Clinical and Experimental Health Sciences

MiR-7-5p May Inhibit AML Cell Proliferation Via SKP2, KLF4, OGTTarget Genes

Ezgi Mehteroğlu[1], İlknur Suer[2], Murat Kaya[3],Şükrü Öztürk[3], Kıvanç Cefle[3], Şükrü Palanduz[3]

[1] İstanbul University, Institute of Health Sciences, Aziz Sancar Experimental Medicine Research Institute, Department of Genetics, İstanbul, Türkiye.
[2] İstanbul University, İstanbul Faculty of Medicine, Department of Medical Genetics, İstanbul, Türkiye.
[3] İstanbul University, İstanbul Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics, İstanbul, Türkiye.

Correspondence Author: İlknur Suer
E-mail: ilknursuer@istanbul.edu.tr
Received: April 14, 2023 **Accepted:** December 16, 2024

ABSTRACT

Objective: Acute Myeloid Leukemia (AML) is distinguished by the differentiation and overgrowth of blast cells. In the current study, we purposed to elucidate the effect of miR-7-5p on AML cellular processes and the expression level of potential target genes.

Methods: miR-7-5p mimic was transfected into AML cells by lipofectamine-mediated method and verified by qRT-PCR. The miR-7-5p's effect on proliferation and apoptosis was investigated by WST-8 and Caspase-3 kit (respectively). miRDB, miRTarBase, Targetscan, miRWalk, https://ongene.bioinfo-minzhao.org/, and http://soft.bioinfo-minzhao.org/lgl/a databases were utilized for in silico identification of possible target genes of miR-7-5p. Relative gene expression of potential target genes was investigated via the qRT-PCR technique.

Results: In the group that is transfected with miR-7-5p, proliferation significantly decreased and apoptosis increased as against the control group. BCL2, SKP2, OGT, KLF4 and EGFR gene expression levels, which were determined as a result of possible target gene analysis by in silico methods and literature search, were investigated in AML cell lines. While the SKP2, KLF4 and OGT expression levels were statistically decreased in the group of transfected with mimic miR-7-5p, no statistically significant change was detected in the expressions of BCL2 and EGFR genes.

Conclusion: miR-7-5p may affect the cancer process in AML by targeting SKP2, KLF4, and OGT genes. It is very important to identify and validate the miR-7-5p target genes, which has the possibility to be a new biomarker in the early diagnosis and therapy of AML. Therefore, our data obtained at mRNA level should be confirmed by further studies.

Keywords: AML cell line, miR-7-5p, *SKP2, KLF4, OGT*

1. INTRODUCTION

Acute Myeloid Leukemia (AML) is a heterogeneous malignant sickness characterized by unlimited overgrowth and accumulation of abnormal blast cells affected by random genetic differentiation in hematopoietic stem cells (1). AML is the most prevalent leukemia in the adult population, accounting for 80% of all cases (2).

MicroRNAs (miRNAs) have critical roles in cancer pathogenesis including AML. They are a subset of non-protein-coding and endogenous single-stranded RNAs with approximately 20 nucleotides (3, 4). They are regulators of gene expression by binding to the 3' end un-translational regions (UTR) of messenger RNAs (mRNAs) target (5, 6). Each of the miRNAs, which are known as regulator of approximately 60% of all human genes, can regulate expression by targeting more than one gene (7, 8).

miR-7 family is highly conserved and is produced by the miRNA precursor 1-3 (9). miR-7-5p is the most studied member of this miRNA family (10, 11). miR-7-5p has been submitted to be associated with many cancers such as colon (12), pancreas (11), melanoma (13), lung (14), breast (15), gastric (16), glioblastoma (17), lymphoma (18). It has been reported that it is down-regulated in most cancer types and mostly acts as a tumor suppressor. It was indicated that miR-7-5p plays a tumor suppressor role in the development of AML by targeting *OSBPL11* (19). However, the relationship between miR-7-5p and AML has not yet been clearly demonstrated. Therefore, more research studies are needed to determine which possible targets of miR-7-5p can influence the cancer process.

From this point of view, the impact of miR-7-5p on cell viability and apoptosis processes in AML cell lines (HL-60 and NB4 cells) was investigated in this study. Then, possible miR-7-5p target genes were determined via *in silico* methods. The qRT-PCR technique was used to affirm the expressions of possible target genes which were selected as a result of *in silico* analysis in AML cells. For this purpose, gene expressions were compared in AML cells transfected

https://doi.org/*10.33808/clinexphealthsci.1279724*

with miR-7-5p mimic and AML cells inoculated with non-targeting miRNA mimic (nt control).

2. METHODS

2.1. Identification of Possible miR-7-5p Targets by Bioinformatics and In Silico Analysis

In the process of determining the possible miR-7-5p target genes, possible target genes were listed through *in silico* programs such as miRDB, miRTarBase, Targetscan, and miRWalk. Then, starting from the genes with the highest target score (score ≥ 85, genes that have strong evidence "validated via Reporter assay, Western-blot, qRT-PCR") was investigated via https://ongene.bioinfo-minzhao.org/ and http://soft.bioinfo-minzhao.org/lgl/ sites.

