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A Bioinformatics Analysis of circRNA/miRNA/mRNA Interactions in Acute Myeloid Leukemia

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ABSTRACT

Objective: Acute myeloid leukemia (AML) is a lethal type of cancer associated with dysregulation of progenitor hematopoietic stem cell behavior and its incidence is, unfortunately, increasing. Although there are various applications in treatment, since most of them are insufficient in early diagnosis, treatment and new prognostic biomarkers should be investigated.

Materials and Methods: In this study, three Gene Expression Omnibus (GEO) datasets Genomic Spatial Event (GSE); GSE94591, GSE116617, and GSE163386) were used to investigate dysregulated expressions of circular RNAs (circRNAs), and the GSE142699 and GSE142698 datasets were analyzed to detect dysregulated expressions of microRNAs (miRNAs) and mRNAs, respectively. Filtering was applied with p value <0.05, log2FC≥0.5 (circRNA), and log2FC≥1 (miRNA and mRNA) from the raw data analyzed using the limma R package (v.3.46.0). We investigated circRNA-miRNA-mRNA interactions using special tools including CSCDv2.0, circBank, miRTarBase, miRDB, multiMiR, miRWalk, DIANA-microT, TarBase, miRanda, and TargetScan. The pathway analysis was performed using KEGG and GO programs. The STRING database and Cytoscape tool were used to construct and view protein interaction. Hub gene analysis was constructed using the MCODE tool. We have utilized the GEPIA tool to determine the Overall Survival of the hub genes.

Results: In our study, 4 circRNAs, 3 miRNAs, and 6 genes that may be closely related to AML were detected.

Conclusion: According to our bioinformatics analysis results, hsa_circ_0012152/miR-199a-5p/*HOXA9* axis could be more important in AML. Therefore, *in vitro and in vivo* investigations are recommended.

Keywords: Acute myeloid leukemia, circular RNA, bioinformatics

INTRODUCTION

Acute myeloid leukemia (AML) is a hematological cancer that affects mostly adults and has a complicated classification and prognosis. There are only a few target therapeutic molecules for AML, and the necessary success in treatment has yet to be reached (1-3) . There is a need for new diagnostic and therapeutic target molecules for AML, which has become more complex due to the wide variety of genetic and epigenetic changes that occur (4). It has been reported that more than 20,000 people are diagnosed with AML every year in the United States and approximately 11,000 people die due to AML (5). Non-coding RNAs regulate gene expression during the post-transcriptional process (3, 6, 7). Long non-coding circular RNAs (circRNAs), which have a wide variety of functions in the cell, control gene expression by sponging miRNAs. circRNAs have a more stable structure than linear RNAs and are among the important research topics of the last 5 years. Dysregulation of the expression of circRNAs has been reported in a wide variety of cancer types, including lung cancer, prostate cancer, gastric cancer, and breast cancer, and also AML (8,

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9). It has been revealed that circRNAs play crucial roles in the pathogenesis of AML through various axes such as circRNA-DLEU2/miRNA-496/*PRKACB* and circ_0040823/miR-516b/*PTEN* (10, 11). In our study, a meta-analysis of circRNA, miRNA, and mRNA datasets in AML was performed with bioinformatics tools and circRNA-miRNA-mRNA axes that may be important in AML was determined.

MATERIALS AND METHODS

circRNA Expression Analysis of AML Datasets

Genomic spatial event (GSE); GSE94591 (4 healthy controls and 6 AML patients / bone marrow samples), GSE116617 (4 healthy controls and 4 AML patients/ bone marrow samples), and GSE163386 (4 healthy controls and 5 AML patients / bone marrow samples) gene expression omnibus (GEO) datasets were used to investigate dysregulated expressions of circRNAs. Filtering was applied with p value <0.05 and log2FC≥0.5 from the raw data analyzed using the limma R package (v.3.46.0). After identifying circRNAs with significant expression changes, their relationship with cancer and AML was investigated in the literature using "AML, hsa_circ_0012152" and "Acute myeloid leukemia, hsa_circ_0012152" parameters in PUBMED and other internet networks.

miRNA and mRNA Expression Analysis of AML Datasets

The GSE142698 and GSE142699 datasets were analyzed to detect dysregulated expressions of mRNAs and miRNAs, respectively. Filtering was applied with p value <0.05 and log2FC≥1 from the raw data analyzed using the limma R package (v.3.46.0).

Determination of circRNA and miRNA Interaction

CSCDv2.0 and circBank databases were used to identify miRNAs that could be sponged via selected circRNAs. The relationship between AML and these detected miRNAs was investigated in the literature using "AML, hsa_circ_0012152, miR-199a-5p" and " Acute myeloid leukemia, hsa_circ_0012152, miR-199a-5p" parameters in PUBMED and other internet networks.

Detection of miRNA-mRNA Relation

The GSE142699 dataset was analyzed to detect dysregulated mRNAs. Filtering was applied with p value <0.05 and log2FC≥1 from the raw data analyzed using the limma R package (v.3.46.0). miRTarBase, miRDB, multiMiR, miRWalk, DIANA-microT, TarBase, miRanda, and TargetScan databases were used for the prediction of selected miRNAs and possible target genes. By evaluating the literature data, genes that may be strongly associated with AML were identified.

Enrichment Analyses Via KEGG and GO Programs

The pathways analysis was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) programs. The search tool for the retrieval of interacting genes/proteins (STRING) database and Cytoscape tool were used to construct and view protein interaction. Hub gene analysis for selected miRNAs' possible target genes was investigated using the molecular complex detection (MCODE) tool with default parameters (9).

