94). Mir-124a is a tumor suppressor that in most hematopoietic malignancies such as ALL has been seen an aberrant expression of miR-124a. The methylation and chromatin condition of mir 124 has been evaluated to detect the epigenetic alterations of miRs in ALL. In fact, the expression of mir 124a in ALL is decreased by promoter hypermethylation and histone modifications. Epigenetic down-regulating mir124a led to an up regulation of cdk6 and retinoblastoma phosphorylation, resulting in abnormal proliferation of ALL cells (95). Moreover, it has been showed that the rate of hypermethylation on miR-124a was 5% and 58.1% in ALL and non- Hodgkin's lymphoma, respectively (96). Another study on 353 ALL cases has shown that 59% of the subjects were hypermethylated in the mir124a gene, and, subsequently, its expression was found to have decreased. However, over expression of miR124a led to a decrease in the growth and reproduction of ALL cells. Furthermore, among methylated and non-methylated cases on miR-124a, the relapse rate was 26 and 57% and mortality was 34 and 56%, respectively (97). MicroR-124a in the other forms of leukemia such as AML is also important. For example, a study in 2014 showed that AML cases with a high expression of miR 124a had low relapse and high survival rates (98).

Other miRNAs

The expression of mir-100, miR-99a and miR-125a demonstrated that are increased in resistant to vincristine ALL. The viability of ETV6-RUNX1 positive REH cells significantly increases. The matched function of miR125 with miR99a and/or mir 100 shows that they are effective in children with ALL resistant to vincristine (99) (figure 4).

One of the important miRNAs is mir204 that decreases in lymphoma B. In vitro, it has shown that an enhanced expression of mir204 would inhibit the growth of B cell lymphoma. Mir-204 could bind to three untranslated regions of the transcription factor of STAT5, which has oncogenic characteristics in B cell lymphoma (100). Moreover, epigenetic silencing of mir-218 regulates the cdk6 expression in pre-B ALL, and up regulates the CDK6 in NALM6 .Targeting the three untranslated regions of cdk6 as an oncogene factor by mir-218 can lead to the inhibiting the translation (101).

Mir 331 is related to leukemia recurrence. The expression of this mir is in contrast to the expression of drug resistant factors such as glycoprotein P. Furthermore, the expression of mir-331 is lower in patients with recurrent leukemia than among patients in the early stages. Hence, dysregulation of mir-331 can lead to leukemia recurrence (102, 103). Another mir is Mir-181a. The enhanced expression of mir-181a plays a key role in the drug resistance characteristic of the CEM-C1 cell line (Glucocorticoid resistant T-ALL cells). Knocking down the mir-181a sensitizes the CEM-C1 cells to campothecin; however, a high expression of miR-181a increases the drug resistance of CCR-CEM. Hence, these results showed that the suppression of the expression of miR-181a can be an inspired therapeutic approach for drug resistance in leukemia (104).

The novel miRNAs

In recent years, new mirs have been evaluated as important factors behind the creation and severity of ALLs. The RT-q PCR of these new micro RNAs showed that they had a different expression profile among ALL and normal hematopoietic cells. For example, in ALL-B compared to CD34+ normal cells of bone marrow, the expression of novel sol-mir30 decreased 4 to 17 folds(105). Moreover, in T-ALL cases the novel-sol mir18 and novel-sol mir16 decreased 5 folds compared to HSC. The known micro RNAs such as mir361-3p,

mir196b and mir708 are highly expressed but in different levels in ALLs. In ALL with hyperdiploidy, mi361-3p is expressed 3 times more than other B-ALL cases. The mir196b in MLL-rearranged is expressed about 500 times more than that in non MLL-rearranged cases, and about 300 and 3000 fold reduction in mir 708 has been demonstrated in MLL-rearranged and T-ALL , respectively (table 2). In TEL-AML1 patients, the novel sol-mir6 is approximately expressed 9 times more than in B-ALL cases without this translocation (106).

