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## REVIEW ARTICLE

# MicroRNAs: Regulatory Biomarkers in Acute Myeloid Leukemia and Graft Versus Host Disease

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### SUMMARY

***Background:*** **MicroRNAs are a group of small non-coding RNAs with about 19 - 22 nucleotides and have a crucial role in different biologic processes such as cell proliferation, differentiation, and cell death at the post-transcriptional level. Disruption in these molecules can play an important role in tumorigenesis, and they can act as oncogenes or tumor suppressors. Acute myeloid leukemia (AML) is a hematologic malignancy with abnormal proliferation and differentiation of immature myeloid cells. MicroRNAs can be considered as biomarkers for diagnosis, prognosis, and treatment in AML patients. One of the treatments in these patients is hematopoietic stem cell transplantation (HSCT), and acute graft versus host disease (aGVHD) is the most common complication of HSCT in these patients. Patients with aGVHD appear with different clinical symptoms. Some microRNAs can predict the risk of aGVHD in these patients.**
***Methods:*** **The resources of this study are from different sites and journals such as ncbi.nlm.nih.gov/pubmed, scopus.com, Blood Journal, British Journal of Haematology, etc.**
***Results:*** **The expression of various microRNAs is different in AML patients. Also, these differences can be observed in patients with aGVHD.**
***Conclusions:*** **Identification of microRNAs can be useful in the diagnosis and prognosis of AML and aGVHD in these patients. In this review, we discuss the role of microRNAs in the pathogenesis of AML and aGVHD in patients who have undergone HSCT.**
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### KEY WORDS

microRNA, acute myeloid leukemia, aGVHD

### LIST OF ABBREVIATIONS

AML - acute myeloid leukemia
aGVHD - acute graft versus host disease
HSCT - hematopoietic stem cell transplantation
NPM1 - nucleophosmin 1
Flt-3 - fms-like tyrosine kinase 3
C/EBPα - CCAAT/enhancer binding protein alpha
MLL - myeloid-lymphoid or mixed-lineage leukemia
NRAS - neuroblastoma RAS viral oncogene homolog
WT1 - Wilms tumor
IDH1 - isocitrate dehydrogenase 1
IDH2 - isocitrate dehydrogenase 2

RUNX1 - runt-related transcription factor 1
PTPN11 - tyrosine-protein phosphatase non-receptor type 11
FAB - French-American-British classification
WHO - World Health Organization
HLA - human leukocyte antigen
CR - complete remission
Tregs - regulatory T cells
APCs - antigen presenting cells
NK cells - natural killer cells
ILC - innate lymphoid cells
MDSC - myeloid derived suppressor cells
MSC - mesenchymal stem cells
SHIP1 - SH2 domain-containing inositol 5´-phosphatase 1
FBXW7 - F-box and WD repeat domain-containing-7
CMP - common myeloid progenitor
GMP - granulocytic-macrophage progenitor
EFS - event-free survival
OS - overall survival
SOCS2 - suppressor of cytokine signaling 2
GAS-7 - growth arrest-specific 7
KLF-5 - Krüppel-like factor 5
ERK5 - extracellular signal regulated kinase 5
DSBs - DNA double-strand breaks
IGF-1R - insulin-like growth factor 1 receptor
IR - insulin receptor
IGF-1 - insulin-like growth factor 1
IGF-2 - insulin-like growth factor 2