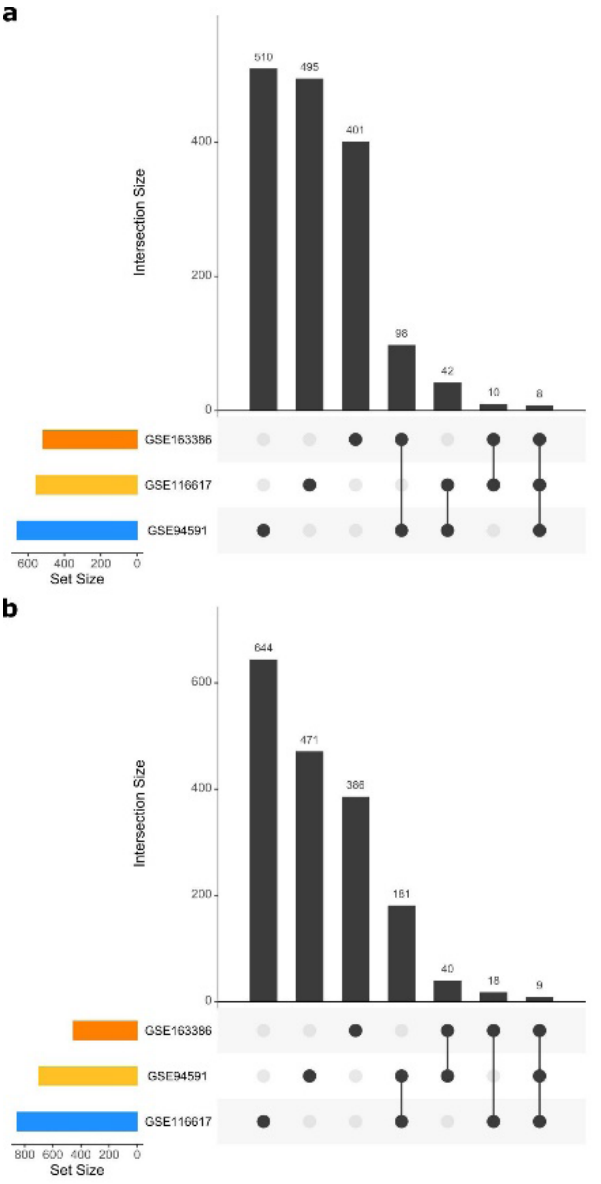

Figure 1. Dysregulated circRNAs detected in the GSE94591, GSE116617 and GSE163386 datasets. a) It was determined that 510 circRNA, 495 circRNA, and 401 circRNA were down-regulated in GSE94591, GSE116617, and GSE163386 datasets respectively. 98 circRNAs overlap in the GSE94591 and GSE163386 datasets. 42 circRNAs overlap in the GSE116617 and GSE94591 datasets. 10 circRNAs overlap in the GSE163386 and GSE116617 datasets. Eight circRNAs overlap in the GSE94591, GSE116617, and GSE163386 datasets. b) It was determined that 644 circRNA, 471 circRNA, and 386 circRNA were up-regulated in GSE116617, GSE94591, and GSE163386 datasets respectively. 181 circRNAs overlap in the GSE94591 and GSE116617datasets. 40 circRNAs overlap in the GSE94591 and GSE163386 datasets. 18 circRNAs overlap in the GSE163386 and GSE116617 datasets. Nine circRNAs overlap in the GSE163386, GSE94591, and GSE116617 datasets.

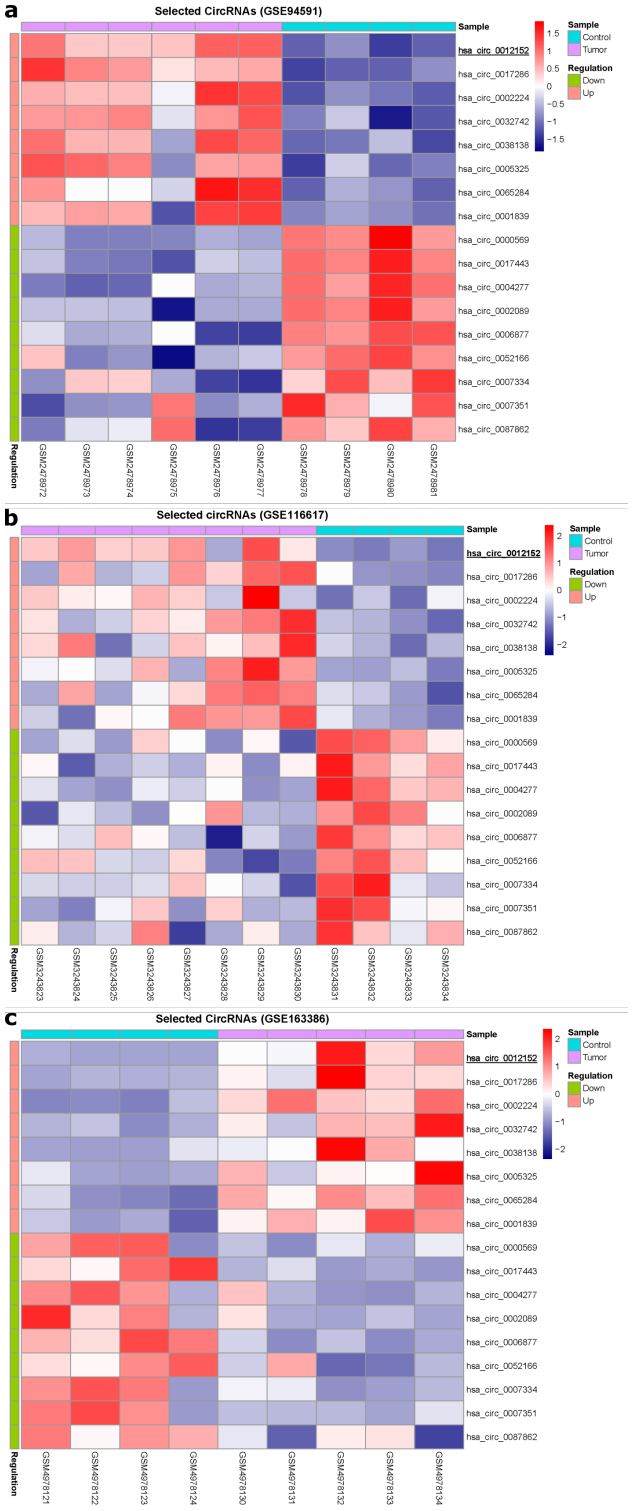

Figure 2. Heatmap of the overlapped down-regulated and up-regulated circRNAs a) GSE94591, b) GSE116617, c) GSE163386.

