Original Article

Eur J Breast Health 2024; 20(2): 102-109
DOI: 10.4274/ejbh.galenos.2024.2023-12-2

Turmeric Inhibits MDA-MB-231 Cancer Cell Proliferation, Altering miR-638-5p and Its Potential Targets

Murat Kaya[1], Asmaa Abuaisha[2], Ilknur Suer[3], Selman Emiroglu[4,5], Fahrunnisa Abanoz[2], Sukru Palanduz[1], Kivanc Cefle[1], Sukru Ozturk[1]

[1]Division of Medical Genetics, Department of Internal Medicine, Istanbul University-Istanbul Faculty of Medicine, Istanbul, Turkey
[2]Department of Genetics, Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Turkey
[3]Department of Medical Genetics, Istanbul University-Istanbul Faculty of Medicine, Istanbul, Turkey
[4]Division of Breast Surgery, Department of General Surgery, Istanbul University-Istanbul Faculty of Medicine, Istanbul, Turkey
[5]Department of Molecular and Medical Genetics, Biruni University Graduate School of Education, Istanbul, Turkey

ABSTRACT

Objective: Recent research suggests curcumin extracted from the turmeric plant may inhibit the proliferation of cancer cells by controlling the expression of microRNAs (miRNAs). The effect of phenolic curcumin on miR-638-5p and potential target gene expressions in the triple negative breast cancer (TNBC) cell line MDA-MB-231 was investigated in this study.

Materials and Methods: GSE154255 and GSE40525 datasets were downloaded and analyzed using GEO2R to identify dysregulated miRNAs in TNBC. To find differently expressed genes in breast cancer (BRCA), The Cancer Genome Atlas Program data was examined. Utilizing *in silico* tools, KEGG, GO, and other enrichment analyses were performed. The databases miRNet, miRTarBase v8.0, and TarBase v.8 were used for miRNA and mRNA matching. Real-time quantitative reverse transcription polymerase chain reaction was used to examine the levels of miRNA and its targets in miRNA mimic transfected/curcumin-treated MDA-MB-231 cultures and controls. The cell viability detection kit-8 method was used to assess cell viability, and the scratch assay was used to conduct migration assessment.

Results: Bioinformatics analysis showed that miR-638-5p was significantly reduced in TNBC patients. Experimental results showed that miR-638-5p was upregulated in MDA-MB-231 treated with curcumin, while the potential target genes of miR-638-5p, *CFL1*, *SIX4*, *MAZ,* and *CDH1* were downregulated. Mimic miR-638-5p transfection inhibited MDA-MB-231 cell proliferation and reduced migration and expression of *CFL1*, *SIX4,* and *MAZ* genes was decreased in mimic miR-638-5p transfected cells.

Conclusion: These findings suggest that curcumin exerts its anticancer effects on MDA-MB-231 cells by modulating the expression of miR-638-5p and its possible target genes.

Keywords: Triple negative breast cancer; bioinformatics; MDA-MB-231; curcumin; miR-638-5p

Cite this article as: Kaya M, Abuaisha A, Suer I, Emiroglu S, Abanoz F, Palanduz S, Cefle K, Ozturk S. Turmeric Inhibits MDA-MB-231 Cancer Cell Proliferation, Altering miR-638-5p and Its Potential Targets. Eur J Breast Health 2024; 20(2): 102-109

Key Points

- This is the first study investigating the curcumin/miR-638-5p/potential target genes in triple negative breast cancer (TNBC) cell line MDA-MB-231.
- The relationship between TNBC and miRNAs/genes was studied using bioinformatics tools and *in vitro* experiments, and many important miRNAs and genes have been identified.
- MiR-638-5p may play an important role in the cancer process through its potential target genes *CFL1, SIX4*, and *MAZ.*

Introduction

The molecular tumor complexity of breast cancer (BRCA) is an important obstacle to the treatment. Even though there are many useful therapies (surgery, radiation therapy, or hormone therapy), BRCA metastases, drug resistance, and relapse result in poor patient survival. Curcumin is the most prominent polyphenol component extracted from the turmeric (rhizomes of Curcuma longa). Vogel and Pelletier of the Harvard College Laboratory first identified curcumin in 1815 (1). Much subsequent research has demonstrated that curcumin is extremely beneficial to health (2). Its cytotoxic efficacy in several cancer cell lines, including BRCA, has been demonstrated. The pleiotropic action of curcumin in cancer cell inhibition is due to its numerous targets, which include signaling pathways, proteins/