## INTRODUCTION

MicroRNAs (miRNAs) are endogenous RNAs, which contain approximately 22 nucleotides and play crucial regulatory roles. They have an important role in regulation of gene expression by targeting the mRNAs [1]. MicroRNAs are involved in different processes such as cell proliferation, cell death, neuronal development, and hematopoietic stem cell differentiation [2].
MicroRNAs are synthesized from primary miRNAs called pri-miRNAs through the function of two RNase III-type proteins, Drosha and Dicer. In the first step, pri-miRNAs are cleaved by Drosha in the nucleus and converted into pre-miRNAs. After that, Dicer cleaves them into smaller molecules in the cytoplasm and finally, miRNAs are formed with about 21 - 25 nucleotides [3]. MicroRNAs target mRNAs, inhibit translation, and downregulate the expression of genes. In fact, their target is the 3´ untranslated region (3´ UTR) of human genes [4].
A high number of studies have been performed about the role of microRNAs in malignancies. It has been indicated that miRNAs can act as oncogenes or tumor suppressors, and they affect all aspects of cancer biology such as proliferation, invasion, and apoptosis [5]. Previous studies have shown that certain microRNAs play an important role in hematopoietic development and differentiation and predict the prognosis of the malignancies. Actually, these molecules affect the transcription factors, which are involved in hematopoiesis [6]. AML is one of the hematologic malignancies, which are characterized by uncontrolled proliferation of immature myeloid cells. Different mechanisms affect the expression of miRNAs and they become dysregulated in AML. Mutations and epigenetic alterations are the most common mechanisms [7]. One of the treatments for AML is HSCT and the most common complication of that is aGVHD. MicroRNAs can be also considered as biomarkers for the risk of aGVHD in AML patients [8,9]. In this study, we summarize the role of microRNAs in the pathogenesis of AML and risk of aGVHD.

### Acute myeloid leukemia

AML is one of the most common hematologic malignancies in adults with high incidence of three to five cases per 100,000 population in the United States [10]. It is caused by abnormal proliferation and differentiation of myeloid stem cells. In fact, genetic mutations and chromosomal translocations can affect the maturation and prevent normal proliferation [11]. Recently, several studies have indicated that two or more genetic mutations as the 2-hit theory plays an important role in creating AML. A series of mutations activate signal transduction pathways, which cause proliferation and action at the level of transcription factors and inhibiting differentiation [12].
Mutations in several genes have an important role in the pathogenesis of AML and identification of these mutations can be useful for prognostic information and better treatment of the disease. Mutations in genes such as nucleophosmin 1 (NPM1), fms-like tyrosine kinase 3 (Flt3), CCAAT/enhancer binding protein alpha (C/EBPα), the myeloid-lymphoid or mixed-lineage leukemia (MLL), the neuroblastoma RAS viral oncogene homolog (NRAS), Wilms tumor (WT1), the isocitrate dehydrogenase 1,2 (IDH1 and IDH2), runt-related transcription factor 1 (RUNX1), tyrosine-protein phosphatase non-receptor type 11 (PTPN11), etc. are recently more significant and have been targeted for novel therapies [13-15].
The French-American-British classification (FAB) and World Health Organization (WHO) have categorized AML into several subtypes. FAB classification was based on morphology and cytochemistry methods and, regarding that, AML was classified into eight major subtypes (M0 to M7). After that, WHO inserted molecular findings into this classification [16,17].
These patients have different clinical signs and symptoms. Cytopenia is remarkable, which causes clinical manifestations and symptoms of anemia such as fatigue, neutropenia, and thrombocytopenia. Thrombocytopenia can cause hemorrhage and excessive bleeding in AML patients [18,19]. Fever, anorexia and weight loss are also common among these patients [10].
For diagnosis of AML, blood and bone marrow smears are the first procedures. Presence of 20% or more blasts

in smears is required for diagnosis of AML, except in certain subtypes. Moreover, immunophenotyping using multicolor flow cytometry and cytogenetics for identification of chromosome abnormalities are used for definitive diagnosis [20].
There are different methods for the treatment of AML. The most common procedures are chemotherapy using various drugs and HSCT [21].