Survival Analysis

We utilized the gene expression profiling interactive analysis (GEPIA) program to determine the overall survival (OS) of the hub genes.

RESULTS

Detected Differentially Expressed circRNAs (DECs)

It was determined that there were 852 downregulated circRNAs and 555 upregulated circRNAs in the GSE116617 dataset, 453 downregulated circRNAs and 517 upregulated circRNAs in the GSE163386 dataset, and 701 downregulated circRNAs and 658 upregulated circRNAs in the GSE94591 dataset. Among these circRNAs, 9 downregulated and 8 upregulated circRNAs overlapped in 3 datasets (Figure 1). These 17 overlapping dysregulated circRNAs expression heatmaps are shown in Figure 2 for the GSE94591 (a), GSE116617 (b), and GSE163386 (c) datasets. As a result of the literature search, 2 downregulated circRNAs (hsa_circ_0002089, hsa_circ_0006877) and 2 upregulated circRNAs (hsa_circ_0012152, hsa_circ_0005325) were selected, which were determined to be closely related to AML and other cancers. Among them, hsa_circ_0012152 was considered to be more closely related to AML. Wilcoxon test results according to tumor and control samples are shown in Figure 3.

Detected Differentially Expressed miRNAs (DEMs)

It was detected that there were 46 downregulated miRNAs and 45 upregulated miRNAs in the GSE142699 dataset. Among these miRNAs, the top 10 downregulated and upregulated miRNAs were determined according to the log2FC value (Figure 4). After that, 2 downregulated miRNAs (miR-199a-5p, miR-376c-3p) that overlap with hsa_circ_0012152 and 1 downregulated miRNA (miR-495-3p), which overlaps with hsa_circ_0005325, were selected. The expression violin plot data of selected miRNAs are shown in Figures 5-a, b, and c and the volcano plot of the GSE142699 dataset is shown in Figure 5-d.

Detected Differentially Expressed mRNAs (DEGs) and Results of KEGG and GO Analysis

A total of 67 downregulated mRNA and 98 upregulated mRNA were detected in the GSE142698 dataset. The potentially targeted genes of these selected miRNAs were found by *in silico* tools; 33 genes for miR-199a-5p, 15 genes for miR-376c-3p, and 38 genes for miR-495-3p were identified. The circRNA–miRNA–mRNA regulatory network is shown in Figure 6a. The target genes' protein-protein interaction networks are shown in Figure 6b and 6c. According to the hub genes, the KEGG and GO pathway analysis results are shown in Figure 7.

Survival Analysis

Survival analysis of 10 possible target genes of miR-199a-5p was investigated. It was determined that Homeobox A9 (*HOXA9*) and Mediator of DNA damage checkpoint 1 (*MDC1*) genes may be important in the survival of AML patients (Figure 8).

