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Review Article

Evaluation of MicroRNA as Minimal Residual Disease in Leukemia: Diagnostic and Prognostic Approach: A Review

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Abstract

Various factors are effective in the development of minimal residual disease (MRD), one of which is MicroRNAs (miRNAs). miRNAs and their dysfunction in gene expression have influential role in the pathogenesis of leukemia. Nowadays, treatments that lead to the suppression or replacement of miRNAs have been developed. Focusing on the role of miRNAs in managing the treatment of leukemia, in this review article we have investigated the miRNAs and signaling pathways involved in the process of apoptosis and cell proliferation, as well as miRNAs with oncogenic function in malignant leukemia cells. Among the studied miRNAs, miR-99a, and miR-181a play an essential role in apoptosis, proliferation and oncogenesis via AKT, MAPK, RAS, and mTOR signaling pathways. miR-223 and miR-125a affect apoptosis and oncogenesis via Wnt/B-catenin, PTEN/PI3K, and STAT5/AKT/ERK/Src signaling pathways. miR-100 also affects both apoptosis and oncogenesis; it acts via IGF1 and mTOR signaling pathways.

Keywords: MicroRNAs; Minimal residual disease; Pathogenesis; Prognosis; Diagnosis

Introduction

Leukaemia is a range of malignant disorders, characterized by disruption of differentiation of blood progenitor cells. This group of malignancy occurs in all age ranges, although its different types have different age distribution in terms of disease incidence (1-3). The clinical manifestations of disease include fatigue, weakness, pallor, bone pain, fever, lymphadenopathy, and arthralgia (4, 5). Acute leukemias are among the most prevalent cancers that affect people of all ages. The cause of acute leukemias is still unknown, although number of genetic and environmental factors have been suggested. A number of genetic and environmental factors have been suggest-

ed, but none has been demonstrated to cause acute leukemias. Genetic significantly influences the emergence of many disorders, and numerous environmental elements, professions, and pastimes have been studied (6-8).

The genetic changes that contribute to the pathogenesis of leukemia vary and include mutations, deletion, insertion, and translocation. Based on the patient's condition and pathophysiology of disease, different treatment strategies are adopted; these include chemotherapy, immunotherapy, target therapy, radiotherapy, and hematopoietic stem cells transplantation (HSCT) (9).

Today, evaluation of minimal residual disease (MRD) is one of the common methods to assess the response to treatment (6, 10). Various techniques have been investigated to evaluate MRD; the use of these methods help to improve the prognosis of patients (6). The term MRD refers to the population of leukemia cells that have survived chemotherapy and radiation therapy and cause the disease to return. To forecast the outcome and choose the level of intensity for additional treatment techniques, MRD must be detected. Significant improvements in the sensitivity of MRD diagnosis have been made thanks to the development of numerous new diagnostic tools, including next-generation sequencing (NGS). Using phenotypic marker patterns or differential gene expression patterns, there are some techniques to diagnose MRD; reverse transcription polymerase chain reaction (RT-PCR), flow cytometry (FCM), or PCR are examples of these techniques. Practical viability and patient demand for improved diagnostic sensitivity will shape future advancements in the clinical treatments (11-13).

Various factors are influential in the development of MRD, one of which is Micro RNAs (miRNAs). These molecules regulate mRNA expression in the posttranscriptional level. miRNAs regulate about half of human genes (14-16). The profile of miRNAs in normal tissues and various diseases has been extensively studied despite limitations. These molecules are actively secreted into the extracellular space by extracellular vesicles (14). This feature along with easy distribution in fluid and their stability has made them a good biomarker for MRD in leukaemia-involved patients (14).

EL-KHAZRAGY et al. emphasized on the pathogenesis role of miR-188a and miR-155a in many types of leukaemia (17). Moreover, miR-31 affects many cellular and developmental processes by targeting the impressive genes of proliferation, apoptosis and oncogenic activity (18-20). They are non-coding RNAs and can affect regulation of biological and pathological processes in many diseases, and the processes of proliferation, apoptosis, and oncogenic activity. Considering that few studies have been done in this field, we investigated the role of miRNAs s in MRD.

miRNAs as oncogenic factor

miRNAs deregulation can lead to the onset and progression of disease (21, 22). Therefore, miRNAs can be used as the potential therapeutic candidates, also studies have shown that these molecules can be used as the diagnostic and prognostic biomarkers in the patients (23, 24).

