Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i4.3590

Research Article

Mir-Let-7i-5p As A Novel Regulator of MYC Gene Expression in Acute Myeloid Leukaemia

Ezalia Esa[1], Wan Muhammad Farhan Syafiq Bin Wan Mohd Nor[2], Elsa Haniffah Mejia Mohamed[2], Wei-Yue Lim[2], Zubaidah Zakaria[1], Yuslina Mat Yusoff[1], Ivyna Pau Ni Bong[1], Yuh-Fen Pung[3], Noor Atiqah Fakharuzi[1], Shamsul Mohd Zain[2]*

1. Haematology Unit, Cancer Research Centre, Institute for Medical Research, Jalan Pahang, Kuala Lumpur, Malaysia
2. Department of Pharmacology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia
3. Division of Biomedical Science, School of Pharmacy, University of Nottingham Malaysia, Semenyih, Selangor, Malaysia

*Corresponding author: shamsul@um.edu.my

ABSTRACT

Acute myeloid leukaemia (AML) is a severe bone marrow malignancy with a high mortality rate. Recent advancements in high-throughput technology and bioinformatics have facilitated the identification of microRNAs (miRNAs) involved in the pathogenesis of AML. This study aimed to investigate the potential role of MicroRNA-let-7i-5p (miR-let-7i-5p) in the progression of AML. MiR-let-7 family has been widely utilised as a tumour inhibitor, suppressing its expression in several types of human cancer. Hence, dysregulation of miR-let-7i-5p is critical to cancer progression. However, the role of miR-let-7i-5p in AML remains unclear. Using the Gene Expression Omnibus (GEO) database, the expression of miR-let-7i-5p in AML patients was investigated. The role of miR-let-7i-5p and its potential target genes in AML was examined using bioinformatic tools. Subsequently, the effect of miR-let-7i-5p was assessed through transfection, followed by the analysis of target gene expression using RT-qPCR. Further investigations employed the MTT and cell cycle assays on the effects of miR-let-7i-5p on AML cell proliferation and cell cycle. According to the GEO database, miR-let-7i-5p was significantly downregulated in AML patients compared to controls. AML development has been associated with miR-let-7i-5p by KEGG pathway analysis. Bioinformatics suggested that miR-let-7i-5p targets MYC in AML cells. Transfection of miR-let-7i-5p into AML cell lines decreased MYC expression, suggesting MYC regulates AML progression. However, MTT and cell cycle assays showed no significant effect of miR-let-7i-5p on AML cell proliferation and cell cycle, indicating the potential involvement of miR-let-7i-5p in alternative pathways in AML pathogenesis. The study highlights the potential significance of miR-let-7i-5p in regulating AML progression.

Key words: Acute myeloid leukaemia, miR-let-7i-5p, MYC, cell proliferation, cell cycle

INTRODUCTION

Acute myeloid leukaemia (AML) has presented a formidable challenge to the medical community. Despite substantial treatment advancements over the past several decades, the prognosis for many patients remains grim (Thol & Heuser, 2021). Fortunately, the recent progress in understanding the underlying pathophysiology of AML and the development of high-throughput technologies and bioinformatics have led to exciting new possibilities for improved diagnostic and therapeutic approaches (Dunlap *et al*., 2012). Furthermore, targeted therapies have been increasingly recognised as viable treatment options due to their established efficacy. This is evidenced by global regulatory bodies' recent approval of numerous FLT3 inhibitors (Arai *et al*., 2022).

A key challenge in developing effective treatments for AML is the high degree of genetic heterogeneity among patients, which necessitates the classification of AML into distinct subtypes to ease the diagnosis and treatment. The type of AML has evolved. Introducing the French-American and British (FAB) classification system in 1976 was a crucial milestone in this field (Bennett *et al*., 1976). Since then, the World Health Organization (WHO) has revised the classification system (Hwang, 2020). Recently, the fifth edition of the WHO classification of AML 2022 has been introduced, emphasizing the importance of genetic and histological covariates in subtyping AML (Khoury *et al*., 2022). With the rapid pace of advancements in high-throughput technologies and data science, researchers can now perform comprehensive genetic analyses of AML patient samples, which promises to enhance the understanding of the disease and inform the development of more targeted treatments. This can be viewed by a recent study demonstrating a comprehensive miRNA analysis on AML patient samples using microarray technology and bioinformatics (Esa *et al*., 2021).

Nonetheless, the alarming statistics from the National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER) in the United States indicate that much work is still needed. In 2022, 20,050 new AML cases and an estimated 11,540 deaths were reported. In addition, the 5-year relative survival rate for AML from 2012-2018 was only 30.5% ('Surveillance, Epidemiology & End Results Program', 2023). Therefore, enhancing the classification of AML through a more comprehensive genetic analysis is crucial to developing better diagnostic and treatment approaches.