Epigenetics

Epigenetics involves molecular alternations at genomic levels to regulate the expression of genes independently of the DNA-Sequence. Epigenetic mechanisms, including DNA methylation, histone modification, chromatin remodeling and non-coding RNAs, are involved in cell processes such as differentiation, proliferation, growth, survival, apoptosis and can be transmitted to daughter cells (128–130). Epigenetic patterns such as methylation profiling would change the development and progression of leukemia like other cancers. The promoter regions of the cell cycle regulator, tumor suppressors, and proto oncogene are hypermethylated but CpG islands associated with the start of the gene of proliferation and survival are hypomethylated. In contrast to gene mutations, hypermethylation could be reversible using demythelation agents and utilized as new therapeutic approaches. Epigenetic modification with importance in disease therapy and prognosis have been found in ALL (131, 132). Today, researchers believe that specific translocations are associated with special malignancies, and specific methylated gene targets have special characters for specific cancer types, because it has been demonstrated that methylation patterns and hot spot methylations differ according to the type of cancer. Hence, such epigenetic modifications could be considered in the diagnosis and the determination of the prognosis in some cases and also used as therapy targets.

As discussed, aberrant epigenetic modifications of genes are observed in a majority of cancers, and changes such as DNA methylation could be specific to ALL and even its sub-groups. Here, we have tried to summarize some of the genes with epigenetic alteration in ALL patients that lead to up and/or down regulation of proteins. Some of these genes are known as calcitonin, ER(133), P15(134-136), P16(136, 137), HIC-1(138), P73(139, 140), and cadherin –E(141), table 3. Further, it has been shown that the calcitonin gene (CALC1) undergoes hypermethylation in 54.3% and 65.7% in AML and ALL cases, respectively, In 70 patients (35 ALL and 35 AML), hypermethylated CALC1 had correlation with poor clinical outcome.

Determination and Prognosis

P73 protein is a tumor suppressor that is functionally and structurally similar to P53 as part of a transcription factor family, which plays a role in apoptosis induction, cell cycle regulation, and inducing the expression of proteins such as P21. Recent studies have shown that in ALL and Burkit lymphoma, an aberrant methylation suppressing P73 gene occurs, leading to tumorgenecity, while in AML, CLL, and non-Hodgkin no methylation is found in CpG islands of P73. In B cell malignancies, the methylation rate is higher than T cells (62% to 17%). Interestingly, no mutation in p73 gene has been found in ALL, however, in response to a demethylation inducer, the expression of p73 increases and results in decreased proliferation and increased apoptosis (139). In another case, DDX51 was introduced to distinguish B-ALL from T-ALL, because different methylation patterns in two diseases. The methylation rate of genes of DDX51 and DLC-1 in B-ALL was 70% and 90%, respectively, while no methylation was found in T-ALL. These two genes could be useful as distinguishing biomarkers in T-ALL and B-ALL diagnosis (144).

There are many genes such as tumor suppressors, which undergoes hypermethylation and subsequent suppression in human cancers. For example, the methylation of the tumor suppressor gene, A1RASSF, has been observed in adult and children's cancers; hence, it can be evaluated in ALL. It has been demonstrated that the A3R2ppp gene in T- ALL and B-ALL is methylated about 69% and 82%, respectively, while in B-ALL the thyroid hormone receptor ß(THRB) and fibulin 2(FBLN2) genes are hyper methylated. Moreover, in approximately 47% of T-ALL promoter region of leucine rich repeat containing 3B (LRRC B3) gene is hyper methylated and methylation of retinoic acid receptor beta 2 (RAR beta2) gene is 50, while 6% methylation associated with RAR beta2 were seen in B-ALL. All these genes are located on the short arm of the chromosome (145). The methylation pattern of Msh homeobox 1(MSX1) and basonuclin 1(BNC1) genes are being used as specific markers for early diagnosis of epithelial cancer (146). It has been shown that BNC1 gene in T-ALL and B-ALL are 77% and 79% metheylated, respectively, while normal lymphocytes are hypo-methylated. Furthermore, the MSX1 methylation rate in T-ALL and B-ALL is 25% and 3%, respectively(147). Therefore, could methylation status of genes including BCN1 and/or MSX1 be considered as a predictive model for ALL?