2.2. Cell Line Culturing Process

HL-60 and NB4 cell lines are known as cell lines derived from human mature acute myeloblastic leukemia cells and they are frequently used in AML studies. NB4 and HL-60 cells were grown under 37°C, 5% CO_2 conditions, and in RPMI-1640 medium prepared by adding 1% antibiotic and 10% FBS. When the cells multiplied by seeding in a 25 cm^2 cell culture flask reached 70-80% density (confluency), they were transferred to a larger 75 cm^2 cell culture flask and passaged. Other cells that were increased during passage were placed in a freezing medium (RPMI-1640 medium containing 10% DMSO) and placed in cryo tubes to be used in further studies. Then, it was stored in a nitrogen tank at – 196°C.

2.3. Mimic Transfection into NB4 and HL-60 Cells

The miR-7-5p mimic was transfected into cell lines. The non-targeting miRNA oligonucleotide sequence, which is known not to target any gene, was purchased commercially and used in the preparation of control cells (nt control) in the study. Transfection procedures were performed according to the Lipofectamine-2000 protocol.

2.4. Isolation of RNA and cDNA Synthesis for Transfection Validation

AML cells, which were transfected with miR-7-5p and nt mimic 24 hours before, were grown in an oven at the appropriate medium, centrifuged at 1500 rpm (5 minutes), and the pellets were dissolved in Trizol solution (Ambion) and the manufacturer's company RNA was isolated according to the protocol.

In order to control whether the transfection process has taken place and to control the expression level of miR-7-5p in cells transfected into HL-60 and NB4 cell lines, first cDNA synthesis followed by the qRT-PCR was conducted with the use of TaqMan primers and probes. In the transfection validation process, 30 ng RNA was used in total for each of the study and control groups. First of all, cDNA synthesis was performed with the TaqMan MicroRNA Reverse Transcription Kit (ThermoFisher Sci) in accordance with the supplier firm's protocol. Afterward, qRT-PCR processes were performed using TaqMan Universal Master Mix II (ThermoFisher Sci.), miR-7-5p RT probe (ThermoFisher Sci.), and RNU43 RT probe (ThermoFisher Sci.) as to the supplier firm's protocol. The experiments were run in duplicate and the data obtained were evaluated by the $2^{-\Delta\Delta Ct}$ method.

2.5. Investigation of the Influence of miR-7-5p on Cellular Processes as Viability and Apoptosis

Cells, seeded in 96-well plates with 3 replications and $3x10^3$ cells in each well, were incubated at 37°C, 5% CO_2. After 24 hours, miR-7-5p and nt mimic transfection was performed into cells according to the Lipofectamine-2000 protocol. Cell viability was evaluated at the 48th and 72nd hours to determine the proliferation of transfected cells. Using the Multiscan FC microplate (ThermoFisher Sci.) reader device, the proliferation of cells was measured with the Cell Viability Detection 8 Kit (CVDK8) (Eco-tech Biotechnology NutriCulture) according to the supplier firm's protocol and measured at 450 nm absorbance. $2x10^5$ NB4 cells were seeded into a 6-well plate for the purpose of determining the apoptosis situation. miR-7-5p mimic and nt mimic were transfected via Lipofectamine-2000 Reagent according to the supplier firm's protocol. 24 hours later from the transfection, miR-7-5p and nt mimic transfected cells were collected. The Human Caspase 3 Instant ELISA Kit (Invitrogen, ThermoFisher Sci.) protocol was applied and the measurement was conducted at 450 nm absorbance via the Multiscan FC microplate reader (ThermoFisher Sci.).

2.6. cDNA synthesis and the qRT-PCR Procedures to Determine Gene Expression Levels

cDNA synthesis was performed with the "RevertAid First Strand cDNA synthesis" kit (ThermoFisher Sci.) using a total of 1000 ng RNA from RNA samples of HL-60 and NB4 cells transfected with miR-7-5p mimic and nt mimic. Relative expression levels of selected genes, in mimic-transfected HL-60 and NB4 cells, were analyzed by the quantitative RT-PCR method. The qRT-PCR was performed according to the "5X HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne)" protocol. The experiments were conducted in duplicate. The βactin gene was used for normalization.

2.7. Statistical Analysis

SPSS 28 software was used in the statistical evaluation of the data obtained in the study. "Student's t-test" was used to compare the study group and the control group, and the data of the analysis results were demonstrated as mean ± standard deviation. The $2^{-\Delta\Delta Ct}$ method was used in the relative quantitation analysis of the qRT-PCR results. Statistically, the data with $p < .05$ were considered significant. GraphPad Prism 9.3 software, Microsoft Excel, and Paint programs were used for the construction of graphics, figures, and tables. The analysis of overall survival (OS) was performed with the "Kaplan-Meier test" using the GEPIA-2 program.

3. RESULTS

3.1. Possible Interacting Genes of miR-7-5p

About 20 of the genes classified as strong possible interacting genes of miR-7-5p in *in silico* databases were found to be among 803 oncogenes on https://ongene.bioinfo-minzhao.org/ (Figure 1a). Among these 20 genes identified as miR-7-5p targets, 5 of them were found in the "http://soft.bioinfo-minzhao.org/lgl/" database (Figure 1b). The expression levels of these 5 genes [B-Cell CLL/ Lymphoma 2 (*BCL2*), S-Phase Kinase Associated Protein 2 (*SKP2*), O-Linked N-Acetylglucosamine (GlcNAc) Transferase (*OGT*), Kruppel Like Factor 4 (*KLF4*) and Epidermal Growth Factor Receptor (*EGFR*) genes] were compared with mimic miR-7-5p and nt transfected AML cells.

