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Original article

Exploring the interplay between microRNA expression and DNA mutation analysis in AML patients

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ABSTRACT

MicroRNAs (miRNAs) are key regulators in Acute Myeloid Leukemia AML, affecting gene expression, including that of CD markers and impacting mutations within leukemic cells. Mutations in AML can alter miRNA profiles, which can affect the expression of CD markers and contribute to disease progression by influencing cellular processes such as differentiation, proliferation, and apoptosis. Here, we examined the interplay of cell surface protein expression (CD markers), DNA mutations, and microRNA expression in AML patients. We included 32 recently diagnosed AML patients, and CD marker expression was evaluated using flow cytometry and molecular techniques. This study aims to delve into this relationship within the context of AML, elucidating its potential implications for diagnosis, prognosis, and therapeutic interventions. Mutations were scrutinized in six patients using Whole-Exome Sequencing (WES), while quantitative PCR (qPCR) was employed to investigate the expression levels of nine microRNAs. Subsequently, a comprehensive interaction network was constructed using Cytoscape software, focusing on genes with significant mutations and their corresponding microRNAs. Cell surface protein expression analysis revealed upregulation of CD45, CD99, CD34, HLA-DR, CD38, CD13, CD33, MPO, CD15 and CD117 in AML patients. The molecular analysis results unveiled mutations in specific genes (FLT3, KIT, PTPN11, BCR, DNMT3A, and NRAS) targeted by nine microRNAs. Notably, eight microRNAs exhibited heightened expression levels. Network analysis highlighted interactions between the PTPN11 gene and six scrutinized microRNAs. Understanding the regulatory dynamics between gene mutations and microRNAs in AML patients is pivotal for unraveling the disease's molecular mechanisms and identifying potential therapeutic targets. Further exploration into the functional roles of microRNAs in gene regulation and AML pathogenesis is warranted to validate their potential as therapeutic targets, diagnostic markers, and advanced treatment strategies.

1. Introduction

AML originates in the bone marrow, where blood cells are produced. It is characterized by the rapid growth of abnormal white blood cells, which cannot function properly. These immature cells, known as myeloblasts or leukemia cells, crowd out healthy blood cells in the bone marrow (BM), decreasing the generation of red blood cells and white blood cells. AML is a heterogeneous disease, manifesting in various forms with genetic mutations and clinical outcomes (Döhner and Döhner, 2008, Testa et al., 2022). AML is the most common type of leukemia in adults, with over 20,000 new cases diagnosed each year in the USA alone (De Kouchkovsky and Abdul-Hay, 2016). The disease can progress rapidly if not treated promptly. The exact cause of AML is often unknown, but factors such as genetic predisposition, exposure to certain chemicals or radiation, and certain medical conditions or treatments may increase the risk of developing the disease. Treatment for AML typically involves chemotherapy, targeted therapy, stem cell transplantation, or a combination of these approaches, depending on the patient's age, overall health, and specific genetic mutations in the leukemia cells. Despite advances in treatment, the overall prognosis for AML remains challenging, with a relatively low 5-year survival rate, highlighting the need for continued research and development of more

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effective therapies (Kabel et al., 2017).

DNA mutations in AML commonly target genes linked to hematopoietic regulation, such as FLT3, DNMT3A, KIT, PTPN11, BCR, NRAS, and others. These genetic alterations have the potential to trigger abnormal cell proliferation, hinder differentiation processes, and confer resistance to apoptosis, all of which contribute to the development of leukemia. Scientists can detect and analyze genetic changes using Next Generation Sequencing (NGS), including single nucleotide variants, insertions/deletions, and structural rearrangements. This comprehensive approach offers insights into the diverse genomic alterations present in AML, providing a detailed understanding of the disease's molecular landscape (Abdel-Wahab, 2012).

Small RNA molecules known as microRNAs (miRNAs) bind to messenger RNA (mRNA) and control the expression of genes by either inhibiting the translation of proteins or degrading mRNA. In AML, specific miRNA expression patterns are linked to disease onset and prognosis. However, understanding how miRNA expression changes during AML progression is limited, as is exploring therapeutic opportunities targeting these miRNA alterations. Dysregulated miRNA expression in AML can act as oncogenes or tumor suppressors, affecting key pathways in leukemia development. By analyzing miRNA expression patterns, researchers can uncover the complex regulatory networks controlled by miRNAs in AML, shedding light on their role in disease progression and potential therapeutic targets (Havelange, 2011).