In a study, aberrant methylation of 10 genes in untreated ALL patients were investigated. The pattern of methylation in these genes was found to be single and in combination. Such genes include MRD1(24.5%), THBS2(20.8%), MYF3(17.6%), ER(16.1%), P15(11.3%), THBS1(8.9%), CD10(4.5%), C-ABL(3.7%), P16(1.3%) Moreover, it demonstrated that methylation of MRD1 with non-being Ph chromosome, methylation of ABL-C with p210 variant of Ph chromosome, methylation of THBS1 with good prognosis and methylation of P73, P15, ABL-C with poor prognosis are associated (148). In 2007, 262 important genes with aberrant methylation in cell lines of ALL reported that 148 genes in promoter and 131 in the other regions had methylation in the CPG islands. Furthermore, methylation of genes,

including ATP-binding cassette, sub-family (ABCB1) with previously reported methylation in ALL, deleted in colorectal cancer (DCC), DEAD (Asp-Glu-Ala-Asp) box polypeptide 51(DDX51), deleted in liver cancer 1(DLC-1), potassium channel subfamily K member 2 (KCNK2), low-density lipoprotein receptor-related protein 1B(LRP1B), NOPE, NK1 Homeobox 1-6(NKX1-6), protocadherin gamma subfamily A, 12(PCDHGA12), Rap 2-interacting protein 9(RPIB9) which previously, its aberrant methylation was shown in AML (145), Solute carrier family 2, facilitated glucose transporter member 19(SLC2A19) has been found that could be involved in patients responses to chemotherapy and as ideal biomarkers for ALL prognosis(149). For example, methylation of ABCB1 gene promoter has been reported to correlate with multi-drug resistance (150).

Interaction of Epigenetics and miRNAs

As mentioned above, miR-124a is a good example for interaction between epigenetics and miRNAs (151). Promoter of the Mir-124a gene is silenced by hyper methylated and histone modification. Such histone alteration includes a decrease in 3HcA, K4H3me3 and increasing K9H3me3, K9H3me2, and K27Hme3. Down-regulation of miR-124a leads to a higher expression of cdk6 and, consequently, increases the cell proliferation in ALL (152). Studies on patients with MLL rearrangement showed that gene disorders had exclusive gene profiling, resulting from histone modification. For example, in patients with t (4, 11), the expression of most genes such as miRNAs were altered through hyper-methylation of CpG islands. miRNA such as 432, 503, 10a, 429, 200a, 200b were influenced by fusions of the MLL gene (153). Some of these miRNA, including 432, a miRNA of tumor suppressor, was down-regulated by epigenetic changes. Further, epigenetic-down-regulated miR-200a led to an increased expression of zeb1 and zeb2 and disease progression. The rate of methylation of

miR-152 was significantly correlated with prognosis. In fact, decreased miR-152 and miR-148 resulted in an inappropriate increase in the expression of DNMT1 and MLL (153). A study on the initial ALL samples demonstrated epigenetically down-regulated expression of miR22, a tumor suppressor with targeting genes such as HDAC4 and C-MYCBP. There is no DNA hyper-methylation in the CpG islands of promoter of miR-22 but increased histone suppressor modification of H3K27me3 at the transcription start site resulted in a decreased expression of miR-22 (154).

From other miRNAs that are reduced in hematological malignancies, miR-203 can be said to have tumor-suppressor activities. it has been indicated that 5% new cases of ALL, 10% AML, 42% CLL have shown a decreased level of miR-203 due to increased methylation (155).

Translocation

Epigenetic alternation could be used in the differentiation and prognosis of ALL subtypes. ALL subtypes that contain chromosomal translocations of the MLL gene have different profiles of methylation in CpG islands (156). The hyper-methylation has been demonstrated in the t(4;11) and t(11;19) while in t(9;11) and the wild type MLL gene, the methylation profile is normal and resembles normal bone marrow samples (157). In t(4;11) and t(11;19) hypomethylation as well as hypermethylation occurs. Hypomethylation has been seen in genes with oncogenic activity such as CDH3, TBX2, ERCC1 and NPR2. A higher expression of these genes could facilitate the tumorgenesis. The MLL gene has DAN methyltransferase activity, which impairs ALL with MLL involvement and can influence the gene expression profile (158). Various types of MLL fusion can recruit different histone methyl transfers leading to improper histone modifications to specific expression and/or suppression of target genes. Hence, the effect of MLL fusion could be a reason for the activation of genes