In patients with leukaemia, increased expression of miR-200c/141 and miR-181a/181b through down regulation of TGFB signalling pathway is clearly related to increased WBC count and poorer prognosis (19, 25, 26). In patients with leukaemia, clonal arrest of B cells occurs in the G0/G1 phase of the cell cycle, associated with clonal proliferation of B cells. This proliferation depends on increasing the expression of miR-22, which leads to phosphatase and tensin homolog (PTEN) down regulation and activation of PI3K/AKT pathway. Accordingly, PI3K inhibitors are used in the treatment of these patients (28-29).

miR-335/ID4 causes Leukemogenesis by activating the PI3K/AKT signalling pathway. Accordingly, aberrant expression of miR-335/ID4 is considered as a prognostic biomarker in leukaemia and can be used for therapeutic purposes (Figs.1,2) (30). miR-335 overexpression results in up regulation of BRCA1 mRNA expression indicating the functional domain of ID4 signalling. The relevance of miR-335 regulation for human

breast cancer has been confirmed in most of sporadic breast cancer samples with significantly reduced levels of miR-335 (31). miR-625 is involved in many biological processes such as invasion, migration, apoptosis, cell proliferation, cell cycle regulation, and drug resistance (32-34). In thyroid cancer, suppressing the miR-625-3p inhibits cell proliferation through inactivating the PI3K/AKT and MEK/ERK signaling pathways (24, 32).

miR-100 and miR-99a play a role through two signalling pathways, the first is the suppression of the IGF1R/mTOR signalling pathway and down regulation of the anti-apoptotic genes, i.e., *MCL1*, which leads to the inhibition of cell proliferation and the apoptosis induction. The second pathway is related to glucocorticoid receptors through the suppression of the FKBP51 signalling pathway, which leads to increased activity of glucocorticoid receptors (35-39).

Increased expression of miR-106b-5p and miR-26b-5p leads to disease progression. The study of several patients with leukaemia has shown the key role of miR-26b-5p in leukaemia (40-42). Its increased expression leads to the inhibition of the TGF-B/SMAD signalling pathway, which results in down expression of *P21-G1P1kinase* inhibitor and high expression of *c-MYC* oncogene. The mentioned events lead to increase in the proliferation of leukemic cells and the leukemic clone; this mechanism is influential in the treatment strategies of leukemic patients (40).

Increased expression of *miR-362-5p* as oncomiR through *GADD45a* down regulation and decreased expression of *miR-320a* as tumour suppressor through PI3K/AKT and NF-kb pathway are involved in the migration and invasion pathway (43). In this way, increasing the expression of *miR-320a* by inhibiting the phosphorylation of mentioned pathways leads to weakening the oncogenic function of bcr/abl (44).

Meanwhile, increasing its expression in THP-1 human leukaemia monocytic cell line produces the opposite result. By targeting DAB2, miR-93 inhibits the PI3K/AKT signalling pathway and increasing *DAB2* expression negatively affects the growth of THP-1 cells (44). miR-125a acts as a tumour suppressor in patients with leukaemia. Increasing its expression by inhibiting the Erb signalling pathway leads to inhibiting the cycle of cell proliferation and increasing the apoptosis rate of leukemic cells (45). On the other hand, in some leukaemia patients, increasing its expression through the signalling pathway leads to resistance to apoptosis and reduced response to treatment. In fact, miR-125a also acts like a double-edged sword, identifying the related signalling pathways leads to better management of the treatment of patients with leukaemia (45-46).

Expression of miR-142-3p as an oncogene increased in leukaemia. Its high level of expression is related to the worse prognosis of disease. This oncogenic role is justified by targeting the cyclic adenosine monophosphate (cAMP), PKA (protein kinase A), GR (glucocorticoid receptor) alpha. Increased expression of *miR-142-3P* leads to decrease in the *cAMP* level and *PKA* activity and eliminates the inhibitory effect of PKA on the proliferation of leukemic cells (47-49).