Article History
Accepted: 6 October 2025
First version online: 28 December 2025
Cite This Article:
Esa, E., Wan Mohd Nor, W.M.F.S.B., Mohamed, E.H.M., Lim, W.Y., Zakaria, Z., Mat Yusoff, Y., Bong, I.P.N., Pung, Y.F., Fakharuzi, N.A. & Zain, S.M. 2025. Mir-Let-7i-5p as a novel regulator of MYC gene expression in acute myeloid leukaemia. Malaysian Applied Biology, 54(4): 146-153. https://doi.org/10.55230/mabjournal.v54i4.3590
Copyright
© 2025 Malaysian Society of Applied Biology

The regulatory function of miRNA in cancer progression, including AML, has been intensively studied for its role in regulating post-transcriptional biological processes by degrading or repressing the translation of its target gene (MacFarlane & Murphy, 2010). For instance, it has been shown that inhibiting miR-9 expression with an antagomir significantly inhibits the proliferation of MLL-rearranged AML cells (Chen *et al*., 2013). With the development of bioinformatics tools, computational miRNA-mRNA prediction followed by in vitro validation has become a standard protocol in the study of miRNA, providing hope in understanding the role of miRNA in AML (Zhang *et al*., 2019). Among the various types of miRNAs, the miR-let-7 family has been widely utilised as a tumour inhibitor, suppressing its expression in several types of human cancer (Barh *et al*., 2010). Song *et al*. demonstrated the inhibitory role of miR-let-7i-5p in the progression of colon cancer by targeting KLK6 (Song *et al*., 2018). However, its biological function and mechanisms in AML remain unknown. Therefore, additional research is required to reveal miRNAs' possible role in AML classification. In this study, we aim to investigate the expression of miR-let-7i-5p in AML cell lines and to examine the association of miR-let-7i-5p expression with AML cellular response. Specifically, we hypothesize that miR-let-7i-5p is downregulated in AML and functions as a tumour suppressor by directly targeting the oncogene MYC. We further hypothesize that the re-expression of miR-let-7i-5p will inhibit the proliferation of AML cells and induce cell cycle arrest, thereby suppressing the malignant phenotype.

MATERIALS AND METHODS

Methods

Cell lines and culture media

B-myelomonocytic leukaemia cell line, MV4-11, and acute monocytic leukaemia cell line, THP-1 (ATCC, USA) were employed. MV4-11 was cultured in Iscove's Modified Dulbecco's Medium (IMDM; Simply Biologics) enriched with 10% of fetal bovine serum (FBS; GIBCO) and 1% of penicillin-streptomycin (GIBCO). THP-1 was cultured in Roswell Park Memorial Institute (RPMI; Simply Biologics) 1640 Medium enriched with 10% of fetal bovine serum (FBS; GIBCO), 1% of penicillin-streptomycin (GIBCO), and 2-mercaptoethanol (2ME). All cell lines were maintained at 37 °C humidified atmosphere in an incubator with 5% CO2 saturation. Transfections of miRNA were deprived of penicillin-streptomycin.

Transfection of miRNAs

The Ambion miR-let-7i-5p precursor and mirVana miRNA mimics Negative Control #1 were purchased from Ambion. To prepare a stock solution of 20 µM, these miRNAs were resuspended in 1x miRNA buffer. MV4-11 and THP-1 cell lines were reversely transfected at a final concentration of 25 nM using Lipofectamine 3000 (Life Technologies) transfection reagent according to the manufacturer's protocols. Briefly, the cells and medium were added to a 96-well plate accordingly. The complexes of miRNA-Lipofectamine were prepared in a 1.5 mL microcentrifuge tube and incubated at room temperature for 20 min. Then, the complexes were aliquoted into the wells. RT-qPCR was used to verify the efficacy of transfection.

RNA extraction and RT-qPCR

Total RNA from AML cells was extracted using the NucleoSpin® miRNA Kit (Macherey-Nagel, Germany) by the manufacturer's recommended protocol. The concentration and purity of the isolated RNA were determined by a Nanodrop spectrophotometer (NanoDrop Technologies Inc., USA). cDNA synthesis of extracted RNA was carried out using QuantiNova Reverse Transcription Kit (Qiagen, United States) according to the manufacturer's instructions. The mRNA expression levels were assessed using RT-qPCR using QuantiNova SYBR Green PCR Kit. As specified in Table 1, oligonucleotide primers were synthesised by Integrated DNA Technologies (IDT). The PCR was performed using the ViiA7 Real-Time PCR Systems (Applied Biosystems, USA). Beta-actin was employed as an internal reference gene for data normalisation.

Table 1. Sequence of primers for RT-qPCR

Gene	Primers	Sequence (5'-3')	GenBank Accession No.	Amplicon size (bp)
JAG1	Forward	TACTGGCACCTGCAGTCACC	NM_000214.3	225
	Reverse	GAAGCAGAACACGGGCGTTG		
DUSP10	Forward	CCCAGCCACTTCACATAGTC	NM_007207.6	78
	Reverse	TACTAAGTCCACCTTTCAACACC		
MYC	Forward	CGTCTCCACACATCAGCACAA	NM_002467.6	68
	Reverse	TCTTGGCAGCAGGATAGTCCTT		
ABL1	Forward	AAGCCGCTCGTTGGAACTC	NM_007313.3	141
	Reverse	AGACCCGGAGCTTTTCACCT		
KMT2D	Forward	ACCATGTGAAGAACAGGAAGAG	NM_003482.4	105
	Reverse	TCACCCTGGCTCAGATTAGA		
β-actin	Forward	TTCCAGCCTTCCTTCTTG	NM_007393.5	182
	Reverse	GGAGCCAGAGCAGTAATC		

*The relative expression levels of the genes were calculated using the 2−ΔΔCT.