Corresponding Author:
Murat Kaya; kmurat@istanbul.edu.tr

Received: 05.12.2023
Accepted: 04.02.2024
Available Online Date: 01.04.2024

enzymes, and microRNAs (miRNAs) (3). miRNAs are single-stranded RNA molecules with around 18-22 nucleotides that act as master regulators of gene expression by binding to their target mRNAs in the cells (4, 5). In 271 species, 38,589 mature miRNAs have been identified, including 2654 mature human miRNAs (6). By recognizing matching sequences at the 3' UTR region of the target mRNA, a single miRNA may affect thousands of genes (7, 8). Many studies, especially in the last 10 years, have demonstrated that dysregulation of miRNA expression is associated with almost every kind of cancer, including BRCA (9, 10). Cancer hallmarks, such as maintaining cell proliferative signaling, apoptosis avoidance, stimulating invasion and metastasis, and triggering angiogenesis have been linked to altered miRNAs (11). Studies show that many natural dietary supplements, including curcumin, have important roles in various cellular processes (12, 13). The results suggest that these may make important contributions to the fight against cancer in the future (14).

This study was conducted to investigate the relationship between BRCA, miRNAs, mRNA and curcumin using *in silico* and *in vitro* methods. Briefly, geo datasets were used to identify miRNAs and genes that may be linked to BRCA. *In silico* tools were used to match the detected miRNAs and target genes. Enrichment analyses of selected miRNAs and genes were performed using various bioinformatics tools. The relationship between the selected miRNA and the target genes were then confirmed in *in vitro* evaluation, and the expression levels of the relevant miRNA and genes were investigated in curcumin-treated cells.

Materials and Methods

Identification of Triple Negative Breast Cancer (TNBC)-Associated miRNAs

Overlapping miRNAs between GSE154255 and GSE40525 datasets, which met the criteria of logFC >2 and logFC >1, respectively, and $p<0.05$ for both datasets, were identified. This was carried out because the GSE154255 dataset contains very few miRNAs with a logFC >2 value, the logFC >1 value was used for this dataset.

Identification of the Effect of Overlapping miRNAs on Overall Survival in BRCA and TNBC

Whether overlapping miRNAs were effective on overall survival (OS) in both BRCA and TNBC was investigated in METABRIC data using kmplot (https://kmplot.com/analysis/) and a significant miRNA was selected for further *in silico* and *in vitro* analysis.

The Detection of Overexpressed Genes in BRCA

To identify significant genes in BRCA, The Cancer Genome Atlas Program (TCGA) BRCA data were searched through the GEPIA2 (http://gepia2.cancer-pku.cn/) web tool. Among the overexpressed genes in TCGA BRCA data, genes that met LogFC >1 and $p<0.05$ criteria were identified.

In silico Investigation of Potential Target Genes of the Selected miRNA

In silico potential target genes of the selected miRNA were identified using the databases miRNet (https://www.mirnet.ca/), miRTarBase v8.0 (https://mirtarbase.cuhk.edu.cn/) and TarBase v.8 (https://dianalab.e-ce.uth.gr/) tools.

Detection of Overlapping Genes Between *in silico* Target Genes of the Selected miRNA and Significant Genes in TCGA BRCA Data

The overlapping genes between the *in silico* potential targets of the selected miRNA and the genes overexpressed in TCGA BRCA and meeting the LogFC >1 and $p<0.05$ criteria were determined.

Enrichment Analysis of Overlapping Genes

The diseases, hub proteins, and pathways most associated with overlapping genes were identified using the Enrichr (https://maayanlab.cloud/Enrichr/) and ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/) tools.

Identification of Genes Associated With BRCA Overall Survival

Employing the kmplot tool, it was determined whether overlapping genes were associated with BRCA OS.