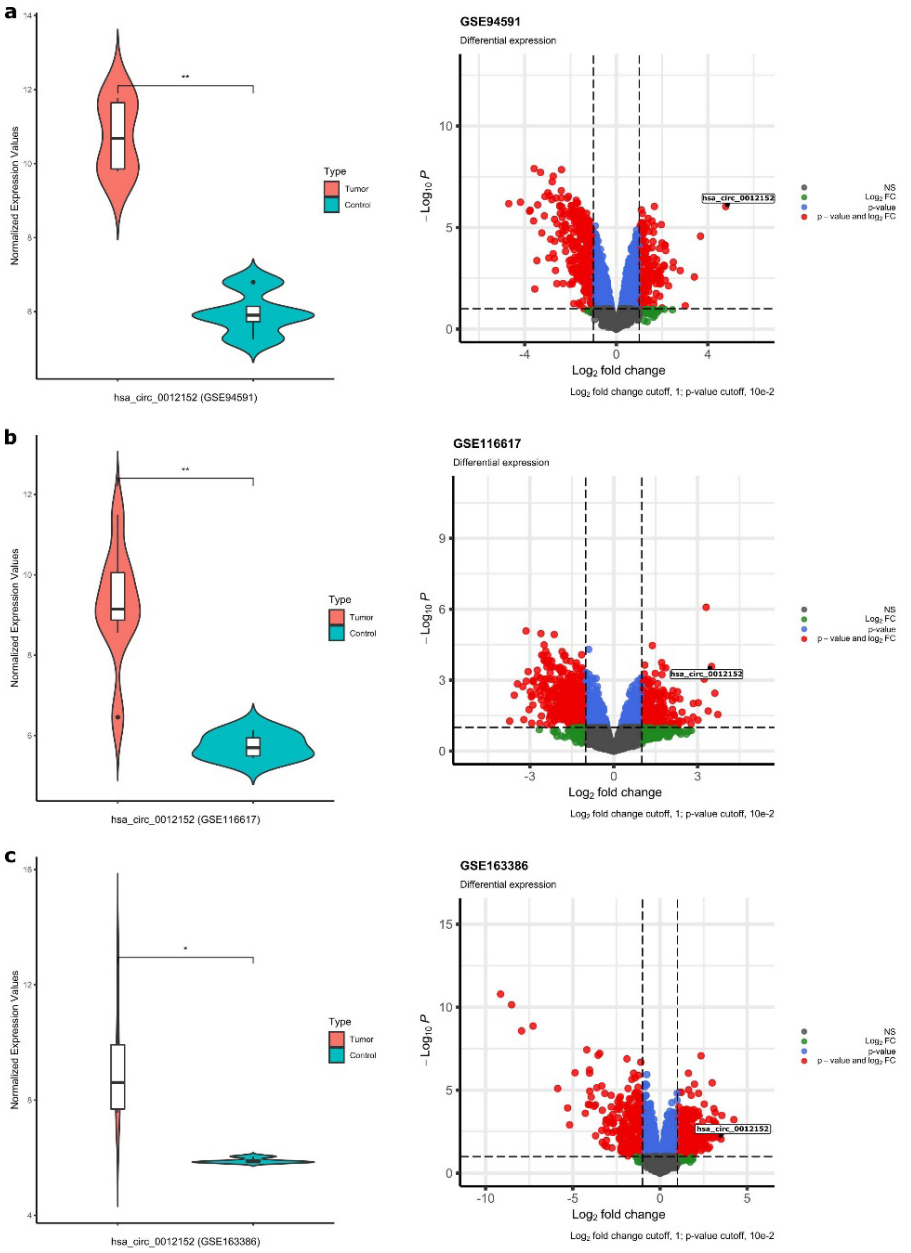

Figure 3. The violin and box plots of hsa_circ_0012152 in a) GSE94591 dataset, b) GSE116617 dataset, c) GSE163386 dataset.

DISCUSSION

Various microarray and RNAseq studies of circRNAs in AML have been performed and it has been determined that thousands of circRNAs were expressed differently in tumor tissue compared to normal tissue. Because it is difficult to study the complex link between these numerous circRNAs, miRNAs, and genes *in vitro and in vivo*, determining the most significant circRNA-miRNA-gene axis in "*in silico*" first will be advantageous. One of the most important reasons for this issue is that each circRNA has the potential to sponge so many miRNAs, and each miRNA has the power to change the expression level of hundreds of genes as well (12). Because of significant advances in bioinformatics, it is now possible to determine circRNA-miRNA-gene relationships *in silico*. Based on the findings of bioinformatics investigations, more precise results can be obtained in "*in vitro and in vivo*" studies.

The expression of hsa_circ_0012152, which we detected to be up-regulated in all 3 datasets in our bioinformatics study, was reported to be up-regulated in AML samples according to both microarray and qRT-PCR results in the study performed by Guo S et al. Moreover, in the same study, it was emphasized that hsa_circ_0012152 may have an expression pattern that distinguishes between AML and ALL (13). It has been reported that hsa_circ_0005325, another prominent circRNA in our

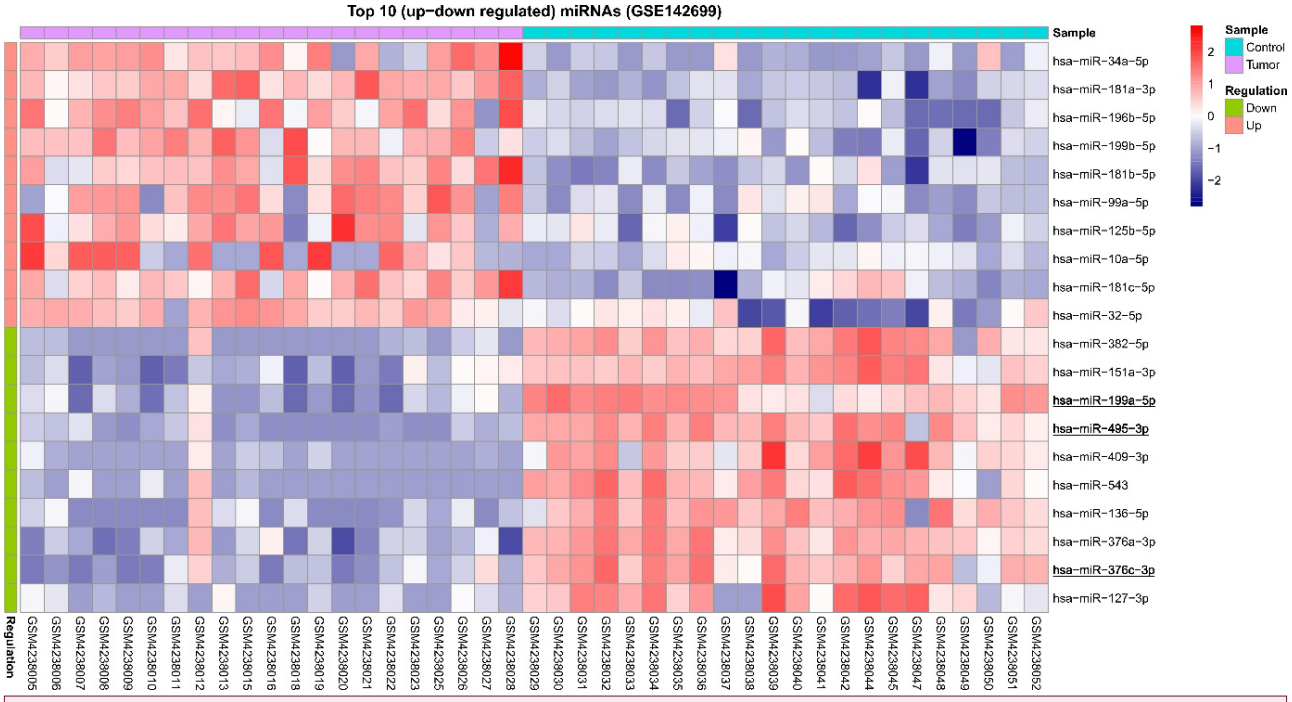

Figure 4. GSE142699 heat map of top 10 selected miRNAs.