Cell proliferation assay

In vitro cell proliferation was assayed using the MTT assay. This assay is based on the reduction of the tetrazolium dye MTT to its insoluble formazan by NADPH-dependent cellular oxidoreductase enzymes. The assay measures cell metabolic activity and is performed to determine the number of cells in a culture (Kuete, Karaosmanoğlu, and Sivas, 2017). Briefly, MV4-11 and THP-1 cells were seeded in culture plates at a density of 1 x 104 viable cells /100 µL/well in triplicate. Then, the cells were transfected with miR-let-7i-5p mimic and a negative control. The negative control consisted of a non-targeting miRNA mimic

with a scrambled sequence that is not expected to bind to any known human mRNA. Subsequently, 10 µL of 5 mg/mL MTT was put into each 96 wells at 37°C. After 4 hr, 100 µL of SDS was added to dissolve the formed purple crystals. The absorbance was measured using a spectrophotometer at a wavelength of 570 nm. The process was repeated for 24 hr, 48 hr, and 72 hr post-transfection.

Flow cytometry assay

For cell cycle analysis, cells were stained with propidium iodide (PI) using the CycleTEST™ Plus DNA Reagent Kit (BD Biosciences, USA). AML cell lines were seeded onto 6-well plates at a 1×10^5 cells/well density at 37°C. 72 hr later, the cells were harvested, and the cell cycle was determined according to the manufacturer's protocol. Briefly, the cells were incubated with 150 µL liquid A at room temperature for 10 min and 150 µL liquid B for 10 min. Cells were subsequently incubated with 120 µL liquid C in the dark for 10 min. Then, the proportion of cells in the G0/G1, S, and G2/M phases was determined by using a FACSCanto II flow cytometer (BD Biosciences). The result was analysed using FlowJo software 10.9 (TreeStar Inc., USA)

Bioinformatics analysis

The present study employed miRNA expression data from GSE142699, which was publicly accessible. The dataset comprised 48 samples, comprising 24 AML samples and healthy samples. The differential analysis was conducted utilising the GEO2R tool, with the control group consisting of healthy samples.

To predict potential target genes of the miRNAs that were differentially expressed, three bioinformatics tools, namely TargetScan (https://www.targetscan.org/vert_80/), TargetMiner (https://www.isical.ac.in/~bioinfo_miu/targetminer20.htm), and miRDB (http://www.mirdb.org/), were employed. Additionally, KEGG pathway analysis was performed using DAVID (DAVID: Functional Annotation Tools, n.d.) (https://david.ncifcrf.gov/tools.jsp) to validate the participation of the predicted target genes in the pathogenesis pathway of AML.

Statistical analysis

Results were expressed as the mean ± SEM. Differences between two groups were statistically analysed using Student's t-test, while differences between multiple groups were statistically analysed using one-way ANOVA (GraphPad Prism 6, GraphPad Software, San Diego, CA, USA). A *p-value*<0.05 is considered statistically significant.

RESULTS

Up-regulation of miR-let-7i-5p Expression in AML Cells

Overexpression of miR-let-7i-5p markedly elevated its expression in both AML cell lines compared with the negative control group. In MV4-11 cells, miR-let-7i-5p expression increased by approximately 2.2-fold ($p<0.05$), whereas in THP-1 cells, the increase was about 9.2-fold ($p<0.01$), indicating a stronger induction (Figure 1).

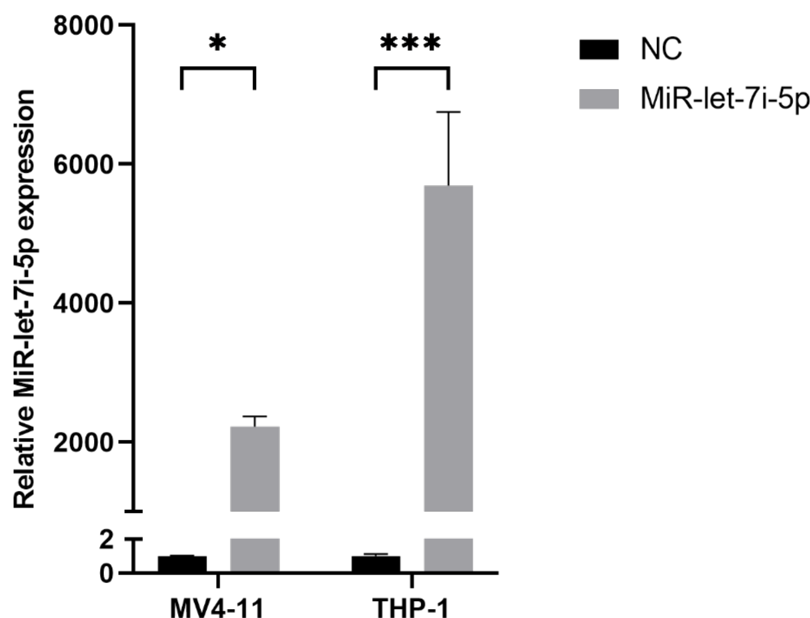

Fig. 1. miR-let-7i-5p overexpression in AML cell lines. Transfection with miR-let-7i-5p mimics markedly increased expression in MV4-11 (~2.2-fold, $p<0.05$) and THP-1 (~9.2-fold, $p<0.01$) cells compared with negative control.

MiR-let-7i-5p expression is downregulated in AML

MiR-let-7i-5p expression is downregulated in AML. To examine the relevance of miR-let-7i-5p in AML, differential analysis was performed on the miRNA expression matrix data of AML patients obtained from the GEO database (GSE142699) using GEO2R. In a cohort comprising 24 AML patients and 24 healthy controls, miR-let-7i-5p expression was significantly lower in AML patients (p=0.0027), showing an approximately 3-fold reduction compared with healthy controls. Figure 2 shows the marked downregulation of miR-let-7i-5p expression in AML compared with healthy controls.