Integrating data on DNA mutations and miRNA expression in AML offers a comprehensive view of the molecular terrain of the disease, revealing the intricate interplay between genetic changes and miRNA-mediated gene regulation. This holistic approach allows researchers to pinpoint crucial driver mutations and aberrantly expressed miRNAs linked to AML development, providing valuable insights into disease pathogenesis, prognosis, and potential therapeutic strategies. Moreover, this integration can unveil novel biomarkers and therapeutic targets, paving the way for precision medicine approaches tailored to individual patients with AML. Overall, combining DNA mutation and miRNA expression data enhances our understanding of AML biology and promises to improve patient care through personalized treatment strategies. Thus, this research aims to examine the interaction between DNA mutations and miRNA expression profiles using NGS technologies. This offers a comprehensive model for understanding the molecular pathways underlying AML development.

Cell surface proteins known as Cluster of Differentiation markers are essential for classifying and differentiating different kinds of blood cells. These proteins are crucial for prognosis, therapy planning, and diagnosing AML. For example, the standard stem cell marker CD34 is frequently linked to hematopoietic progenitor cells and is involved in the pathogenesis and response to treatment of acute myeloid leukemia. Additionally, some treatments, such as antibody-drug conjugates, can target the expression of particular CD markers, such as CD33. This is an essential and dynamic topic of current research since recent studies in the field of hematological malignancies provide fresh perspectives into the landscape of CD marker expression and their therapeutic applications in AML (Hikmat et al., 2023).

2. Materials and methods

2.1. Sample collection

The Human Ethics Committee of Salahaddin University, Erbil's College of Science, approved and authorized the current study (Approval No: 1770, Date: 14/6/2021). Every participant provided written, informed consent for publishing the study's data. The present observational cross-sectional investigation was conducted at Nanakaly Hospital for Blood Diseases, situated in Erbil, Kurdistan region, Iraq, specifically within its clinical flow cytometry unit, from September 1, 2021, to April 1, 2022. The study enrolled a total of 32 cases, selected through a non-random convenient sampling approach, wherein every instance of new-onset de novo AML presenting at Nanakaly Hospital was included, irrespective of age or gender. Upon completing history-taking and physical examination, each patient provided a blood sample of 4–5 cc of peripheral blood (PB). These samples were then placed in EDTA tubes for immunophenotyping analysis of cytoplasmic/nuclear or surface antigenic markers via flow cytometry, employing three laser light sources and NGS analysis.

2.2. Immunophenotyping through flow cytometry

Multicolored flow cytometry was used to perform immunophenotyping using peripheral blood samples and bone marrow aspirate. For the CD45-gating strategy, 15,000 events were used to plot and determine the blast cells' immunophenotype. An acute leukemia flow panel containing 28 antibodies was applied to the screen. A BD FACSCanto II flow cytometer (BD Biosciences) was used to collect the data, and FlowJo software version 10.9.0 (TreeStar Inc., San Francisco, CA, USA) was utilized for analysis.

2.3. Making libraries and isolating DNA

DNA was extracted from peripheral leukocytes using a QIAamp DNA blood kit (QIAGEN), following the manufacturer's instructions. The library was created via the Twist Human Core Exome Enzymatic Fragmentation (EF) Multiplex Complete kit. The MGIEasy FS DNA Library Prep Kit was applied to synthesize circular DNA and prepare the library for MGI system sequencing.

2.4. Evaluation of NGS data

The library was sequenced with the (MGI-DNBSEQ-G400, China) devices, producing 150 bp paired-end reads with 100X mean target coverage. NGS generated raw fastq files as its output. FastQC software was used to determine the quality of this file. The Burrows-Wheeler Aligner (BWA) program was then used to align the reads to the reference human genome (hg19). The software Genome Analysis Toolkit (GATK) was used to detect variants. Variants in circular DNA have been identified using the Integrative Genomic Viewer (IGV) program, which further assisted in preparing the library for MGI sequencing.