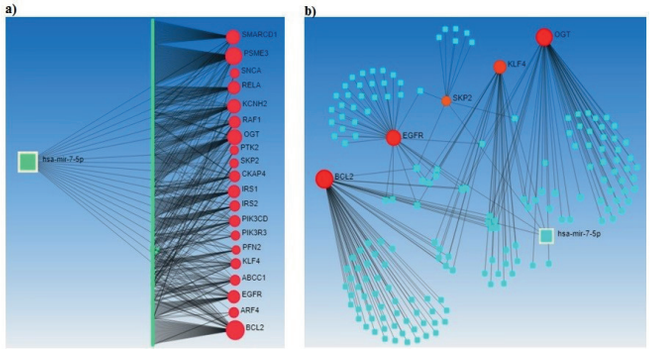

Figure 1. *Bioinformatics analysis results according to searching the miRDB, miRTarBase, Targetscan, miRWalk, https://ongene.bioinfo-minzhao.org/ and http://soft.bioinfo-minzhao.org/lgl/a) databases. a) 20 genes identified to be miR-7-5p targets (517 nodes 622 edges). b) Among these 20 genes, 5 genes were selected for in vitro study (168 miRNAs nodes with 202 edges)*

3.2. Validation of miRNA Mimic Transfection

Verification of the miRNA mimic transfection was showed that the amount of miR-7-5p and nt control mimics transfected into HL-60 and NB4 cells by Lipofectamine-mediated methods increased statistically in the cells and the transfection was successful (Figure 2).

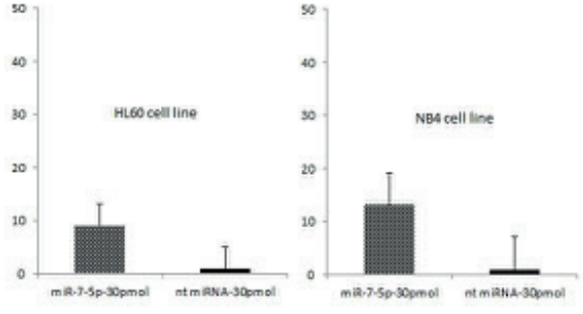

Figure 2. *Transfection validation of miRNA mimic-transfected HL-60 and NB4 cells*

3.3. Impact of miR-7-5p on Cell Proliferation

As a result of the cell proliferation experiment performed to determine the impact of miR-7-5p on the proliferation of cells, the miR-7-5p transfected group in HL-60 and NB4 cells compared to the nt control group at both 48th hour (p=.04; p=.01), It was determined that there was a significant decrease in cell proliferation in both (respectively) and 72 hours (p=.028; p=.002, respectively) (Figure 3).

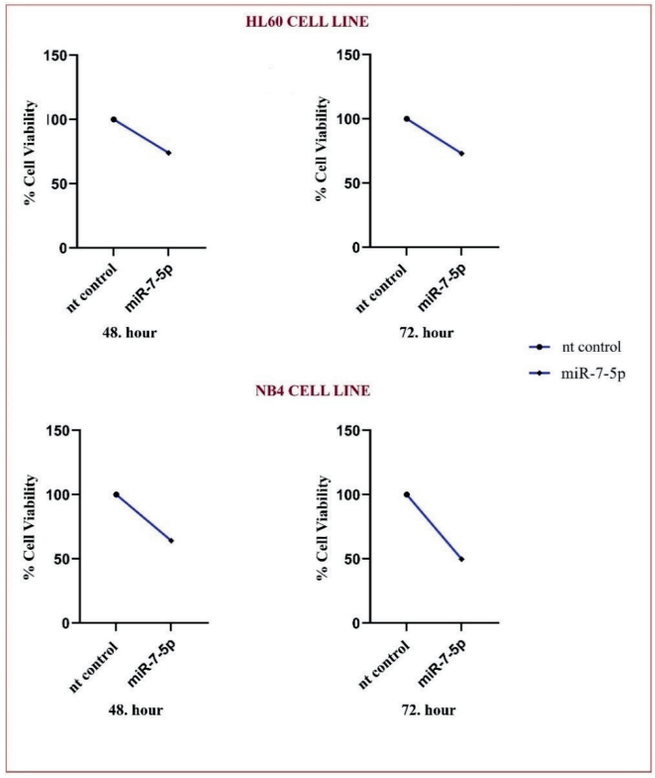

Figure 3. *Proliferation changes in HL-60 and NB4 cells at 48. and 72. Hours*

3.4. Impact of miR-7-5p on Apoptosis

Caspase-3 level was evaluated to determine the impact of miR-7-5p on the apoptosis process and it was found that the cell death in the miR-7-5p transfected group in NB4 cells increased statistically significantly compared to the nt control group (p=.003) (Figure 4).