In vitro Studies

Cell Culture

For cell culture, the TNBC cell line, MDA-MB-231, was cultivated in Dulbecco's Modified Eagle's Medium (DMEM) (EcoTech Biotechnology, Erzurum, Turkey) with 1% penicillin (Invitrogen, Thermo Fisher Scientific Inc., Waltham, MA, USA) 10% fetal bovine serum (FBS) (EcoTech Biotechnology, Turkey) in a humidified incubator (Sanyo) with 5% CO_2 at 37 °C.

Curcumin Treatment

Highly purified curcumin (Bio Basic Inc., Canada) was dissolved in dimethyl sulfoxide (DMSO) (1 mg/mL). Curcumin was prepared at different concentrations (1 μM, 3 μM, 5 μM, 10 μM, 30 μM and 50 μM). As curcumin was dissolved in DMSO, the control cells were treated with DMSO at the same quantities as the experimental groups. Cells were maintained in 6-well or 96-well plates (Nest Biotechnology Co., China) for 24 hours at 37 °C. The 50% inhibition concentration (IC50) value of curcumin was determined (10 μM). For further investigation, this value was used to treat MDA-MB-231 cells.

miR-638-5p Mimic Transfection

MDA-MB-231 cells were seeded at sixty percent confluency into 96-well or 6-well cultivation plates. Afterward, using the supplier's protocol for transient overexpression of miR-638-5p, cells were transfected with 30 pM miR-638-5p mimic (5′-AGGGAUCGCGGGCGGGUGGCGGCCU-3′) (Thermo Fisher Scientific), or non-targeting (NT) control miRNA using lipofectamine 2000 (Invitrogen). Following 24 hours of culture, transfected cells were used for functional assays.

RNA Isolation, cDNA Synthesis Process, and Quantitative Real-time PCR

Total RNA was extracted from curcumin-treated and miR-638-5p transfected cells and control cultures using TRIzol (Invitrogen). A NanoDrop spectrophotometer (Thermo) was used to assess the quality and quantity of the RNA samples. To examine the expression of selected genes or miRNAs, equal amounts of RNA from the specimens were reverse-transcribed into cDNA using a cDNA Reverse Transcription Kit (Invitrogen, Thermo Fisher Scientific) or TaqMan Kit (Invitrogen, Thermo Fisher Scientific), respectively. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) reactions were carried out via 5x HOT FIRE qPCR Mix Plus (Solis Bio-Dyne Co, Estonia) or TaqMan Advanced Master Mix (Invitrogen, Thermo Fisher Scientific). Table 1 shows the primer sequences used for qRT-PCR experiments. *B-actin* or RNU43 expression were used to normalize gene or miRNA expression. All reactions were performed at least twice. The $2^{-\Delta\Delta Ct}$ method was employed to calculate the relative expressions of the genes and miRNAs that were investigated.

Detection of Cell Viability Using Cell Viability Detection Kit-8

Cell viability was determined via the cell viability detection kit-8 (CVDK-8) assay (EcoTech Biotechnology) MDA-MB-231 cells (3 x 10^3 cells per well) were seeded into 96-well plates in five replicates and incubated for 24 hours. Then the cells were transfected with lipofectamine 2000 (Invitrogen) reagent to express miR-638-5p mimic or NT miRNA. After 24 hours, each well was treated with CVDK-8 reagent, and the plates were incubated for three hours. A Multiskan spectrophotometer (Thermo Fisher Scientific) was used to measure absorbance at 450 nm.

Detection of Cell Proliferation Using the Viability Imaging Method

After enzyme-linked immunoabsorbance (ELISA) evaluation for cell viability, the 96 well plate was inverted and the liquid was removed. Then the wells were washed with PBS. After removal of the PBS, a light microscope image was taken at x10 and recorded.

Scratch Assay

5 x10^5 MDA-MB-231 cells in DMEM with 10% FBS were seeded in 6-well plates. When the cells reached 95% confluency, scratches were made with a 10 µL pipette tip. After removing the medium from the plate and washing with PBS, the attached cells were cultured in DMEM. The cells that migrated to the “wound area” were measured from multiple microscopic areas, and images were captured at 0 and 24 hours with a light microscope at x100 magnification.