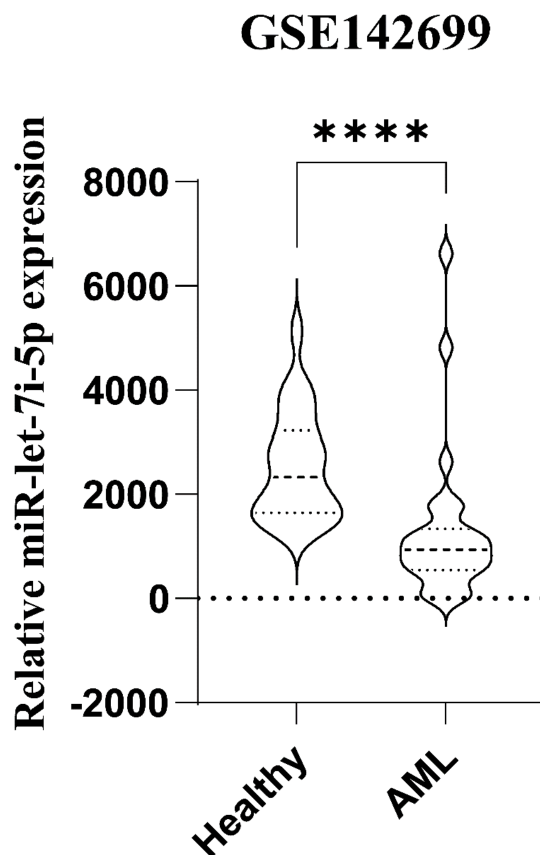

Fig. 2. MiR-let-7i-5p expression is downregulated in AML. (Violin plot of the differentially expressed miRNAs in AML from the GEO dataset (GSE142699). MiR-let-7i-5p expression in AML patients was significantly downregulated compared to healthy controls.

MYC is a putative target gene of miR-let-7i-5p in AML.

Identification and validation of MYC as a putative miR-let-7i-5p target in AML

Putative targets of miR-let-7i-5p were predicted using TargetScan, miRDB, and TargetMiner. Genes predicted by all three tools were retained, resulting in 264 overlapping candidates. These were cross-referenced with the GSE142700 dataset to identify transcripts significantly dysregulated in AML (adjusted p<0.05). Given the ~3-fold downregulation of miR-let-7i-5p in AML, candidates upregulated in AML were prioritised (Figure 3A).

KEGG pathway enrichment analysis of the 264 candidates using DAVID revealed significant enrichment in the PI3K–Akt signalling pathway (38 genes, $p=1.58\times10^{-4}$), p53 signalling pathway (21 genes, $p=3.16\times10^{-4}$), and JAK–STAT signalling pathway (17 genes, $p=1.58\times10^{-3}$) (Figure 3A). Among these, MYC emerged as a top candidate based on: (i) prediction by all three tools, (ii) significant upregulation in AML, and (iii) central involvement in multiple enriched pathways.

To experimentally evaluate MYC regulation, MV4-11 and THP-1 cells were transfected with either miR-let-7i-5p mimics or a negative control. Transfection with miR-let-7i-5p mimics significantly reduced MYC expression in MV4-11 (p=0.0047) and THP-1 (p=0.0015) (Figure 3C).

In parallel, four additional predicted targets (JAG1, KMT2D, DUSP10, and ABL1), previously suggested by Esa *et al*. (2021), were examined. Transfection of miR-let-7i-5p mimics did not significantly alter the expression of these genes in either cell line (p>0.05), indicating that MYC, but not these candidates, is likely a primary functional target of miR-let-7i-5p in AML (Figure 3B).