The expression of *EXT1* decreases in patients with leukaemia; it is inversely related to the expression of miR-665 (50). Furthermore, decrease in the expression of *EXT1* and increase in the expression of miR-665 are associated with poor prognosis in these patients. *EXT1* through inactivation of the pathway ERK1/2 signalling leads to apoptosis induction in AML cells (50). In patients with leukaemia, increased expression of *miR-204* leads to the suppression of proliferation, migration, and cell invasion and induction of apoptosis in leukemic cells. Hepatic growth factor (HGF) is up regulated in leukemic cells (51). By directly targeting HGF, miR-204 regulates the c-MYT signalling pathway; in fact, it has an inhibitory effect on the progression of AML through the HGF/c-MYT pathway (52, 53).

MiR-181a directly binds to 3'UTRs, which results in *KRAS*, *NRAS*, and *MAPK1* downregulation and decreased leukemic cell proliferation in AML. Inhibited colony formation, decreased proliferation, and greater sensitivity to chemotherapy are all effects of elevated miR-181a expression (Fig.1) (54, 55).

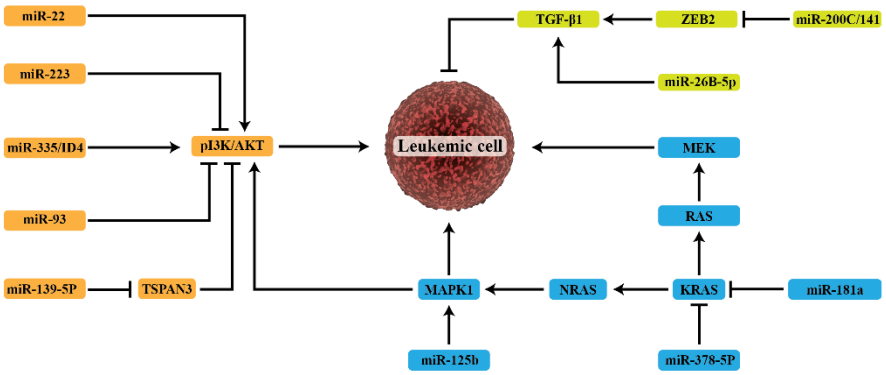

Fig. 1: Role of miRNAs in MRD: miR-181a , miR-100 and miR-99a play an important role in MRD through KRAS, mTOR, and Akt signaling pathways. Activation of Wnt/B-catenin and inhibition of PI3K/AKT signaling pathways by miR-139-5P leads to proliferation of leukemic cells (Original Figure)

Involved miRNAs in apoptosis

Leukaemia cells have abnormalities in one or more of apoptosis signalling pathways (Table1, Fig.2) (56). miR-3151, as a tumour growth suppressing factor in patients with leukaemia, undergoes a decrease in gene expression and hyper methylation; by increasing the activity of MEK/ERK and PI3K/AKT pathways and increasing the expression of *MCL1*, it protects the malignant cells against apoptosis (52, 56, 57).

miR-378 dysregulation occurs in various types of leukaemia. The level of miR-378 is clearly higher in patients with leukaemia. Increased expression of miR-378 in malignant cells leads to increased cell proliferation and drug resistance. Apoptosis is inhibited in cells transfected with miR-378. Increase in miR-378 expression is actually associated with up regulation of stem cell markers of *OCT4* and *c-Myc* (58). Meanwhile, in colorectal cancer miR-378-5P leads to inhibition of cell proliferation and induction of apoptosis through the RAS/RAF/MEK signalling pathway (59). Increased expression of miR-34 in leukaemia patients leads to apoptosis through inhibition of PI3K/Akt signalling pathway, and inhibits autophagy in leukaemia cells (60).

In malignant cells, miR-99a down regulation and methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) up regulation occurs in some types of leukaemia. In fact, MTHFD2 knocking down and increased expression of miR-99a lead to inhibition of cell proliferation and induction of apoptosis (61, 62). miR-99a induces apoptosis and inhibits cell proliferation by inhibiting the AKT signalling pathway by targeting the *MTHFD2* (61).