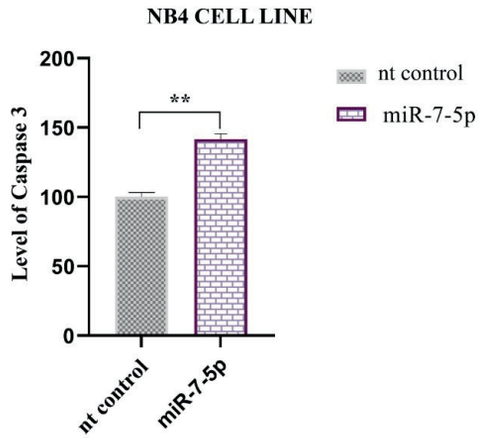

Figure 4. *Effect of miR-7-5p on apoptosis in NB4 cells (** p < .01)*

3.5. miR-7-5p Relation With Expression Levels of OGT, SKP2, BCL2, KLF4 and EGFR

The expression data obtained as a result of qRT-PCR used to look at the expression levels of *OGT*, *SKP2*, *BCL2*, *KLF4,* and *EGFR* genes, which are determined as possible targets of miR-7-5p, were evaluated by the relative quantitation method and analyzed via Student's t-test using βactin gene for normalisation. While the p values obtained in the analyzes were less than 0.05 were considered statistically important, those greater than 0.05 were not considered statistically important. The primer sequences used for qRT-PCR to investigate the expressions of the relevant genes were shown in Table 1. In conclusion, the relative expression levels of *OGT* (p=.012; p=.017), *SKP2* (p=.019; p=.036) and *KLF4* (p=.003; p=.027) genes were reduced in miR-7-5p transfected cells compared to the control group in HL-60 and NB4 cell lines. However, no statistically significant change was detected in the relative expressions of *BCL2* (p=.652; p=.174) and *EGFR* (p=.201; p=.698) genes in miR-7-5p transfected cells compared to the control group in both cell lines (Figure 5). All mean and p values of the gene expression levels were shown in Table 2.

Table 1. *Primer sequences*

Genes	Sequences	Base	Reference
BCL2-F	5′-GATGTGATGCCTCTGCGAAG-3′	20	(43)
BCL2-R	5′-CATGCTGATGTCTCTGGAATCT-3′	22	
SKP2-F	5′-ATGCCCCAATCTTGTCCATCT-3′	21	(44)
SKP2-R	5′-CACCGACTGAGTGATAGGTGT-3′	21	
OGT-F	5′-TGTCACCCTTGACCCAAACTT-3′	21	(45)
OGT-R	5′-GGCACGAAGATAAGCTGCCA-3′	20	
KLF4-F	5′-CCCAATTACCCATCCTTCCTG-3′	21	(46)
KLF4-R	5′-GTCTTCCCCTCTTTGGCTTG-3′	20	
EGFR-F	5′-ATGGTCAAGTGCTGGATG-3′	18	(47)
EGFR-R	5′-GAGGAAGGTGTCGTCTATG-3′	19	
βactin-F	5′-GCCTCGCCTTTGCCGATC-3′	18	(46)
βactin-R	5′-CCCACGATGGAGGGGAAG-3′	18	

Table 2. *Mean and p values of gene expression levels*

Genes	Expression levels in HL-60 cell line		Expression levels in NB4 cell line	
	mean	p-value	mean	p-value
OGT	0,248	.012	0,356	.017
SKP2	0,094	.019	0,534	.036
KLF4	0,386	.003	0,229	.027
BCL2	1,096	.652	1,207	.174
EGFR	0,287	.201	1,325	.698

**significant (p < .05)*

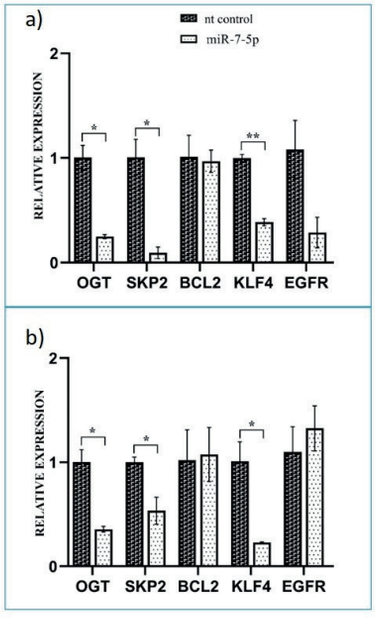

Figure 5. *Comparison of the expression levels of OGT, SKP2, KLF4, BCL2, and EGFR genes in miR-7-5p mimic and non-targeting miRNA mimic transfected AML cells a) HL-60 cell line b) NB4 cell line*

2.6. Overal Survival (OS) Analysis

The overall survival analysis result showed that the *OGT* gene had an indicative role in poor OS in AML patients (Figure 6).

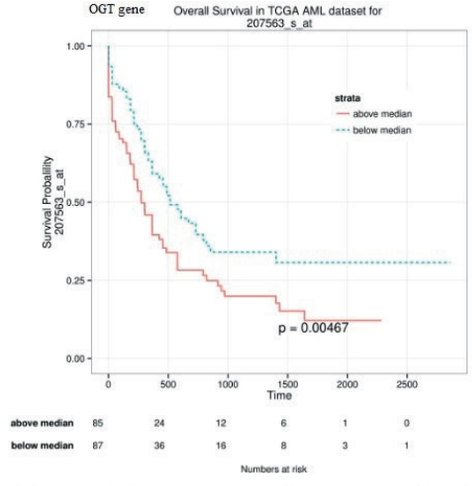

Figure 6. *OGT gene influence on overall AML survival in TCGA. Points to consider when analyzing: Group cutoff: Median, Cutoff – High (%): 50, Cutoff-Low (%): 50, Hazards Ratio (HR): Yes, 95% Confidence Interval: Yes, Axis Units: Months*