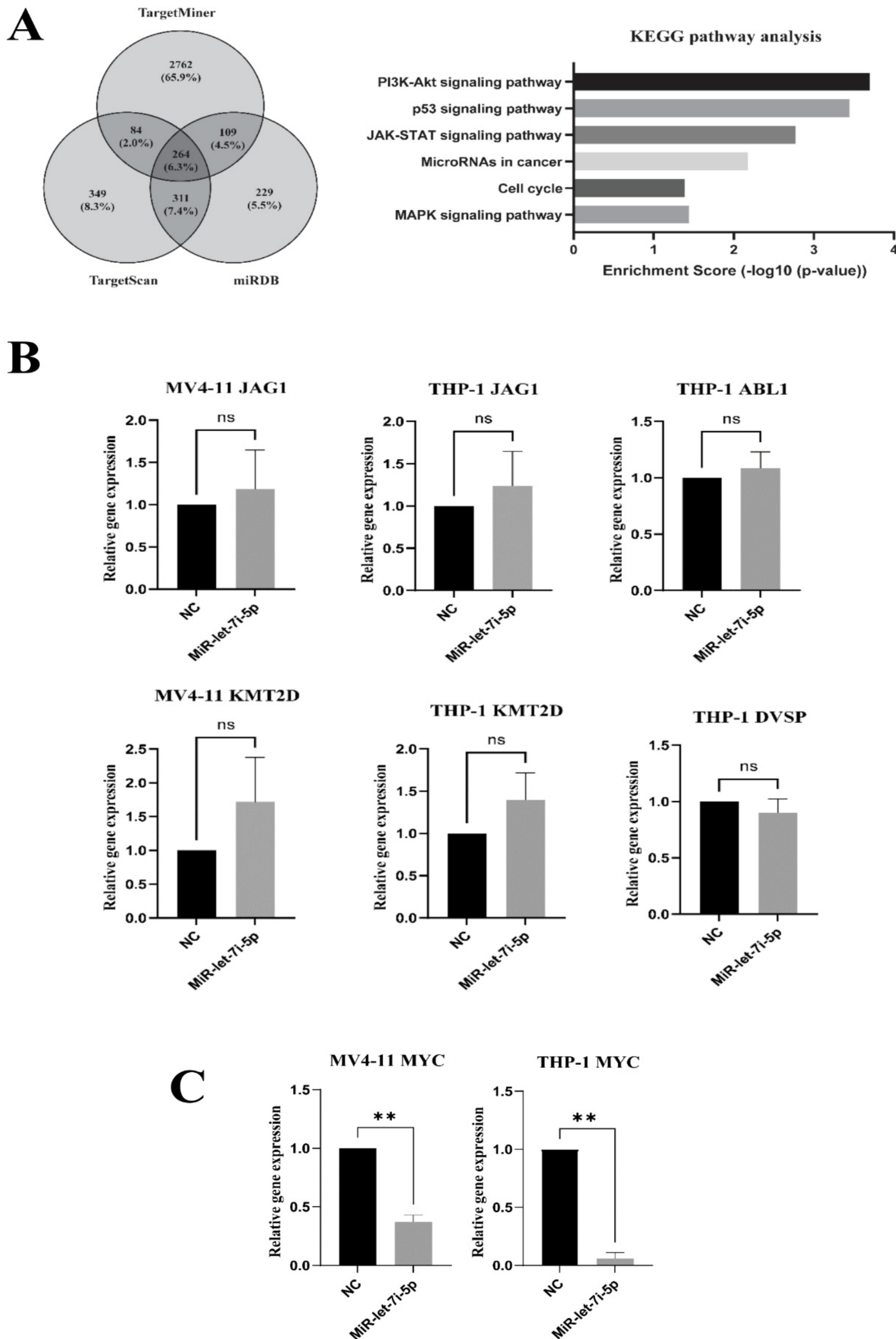

Fig. 3. MYC is a putative target gene of miR-let-7i-5p in AML. (A) Putative targets for miR-let-7i-5p were predicted using TargetScan, release 8.0, miRDB, TargetMiner, and the GSE142700 dataset. (B) RT-qPCR detected the JAG1, KMT2D, DVSP, and ABL1 gene expression in miR-let-7i-5p-treated AML cell lines (MV-4-11 and THP-1). The changes in the expression level of JAG1 and KMT2D were detected in both cell lines, but the differences were not significant (p>0.05). The changes in the expression level of DVSP and ABL1 were only detected in THP-1, and the changes were not significant (p>0.05). (C) The MYC gene expression was detected by RT-qPCR in miR-let-7i-5p-treated AML cell lines (MV-4-11 and THP-1). MYC expression in miR-let-7i-5p-treated cells was downregulated compared to the negative control in MV-4-11 (p=0.0047) and THP-1 (p=0.0015).

Effect of miR-let-7i-5p on cell proliferation and cell cycle.

To understand the effects of miR-let-7i-5p on the proliferation capability of AML cells, MV4-11 and THP-1 were transfected with either miR-let-7i-5p or a negative control. MTT assay showed that transfection of miR-let-7i-5p mimic has no significant effect on the proliferation rate of MV-4-11 and THP-1 cells, as shown in Figure 4 (A).

Furthermore, flow cytometry with PI single staining was conducted to study the effects of miR-let-7i-5p on AML cell cycle distribution. Relative to the negative control group, the cell cycle phases of MV4-11 and THP-1 upon transfection with the miR-let-7i-5p mimic group were not significantly changed. Transfection with miR-let-7i-5p mimic did not modify the population percentages in the cell cycle phases MV4-11 and THP-1 compared to their control, as shown in Figure 4 (B).

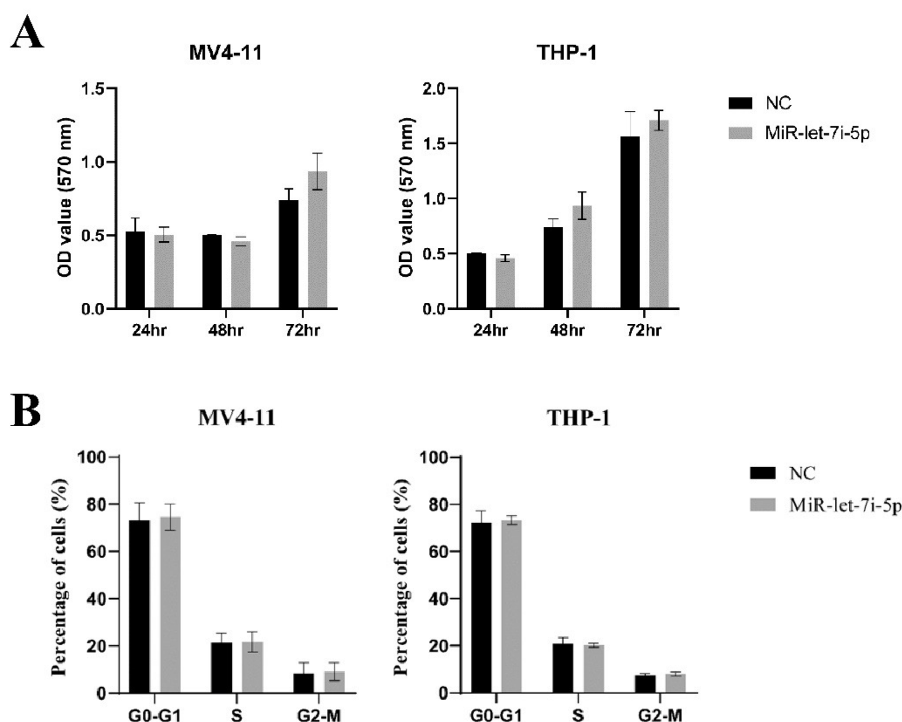

Fig. 4. Effect of miR-let-7i-5p on cell proliferation and cell cycle. (A) Proliferation assay revealed that the transfection of miR-let-7i-5p mimics slightly promoted cell proliferation in MV-4-11 and THP-1 cells compared to the negative control. However, the changes are not significant. (B) Cell cycle progression was assessed in MV4-11 and THP-1 transfected with miR-let-7i mimic compared with negative controls (NC). There were no significant changes in the cell cycle between transfected cells and the controls for both MV4-11 and THP-1.