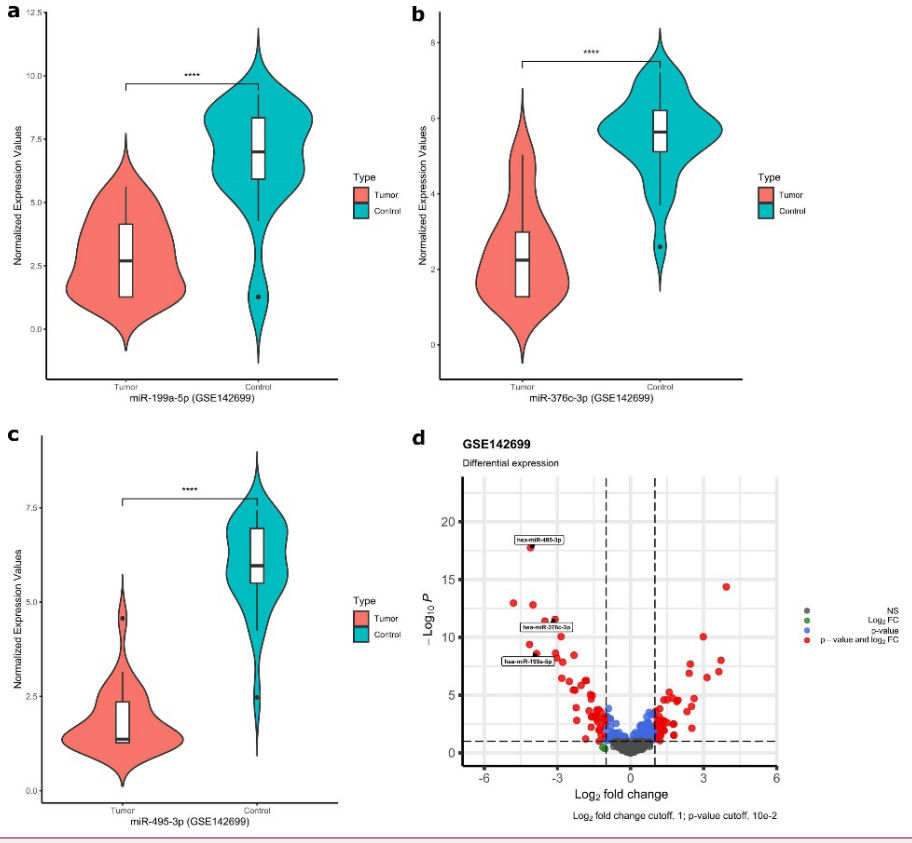

Figure 5. Expression violin plot data of selected miRNAs a) miR-199a-5p, b) miR-376c-3p, c) miR-495-3p, d) GSE142699 volcano plot.