4. DISCUSSION

AML is a heterogeneous form of malignant leukemia mostly seen in adults, characterized by excessive accumulation of immature myeloblast cells in the bone marrow/blood (20). One of the factors that play an active role in the formation processes of various cancers, incorporating leukemias, is miRNA deregulation. miRNAs function as both oncogenic and tumor suppressors in the formation processes of various diseases, including cancers (21, 22). Rearrangement of expression levels in target genes of miRNAs offers potential treatment alternatives for various diseases. It is thought that it will make a great contribution to the development of new treatment strategies in addition to the treatments used today, especially with the rearrangement of the expressions of miRNAs in cancer types (23, 24). In the studies, oncogenic genes are silenced with the rearrangement of miRNA expressions or it is aimed to have a stopping effect in the cancer development process by providing the re-expression of tumor suppressor genes with reduced expression.

miR-7-5p is the most well-known member of the highly conserved miR-7 family (9). It is known that this miRNA behaves as a tumor repressor in many cancers and plays a reduced role in cancer pathogenesis. In the literature, it has been shown in lung cancer (25) and cholangiocarcinoma (26) studies that miR-7-5p plays a role in inhibiting diverse cellular procedures such as cell proliferation, growth, migration, and metastasis, promoting apoptosis and maintaining cell cycle control.

In this project, a substantial decrease in cell proliferation was detected in HL-60 and NB4 cell lines transfected with miR-7-5p mimic in comparison to the control group. Similarly, as a result of the apoptosis experiment, it was determined that miR-7-5p increased the amount of Caspase-3 and drove cells to apoptosis in NB4 cells. Only NB4 cells were used in the apoptosis assay because of the limitation of the project budget., therefore, it could not be compared with the apoptosis process in HL-60 cells. Therefore, apoptosis changes in HL-60 cells should be controlled in further studies so that the effect of miR-7-5p on genes can be better understood by comparing them with the changes in NB4 cells. The expression levels of *BCL2*, *SKP2*, *OGT*, *KLF4*, and *EGFR* genes which were selected as possible targets of miR-7-5p via the bioinformatics approach and *in silico* analysis, were investigated. *SKP2*, *KLF4,* and *OGT* gene expression levels in the miR-7-5p mimic transfected group were found to be statistically significantly decreased compared to the nt control mimic transfected group. But then, it was concluded that there was no substantially significant change in *BCL2* and *EGFR* gene expression levels.

The *SKP2* gene is included in the modulation of the cell cycle and is known to act as a proto-oncogene in tumor pathogenesis in various cancer types known to be overexpressed (27). By directly targeting the expression of the *SKP2* gene, which has a high expression level in breast cancer, miR-7-5p could reduce drug-resistant cancer cell proliferation and epithelial-mesenchymal transition (28). Studies have shown that *SKP2* is a highly oncogenic protein and is strongly associated with AML prognosis (29, 30, 31). On the other hand, there is also a study in the literature reporting that *SKP2* has a weak relationship with AML and that this gene does not play an active role in AML prognosis (32). In our study, we found that *SKP2* was up-regulated in AML cells and its expression was significantly decreased following miR-7-5p transfection. Our findings support the previous studies showing a high association between *SKP2* and AML pathogenesis. Furthermore, it was determined that miR-7-5p and *SKP2* showed a negative correlation in AML cells, as in many other types of cancer, and miR-7-5p played a role in reducing cancer cell proliferation.

The *KLF4* gene is involved in cell cycle control, differentiation, and many developmental processes. In a study on colon cancer in which *KLF4* was reported to play an oncogenic role, the connection between miR-7-5p and *KLF4* was confirmed and it was shown that this miRNA partially reduced the invasion and migration of cancerous cells (33). A study in the literature on AML showed that *KLF4* has high expression levels and promotes monocytic differentiation (34). According to our current study, miR-7-5p and *KLF4* showed a negative correlation in AML cells same as in these literature data.

Disruption in the expression of the *OGT* gene, which is identified to be included in the coordinating of cellular response pathways to stress and cell cycle regulation, has been associated with diabetes, cancer, and cardiac complications (35, 36, 37). Inhibition of O-linked-N-acetylglucosaminylation has been indicated to increase apoptosis tendency in AML cells. Therefore, it is thought that *OGT* may have the potential to be an important target for the formation of a potential therapy for AML patients (38). A study conducted on AML showed that *OGT* expression level was high in AML cells and cell proliferation decreased and apoptosis increased with the inhibition of *OGT* (39). The relevancy between *OGT* and miR-7-5p was also examined on AML cells in our study and it was found a correlation with the literature. Also our current study's overall survival (OS) analysis showed that the *OGT* gene had an indicative role in poor OS in AML patients (Figure 6).

A miRNA can have more than one target gene, and a gene can be targeted by more than one miRNA (40). According to studies in the literature, *EGFR* and *BCL-2* genes are overexpressed in several cancer types including AML and these genes are highly effective for cancer progress. In addition to this, they have potential biomarker properties for cancers (41,42). They can be targeted by miRNAs, mRNAs, or signaling pathways and their expression levels can change with the targeting. Therefore, the roles of these genes in cancer development can be knocked out or knocked down with several procedures. In this case, direct targeting of genes and changing their expression is very important. If there is no direct or indirect relationship between those targeting the gene and the target genes, it is not possible to silence the gene. As in our study, the miR-7-5p directly targeted the *SKP2*, *KLF4,* and *OGT* genes, causing their downregulation. The fact that no statistically significant changes were detected in *BCL2* and *EGFR* genes whose expression levels were examined may suggest that these genes may be under the control of miRNAs other than miR-7-5p in AML cells.