associated with uncontrolled cell proliferation and inactivation of genes involved in normal differentiation of B cells(159). Furthermore, the results suggested that the hyper-methylation rate in t(11;19), t(4;11) impacted on disease relapse; hence, the greater the methylation rate the greater is the relapse risk. The study showed that in other subtypes of ALL such as MLL gene involvement, hypermethylation of tumorgenicity mediated genes occur, and could probably be used as a therapeutic target, so that the proliferation, apoptosis and prognosis of genetic subtypes could be evaluated by using demethylasing agents (160).

Chromosomal translocations that occur in the genomic region are unstable because of hypomethylation. For example, translocations demonstrated in ALL, such as BCR22q and AML21q22, are seen in hypomethylation regions. Furthermore, chromosomal deletions, including 10p, 5p, 20q, are observed in non-hypermethylated regions (144).

Conclusion and perspectives

As mentioned, ALL is by far the most common form of leukemia during childhood and it embraces many adulthood hematological malignancies. Owing to its cellular origin variability, wall-to-wall therapies are difficult and treatments should be categorized based on molecular and genetic disruption. Hence, profound knowledge of genetic disorders and underlying molecular mechanisms is necessary to achieve efficient therapies and optimal treatment rates. Epigenetic mechanisms and miRNAs, as vital factors in gene expression, are important enough to attract significant notice. As experienced in clinical and experimental models, gene expression regulation, oncogenic and tumor suppressor functions, and discerning altered miRNome profiles such as miR-92a, miR-100, miR125a-5p, and let-7e, which mainly have alterations compared to normal cells, could be exploited for diagnosis and prognosis in patients. These factors can have direct therapies to the requisite therapeutic point, thus delivering patients from suffering and abortive tabs. In recent years, most cancer-

associated studies have focused on protein tumor suppressors such as p16 and p15, cell cycle checkpoint regulators, and oncogenes disturbances, whereby the unnatural cellular signaling results in abnormal proliferation and differentiation. Along the same lines, epigenetic factors mainly regulate gene expression by histone modification, DNA methylation, and noncoding RNA. So, our review suggests that epigenetic alterations can be specific to malignancies and there are some aberrant epigenetic alterations in ALL subtypes, such as in calcitonin, ER, and p15. One of the sacrifices of aberrant epigenetic alterations involves miRNAs like miR-9 and miR-125, which can be downregulated subsequently to inopportune hyper-methylation. It was also shown that epigenetic factors and miRNAs could affect acute lymphoblastic leukemia, and both could jointly play a critical role in pathogenesis and, subsequently, the diagnosis and prognosis of this disorder.

Owing to these themes, we can finally mention that the epigenetic alterations and miRNAs are novel implements of biological identifications, which could be specified in ALL, and considered as diagnostic and therapeutic targets.

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Figure Captions

Figure 1. Schematic representation of the steps of micro-DNA molecule synthesis inside and outside the cell

Figure 2. IRF4 plays a role in the various stages of B cell differentiation either normal or cancerous.
Activation of B cells via BCR, CD40L and cytokine stimulation leads to IRF4 expression from the NFkB and STAT streams. MITF, which is an IRF4 suppressor, is currently inactive at this stage of B cell development. In addition to activating Myc and PRDM1, IRF4 also stimulates itself, which results in the initiation of cell division. PRDM1 as the IRF4 downstream activator is also regulate by miR125b.

Figure3. The selective and preferential expression of miR125b in the germinal center centroblasts can stimulate BCL6 function, which is a direct c-Myc suppressor. The presence of Bcl6 is essential for germinal center responses, due to enhancement of cell cycle progression, somatic hypermutation, and affinity maturation while IRF4 and PRDM1 are essential for plasma cell differentiation. With the effect of miR125b, the cell prolifrate and gradually becomes malignant.