2.5. Preparation and quantification of mRNA and miRNA

MicroRNA and mRNA extraction were performed using the FavorPrepTM MicroRNA Isolation Kit and FavorPrepTM mRNA Isolation Kit (both from FAVORGEN BIOTECH CORP, Taiwan), following the manufacturer's instructions. Total microRNA and mRNA quality were measured with a NanoDrop spectrometer (Biometrics, OneDrop TOUCH Pro/Lite Micro-Volume Spectrophotometer, Wilmington, USA), measuring the 260/280 nm wavelength ratio. cDNA synthesis of MicroRNAs (using reverse transcriptase with microRNA-specific stem-loop primers) and mRNA was conducted using the AddScript cDNA Synthesis Kit (add bio), as per the manufacturer's instructions, targeting 9 specific MicroRNAs (miRNA15a, miRNA16, miRNA21, miRNA125a, miRNA125b, miRNA155, miRNA192, miRNA221, miRNA497). Specific RT primers were used to detect each microRNA, while oligo (dT) primers were used with mRNA for housekeeping gene detection (Table 1). PCR reactions were performed, and qRT-PCR was conducted to determine microRNA expression using the SYBR green method following the manufacturer's guidelines (AddScript RT-PCR SYBR Master (2x conc.), Korea). A universal reverse primer and a specific forward primer for each microRNA were employed in the qRT-PCR reaction (Table 1); all primer sequences used in this study were provided in each PCR tube and held a total volume of 25 μl for the RT-PCR process. The components were as follows: 2 μl of cDNA template RT+/RT-/water/patient sample/control; 9 μl of PCR water; 0.5 μl of each of the 100 pmol/μl forward and reverse primers; and 13 μl of SYBR Green PCR Master Mix as described in

Table 1
List of RT-PCR, microRNA forward, and universal primer sequences.

No	microRNAs	Primer Sequence	Primer Type
1	**miRNA-15a**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACAAAC-3	Revers Transcript
2	**miRNA-16**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCCAA-3	
3	**miRNA-21**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAACA-3	
4	**miRNA-125a**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCACAG-3	
5	**miRNA-125b**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC-3	
6	**miRNA-155**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCCTAT-3	
7	**miRNA-192**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACTGG-3	
8	**miRNA-221**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAAACCCATCACAA-3	
9	**miRNA-497**	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAACCA-3	
10	**miRNA-15a**	5′-GCGGCTAGCAGCACATAATGG-3	Forward
11	**miRNA-16**	5′-TAGCAGCACGTAAATATTGGCG-3	
12	**miRNA-21**	5′-GCCCGCTAGCTTATCAGACTGATG-3	
13	**miRNA-125a**	5′-TCCCTGAGACCCTTTAACCT-3	
14	**miRNA-125b**	5′-CGTCCCTGAGACCCTAACTT-3	
15	**miRNA-155**	5′-CTCAGACTCGGTTAATGCTAATCGTGATAGG-3	
16	**miRNA-192**	5′-CTGACCTATGAATTGACAGCCA-3	
17	**miRNA-221**	5′-GTTGGTGGGAGCTACATTGTCTGC-3	
18	**miRNA-497**	5′-TCGGGCAGCAGCACACTGTG-3	
21	**All miRNAs**	5′-GTGCAGGGTCCGAGGT-3′	Universal Reverse
22	**β-actin**	Forward 5′-ACTCGTCATACTC CTGCT-3′ Reverse 5′-GAAACTACCTTCAACTCC-3′	Housekeeping gene

(Khan et al., 2023).

2.6. Analysis of miRNA-mRNA integration

We used Cytoscape software to create a network depicting the interaction between miRNAs and genes. The prediction of target genes for miRNAs was conducted through TargetScanHuman.

2.7. Statistical analysis

NCSS 2021 (version 21.0.3) and Graph Pad Prism statistical software (version 9.01, Graph Pad, San Diego, CA, USA) were used to perform the statistical tests. A P-value of less than 0.05 was taken to be statistically significant for every test. The relative quantity of each microRNA25 was calculated using the $2-\Delta\Delta Ct$ method(Kim et al., 2021). The result has been normalized using β-actin as an endogenous control to adjust for changes in cDNA levels.