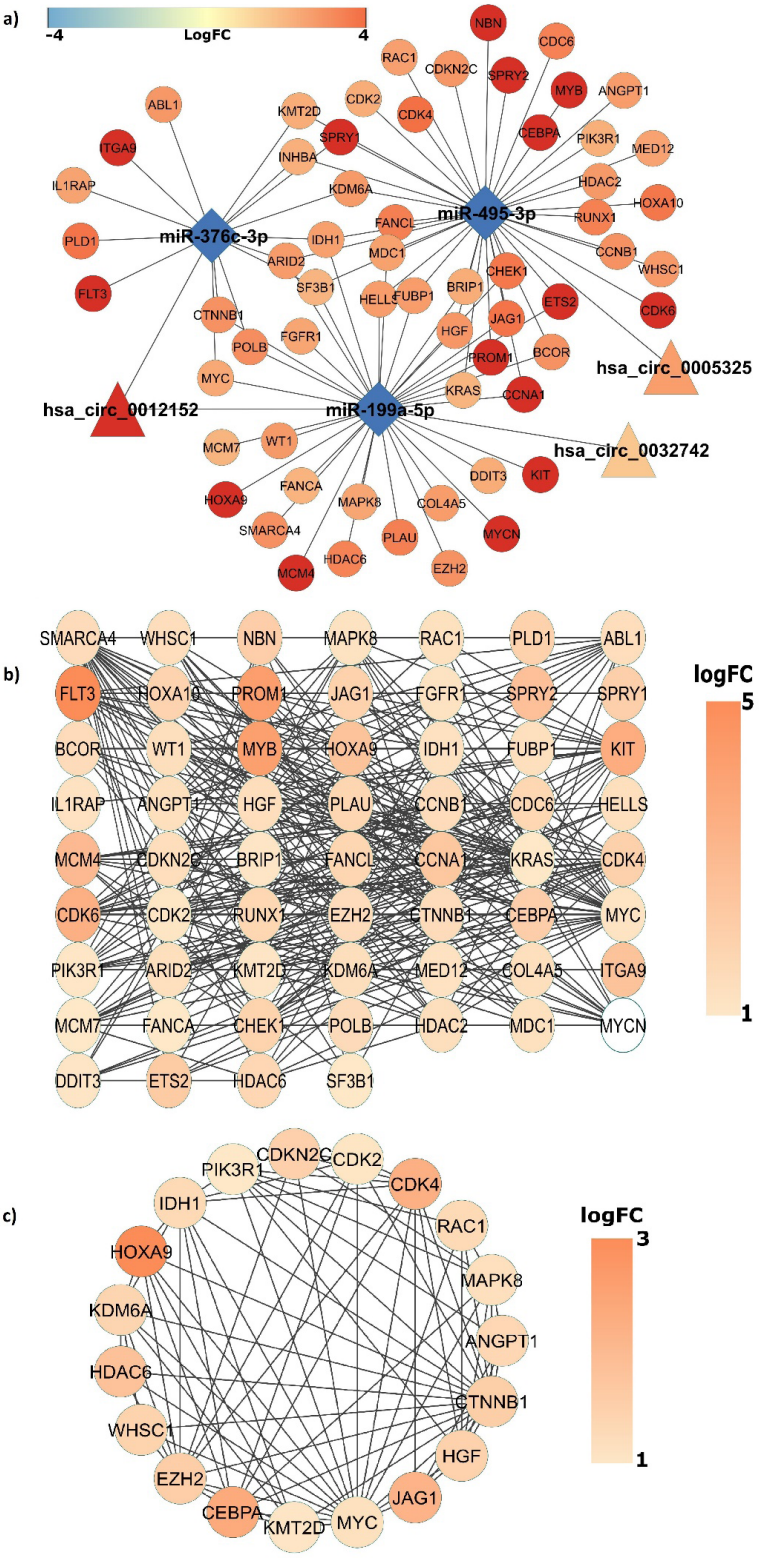

Figure 6. circRNA–miRNA–mRNA regulatory network and protein-protein interaction network of the target genes. a) The network consisting of three cricRNAs (hsa_circ_0012152, hsa_circ_0005325, and hsa_circ_0032742), three miRNAs (miR-376c-3p, miR-495-3p, and miR-199a-5p), and 62 genes was generated by Cytoscape 3.9.0. b) PPI network of the 60 target genes that exert momentous roles in AML. c) The hub genes were identified by the MCODE tool.

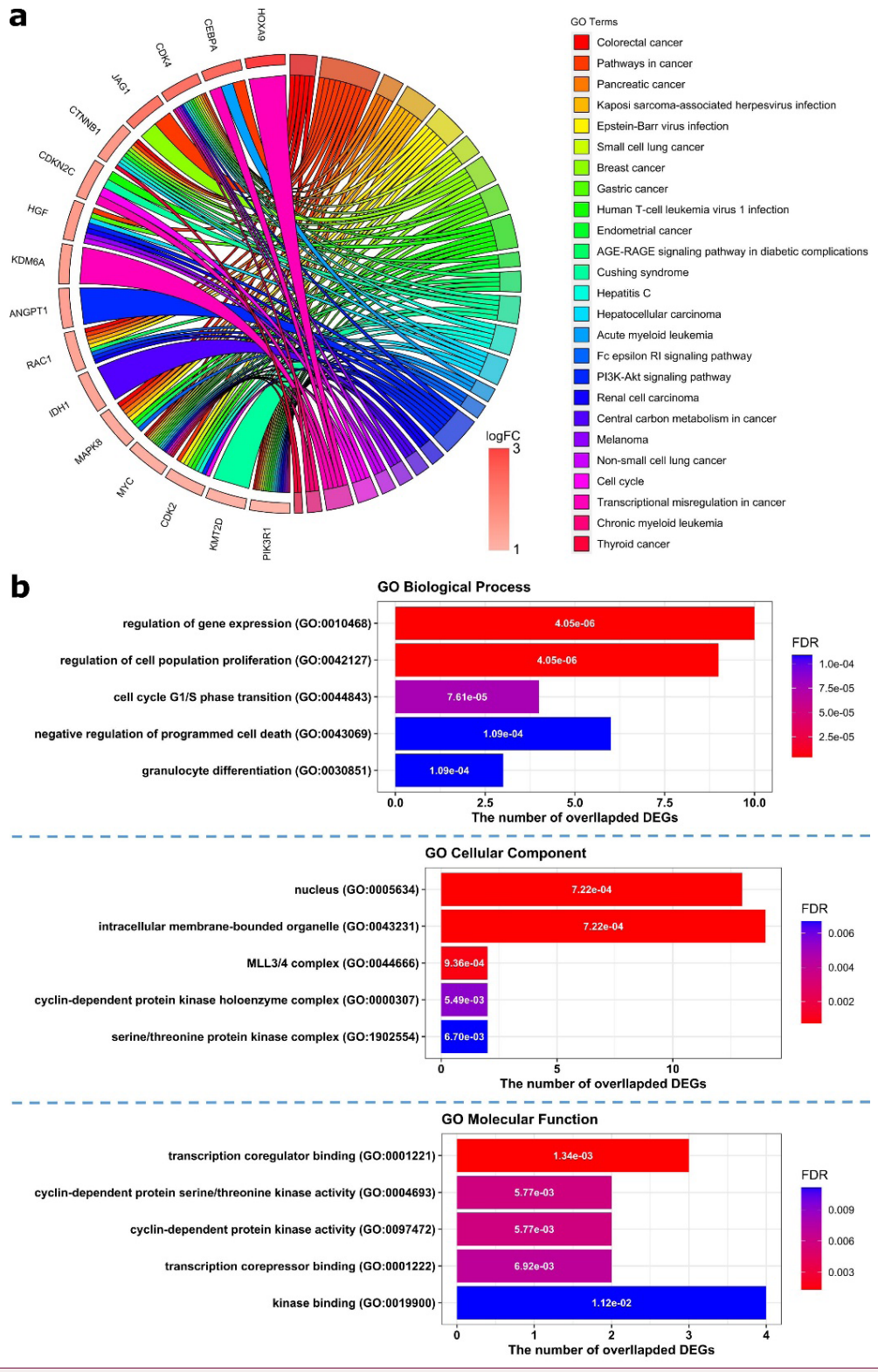

Figure 7. KEGG and GO pathway analysis results. a) The significantly enriched KEGG pathways with a FDR < 0.05. Cohort plot shows that the sixteen hub genes are correlated via ribbons with their assigned KEGG terms. b) Top five GO enrichment annotations of the sixteen hub genes (biological process, cellular component, molecular function). FDR is calculated using the Benjamini-Hochberg method to adjust the multiple hypothesis testing. KEGG: Kyoto encyclopedia of hub-genes and genomes; GO: Gene ontology; DEGs: Differentially expressed genes; FDR: False discovery rate.