Figure4. All miRNAs involved in stimulating or inhibiting ALL types. The red lines indicate the inhibitory effects of miRNAs and green lines indicating the stimulatory effects of miRNAs on the development of this cancer. The brown circles report the pathway for the effect of miRNAs.

Table 1. Main genetic abnormality in ALL.

Abnormality	**Prognosis**	**Refe**
t(9;22)[*BCR-ABL*]	poor	(10)
t(1;19)(q23;p13) [*E2A-PBX1*]	poor	(10)
Cryptic t(12;21)[TEL-AML1]	favorable	(11)
Rearrangement of 11q23 t(4;11)(q21;q23)[MLL-AF4] t(11;19)(q23;p13.3)[MLL-ENL] t(9;11)(q23;p13)	poor favorable poor	(12) (13) (14)
t(8;14)(q24;q32)[IGH-MYC] t(8;22)(q24;q11)[IGL-MYC] t(2;8)(q11-q12;p24)[IGL-MYC]	poor poor poor	(15) (16) (17)
TCR loci translocation t(11;14)(p13;q11)[TCR-RBTN2] t(11;14)(p15;q11)[TCR-TTG1] inv (14)(q11;q32.3) t(8;14)(q24;q11) t(10;14)(q24;q11) t(1;14)(p32-p34;q11]	 intermediate intermediate intermediate poor favorable favorable	 (18) (19) (20) (21) (21) (22)

del(6p)	favorable	(23)
Hypodiploidy	poor	(24)
pesudodiploidy	Very poor	(25)
Hyperdiploidy> 50	favorable	(26)
9p abnormalities	poor	(27)
t(1;19)	favorable	(28)

Table 2. Down regulated miRNAs in ALL.

Down-regulated miRNAs	Role in ALL	Ref
Let7b	Precursor B-ALL with 11q23/MLL- translocation Let7b has role in Children with MLL- rearranged ALL through increase in expression of C-MYC	(71)
Let7c	Precursor B-ALL with 11q23/MLL-translocation Decreased level of LET7c, LET7b are seen different acute leukemia	(111)
Mir223	Interestingly, miR-92a would reduce in the patient plasma with acute leukemia	(122, 123)
Mirr92a		(124)
Mir125b *Mir99a* *Mir100* *Let7c*	Increased pro-B cells viability in ALL with TEL-AML1 translocation . Increased Differentiation of myeloid progenitors through expression of miR-125b	(111)
Mir126 *Mir345* *Mir222* *Mir551*	Decreased in CNS-recurrent ALL compared to non- CNS-recurrent ALL Can be as biomarker of relapse of CNS in ALL	(115) (115)

Mir320a	Precursor B-ALL with t(12;21)/TEL–AML1	(125)
miR-494	Precursor B-ALL with t(12;21)/TEL–AML1	(125)
miR-708	Precursor B-ALL with 11q23/MLL-translocation T-ALL	(111)
novel sol-miR-23 a	Precursor B-ALL	(109)
Mir204	Expression of this miR led to inhibit the proliferation of B-lymphoma.	(100)
Mir124a	Epigenetic-decreased miR-124a led to up-regulation of cdk6 and retinoblastoma phosphorylation that resulted in abnormal proliferation of ALL cells	(126)
Mir365	Decrease in TCF3-rearranged BCP-ALL miR-365, miR-126, and miR-24 regulate cell-cycle development and apoptosis in various tumors	(127)

Table 3. **Epigenetics of acute lymphocytic leukemia.**

Gene	Chromosomal location	Role	Epigenetic modification	Ref
GIPC2	1p31	Prostanoid signalling	Hypermethylation 100% methylated in analyzed regions	(10)
RSPO1	1p34	Wnt signalling	Hypermethylation 100% methylated in analyzed regions	(10)
Calcitonin	11p15	Calcium metabolism	Hypermethylation 62% (42–93) methylated in analyzed regions	(10)
ER	6q25	Estrogen receptor	47% (36–94) methylated in analyzed regions	(142)
P15	9p21	Cell cycle regulator Tumor suppressor	38% (17 of 45) methylated in 5' CpG island	(142)
P16	9p2l	Cell cycle regulator Tumor suppressor	4% (2 of 49) Methylated rarely	(142)
P73	1p36	Transcription factor Cell cycle	(18-31)20% methylated in analyzed regions	(131)

		regulation and inducing apoptosis and oncogene		
HIC-1	17p13	Tumor suppressor and growth regulator by regulating SIRT-1	methylated in virtually all recurrent acute lymphocytic leukemia	(143)
E-Cadherin	16q22	Cell adhesion	39 (37–54)% methylation	(143)