**Hematopoietic stem cell transplantation**
HSCT is one of the procedures to treat different diseases such as hematologic malignancies. This method is performed using hematopoietic stem cells, which are extracted from bone marrow, peripheral blood, or umbilical cord blood. For HSCT, compatibility of human leukocyte antigens (HLA) between donor and recipient is necessary, but it can be performed across ABO incompatibility [22,23].
HSCT is one of the most effective post-remission treatments in AML, and it is commonly used in patients at high risk of relapse. HSCT can be performed at first complete remission (CR1) or second complete remission (CR2), depending on how much the patient is at risk of relapse [24]. Before HSCT, preparative myeloablative regimens are used, which usually include cyclophosphamide with busulfan or total body irradiation [25].

**Graft versus host disease**
GVHD is a common and serious complication, which occurs after HSCT and affects different organs such as skin, liver, gastrointestinal tract, and lungs [8]. GVHD can be acute or chronic. Acute GVHD (aGVHD) usually occurs before day 100 of HSCT, whereas chronic GVHD (cGVHD) happens after that time [26]. Severity of aGVHD is based on the involvement level of targeted organs and is divided into four grades; grade I (mild), II (moderate), III (severe), and IV (very severe) [27]. Skin is usually the first organ that is involved and appears as erythematous rashes. Gastrointestinal manifestations include diarrhea, nausea, vomiting, and abdominal pains. Hepatic involvement causes hyperbilirubinemia and increased liver enzymes [28]. One of the most important reasons for GVHD is the response of donor T cells to proteins on host cells, especially HLA molecules [29]. The immune cells, which are involved in GVHD are T-cell subsets (CD4+ and CD8+ T cells), regulatory T cells (Tregs), antigen presenting cells (APCs), and natural killer cells (NK cells) [26].
There are several agents, which are used for the prevention and treatment of GVHD. High dose of corticosteroids such as intravenous methylprednisolone is used as the first-line treatment [30]. However, some patients show resistance to steroids. Therefore, anti-thymocyte globulin and different monoclonal antibodies are usually used in these patients [28]. Recently, some studies have indicated that regulatory cell populations play an important role in immune homeostasis and can reduce GVHD. These cells are Tregs, NK, and NKT cells, innate lymphoid cells (ILC), myeloid derived suppressor cells (MDSC), and mesenchymal stem cells (MSC) [31].
Diagnosis of GVHD is still based on clinical symptoms such as skin rash, diarrhea, and elevation of bilirubin. Also, skin and liver biopsies and measurement of plasma biomarkers such as several cytokines and interleukins are used as confirmatory methods for diagnosis [32,33]. Recent studies have demonstrated that microRNAs can be considered biomarkers for diagnosis and prognosis of GVHD and used as therapeutic goals [9].

**MicroRNAs**
MicroRNAs (miRNAs) are small non-coding RNAs, which contain 19 - 22 nucleotides and play an important role in gene expression. They can degrade the targeted mRNAs or inhibit translational process. These molecules are involved in different processes such as cell development and differentiation, immunomodulation, neural development, and cell cycle (Figure 1) [34,35]. The expression level of microRNAs is different in various diseases. Also, they can act as oncogenes or tumor suppressors [36].

**MicroRNAs and AML**
Based on previous studies, the expression of microRNAs is different in AML patients (Table 1). MiR-155 is one of the microRNAs, which is overexpressed in AML patients, especially in those with FLT3-ITD mutations [37]. The SH2 domain-containing inositol 5´-phosphatase 1 (SHIP1) is one of the targets of miR-155, which is considered as a negative regulator of the PI3K/AKT signaling pathway and a suppressor of hematopoietic transformation [38]. A study has shown that in AML patients, especially AML-M4 and M5, overexpression of miR-155 can decrease the level of SHIP1, and miR-155 acts as an onco-miR [39].
The expression of miR-196b, which is located on chromosome 7, is increased in AML patients, especially in patients with t(11q/23)/MLL. Previous studies, which were performed on miR-196b, have indicated that overexpression of this microRNA inhibits differentiation of progenitor cells in bone marrow and causes an increase in the proliferative process. Therefore, it can be a therapeutic target in AML patients with MLL mutations [40, 41].
A study performed by Gong et al. has indicated that the family members, miR-29a, -29b, and -29c, are downregulated in AML patients compared to healthy controls. Overexpression of these microRNAs in AML cell lines can inhibit cell proliferation and enhance cell apoptosis and act as tumor suppressors by targeting AKT2 and CCND2 mRNAs [42].
In 2016, Xiao and colleagues performed a study and investigated the expression level of miR-223 in AML patients. They showed that this microRNA is downregulated in AML compared to healthy controls and causes poor outcomes in these patients [43]. F-box and WD repeat domain-containing-7 (FBXW7) is an F-box protein