DISCUSSION

AML is a heterogeneous group of aggressive hematologic cancers characterised by the clonal expansion of myeloid blasts in the bone marrow and peripheral circulation. The pathogenesis of AML involves multiple genetic and epigenetic alterations that lead to abnormal cell proliferation and differentiation, impaired apoptosis, and disrupted signalling pathways. AML is a complex disease with various contributing factors (Kantarjian *et al*., 2021). Several studies have reported aberrant miRNA expression profiles in AML, which may contribute to disease progression and poor prognosis. For example, elevated levels of miRNAs such as miRNA-149*, miRNA-126, miR-376, and miR-21 in AML patients are associated with an increased risk of relapse and poor prognosis (Trino *et al*., 2018). Overexpression of these miRNAs has been shown to promote cell proliferation, inhibit apoptosis, and contribute to the aggressive nature of AML cells. These findings highlight the potential significance of miRNAs in AML pathogenesis and suggest that targeting these miRNAs may represent a promising therapeutic strategy for this disease.

Although several miRNAs have been implicated in the pathogenesis of AML, their precise function in the disease remains poorly understood. Recent research by Esa and colleagues has suggested that miR-let-7i-5p may play a role in AML development (Esa *et al*., 2021). Previous studies have demonstrated that miR-let-7i-5p functions as a tumour suppressor in other cancers by modulating specific genes, such as glioblastoma and bladder cancer (Qin *et al*., 2019; Sun *et al*., 2019). However, its role in AML is yet to be fully explored. To investigate the potential role of miR-let-7i-5p in AML, we analysed its expression levels in AML patients using the GEO database (GSE142699). Our findings indicated that miR-let-7i-5p is significantly down-regulated in AML patients compared to healthy control patients. This downregulation suggests that miR-let-7i-5p may function as a tumour suppressor in AML and that its dysregulation may contribute to the development and progression of AML by promoting the overexpression of target genes implicated in cell proliferation, differentiation, and survival pathways. Further research is needed to fully understand the mechanisms by which miR-let-7i-5p functions in AML and to explore its potential as a therapeutic target for the disease.

This study used bioinformatics databases to predict the target genes of miR-let-7i-5p, including KMT2D, ABL1, DVSP, and JAG1 (Esa *et al*., 2021), and identified MYC as the most probable target gene. MYC is a proto-oncogene involved in cell cycle progression, apoptosis, cellular transformation, DNA damage response, and haematopoiesis. Its dysregulation has been linked to the progression of various cancers, including AML (Ahmadi *et al*., 2021). KEGG pathway analysis revealed that MYC is involved

in the PI3K-Akt, p53, JAK-STAT, and MAPK signalling pathways, cancer, and cell cycle. The PI3K-Akt pathway, upregulated in AML (Nepstad *et al*., 2020), had the highest enrichment score. To experimentally validate these predictions, RT-qPCR analysis demonstrated that miR-let-7i-5p mimics significantly downregulated MYC expression in AML cell lines. Taken together, the combined bioinformatic (target prediction & KEGG enrichment) and experimental (RT-qPCR) results suggest that the miR-let-7i-5p/MYC/PI3K-Akt axis may play a role in the pathogenesis of AML.To investigate the effect of miR-let-7i-5p on MYC gene expression, RT-qPCR was used, and the results showed that miR-let-7i-5p significantly downregulated MYC expression. This finding suggests that miR-let-7i-5p may act as a tumour suppressor gene by downregulating the proto-oncogene MYC in AML. However, the involvement of the miR-let-7i-5p/MYC/PI3K–Akt axis remains hypothetical, and further studies are needed to clarify its mechanisms and potential therapeutic relevance. Numerous miRNAs have been investigated concerning their role in cell proliferation in human leukaemia cells, with some found to promote and others to inhibit cell proliferation. Lu *et al*. demonstrated that miR-301b-3p inhibits AML cell proliferation by targeting FOXF2, while miR-338-3p inhibits glioma cell proliferation by targeting MYT1L in glioma malignancy(Yu *et al*., 2022; Lu *et al*., 2022). These studies indicate that miRNAs play an essential function in the proliferation of cancer cells. Previous research has implicated let-7i-5p in the proliferation of cancer cells, including clear cell renal cell carcinoma and colon cancer (Liu *et al*., 2021; Song *et al*., 2018). Nonetheless, the role of miR-let-7i-5p in AML proliferation remains unsupported by evidence. To investigate the miR-let-7i-5p/MYC/PI3K-Akt axis, an MTT proliferation assay was utilised to assess the impact of miR-let-7i-5p on reducing cell proliferation. However, the results were unexpected, as transfection of miR-let-7i-5p into cells only slightly increased compared to the negative control. Thus, the changes were considered insignificant, suggesting that the MYC gene may have different functions in AML other than cell proliferation.