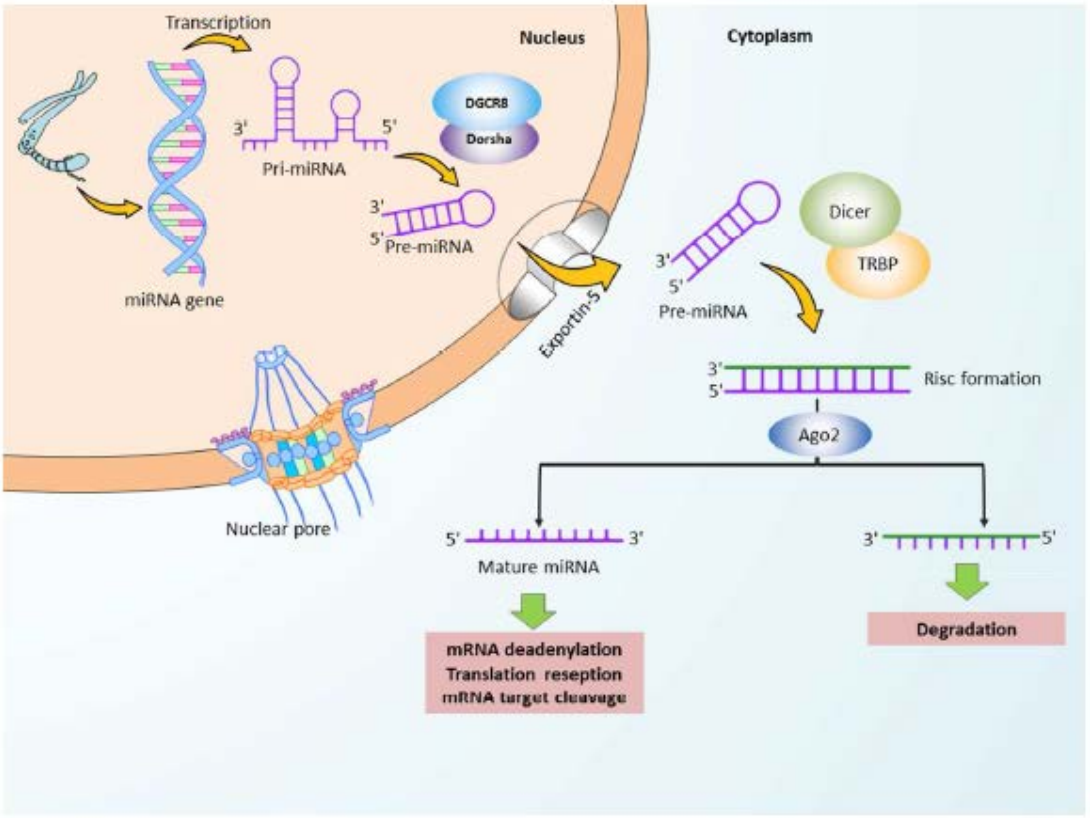

Figure 1