5. CONCLUSION

In conclusion, in our study in which the relevancy of miR-7-5p and AML was examined by cell proliferation, gene expression, and Caspase-3 experiments in HL-60 and NB4 cells, it was determined that the expressions of *SKP2*, *KLF4,* and *OGT* genes were modulated via miR-7-5p at the mRNA level. It is important to confirm these findings with further studies. The limitations of our study include the absence of tissue confirmation. Our results need confirmation of the mRNA and protein levels in patient tissue samples. There are several possible targets of miR-7-5p identified via *in silico* methods. It is very crucial

to conclude the definitive interacting genes of miR-7-5p, which may have important potential as a biomarker in processes such as early diagnosis, prognosis follow-up, and discovery of novel treatment approaches for AML.

Acknowledgments: *The authors thank Istanbul University Scientific Research Projects Unit for their project support (Project no: 37653).*
Funding: *The present work was supported by the fund of Istanbul University Scientific Research Projects (Project no: 37653).*
Peer-review: *Externally peer-reviewed.*
Conflict of interest: *The authors declared no conflict of interest.*
Ethics: *Ethical approval was not obtained as a commercially produced cell line was used in the study.*
Author Contribution:
Research idea: IS, MK
Design of the study: IS, MK, SP
Acquisition of data for the study: EM, IS, MK
Analysis of data for the study: EM, IS, MK
Interpretation of data for the study: IS, MK, SO, KC, SP
Drafting the manuscript: EM, IS, MK
Revising it critically for important intellectual content: IS, MK, SO, KC, SP
Final approval of the version to be published: EM, IS, MK, SO, KC, SP

REFERENCES

[1] Short NJ, Ravandi F. How close are we to incorporating measurable residual disease into clinical practice for acute myeloid leukemia? Haematologica. 2019;104(8):1532-41. https://doi.org/10.3324/haematol.2018.208454

[2] Vakiti A, Mewawalla P. Acute Myeloid Leukemia. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2023, StatPearls Publishing LLC.; 2023.

[3] Kaya M, Suer I. The effect of miR-34a-5p on overexpressed AML associated genes. J Ist Faculty Med. 2023;86(1):59-68. https://doi.org/10.26650/IUITFD.1168793

[4] Suer I, Guzel E, Karatas OF, Creighton CJ, Ittmann M, Ozen M. MicroRNAs as prognostic markers in prostate cancer. Prostate. 2019;79(3):265-71. https://doi.org/10.1002/pros.23731.

[5] Suer I, Kaya M. Is the AURKB Gene Involved in Aml Cell Proliferation Since It is Targeted by miR-34a-5p and let-7b-5p? Konuralp Medical Journal. 2023. DOI:10.18521/ktd.1171549.

[6] Kaya M, Karatas OF. The Relationship Between Larynx Cancer and MicroRNAs. Van Med J 2020;27(4):535-41. https://doi.org/10.5505/vtd.2020.80947.

[7] Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009;19(1):92-105. https://doi.org/10.1101/gr.082701.108

[8] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215-33. https://doi.org/10.1016/j.cell.2009.01.002.

[9] Kalinowski FC, Brown RA, Ganda C, Giles KM, Epis MR, Horsham J, Leedman PJ. microRNA-7: a tumor suppressor miRNA with therapeutic potential. Int J Biochem Cell Biol. 2014;54:312-7. https://doi.org/10.1016/j.biocel.2014.05.040.

[10] Gu DN, Jiang MJ, Mei Z, Dai JJ, Dai CY, Fang C, Huang Q, Tian L. microRNA-7 impairs autophagy-derived pools of glucose to suppress pancreatic cancer progression. Cancer Lett. 2017;400:69-78. https://doi.org/10.1016/j.canlet.2017.04.020.

[11] Zhu W, Wang Y, Zhang D, Yu X, Leng X. MiR-7-5p functions as a tumor suppressor by targeting SOX18 in pancreatic ductal adenocarcinoma. Biochem Biophys Res Commun. 2018;497(4):963-70. https://doi.org/10.1016/j.bbrc.2018.02.005.

[12] Zeng CY, Zhan YS, Huang J, Chen YX. MicroRNA-7 suppresses human colon cancer invasion and proliferation by targeting the expression of focal adhesion kinase. Mol Med Rep. 2016;13(2):1297-303. https://doi.org/10.3892/mmr.2015.4643.

[13] Giles KM, Brown RA, Epis MR, Kalinowski FC, Leedman PJ. miRNA-7-5p inhibits melanoma cell migration and invasion. Biochem Biophys Res Commun. 2013;430(2):706-10. https://doi.org/10.1016/j.bbrc.2012.11.086.

[14] Xiao H. MiR-7-5p suppresses tumor metastasis of non-small cell lung cancer by targeting NOVA2. Cell Mol Biol Lett. 2019;24:60. https://doi.org/10.1186/s11658-019-0188-3.

[15] Shi Y, Luo X, Li P, Tan J, Wang X, Xiang T, Ren G. miR-7-5p suppresses cell proliferation and induces apoptosis of breast cancer cells mainly by targeting REGγ. Cancer Lett. 2015;358(1):27-36. https://doi.org/10.1016/j.canlet.2014.12.014.

[16] Yang Z, Shi X, Li C, Wang X, Hou K, Li Z, Zhang X, Fan Y, Qu X, Che X, Liu Y. Long non-coding RNA UCA1 upregulation promotes the migration of hypoxia-resistant gastric cancer cells through the miR-7-5p/EGFR axis. Exp Cell Res. 2018;368(2):194-201. https://doi.org/10.1016/j.yexcr.2018.04.030.