3. Results

3.1. Immunophenotypic analysis of AML

Cell surface markers, also called antigens, are detected on cells through flow cytometry immunophenotyping, aiding physicians in diagnosis. Immunophenotyping in flow cytometry analysis reveals a characteristic profile of AML, marked by the presence of common markers CD45, CD99, CD34, HLA-DR, CD38, and myeloid-specific markers CD33, CD13, and MPO, alongside CD15 and CD117. Additionally, AML cases with a monocytic component exhibit CD11c, CD64, CD14, and CD36 expression. Notably, an aberrant phenotype is characterized by elevated CD99, CD33, and CD45 expression levels, observed in approximately 90.56 %, 71.93 %, and 70.25 % of cases, respectively. In essence, leukemic cells in AML display increased expression of CD45, CD99, CD34, HLA-DR, and CD38 while demonstrating reduced expression of B-lymphoid-specific markers CD10, CD19, CD20, CD22, CD79a, and T and NK lymphoid specific markers CD1a, CD2, CD3, CD4, CD5, CD7, and CD56 (Fig. 1).

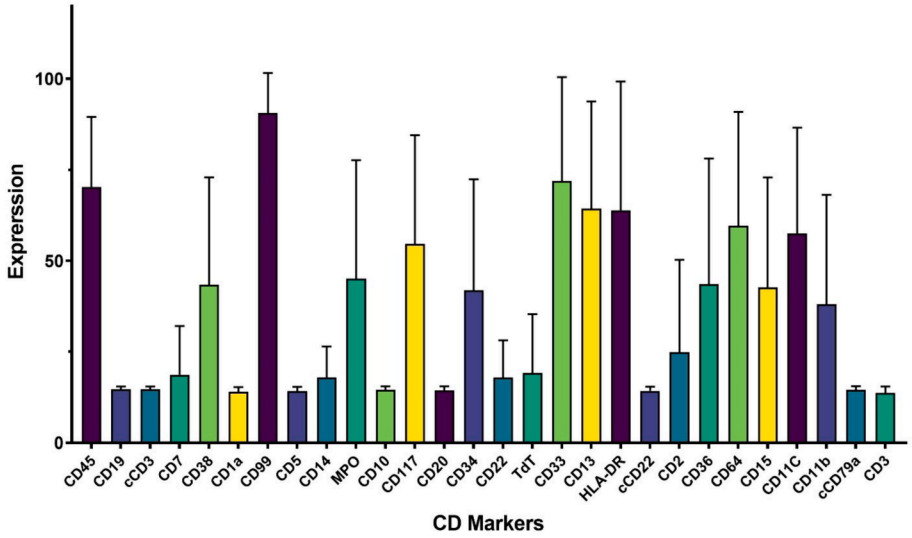

Fig. 1. Diagnosis of AML based on reactivity with various cell surface markers. The bar chart displays the expression levels of various CD markers, measured through flow cytometry. Each bar represents a specific CD marker and its height corresponds to the average expression level within a population of cells. The error bars represent the mean ± standard deviation, indicating the variability in expression levels.

3.2. Quality assessment of NGS data

An average of 21,97 M reads was obtained. 97,7 % of all reads were filter-passed aligned unique average reads (21,46 M) per sample. Among those reads 71 % were on bait reads. The mean bait coverage was 122. Percent of target bases covered 10x or more was an average of 99,3 %. The percentage of target bases covered 30x or more was 97,2 %. The percentage of target bases covered 50x or more was 82,4 %.

3.3. Gene mutations detected by NGS

Mutations were assessed in six genes (FLT3, KIT, PTPN11, BCR, DNMT3A, and NRAS) through Whole-Exome Sequencing (WES) in AML patients. Eight variants were detected across six patients, each exhibiting at least one mutation. FLT3 was the most frequently mutated gene (25 %), followed by KIT (12.5 %), PTPN11 (12.5 %), BCR (12.5 %), DNMT3A (12.5 %), FLT3-ITD (12.5 %), and NRAS (12.5 %). These variants comprised five missense mutations, one frameshift mutation, and two synonymous mutations. Six variants were identified as somatic mutations, while two were heterozygous mutations. Table 2 provides a detailed overview of the variant characteristics.