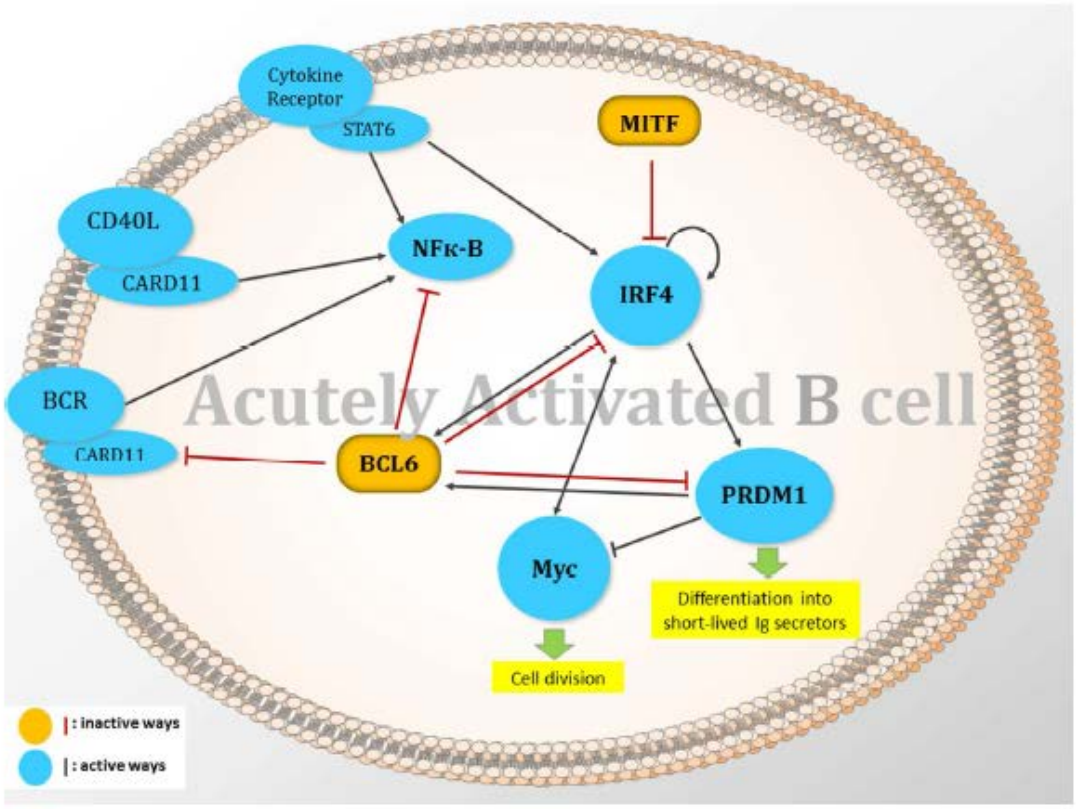

Figure 2

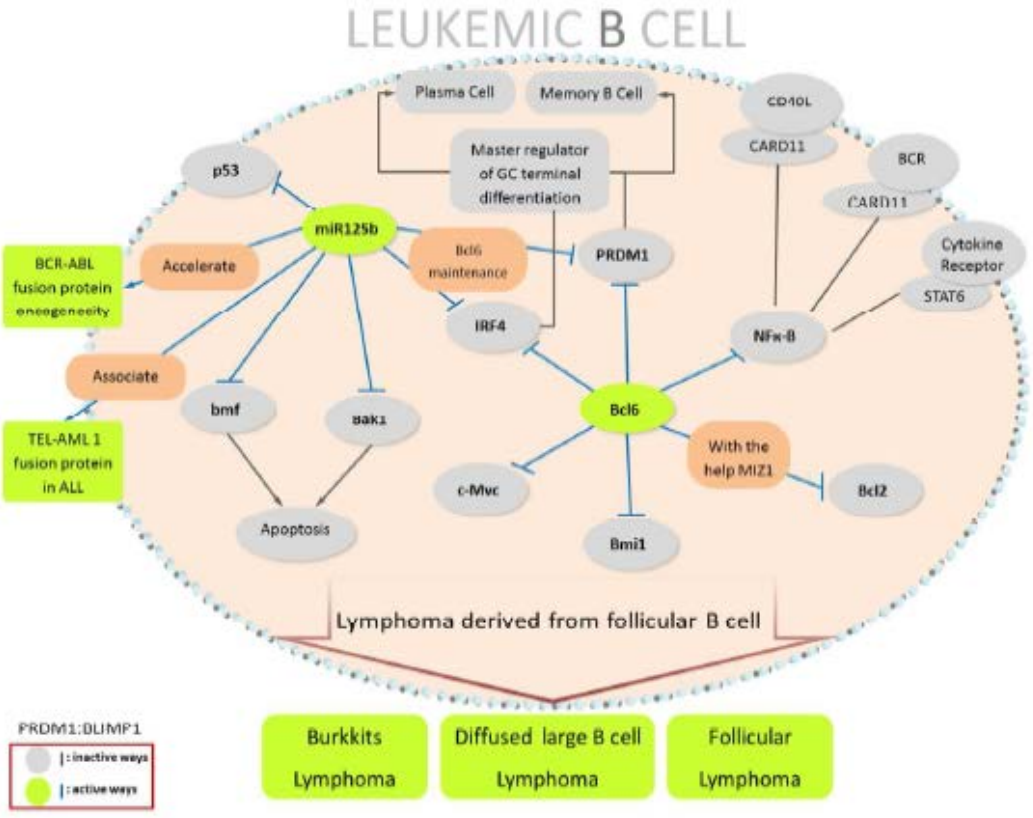