[17] Liu Z, Liu Y, Li L, Xu Z, Bi B, Wang Y, Li JY. MiR-7-5p is frequently downregulated in glioblastoma microvasculature and inhibits vascular endothelial cell proliferation by targeting RAF1. Tumour Biol. 2014;35(10):10177-84. https://doi.org/10.1007/s13277-014-2318-x.

[18] Sorrentino D, Frentzel J, Mitou G, Blasco RB, Torossian A, Hoareau-Aveilla C, Pighi C, Farcé M, Meggetto F, Manenti S, Espinos E, Chiarle R, Giuriato S. High Levels of miR-7-5p Potentiate Crizotinib-Induced Cytokilling and Autophagic Flux by Targeting RAF1 in NPM-ALK Positive Lymphoma Cells. Cancers (Basel). 2020;12(10). https://doi.org/10.3390/cancers12102951.

[19] Jiang D, Wu X, Sun X, Tan W, Dai X, Xie Y, Du A, Zhao Q. Bone mesenchymal stem cell-derived exosomal microRNA-7-5p inhibits progression of acute myeloid leukemia by targeting OSBPL11. J Nanobiotechnology. 2022;20(1):29. https://doi.org/10.1186/s12951-021-01206-7.

[20] Pelcovits A, Niroula R. Acute Myeloid Leukemia: A Review. R I Med J (2013). 2020;103(3):38-40.

[21] Testa U, Pelosi E. MicroRNAs expressed in hematopoietic stem/progenitor cells are deregulated in acute myeloid leukemias. Leuk Lymphoma. 2015;56(5):1466-74. https://doi.org/10.3109/10428194.2014.955019.

[22] Capik O, Sanli F, Kurt A, Ceylan O, Suer I, Kaya M, Ittmann M, Karatas OF. CASC11 promotes aggressiveness of prostate cancer cells through miR-145/IGF1R axis. Prostate Cancer Prostatic Dis. 2021;24(3):891-902. https://doi.org/10.1038/s41391-021-00353-0.

[23] Nair R, Salinas-Illarena A, Baldauf HM. New strategies to treat AML: novel insights into AML survival pathways and combination therapies. Leukemia. 2021;35(2):299-311. https://doi.org/10.1038/s41375-020-01069-1.

[24] Liu Y, Cheng Z, Pang Y, Cui L, Qian T, Quan L, Zhao H, Shi J, Ke X, Fu L. Role of microRNAs, circRNAs and long noncoding RNAs in acute myeloid leukemia. J Hematol Oncol. 2019;12(1):51. https://doi.org/10.1186/s13045-019-0734-5.

[25] Li Q, Wu X, Guo L, Shi J, Li J. MicroRNA-7-5p induces cell growth inhibition, cell cycle arrest and apoptosis by targeting PAK2 in non-small cell lung cancer. FEBS Open Bio. 2019;9(11):1983-93. https://doi.org/10.1002/2211-5463.12738.

[26] Tang Y, Tang Z, Yang J, Liu T, Tang Y. MicroRNA-7-5p Inhibits Migration, Invasion and Metastasis of Intrahepatic Cholangiocarcinoma by Inhibiting MyD88. J Clin Transl Hepatol. 2021;9(6):809-17. https://doi.org/10.14218/JCTH.2021.00021.

[27] Gstaiger M, Jordan R, Lim M, Catzavelos C, Mestan J, Slingerland J, Krek W. Skp2 is oncogenic and overexpressed in human cancers. Proc Natl Acad Sci U S A. 2001;98(9):5043-8. https://doi.org/10.1073/pnas.081474898.

[28] Wang HF, Dong ZY, Yan L, Yang S, Xu HN, Chen SL, Wang WR, Yang QL, Chen CJ. The N-terminal polypeptide derived from vMIP-II exerts its antitumor activity in human breast cancer through CXCR4/miR-7-5p/Skp2 pathway. J Cell Physiol. 2020;235(12):9474-86. https://doi.org/10.1002/jcp.29755.

[29] Dan W, Zhong L, Zhang Z, Wan P, Lu Y, Wang X, Liu Z, Chu X, Liu B. RIP1-dependent Apoptosis and Differentiation Regulated by Skp2 and Akt/GSK3β in Acute Myeloid Leukemia. Int J Med Sci. 2022 Mar 6;19(3):525-536. https://doi.org/10.7150/ijms.68385. PMID: 35370472; PMCID: PMC8964317.

[30] Thacker G, Mishra M, Sharma A, Singh AK, Sanyal S, Trivedi AK. CDK2-instigates C/EBPα degradation through SKP2 in Acute myeloid leukemia. Med Oncol. 2021 May 17;38(6):69. https://doi.org/10.1007/s12032-021-01523-9. PMID: 34002296.

[31] Thacker G, Mishra M, Sharma A, Singh AK, Sanyal S, Trivedi AK. E3 ligase SCFSKP2 ubiquitinates and degrades tumor suppressor C/EBPα in acute myeloid leukemia, Life Sciences (2020), https://doi.org/10.1016/j.lfs.2020.118041.