Increased expression of *miR-140-5p* leads to decreased cell proliferation and induction of apoptosis through sine oculis homebox 1(SIX1) in leukemic cells (63). In fact, inhibition of *SIX1* expression by inhibiting the STAT3 signalling pathway clearly leads to cell proliferation decrement in leukemic cells and induction of apoptosis through increased expression of proteins related to apoptosis, such as Bcl-2, Bax, and caspase-3. This path can be used as a therapeutic goal in the treatment of leukaemia (63).

miR-582-5p in patients with leukaemia leads to inhibition of cell proliferation, induction of apoptosis and suppression of cell invasion and migration. Its increased expression leads to the expression of two markers involved in cell apoptosis, namely Cleaved caspase-3 and Cleaved PARP proteins (64). miR-34 in leukemic cells is associated with poor prognosis and reduced response to treatment. In fact, miR-34a expression through targeting the pathway Janus kinase 1-signal transducer and activator of transcription 2-p53 axis signalling leads to stimulation of cell death in leukemic cells (64, 65).

By targeting the 3' UTR of FKBP51, the expression of *miR-100* reduces and this effect leads to

cell proliferation and inhibition of apoptosis (66). The expression of miR-125a decreases in patients with leukaemia (28). By increasing its expression, the activity of the ErbB signalling pathway decreases and leads to inhibition of cell proliferation and induction of apoptosis. Mubritinib is used to treat some types of leukaemia; it inhibits the ErbB pathway and increases the expression of miR-125a (45).

miR-486-5p is clearly down regulated in leukemic cells. Its increased expression leads to apoptosis induction, increased caspase-3 activity in malignant cells, and decreased *FOXO1* expression (67). *RaIA* is a downstream molecule and a component of the bcr-abl fusion protein in the RAS signalling pathway (24). This molecule is the target of miR-181a in leukemic cells. Increased expression of miR-181a leads to cell proliferation suppression and stopping cell proliferation in the stage of G2 and induction of apoptosis in the K562 leukemic cells (68).

miR-1271-5p is down regulated in patients with leukaemia. Increasing its expression leads to induction of apoptosis in leukemic cells. The translational factor ZIC2 is the direct target of *miR-1271-5p*; it decreases cell proliferation and increases apoptosis through the reduction of *ZIC2* expression. It also plays the same role in ovarian cancer by inhibiting the NOTCH signalling pathway, and leads to the inhibition of proliferation (5, 69). Increased expression of *miR-451a* in patients with various types of leukaemia leads to cell proliferation inhibition; it also results in down regulation of *c-Myc* oncogene through IL-6R and inhibition of activation of JAK and STAT3 signalling pathways (70).

Table 1: Summary of miRNAs involved in apoptosis

miRNAs	*Altered expression*	*Target*	*Mechanism*	*Ref.*
miR-9	↑	*Hes1*	*Hes1* inhibiting miR-9 and reduce growth cell	(71)
miR-21	↑	*PTEN/AKT pathway*	Inhibiting *miR-21* cause apoptosis	(72)
miR-29b	↓	*MCL-1 CXXC6 CDK6*	It leads to proliferation and inhibiting apoptosis	(73)
miR-125b	↑	*NF-κB*	It inhibits the invasion, proliferation, and death of human leukemic cells.	(74)
miR-126	↑	*PLK2*	It increases cell viability and prevents cell death	(75)
miR-135a	↓	*HOXA10*	*miR-135a* overexpression induces cellular death while suppressing cell growth and the cell cycle.	(76)
miR-143	↑	*ERK5*	Apoptosis is induced by an increase in the granulocyte surface marker Ly6G and a more developed morphology toward granulocytes.	(77)
miR-150	↓	*EIF4B, FOXO4, PRKCA, TET3*	In both vitro and vivo, it promotes cell proliferation and prevents apoptosis.	(78)