study, is also upregulated in lung adenocarcinoma cells (14) and hepatic fibrosis (15). It has been shown that hsa_circ_0002089, which was detected to be significantly down-regulated in our AML datasets, is also among the top 10 down-regulated circRNAs in gastric cancer (16). hsa_circ_0006877 (circLDLR), which was found to be decreased in AML in our study data, has also been shown in the literature to be associated with various cancers such as papillary thyroid carcinoma and hepatocellular carcinoma (17, 18). In addition, it has been demonstrated that the hsa_circ_0006877 expression level decreases in some diseases other than cancer, such as polycystic ovary syndrome(19). Of these 4 circRNAs with significant expression changes in all 3 datasets, hsa_circ_0012152 and hsa_circ_0005325 were also found to interact with the miRNAs detected by the analysis of the GSE142699 dataset in our study. We also determined that hsa_circ_0005325 could be the sponge for miR-495-3p. In the literature, the LGMN pseudogene has been reported to promote tumor progression by acting as a sponge for miR-495-3p in glioblastoma cancer (20). In AML, it has been reported that miR-495-3p may have a pivotal role in patients with cytogenetically normal (21). It has been reported that miR-376c-3p, one of the two miRNAs determined to interact with hsa_circ_0012152, may affect the cell cycle in neuroblastoma cells via cyclin D1 (22). It has been shown that miR-199a- 5p may have a crucial role in many cancer types, including AML. For example, miR-199a-5p was sponged by Linc00662 in bladder cancer and its role in tumor development has been reported (23). In another study, it was shown that this miRNA is involved in the regulation of cancer stemness via the HOTAIR/Sp1 axis in cutaneous-squamous cell carcinoma (24). It has been shown that miR-199a-5p is effective in the sensitivity of AML cells to Adriamycin via the *DRAM1* gene (25). It has been stated that miR-199a-5p plays a role in the regulation of the chemoresistance process, and it has been emphasized that it may be an important therapeutic target miRNA in drug-resistant AML (26). Many of the 33 genes in the GSE142699 dataset identified as targets of miR-199a-5p have been reported to be associated with cancer and AML. For example, the *MDC1* gene, which was determined as the hub gene among these genes, was found to be closely related to many cancers (27, 28). In the study by Ruff et al., it was suggested that *MDC1* may be an important biomarker in carcinogenesis (29). Another hub gene, *HOXA9*, which is among the important targets of the miR-199a-5p, has been reported as a director of the prognosis of the disease by playing a role in the increase of blood cells in AML (30).

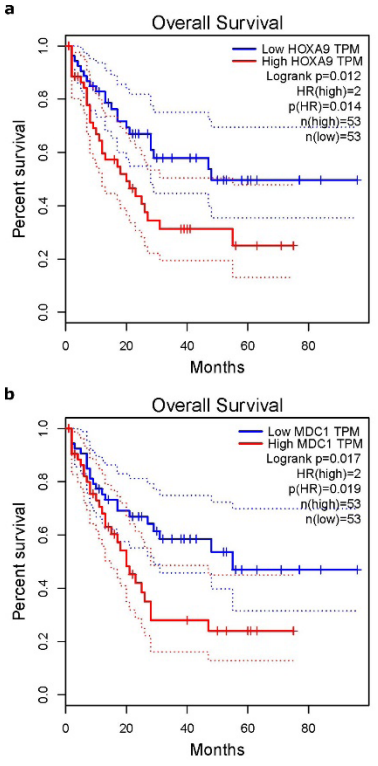

Figure 8. Survival analysis of the *HOXA9* (a) and MDC1 (b) in AML patients.

CONCLUSION

In this study, a meta-analysis of circRNA, miRNA, and mRNA datasets in AML was performed with bioinformatics tools and circRNA-miRNA-mRNA axes that may be important in AML were determined. As a result of all *in silico* evaluations and a detailed literature review, it was understood that hsa_circ_0012152, miR-199a-5p and *HOXA9* may be important for AML. In summary, it was thought that the role of the hsa_circ_0012152/miR-199a-5p/*HOXA9* axis in AML should be investigated with further studies *in vitro and in vivo*.

Ethics Committee Approval: The results of the study were obtained using public Geo Datasets. Since these data are bioinformatics analysis data and there is no clinical or experimental studies have been conducted, ethics committee approval is not required.

Author Contributions: Conception/Design of Study- C.E., M.K., I.S.; Data Analysis: C.E.; Interpretation and Drafting Manuscript- I.S., M.K.; Critical Revision of Manuscript- C.E., M.K., I.S.; Final Approval – C.E., M.K., I.S.

Conflicts of Interest: The authors declare no conflict of interest.

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REFERENCES

1. Kim TK, Gore SD, Zeidan AM. Epigenetic therapy in acute myeloid leukemia: Current and future directions. Semin Hematol 2015; 52(3):172-83. [CrossRef]
2. Zhang S, Liu M, Yao Y, Yu B, Liu H. Targeting LSD1 for acute myeloid leukemia (AML) treatment. Pharmacol Res 2021; 164: 105335. [CrossRef]
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. [CrossRef]