[32] Min YH, Cheong JW, Lee MH, Kim JY, Lee ST, Hahn JS, Ko YW. Elevated S-phase kinase-associated protein 2 protein expression in acute myelogenous leukemia: its association with constitutive phosphorylation of phosphatase and tensin homologue protein and poor prognosis. Clin Cancer Res. 2004 Aug 1;10(15):5123-30. https://doi.org/10.1158/1078-0432.CCR-04-0136. PMID: 15297415

[33] Dong M, Xie Y, Xu Y. miR-7-5p regulates the proliferation and migration of colorectal cancer cells by negatively regulating the expression of Krüppel-like factor 4. Oncol Lett. 2019;17(3):3241-6. https://doi.org/10.3892/ol.2019.10001.

[34] Noura M, Morita K, Kiyose H, Matsuo H, Nishinaka-Arai Y, Kurokawa M, Kamikubo Y, Adachi S. Pivotal role of DPYSL2A in KLF4-mediated monocytic differentiation of acute myeloid leukemia cells. Sci Rep. 2020;10(1):20245. https://doi.org/10.1038/s41598-020-76951-0.

[35] Fardini Y, Dehennaut V, Lefebvre T, Issad T. O-GlcNAcylation: A New Cancer Hallmark? Front Endocrinol (Lausanne). 2013;4:99. https://doi.org/10.3389/fendo.2013.00099.

[36] Krause MW, Love DC, Ghosh SK, Wang P, Yun S, Fukushige T, Hanover JA. Nutrient-Driven O-GlcNAcylation at Promoters Impacts Genome-Wide RNA Pol II Distribution. Front Endocrinol (Lausanne). 2018;9:521. https://doi.org/10.3389/fendo.2018.00521.37.

[37] Slawson C, Hart GW. O-GlcNAc signalling: implications for cancer cell biology. Nat Rev Cancer. 2011;11(9):678-84. https://doi.org/10.1038/nrc3114.

[38] Asthana A, Ramakrishnan P, Vicioso Y, Zhang K, Parameswaran R. Hexosamine Biosynthetic Pathway Inhibition Leads to AML Cell Differentiation and Cell Death. Mol Cancer Ther. 2018;17(10):2226-37. https://doi.org/10.1158/1535-7163.MCT-18-0426.

[39] He N, Ma D, Tan Y, Liu M. Upregulation of O-GlcNAc transferase is involved in the pathogenesis of acute myeloid leukemia. Asia Pac J Clin Oncol. 2022;18(5):e318-e28. https://doi.org/10.1111/ajco.13685.

[40] Suer I, Kaya M, Ozgur E. The Effect of miR-34a-5p and miR-145-5p Ectopic Expression on Cell Proliferation and Target Gene Expression in the MDA-MB-231 Cell Line. NKMJ. 2021;9(2):166-73. https://doi.org/10.4274/nkmj.galenos.2021.770512.

[41] Mahmud H, Kornblau SM, Ter Elst A, Scherpen FJ, Qiu YH, Coombes KR, de Bont ES. Epidermal growth factor receptor is expressed and active in a subset of acute myeloid leukemia. J Hematol Oncol. 2016 Aug 3;9(1):64. https://doi.org/10.1186/s13045-016-0294-x. PMID: 27488458; PMCID: PMC4971659

[42] Wei Y, Cao Y, Sun R, Cheng L, Xiong X, Jin X, He X, Lu W, Zhao M. Targeting Bcl-2 Proteins in Acute Myeloid Leukemia. Front Oncol. 2020 Nov 5;10:584974. https://doi.org/10.3389/fonc.2020.584974. PMID: 33251145; PMCID: PMC7674767

[43] Golestani Eimani B, Sanati MH, Houshmand M, Ataei M, Akbarian F, Shakhssalim N. Expression and prognostic significance of bcl-2 and bax in the progression and clinical outcome of transitional bladder cell carcinoma. Cell J. 2014;15(4):356-63.

[44] Zhao H, Pan H, Wang H, Chai P, Ge S, Jia R, Fan X. SKP2 targeted inhibition suppresses human uveal melanoma progression by blocking ubiquitylation of p27. Onco Targets Ther. 2019;12:4297-308. https://doi.org/10.2147/OTT.S203888.

[45] Zhang C, Xie F, Li L, Zhang C, Zhang Y, Ying W, Liu L, Yan X, Yin F, Zhang L. Hepatocyte nuclear factor 1 alpha (HNF1A) regulates transcription of O-GlcNAc transferase in a negative feedback mechanism. FEBS Lett. 2019;593(10):1050-60. https://doi.org/10.1002/1873-3468.13381.

[46] Suer I, Karatas OF, Yuceturk B, Yilmaz M, Guven G, Buge O, Cansiz H, Ozen M. Characterization of stem-like cells directly isolated from freshly resected laryngeal squamous cell carcinoma specimens. Curr Stem Cell Res Ther. 2014;9(4):347-53. https://doi.org/10.2174/1574888x09666140330201632.

[47] Zhang B, Tian Y, Jiang P, Jiang Y, Li C, Liu T, Zhou R, Yang N, Zhou X, Liu Z. MicroRNA-122a Regulates Zonulin by Targeting EGFR in Intestinal Epithelial Dysfunction. Cell Physiol Biochem. 2017;42(2):848-58. https://doi.org/10.1159/000478629.

How to cite this article: Mertoğlu E, Suer İ, Kaya M, Öztürk Ş, Cefle K, Palanduz Ş. MiR-7-5p May Inhibit AML Cell Proliferation Via SKP2, KLF4, OGTTarget Genes. Clin Exp Health Sci 2025; 15: 8-14. https://doi.org/10.33808/clinexphealthsci.1279724