3.4. The differential expression patterns of miRNAs by qPCR

The relative fold change analysis for the different microRNAs between the groups revealed that eight microRNAs displayed significant differences between AML and the healthy group. In addition, the difference in microRNA-15a expression was non-significant in this comparison. Also, the results of qPCR data analysis showed that all microRNAs were overexpressed in AMLs compared to normal controls (Table 3).

3.5. Data integration and analysis

To explore the regulatory dynamics between microRNAs (miRNAs) and the mutated genes, we constructed an interaction network illustrating their associations (see Fig. 2). In this network, hsa-miR-16 and hsa-miR-497 assumed pivotal roles as regulators, each directing their influence towards four unique genes. PTPN11 garnered attention as a central gene node, subject to regulation by six distinct microRNAs. KIT and BCR were also identified as second genes controlled by four distinct microRNAs. Moreover, the interaction of FLT3, NRAS and DNMT3A genes was regulated through three different microRNAs.

4. Discussion

The diagnosis and classification of AML require a meticulous approach, as the World Health Organization (WHO) advised, encompassing a comprehensive blend of clinical history, morphological evaluation, cytogenetic/molecular genetic analysis, and immunophenotyping. Nonetheless, the absence of cytogenetic/molecular analyses in numerous healthcare facilities underscores the utility of cytomorphological evaluation supplemented by immunophenotyping, which proves adequate for expedited diagnosis and classification of AML

Table 3
The microRNA expression pattern between AML patients and healthy controls.

No.	microRNAs	Expression level		p-value
		AML patients	Control group	
1	**microRNA 15a**	**1.428 ± 0.441**	**2.268 ± 0.5444**	**0.4551**
2	**microRNA 16**	**3.126 ± 1.426**	**8.11 ± 2.154**	**0.0051**
3	**microRNA 21**	**16.92 ± 9.407**	**44.61 ± 14.22**	**0.0003**
4	**microRNA 125a**	**2.016 ± 0.776**	**7.856 ± 2.117**	**0.0256**
5	**microRNA 125b**	**18.46 ± 9.371**	**20.3 ± 10.69**	**0.0115**
6	**microRNA 155**	**4.57 ± 2.099**	**15866 ± 4047**	**0.0001**
7	**microRNA 192**	**4.485 ± 2.693**	**97.14 ± 16.07**	**0.0001**
8	**microRNA 221**	**2.17 ± 0.6075**	**25.5 ± 5.199**	**0.0001**
9	**microRNA 497**	**3.511 ± 1.35**	**15.4 ± 3.539**	**0.0001**

This table illustrates the expression patterns of microRNAs in AML patients compared to healthy samples. It visually depicts the fold changes observed for specific microRNAs, highlighting any statistically significant findings with a star symbol.

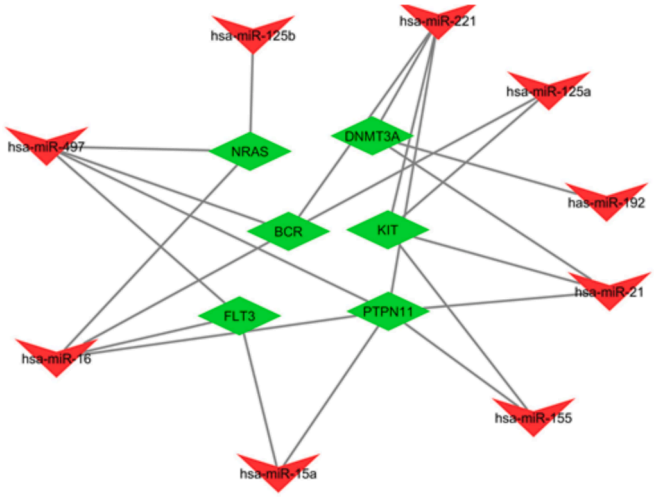

Fig. 2. The miRNA-gene interaction network was constructed with Cytoscape software.