Involved miRNAs in proliferation

Various molecular, genetic, and cytogenetic abnormalities are effective in the clonal expansion and proliferation of blood progenitor cells (Table.2, Fig.2). Aberrant expression of *miR-181a* in leukemic cells clearly leads to increased cell proliferation through activation of Akt signalling pathway (79). The expression of *miR-1193* in leukemic cells significantly reduces the proliferation and invasion of malignant cells. In breast cancer,

miR-1193 reduces the proliferation of malignant cells through the activation of the PI3K/Akt signalling pathway (80). In patients with leukaemia, increase in the expression of *miR-99a* and miR-100 leads to the inhibition of cell proliferation by inactivating the mTOR signalling pathway and inhibiting the lGF1R gene expression (35).

In leukemic cells, miR-139-5p leads to the inhibition of cell proliferation by inactivating the Wnt/B-catenin signalling pathway (81). In patients with leukaemia, the expression of *miR-582-5p* leads to inhibition of cell proliferation and induction of apoptosis. In patients with non-small cell lung cancer (NSCLC), miR-582-5p plays the same role by inhibiting the hippo-yap/taz signalling pathway (64). The increased expression of *miR-141-5p* reduces the proliferation of leukemic cells and accelerates apoptosis. While expression in prostate cancer leads to the development of metastasis through the activation of the NF-KB signalling pathway (82). miR-132 and miR-212 lead to increased proliferation and invasion of leukemic cells; they play the same role in various cancers by inhibiting the TGF beta and PI3K signalling pathways (83).

miR-125ba leads to the acceleration of cell proliferation of leukemic cells by increasing the activity of PI3K/Akt, NF-kB and MAPK signalling pathways (84). In patients with leukaemia, *miR-182* expression reduction widely leads to decrease in cell proliferation, and its expression decrease by inhibiting the TLR4/NF-kB signalling pathway in malignant breast cancer cells; it leads to decrease in cell proliferation and invasion (85). In leukemic cells, the expression of *miR-15a* and *miR-16* leads to the proliferation of malignant cells (16). In prostate cancer, miR-15a/16 inhibits the TGF-beta signalling pathway and leads to disease progression and metastasis (86). On the other hand, in patients with papillary thyroid cancer, increased expression of miR-15 inhibits the proliferation of malignant cells through inhibiting the RET/Akt signalling pathway; so, its dual role can play an important role in managing the treatment of patients with leukaemia (86).

Table 2: Summary of miRNAs involved in proliferation

miRNAs	***Altered expression***	***Target***	***Mechanism***	***Ref.***
miR-9	↓	*HMGA2 LIN28B*	Cause cell proliferation	(91)
miR-9	↓	*RUNX1, RUNX1T1, RUNX1-RUNX1T1*	Inhibiting differentiation, and cause proliferation	(92)
miR-9	↓	*SIRT1*	Cause reduce proliferation of cells	(93)
miR-9	↓	*FOXO1 FOXO3*	Cause cell proliferation	(94)
miR-21	↑	*PTEN/AKT pathway*	Cause reduces proliferation of cells	(72)
miR-29b	↓	*SP1*	Cause cell proliferation.	(95)
miR-34a	↓	*E2F3*	Boost proliferation while reducing differentiation.	(96)
miR-135a	↓	*HOXA10*	Inhibiting differentiation, and cause proliferation	(76)

In leukaemia, miR-124 leads to the acceleration of cell proliferation and resistance to glucocorticoid treatment. Also, in bladder cancer by inhibiting the STAT3 signalling pathway, it leads to apoptosis induction and increased cell proliferation (87). miR-223 leads to decrease in the

growth rate and suppresses the proliferation of leukemic cells; it performs this role by inhibiting the Akt/mTOR/p70S6K signalling pathway (88). miR-205 leads to the inhibition of cell proliferation in leukemic cells. In gastric cancer, it plays this role by inhibiting the Akt signalling pathway (89-90).