**Table 1. MicroRNAs involved in AML.**

| MicroRNAs | Expression level | Predicted target | Ref |
|---|---|---|---|
| miR-155 | up-regulate | SHIP1 | [39] |
| miR-196b | up-regulate | MLL | [40] |
| miR-29 | down-regulate | AKT2, CCND2 | [42] |
| miR-223 | down-regulate | FBXW7 | [43] |
| miR-34a | down-regulate | E2F3 | [46] |
| miR-181a-3p | up-regulate | NEMO/IKBKG | [47] |
| miR-125 | up-regulate | NF-κB | [51,52] |
| miR-193b | down-regulate | MAPK | [56] |
| miR-486 | up-regulate | JAK-STAT3 | [59] |
| miR-362-5p | up-regulate | GAS-7 | [60] |
| miR-21 | up-regulate | KLF-5 | [61] |
| miR-143 | up-regulate | ERK5 | [65] |
| miR-107 | down-regulate | RAD51 | [67] |
| miR-149-5p | up-regulate | FasL | [70] |
| miR-4792 | up-regulate | Kindlin-3 | [71] |
| miR-628 | down-regulate | IGF-1R | [75] |

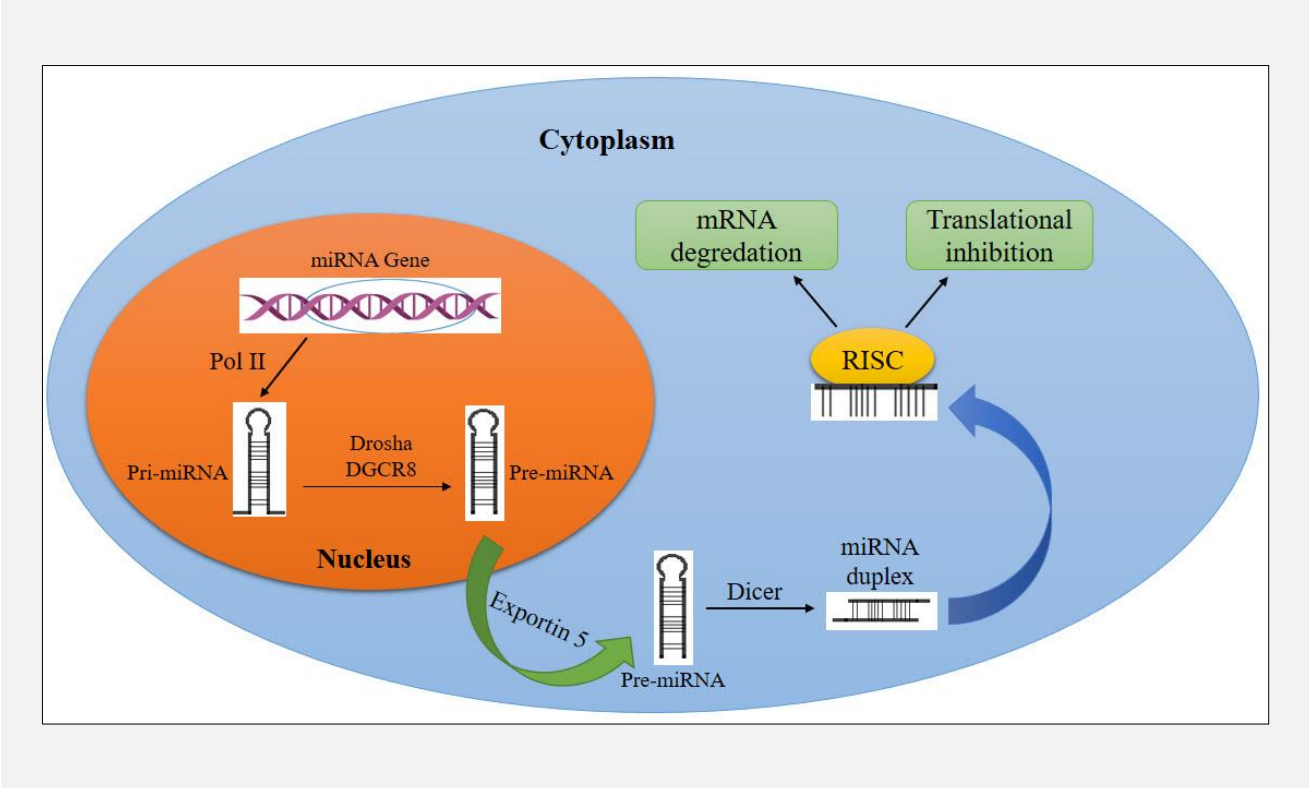


**Figure1. MicroRNA formation and function.**

in the SCF E3 ligase complex, which regulates different processes such as cell proliferation, differentiation, cell cycle, etc. by targeting various substrates for degradation. Therefore, downregulation of FBXW7 causes the inhibition of cell proliferation through degradation of key regulators of cell cycle [44]. Xiao et al. showed that FBXW7 is significantly downregulated by transfection of miR-223 into AML-HL60 cell line and causes the suppression of cell proliferation and enhancement of cell apoptosis because FBXW7 is targeted by miR-223 [43].

C/EBPα is a transcription factor, which is required for granulopoiesis. It is necessary for differentiation such as transition from the common myeloid progenitor (CMP) to the granulocytic-macrophage progenitor (GMP) [45]. In a study, Pulikkan et al. showed that miR-34a is one of the targets of C/EBPα in granulopoiesis, and in AML patients with C/EBPα mutation, miR-34a is downregulated and acts as a tumor suppressor. In fact, miR-34a targets E2F3, a transcription factor involved in granulopoiesis [46].