(de Morais et al., 2022). the present study evaluated the levels of various cytokines in AML using flow cytometry for diagnostic purposes. Flow cytometric immunophenotypic analysis was conducted on all patients. The expression levels of CD99, CD33, CD45, CD13, HLA-DR, CD64, CD11c, and CD117 ranged from 54.58 % to 90.56 % in all cases. Meanwhile, MPO, CD38, CD36, CD15, CD34, CD11b, and CD2 exhibited expression levels between 24.48 % and 45.06 %, with the remaining CD markers showing expression levels below 20 % in all cases. These findings were consistent with previous studies on AML (Rasheed et al., 2021, Piñero et al., 2022). Previous researchers noted that markers such as MPO, CD13, CD33, CD15, and CD117 are specific to myeloid cells, while CD11c, CD64, CD14, and CD36 indicate monocytic lineage. Additionally, markers like CD19, CD22, CD10, CD79a, and CD20 are associated with B-lymphoid cells, while CD1a, CD2, CD3, CD4, CD5,

Table 2
Single nucleotide variants found in six AML patients.

No.	Gene names	DNA variant	Protein Variant	Zygosity	Variants classification
1	FLT3	0c.2508_2510: del ATG>?	p.I836del	41 % Somatic	Likely Pathogenic
2	KIT	0c.54733270: T > C	Intronic	71 % Somatic	Uncertain Significance
3	PTPN11	0c.523: A > G	p.M175V	Heterozygote	Pathogenic
4	BCR	0c.3184: G > A	p.D1062N	Heterozygote	Uncertain Significance
5	DNMT3A	0c.2645: G > A	p.Arg882His	22 % Somatic	Pathogenic
6	NRAS	0c.38: G > A	p.Gly13Asp	39 % Somatic	Pathogenic
7	FLT3	ITD Variant	ITD	27 % Somatic	Pathogenic
8	FLT3	0c.1789: T > A	p.Tyr597Asn	18 % Somatic	Uncertain Significance

CD7, and CD56 are linked to T-lymphoid/natural killer cells. Moreover, CD34, CD45, CD99, HLA-DR, Terminal deoxynucleotidyl transferase (TdT), and CD38 are commonly used as progenitor markers for diagnosing and classifying AML (Weir and Borowitz, 2001, Peters and Ansari, 2011). The expression of nine microRNAs in AML leukemia patients was evaluated. Notably, eight of these microRNAs exhibited a substantial increase in expression, while one showed no significant difference, as depicted in Table 3. Concurrently, we utilized NGS techniques to characterize the mutations (variants) in various genes, revealing multiple mutations in certain gene loci.

Furthermore, we constructed a comprehensive miRNA-gene interaction network to visually represent the intricate relationships among six genes and the nine microRNAs. This approach facilitated a holistic understanding of the regulatory interactions between microRNAs and their target genes within the context of AML pathology. Consequently, by identifying these target genes, a clearer insight into the specific roles of microRNAs in the pathogenesis of AML can be gained, enriching our understanding of the disease mechanisms at the molecular level. The diverse genetic and molecular landscape of AML contributes to differences in disease presentation, progression, and response to therapy among patients. Understanding this heterogeneity is crucial for developing personalized treatment approaches tailored to individual patients' molecular profiles and disease characteristics (Ishikawa, 2023). Identifying pivotal molecular events, such as DNA mutations and aberrant microRNA expression, is essential for understanding AML's underlying mechanisms. Dysregulated microRNA expression in AML contributes to leukemia development and progression, offering diagnostic and prognostic potential (Jurgec et al., 2022). Next-Generation Sequencing (NGS) has unveiled recurrent DNA mutations and gene expression patterns in AML, revealing its genetic complexity. NGS-based transcriptomic profiling has identified novel gene signatures associated with AML subtypes and progression. DNA mutations can influence microRNA expression, altering target gene regulation and contributing to AML pathogenesis (Makkar et al., 2023). Given the critical nature of this concern and the common occurrence of late disease detection, our focus lies in devising methodologies to enable early and prompt diagnosis of AML.