The study had several limitations. First, it lacked actual patient samples and relied solely on bioinformatics analysis of publicly available databases, which may introduce bias. Second, the findings were not validated using additional methods such as luciferase reporter assays to confirm the predicted target genes, nor were they confirmed at the protein level. Third, while our results indicated that miR-let-7i-5p is downregulated in AML patients compared to healthy controls and that MYC is a putative target downregulated by miR-let-7i-5p, both the MTT proliferation assay and the cell cycle analysis showed no significant effects of miR-let-7i-5p overexpression on AML cells. This suggests that MYC may exert its role in AML through mechanisms other than regulating proliferation or cell cycle progression, such as apoptosis, differentiation, or other signalling pathways. Future studies should explore these alternative functions using assays for apoptosis, migration, or differentiation, and further validate the miR-let-7i-5p/MYC interaction in vivo through animal models.

CONCLUSION

In summary, this study provides preliminary but important insights into the potential regulatory relationship between miR-let-7i-5p and MYC in AML. While several limitations remain, including the lack of protein-level validation and in vivo confirmation, the findings suggest that this miRNA-mRNA interaction could play a role in AML pathogenesis. Future studies are needed to validate the interaction and clarify the specific cellular responses influenced by MYC modulation, such as apoptosis, differentiation, or other signalling processes. In particular, MYC-specific siRNA knockdown experiments should be performed to determine whether MYC suppression alone can recapitulate the phenotype observed with miR-let-7i-5p overexpression. Such studies, combined with in vivo models, would provide more definitive evidence of the biological and clinical relevance of the miR-let-7i-5p/MYC axis in AML.

ACKNOWLEDGEMENTS

This study was funded by the Ministry of Higher Education Fundamental Research Grant Scheme FP027-2018A and the National Health Institute NMRR-19-2148-48481. We also express our appreciation to the Director General of Health, Ministry of Health, Malaysia, for his permission to publish this article. We thank the Deputy Director General of Health (Research and Technical Support, Ministry of Health) and the Director of the Institute for Medical Research (IMR) Malaysia for their continuous support.

ETHICAL STATEMENT

This study was exempted by the Medical Research and Ethics Committee, which serves as the regional ethical board within the Ministry of Health in Malaysia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahmadi, S.E., Rahimi, S., Zarandi, B., Chegeni, R. & Safa, M. 2021. MYC: A multipurpose oncogene with prognostic and therapeutic implications in blood malignancies. Journal of Hematology & Oncology, 14(1): 1–49. https://doi.org/10.1186/S13045-021-01111-4

Arai, Y., Chi, S.G., Minami, Y. & Yanada, M. 2022. FLT3-targeted treatment for acute myeloid leukemia. International Journal of Hematology, 116(3): 351–363. https://doi.org/10.1007/S12185-022-03374-0

Barh, D., Malhotra, R., Ravi, B. & Sindhurani, P. 2010. MicroRNA Let-7: An emerging next-generation cancer therapeutic. Current Oncology, 17(1): 70–80. https://doi.org/10.3747/CO.V17I1.356

Bennett, J.M., Catovsky, D., Daniel, M.-T., Flandrin, G., Galton, D.A.G., Gralnick, H.R. & Sultan, C. 1976. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. British Journal of Haematology, 33(4): 451–458. https://doi.org/10.1111/J.1365-2141.1976.TB03563.X

Chen, P., Price, C., Li, Z., Li, Y., Cao, D., Wiley, A., He, C., Gurbuxani, S., Kunjamma, R.B., Huang, H., Jiang, X., Arnovitz, S., Xu, M., Hong, G.M., Elkahloun, A.G., Neilly, M.B., Wunderlich, M., Larson, R.A., Le Beau, M.M., Mulloy, J.C., Liu, P.P.,

Rowley, J.D. & Chen, J. 2013. miR-9 is an essential oncogenic microRNA specifically overexpressed in *mixed lineage leukemia*-rearranged leukemia. Proceedings of the National Academy of Sciences of the United States of America, 110(28): 11511–11516. https://doi.org/10.1073/PNAS.1310144110

Dunlap, J., Beadling, C., Warrick, A., Neff, T., Fleming, W.H., Loriaux, M., Heinrich, M.C., Kovacsovics, T., Kelemen, K., Leeborg, N., Gatter, K., Braziel, R.M., Press, R., Corless, C.L. & Fan, G. 2012. Multiplex high-throughput gene mutation analysis in acute myeloid leukemia. Human Pathology, 43(12): 2167–2176. https://doi.org/10.1016/J.HUMPATH.2012.03.002

Esa, E., Hashim, A.K., Mohamed, E.H.M., Zakaria, Z., Abu Hassan, A.N., Mat Yusoff, Y., Kamaluddin, N.R., Abdul Rahman, A.Z., Chang, K.M., Mohamed, R., Subbiah, I., Jamian, E., Ho, C.S.L., Lim, S.M., Lau, P.C., Pung, Y.F. & Zain, S.M. 2021. Construction of a microRNA-mRNA regulatory network in de novo cytogenetically normal acute myeloid leukemia patients. Genetic Testing and Molecular Biomarkers, 25(3): 199–210. https://doi.org/10.1089/gtmb.2020.0182

Hwang, S.M. 2020. Classification of acute myeloid leukemia. Blood Research, 55(Suppl): S1–S1. https://doi.org/10.5045/BR.2020.S001

Kantarjian, H., Kadia, T., DiNardo, C., Daver, N., Borthakur, G., Jabbour, E., Garcia-Manero, G., Konopleva, M. & Ravandi, F. 2021. Acute myeloid leukemia: Current progress and future directions. Blood Cancer Journal, 11(2): 41. https://doi.org/10.1038/S41408-021-00425-3