Certain microRNAs can be considered as diagnostic and prognostic biomarkers in AML patients. For instance, miRNA-181a-3p expression is significantly increased in AML patients, especially in M1, M2, M3, and M4 subtypes compared to healthy controls. These patients have a better prognosis at the time of diagnosis [47]. In fact, this microRNA inhibits NF-κB signaling pathway by targeting NEMO/IKBKG [48]. NF-κB is a transcription factor that plays a regulatory role in cell proliferation, invasion, and apoptosis, and has a progressive role in AML, in which chromosomal translocations and gene mutations increase its activity [49,50]. MiR-125a and -125b are other microRNAs, which are overexpressed in AML patients inhibiting AML cells invasion and proliferation and causing apoptosis by targeting NF- κB signaling pathway [51,52].

Expression of certain microRNAs can predict good or bad prognosis in AML patients. For example, in AML patients who receive chemotherapy alone, high expression of miR-93 and miR-98 is associated with longer event-free survival (EFS) and overall survival (OS), and they are considered as good prognostic factors in these patients. However, these associations are not observed in patients undergoing allo-HSCT [53,54]. On the other hand, dysregulation of miR-328 causes poor clinical outcome in AML patients compared to healthy controls and those who receive treatment [55]. In another study, Bhayadia et al. investigated the role of miR-193b in the prognosis of AML. They showed that this microRNA acts as a tumor suppressor by targeting involved genes in the MAPK signaling pathway, but low expression of miR-193b in AML patients is associated with poor prognosis and lower OS in these patients [56].