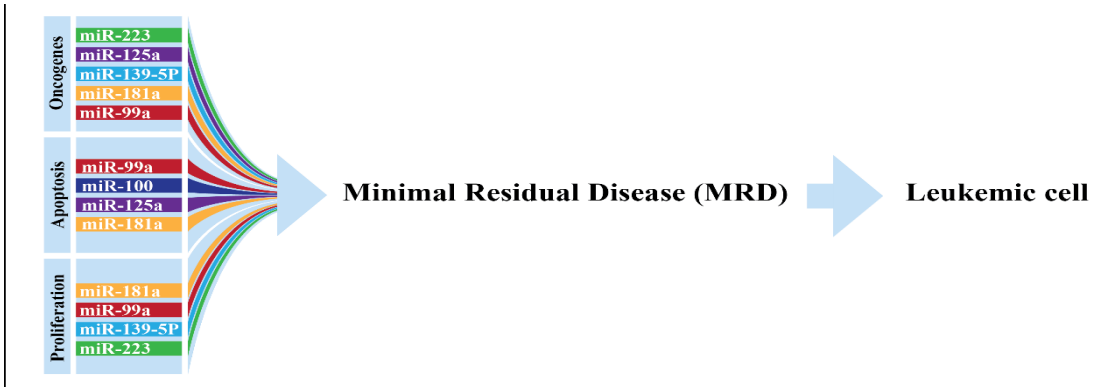

Fig. 2: Role of miRNAs in proliferation, apoptosis and oncogenes related MRD. miR-223, miR-125a, miR-139-5p, miR-181a, and miR-99a are involved as oncogenes. miR-99a, miR-100, miR-125a, and miR-181a are also involved in apoptosis. miR-181a, miR-99a, miR-139-5p, and miR-223 are involved in proliferation (Original Figure)

Strategy and potential therapeutic

Clinical trials and ongoing researches are actively working to personalize the therapy through the discovery of molecular targets, identification of patient- and disease-specific risk factors, and the identification of potent medication and modality combinations. In Table 3, the drugs used as target therapy in leukemia and their mechanisms are listed. One of the therapeutic strategies to prevent MRD is targeting the miRNAs (97). In many cases, these paths are double-edged swords. On the one hand, it inhibits MRD. In addition, targeting the miRNAs can also affect the activity of LncRNAs. The factors in the downstream of miRNAs play a role in the sensitivity of cells to chemotherapy. Some of these factors play a role in the sensitivity of cells to apoptosis (98).

Table 3: Drugs used as targeted therapy and their mechanisms in leukemia

Drug	*Mechanism*	*miRNA*	*Ref.*
Venetoclax	Selective small molecule BCL2 inhibitor blocks the anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein, and causes apoptosis of CLL cells. Overexpression of Bcl-2 has been linked to increased resistance to chemotherapy.	miRNA-15/16	(99)
Dasatinib-Blinatumomab	Inhibiting BCL-2 and cause induced apptosis	*miRNA-378 and miRNA-17 miRNA-21-5p*	(100)
Asciminib	Dasatinib targeting BCR/Abl , Src, c-Kit, ephrin receptors. Blinatumomab binding to CD3 and CD19.	*miR-139-5p*	(68)
Acalabrutinib	Targeting the ABL myristoyl pocket.	*miR-210*	(47)
Vemurafenib	It blocks Bruton's tyrosine kinase, and caused delaying progression of the cancer.	*miR-204-5p and miR-211-5p*	(59)
Ivosidenib	Inhibiting BRAF and cause apoptosis	*miR-183*	(52)

Conclusion

Among the studied miRNAs, miR-99a and miR-181a play an essential role in apoptosis, proliferation, and oncogenesis via AKT, MAPK, RAS, and mTOR signaling pathways; while miR-223 and miR-125-a have function in apoptosis and oncogenesis via Wnt/B-catenin, PTEN/PI3K, STAT5/AKT/ERK/Src signaling pathways. miR100 is also common between apoptosis and oncogenesis, and act via IGF1 and mTOR signaling pathways. Paying attention to their dual role in causing apoptosis and proliferation and as an oncogenic factor, they could be used to design specific treatment methods, diagnostic panels, and screening programs in the high-risk patients.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

This is a review study, and it is not an original. Data availability is corresponding author responsibility.

Consent for publication

Not applicable.

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