4. Voso MT, Ottone T, Lavorgna S, Venditti A, Maurillo L, Lo-Coco F, et al. MRD in AML: The role of new techniques. Front Oncol 2019; 9: 655. [CrossRef]
5. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022; 72(1): 7-33. [CrossRef]
6. Capik O, Sanli F, Kurt A, Ceylan O, Suer I, Kaya M, et al. CASC11 promotes aggressiveness of prostate cancer cells through miR-145/IGF1R axis. Prostate Cancer Prostatic Dis 2021; 24(3): 891-902. [CrossRef]
7. Kaya M, Karatas OF. The relationship between larynx cancer and microRNAs. Van Med J 2020; 27(4): 535-41. [CrossRef]
8. Li X, Yang L, Chen LL. The biogenesis, functions, and challenges of circular RNAs. Mol Cell 2018; 71(3): 428-42. [CrossRef]
9. Cheng Y, Su Y, Wang S, Liu Y, Jin L, Wan Q, et al. Identification of circRNA-lncRNA-miRNA-mRNA competitive endogenous rna network as novel prognostic markers for acute myeloid leukemia. Genes (Basel) 2020; 11(8). [CrossRef]
10. Wu DM, Wen X, Han XR, Wang S, Wang YJ, Shen M, et al. Role of circular RNA DLEU2 in human acute myeloid leukemia. Mol Cell Biol 2018; 38(20). [CrossRef]
11. Wang N, Yang B, Jin J, He Y, Wu X, Yang Y, et al. Circular RNA circ_0040823 inhibits the proliferation of acute myeloid leukemia cells and induces apoptosis by regulating miR-516b/PTEN. J Gene Med 2022; 24(3): e3404. [CrossRef]
12. 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; 15(1): 16-23 [CrossRef]
13. Guo S, Li B, Chen Y, Zou D, Yang S, Zhang Y, et al. Hsa_circ_0012152 and Hsa_circ_0001857 accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Front Oncol 2020; 10: 1655. [CrossRef]
14. Yan YY, Yang JT, Pathak JL, Wang HY, Zha J, Wei YX, et al. CircRNA_104889 promotes lung adenocarcinoma cell invasion via sponging miR4458. Cancer Cell International 2020; 20(1). [CrossRef]
15. Yang YR, Hu S, Bu FT, Li H, Huang C, Meng XM, et al. Circular RNA CREBBP suppresses hepatic fibrosis via targeting the hsa-miR-1291/LEFTY2 axis. Front Pharmacol 2021; 12: 741151. [CrossRef]
16. Shao Y, Li J, Lu R, Li T, Yang Y, Xiao B, et al. Global circular RNA expression profile of human gastric cancer and its clinical significance. Cancer Med 2017; 6(6): 1173-80. [CrossRef]
17. Jiang YM, Liu W, Jiang L, Chang H. CircLDLR promotes papillary thyroid carcinoma tumorigenicity by regulating miR-637/LMO4 Axis. Dis Markers 2021; 2021: 3977189. [CrossRef]
18. Jia Y, Li S, Zhang M, Zhang Z, Wang C, Zhang C, et al. Circ_LDLR knockdown suppresses progression of hepatocellular carcinoma via modulating miR-7/RNF38 axis. Cancer Manag Res 2021; 13: 337-49. [CrossRef]
19. Huang X, Wu B, Chen M, Hong L, Kong P, Wei Z, et al. Depletion of exosomal circLDLR in follicle fluid derepresses miR-1294 function and inhibits estradiol production via CYP19A1 in polycystic ovary syndrome. Aging (Albany NY). 2020; 12(15): 15414-35. [CrossRef]
20. Liao K, Qian Z, Zhang S, Chen B, Li Z, Huang R, et al. The LGMN pseudogene promotes tumor progression by acting as a miR-495-3p sponge in glioblastoma. Cancer Lett 2020; 490: 111-23. [CrossRef]
21. Esa E, Hashim AK, Mohamed EHM, Zakaria Z, Abu Hassan AN, Mat Yusoff Y, et al. Construction of a microRNA-mRNA regulatory network in de novo cytogenetically normal acute myeloid leukemia patients. Genet Test Mol Biomarkers 2021; 25(3): 199-210. [CrossRef]
22. Bhavsar SP, Løkke C, Flægstad T, Einvik C. Hsa-miR-376c-3p targets Cyclin D1 and induces G1-cell cycle arrest in neuroblastoma cells. Oncol Lett 2018; 16(5): 6786-94. [CrossRef]
23. Ma X, Wen Y, Wang Y, Zhang M, Shi L, Wang C, et al. Linc00662 plays an oncogenic role in bladder cancer by sponging miR-199a-5p. Am J Transl Res 2021; 13(11): 12673-83.
24. Chen J, Hou SF, Tang FJ, Liu DS, Chen ZZ, Zhang HL, et al. HOTAIR/Sp1/miR-199a critically regulates cancer stemness and malignant progression of cutaneous squamous cell carcinoma. Oncogene 2022; 41(1): 99-111. [CrossRef]
25. Li Y, Sun Y, Miao M, Shi X, Yang W, Liu ZG. [MiR-199a-5p Affects sensitivity of acute myeloid leukemia to adriamycin by targeting DRAM1]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2020; 28(4): 1096-104.
26. Li Y, Zhang G, Wu B, Yang W, Liu Z. miR-199a-5p represses protective autophagy and overcomes chemoresistance by directly targeting DRAM1 in acute myeloid leukemia. J Oncol 2019; 2019: 5613417. [CrossRef]
27. Singh N, Bhakuni R, Chhabria D, Kirubakaran S. MDC1 depletion promotes cisplatin induced cell death in cervical cancer cells. BMC Res Notes 2020; 13(1): 146. [CrossRef]
28. Liu X, Dong R, Jiang Z, Wei Y, Li Y, Wei L, et al. MDC1 promotes ovarian cancer metastasis by inducing epithelial-mesenchymal transition. Tumour Biol 2015; 36(6): 4261-9. [CrossRef]
29. Ruff SE, Logan SK, Garabedian MJ, Huang TT. Roles for MDC1 in cancer development and treatment. DNA Repair (Amst). 2020; 95: 102948. [CrossRef]
30. Talarmain L, Clarke MA, Shorthouse D, Cabrera-Cosme L, Kent DG, Fisher J, et al. HOXA9 has the hallmarks of a biological switch with implications in blood cancers. Nat Commun 2022; 13(1): 5829. [CrossRef]