Previous studies have shown that major energy substrates for leukemia growth are glucose, fructose and glutamine, and play an important role in tumorigenesis [57]. In a study, Lin and colleagues have indicated that high expression of miR-532 in AML causes a decrease in cellular energy for leukemia growth and leads to favorable outcomes in these patients [58].

In a study, Sha et al. investigated the role of miR-486 in the pathogenesis of AML and they found that this microRNA is upregulated in AML patients. This overexpression leads to an increase the activity of JAK-STAT3 signaling pathway and causes AML cells proliferation by silencing the suppressor of cytokine signaling 2 (SOCS2) [59]. Furthermore, miR-362-5p and miR-21 are other microRNAs, which are overexpressed in blood samples of AML patients and cause proliferation and invasion of AML cells by targeting growth arrest-specific 7 (GAS-7) and Krüppel-like factor 5 (KLF-5), respectively [60,61]. Some microRNAs can be considered as the regulators of myeloid differentiation. MiR-10a and -10b are the examples of these molecules. In a study, Bi et al. showed that the expression of miR-10a/b is increased in AML patients, especially in M1, M2, and M3 subtypes. Overexpression of these microRNAs results in the proliferation of immature cells and suppression of mature cell differentiation, and finally, causes the occurrence of AML [62].

Extracellular signal-regulated kinase 5 (ERK5) is a member of MAPK family, which is involved in cell proliferation and differentiation [63]. Recent studies have shown that ERK5 can also play an essential role in growth of leukemic cells and cause resistance to chemotherapy [64]. In AML, miR-143 is overexpressed and leads to the reduction of proliferation and increased apoptosis of leukemic cells by targeting ERK5 [65].

RAD51 is an important gene, which is located on chromosome 15 and plays a crucial role in repairing DNA double-strand breaks (DSBs) and causes the stability of DNA molecules. Therefore, changes in the RAD51 gene cause several chromosomal breaks and lead to different malignancies [66]. In 2018, Huang et al. found that miR-107 is significantly downregulated in AML patients compared to healthy controls. On the other hand, they transfected miR-107 mimics into cell-lines such as HEL and TF-1, and the expression of miR-107 was remarkably increased. They showed that upregulation of miR-107 can inhibit the expression of RAD51 in cell-lines, but the expression of apoptosis-associated genes is upregulated. This situation can cause the death of AML cells [67].

MiR-133 is considered as a tumor suppressor in different human cancers. The expression evaluation of this microRNA in AML patients has shown that it is downregulated in AML patients compared to normal individuals. This situation is associated with poor prognosis in these patients. On the other hand, the expression of miR-133 is elevated in AML patients with favorable response to chemotherapy or achieving complete remission [68].

CD95 (Fas/APO-1) is a death receptor, which mediates apoptosis when it binds to its ligand, CD95L (FasL), and plays an important role in homeostasis. A number of apoptotic factors such as caspases are required for apoptosis, which are recruited by CD95 [69]. In a study,

Tian et al. investigated the expression of miR-149-5p in AML patients and cell line THP-1. They showed that miR-149-5p expression is significantly increased in patients and THP-1 cell line. Probably, FasL is the target of this microRNA and it is negatively associated with miR-149-5p. Therefore, suppression of miR-149-5p can induce apoptosis in AML cells by targeting FasL and activating apoptotic factors [70]. MiR-4792 is another microRNA that can inhibit AML cell proliferation and induce apoptosis by targeting kindlin-3. In fact, overexpression of miR-4792 causes downregulation of kindlin-3 and inhibition of cancerous cell proliferation [71]. Kindlin-3 is expressed in different cells such as hematopoietic and endothelial cells, leukocytes, and platelets. It has a role in leukocyte migration and platelet aggregation [72]. Recent studies have shown that it can be considered as a tumor suppressor or oncogene in cancers [73].