Figure 3

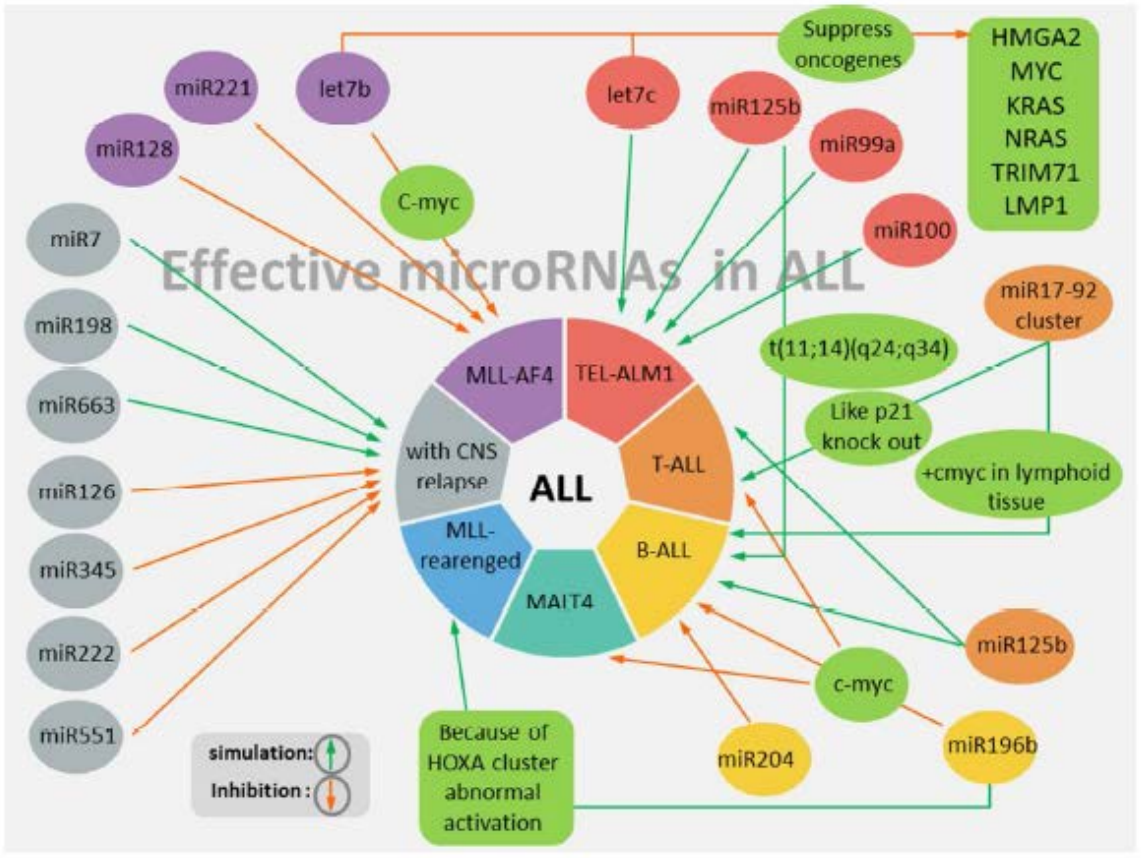

Figure 4