Khoury, J.D., Solary, E., Abla, O., Akkari, Y., Alaggio, R., Apperley, J.F., Bejar, R., Berti, E., Busque, L., Chan, J.K.C., Chen, W., Chen, X., Chng, W.J., Choi, J.K., Colmenero, I., Coupland, S.E., Cross, N.C.P., De Jong, D., Elghetany, M.T., Takahashi, E., Emile, J.F., Ferry, J., Fogelstrand, L., Fontenay, M., Germing, U., Gujral, S., Haferlach, T., Harrison, C., Hodge, J.C., Hu, S., Jansen, J.H., Kanagal-Shamanna, R., Kantarjian, H.M., Kratz, C.P., Li, X.Q., Lim, M.S., Loeb, K., Loghavi, S., Marcogliese, A., Meshinchi, S., Michaels, P., Naresh, K.N., Natkunam, Y., Nejati, R., Ott, G., Padron, E., Patel, K.P., Patkar, N., Picarsic, J., Platzbecker, U., Roberts, I., Schuh, A., Sewell, W., Siebert, R., Tembhare, P., Tyner, J., Verstovsek, S., Wang, W., Wood, B., Xiao, W., Yeung, C. & Hochhaus, A. 2022. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia, 36(7): 1703–1719. https://doi.org/10.1038/s41375-022-01613-1

Kuete, V., Karaosmanoğlu, O. & Sivas, H. 2017. Anticancer activities of African medicinal spices and vegetables. In: Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases. V. Kuete (Ed.). Academic Press, London. pp. 271–297. https://doi.org/10.1016/B978-0-12-809286-6.00010-8

Liu, Y., Hu, X., Hu, L., Xu, C. & Liang, X. 2021. Let-7i-5p enhances cell proliferation, migration and invasion of ccRCC by targeting HABP4. BMC Urology, 21(1): 1–11. https://doi.org/10.1186/S12894-021-00820-9

Lu, Y., Zhong, L., Luo, X., Liu, C., Dan, W., Chu, X., Wan, P., Zhang, Z., Wang, X., Liu, Z. & Liu, B. 2022. MiRNA-301b-3p induces proliferation and inhibits apoptosis in AML cells by targeting FOXF2 and regulating Wnt/β-catenin axis. Molecular and Cellular Probes, 63: 101805. https://doi.org/10.1016/J.MCP.2022.101805

MacFarlane, L.A. & Murphy, P.R. 2010. MicroRNA: Biogenesis, function and role in cancer. Current Genomics, 11(7): 537-554. https://doi.org/10.2174/138920210793175895

Nepstad, I., Hatfield, K.J., Grønningsæter, I.S. & Reikvam, H. 2020. The PI3K-Akt-mTOR signaling pathway in human acute myeloid leukemia (AML) cells. International Journal of Molecular Sciences, 21(8): 2907. https://doi.org/10.3390/IJMS21082907

Qin, M.M., Chai, X., Huang, H.B., Feng, G., Li, X.N., Zhang, J., Zheng, R., Liu, X.C. & Pu, C. 2019. let-7i inhibits proliferation and migration of bladder cancer cells by targeting HMGA1. BMC Urology, 19(1): 70. https://doi.org/10.1186/S12894-019-0485-1

Song, J., Wang, L., Ma, Q., Yang, Y., Yang, Z., Wang, B. & He, N. 2018. Let-7i-5p inhibits the proliferation and metastasis of colon cancer cells by targeting kallikrein-related peptidase 6. Oncology Reports, 40(3): 1459–1466. https://doi.org/10.3892/OR.2018.6577

Sun, X., Xue, H., Xiong, Y., Yu, R., Gao, X., Qian, M., Wang, S., Wang, H., Xu, J., Chen, Z., Deng, L. & Li, G. 2019. GALE promotes the proliferation and migration of glioblastoma cells and is regulated by miR-let-7i-5p. Cancer Management and Research, 11: 10539–10554. https://doi.org/10.2147/CMAR.S221585

Surveillance, Epidemiology, and End Results Program. 2023. Surveillance, Epidemiology, and End Results Program [WWW Document]. URL https://seer.cancer.gov/ (accessed 12.26.24).

Thol, F. & Heuser, M. 2021. Treatment for relapsed/refractory acute myeloid leukemia. HemaSphere, 5(6): E572. https://doi.org/10.1097/HS9.0000000000000572

Trino, S., Lamorte, D., Caivano, A., Laurenzana, I., Tagliaferri, D., Falco, G., Vecchio, L.Del, Musto, P. & De Luca, L. 2018. MicroRNAs as new biomarkers for diagnosis and prognosis, and as potential therapeutic targets in acute myeloid leukemia. International Journal of Molecular Sciences, 19(2): 460. https://doi.org/10.3390/IJMS19020460

Yu, Z., Liu, Y., Li, Y., Zhang, J., Peng, J., Gong, J., Xia, Y. & Wang, L. 2022. miRNA-338-3p inhibits glioma cell proliferation and progression by targeting MYT1L. Brain Research Bulletin, 179: 1–12. https://doi.org/10.1016/J.BRAINRESBULL.2021.11.016

Zhang, N., Hu, G., Myers, T.G. & Williamson, P.R. 2019. Protocols for the analysis of microRNA expression, biogenesis and function in immune cells. Current Protocols in Immunology, 126(1): e78. https://doi.org/10.1002/CPIM.78