The utilization of Whole-Exome Sequencing (WES) to assess mutations in six specified genes (FLT3, KIT, PTPN11, BCR, DNMT3A, and NRAS) presents a comprehensive approach to understanding the genetic landscape of AML. WES offers a high-throughput method for analyzing the protein-coding regions of the genome, which are known to harbor a significant portion of disease-causing mutations (Seaby and Ennis, 2020). By focusing on these specific genes, the study aimed to identify genetic variations that may contribute to AML pathogenesis, potentially uncovering novel therapeutic targets or prognostic markers. This approach provides valuable insights into the molecular mechanisms underlying AML and lays the groundwork for personalized treatment strategies tailored to the genetic profile of individual patients. In parallel, the investigation into the expression levels of nine microRNAs (miRNA15a, miRNA16, miRNA21, miRNA125a, miRNA125b, miRNA155, miRNA192, miRNA221, and miRNA497) offers a deeper understanding of the regulatory networks involved in AML development and progression. MicroRNAs play pivotal roles in post-transcriptional gene regulation, influencing various cellular processes in cancer, including cell proliferation, differentiation, and apoptosis (Liz and Esteller, 2016). This integrated approach allowed for a comprehensive exploration of the molecular mechanisms driving AML, facilitating the identification of novel therapeutic targets for improved disease management. The study sought to unravel the intricate molecular networks underlying AML pathogenesis by examining the relationship between microRNA expression and DNA mutations. The study involved six patients and observed a direct correlation between elevated microRNA expression and mutations in their target genes. These findings are consistent with prior research documenting similar associations, providing further support for the role of microRNAs in AML pathogenesis.

The FLT3 gene encodes the FMS-like receptor tyrosine kinase-3 (RTK), crucial in regulating cell proliferation, survival, and differentiation in hematopoietic cells. Mutations in the FLT3 gene, particularly FLT3-ITD (internal tandem duplication) and FLT3-TKD (tyrosine kinase domain) mutations are common genetic abnormalities observed in acute myeloid leukemia (AML) patients. These mutations result in constitutive activation of FLT3 signaling pathways, leading to uncontrolled growth and survival of leukemic cells (Parcells et al., 2006, Müller and Schmidt-Arras, 2020). Several studies have reported that hsa-miR-15a, hsa-miR-16, and hsa-miR-497 can directly target FLT3 mRNA, thereby modulating its expression levels. Specifically, these miRNAs have been shown to downregulate FLT3 expression, potentially inhibiting FLT3 signaling and leukemic cell proliferation. Dysregulation of these miRNAs, leading to aberrant FLT3 expression, has been implicated in AML pathogenesis and progression (Dong et al., 2009, Rossi et al., 2013, Sueur et al., 2020).

The KIT gene encodes a 145-kDa transmembrane glycoprotein and belongs to the type III-RTK family. When the KIT receptor binds to its ligand, stem cell factor (known as KIT ligand), it undergoes dimerization and subsequent autophosphorylation at specific tyrosine sites. This activation leads to the initiation of various signal transduction pathways crucial for regulating cell proliferation, survival, self-renewal, and differentiation. Somatic mutations resulting in the gain of function of the KIT receptor are found in approximately 10 % of acute myeloid leukemia (AML) patients. However, these mutations are more commonly observed in patients with CBF-AML rearrangements. In this subgroup of AML, the presence of KIT mutations may contribute to a poorer prognosis despite the typically favorable outlook associated with this AML subtype (Gu et al., 2018, Tarlock et al., 2019). Fig. 2 shows that this gene is the target for hsa-miR-21, hsa-miR-221, and hsa-miR-125a, which aligns with the research of Moussa Agha et al., 2020 (Moussa Agha et al., 2020).

In AML, the non-receptor protein tyrosine phosphatase PTPN11, also known as SHP2, plays a significant role. PTPN11 is involved in various signaling pathways crucial for cellular proliferation, survival, and differentiation. PTPN11 mutations are observed in nearly 5 % to 10 % of AML cases, with a higher frequency noted in older patients, particularly those with secondary AML (Kishtagari and Levine, 2021).