Insulin-like growth factor 1 receptor (IGF-1R) is a member of insulin receptor (IR) family. Insulin-like growth factor 1 (IGF-1) or insulin-like growth factor 2 (IGF-2) are considered as the ligands of IGF-1R. The reactions between ligand and receptor cause the activetion of the MAPK and PI3K/AKT signaling pathways. IGF-1R is expressed in normal tissues and plays an important role in different physiological functions. Recent studies have shown that IGF-1R is overexpressed in certain tumors and hematologic malignancies, and inhibition of this molecule was recently used in clinical trials [74].

**MicroRNAs and GVHD**

According to recent studies, one of the treatments in different hematologic malignancies such as AML is HSCT. GVHD is still the most common complication which occurs after HSCT. In a review study, Paczensy et al. demonstrated that there are several biomarkers which indicate the risk of GVHD after HSCT, prognosis, and the treatment responsiveness. One of these biomarkers is microRNA [9,75]. Therefore, determining these molecules can be useful in identifying patients who develop GVHD.

A high number of studies have evaluated the expression of different microRNAs in GVHD and observed various results. In a study, Saadi et al. investigated the expression of miR-92b, miR-551, and miR-1275 in AML patients who have undergone HSCT and developed aGVHD, but no significant increase was observed in these microRNAs. Although they detected that the expression of miR-1275 and miR-92b is increased in patients with high-grade aGVHD, it was not statistically significant [76]. In another study, they investigated the expression of miR-222 and miR-181b in AML patients and their correlation with the occurrence of aGVHD. Both microRNAs were upregulated in patients with aGVHD compared to those without aGVHD, but these differences were not statistically significant between the two groups. Therefore, they have stated that these microRNAs cannot be considered as biomarkers for developing aGVHD. However, they have suggested that this study should be performed again with more patients [77].

In 2015, Wang et al. evaluated the level of miR-586 in plasma samples of patients with different diseases after HSCT. They observed the increased level of miR-586 expression in patients with aGVHD, but the level of this microRNA can be affected by infections, which are caused by aGVHD [78].

MiR-181a is another important microRNA, which is considered a biomarker for aGVHD after HSCT. Previous studies have estimated the expression of miR-181a in hematologic malignancies such as AML, CML, etc. after HSCT and demonstrated that this microRNA is overexpressed in aGVHD patients compared to non-aGVHD. Also, they showed that the serum level of miR-181a is associated with the severity of aGVHD and elevated in grade III and IV compared to grade I and II [79].

In a study, Xiao and colleagues investigated the expression level of 6 miRNAs, such as miR-423, miR-199a-3p, miR-93*, miR-377, miR-155, and miR-30a, in patients with malignant diseases such as AML, CML, CLL, etc. with and without aGVHD. They found that the expression of these microRNAs is significantly upregulated in the plasma of aGVHD patients when compared to non-GVHD patients. Also, they showed that miR-423, miR-199a-3p, miR-93*, and miR-377 can be used as predictors of aGVHD because they are elevated before the onset of aGVHD [80].

## CONCLUSION

MicroRNAs recently have been considered as important factors in the pathogenesis of human cancers and hematologic malignancies such as AML. They can also be used as biomarkers for aGVHD in AML patients who have undergone HSCT. The expression profile of microRNAs can be used for the categorization of AML, determination of prognosis and diagnosis, and also, monitoring the response to treatment in these patients. These molecules can act as oncogenes or tumor suppressors. In this study, we discussed the expression of different microRNAs in AML patients and aGVHD and their crucial roles in proliferation, invasion, and apoptosis of leukemic cells. Investigation of these molecules can predict the prognosis and outcome of the disease. Today, studying microRNAs in AML patients is widely carried out, but the functional mechanism of these molecules still remains unclear. Therefore, more studies on different groups of patients with various subtypes and cytogenetics are required to evaluate the key role of miRNAs in AML and aGVHD.

**Declaration of Interest:**
The authors declare that there are no conflicts of interest.

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