Moreover, evidence suggests a potential regulatory relationship between PTPN11 and certain microRNAs (miRNAs), specifically those overexpressed in AML. These miRNAs, known as hsa-miRNAs, may target PTPN11 mRNA and modulate its expression levels, thus influencing the activity of PTPN11 and its downstream signaling pathways. Dysregulated expression of these miRNAs could potentially lead to the pathogenesis of AML via modifying the balance of signaling networks controlled by PTPN11 (COSTA, E. A. S. , 2016).

The DNMT3A gene encodes DNA methyltransferase 3A, an enzyme involved in DNA methylation, a critical epigenetic modification regulating gene expression. Mutations in the DNMT3A gene are commonly observed in AML patients, occurring in approximately 20–30 % of cases (A. Khrabrova et al., 2019). Recent studies have implicated several microRNAs (miRNAs) in AML pathogenesis, including hsa-miR-192, hsa-miR-221, and hsa-miR-21. These miRNAs are dysregulated in AML patients and may affect disease progression and treatment response (Wallace and O'Connell, 2017, Fletcher et al., 2022). Our results agree with the results of the present study.

Mutations in the NRAS gene are frequently observed, particularly in patients with specific disease subtypes. NRAS is a proto-oncogene that encodes a GTPase protein, which plays a crucial role in cell signaling pathways, regulating cell growth, differentiation, and survival. Mutations in NRAS lead to constitutive activation of these signaling pathways, contributing to the development and progression of AML (Wang et al., 2020).

Recent investigations have unveiled a variety of microRNAs that are dysregulated in AML and play roles in disease pathogenesis. Among these miRNAs are hsa-miR-16, hsa-miR-497, and hsa-miR-125, which

have been implicated in regulating NRAS gene expression and cellular processes relevant to cancer development and progression (Hussen et al., 2021, Kipkeeva et al., 2022).

The BCR gene is a key player in the pathogenesis of several cancers, including AML. It encodes a protein regulating cell growth, differentiation, and survival. Dysregulation of the BCR gene, often through chromosomal translocations, can lead to the formation of oncogenic fusion proteins that drive leukemogenesis (Boucher et al., 2023). In the context of AML, aberrant expression of microRNAs has been implicated in disease progression. Specifically, miRNAs such as hsa-miR-16, hsa-miR-497, and hsa-miR-125 have been identified as potential regulators of gene expression and cellular processes relevant to cancer development. These miRNAs may target the BCR gene, influencing its expression levels and activity. Dysregulation of hsa-miR-16, hsa-miR-497, and hsa-miR-125 could thus contribute to aberrant BCR signaling and AML pathogenesis (Szczepanek, 2020). Understanding the regulatory interactions between genes and these miRNAs in AML patients is crucial for elucidating the molecular mechanisms underlying the disease and identifying potential therapeutic targets. Targeting the dysregulated expression of these miRNAs or modulating gene activity may represent promising strategies for AML treatment. Further investigation into the functional roles of hsa-miRNAs in gene regulation and AML pathogenesis is needed to validate their potential as therapeutic targets or diagnostic biomarkers.

5. Conclusions

The interplay between flow cytometry (CD marker expression) and molecular (microRNA expression and DNA mutations) techniques in AML provides a promising avenue for identifying novel therapeutic targets. Dysregulated microRNAs and the mutations they influence represent potential targets for therapeutic intervention in AML treatment. Targeting aberrantly expressed microRNAs and their downstream pathways and addressing specific DNA mutations makes it possible to develop more precise and tailored therapeutic strategies. This approach can disrupt the molecular mechanisms driving AML progression, improving treatment outcomes and overcoming resistance to conventional therapies. Furthermore, by targeting multiple components of the dysregulated molecular network, including microRNAs and DNA mutations, a synergistic therapeutic effect may be achieved, leading to enhanced efficacy and reduced side effects. Overall, exploring the interplay between microRNA expression and DNA mutations opens new avenues for developing innovative and personalized treatments for AML.

CRediT authorship contribution statement

Rastee H. Saeed: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zirak Faqe Ahmed Abdulrahman:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Dara K. Mohammad:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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