Statistical Analysis

Publicly available data were used in part of the bioinformatics the study, and in the miRNA analysis, those with logFC >2 for GSE154255, logFC >1 for GSE40525, and $p<0.05$ for both datasets were selected. For genes, among the TCGA BRCA data, those with logFC >+1 and $p<0.05$ values were considered significant. In terms of the *in vitro* studies, all data is shown as the mean ± standard deviation of a minimum of two independent experiments that yielded comparable results. Student’s t-test was used to analyze significant differences using GraphPad Prism 7.0. A difference that was statistically significant was indicated by $p<0.05$.

Results

Bioinformatics Analysis

TNBC-Associated miRNAs

The geo dataset analysis revealed 16 downregulated miRNAs to be common in both geo datasets (Table 2).

The Prognostic Importance of Selected miRNA

The KMplot survival evaluation revealed that decreased expressions of miR-638-5p and miR-139-3p had an effect on the OS of BRCA patients in general and also for the TNBC subtype of BRCA (Figure 1). As miR-638-5p was found to be more closely associated with BRCA on literature review, it was chosen for the remaining *in silico* analyses and the *in vitro* study.

Overexpressed Genes in TCGA BRCA Data

Analysis of TCGA BRCA data identified 248 genes which met the LogFC >+2 and $p<0.05$ criteria.

Detection of Potential Target Genes of the Selected miRNA

Using miRNet (miRTarBase v8.0 and TarBase v.8), it was found that miR-638-5p could potentially target 1416 genes (Figure 2).

Detection of Overlapping Genes

Thirteen genes were found to overlap between the TCGA data and potential *in silico* targets of miR-638-5p (Table 3).

Enrichment Analysis Results

It was found that the thirteen overlapping genes were linked to various cancers, particularly BRCA, and that these genes are associated with cancer-related pathways, such as those involved in cell division and chromosome segregation, as well as being related to hub proteins which are closely associated with BRCA (Figure 3 and Figure 4).

The Prognostic Importance of Selected Genes

The prognostic importance of four hub genes in BRCA patient survival was investigated. It was revealed that the differential expression of *SIX4* and *CDH1* influenced patient survival (Figure 5).

In vitro Investigations

Cell Viability Assay Results

Both ELISA absorbance measurement results and the viability imaging method results showed that curcumin treatment or miR-638-5p mimic transfection significantly reduced the proliferation of MDA-MB-231 cells at 24 hours ($p<0.01$) (Figure 6 and Figure 7).

Table 1. Primer sequences for qRT-PCR

Gene	Forward	Reverse	Ref.
CDH1	5’-AGAACGCATTGCCACATACA-3’	5’-TGCTTAACCCCTCACCTTGA-3’	(30)
MAZ	5’-GGATCACCTCAACAGTCACGTC-3’	5’-GGCACTTTCTCCTCGTGTCGTA-3’	(31)
SIX4	5’-AGCAGCTCTGGTACAAGGC-3’	5’-CTTGAAACAATACACCGTCTCCT-3’	(25)
CFL1	5’-TGCTGCCAGATAAGGACTGC-3’	5’-CTCTTAAGGGGCGCAGACTC-3’	(32)
SMC1A	5’-TGATGCTGCCTTGGATAACA-3’	5’-TTCGACCTCACCAAGTACCC-3’	(33)
β-actin	5’-GCCTCGCCTTTGCCGATC-3’	5’-CCCACGATGGAGGGGAAG-3’	(34)

Primer list of selected putative target genes of miR-638-5p for in vitro study. Ref.: Reference. β-actin gene was used internal control (housekeeping gene); qRT-PCR: Real-time quantitative reverse transcription polymerase chain reaction

Scratch Assay Results

Scratch assay results showed that curcumin treatment at a concentraton of 10 μM significantly diminished the cell migration of MDA-MB-231 compared to the untreated control group at 24 hours of evaluation. Furthermore, at 24 hours, miR-638-5p mimic transfection reduced cell migration compared to the NT miRNA mimic group (Figure 8).

qRT-PCR Results

The effect of curcumin treatment or miR-145-5p mimic transfection on the expression of the selected genes were investigated using qRT-PCR. The selected *CDH1*, *MAZ*, *SIX4*, *CFL1*, and *SMC1A* genes were quantified using the primers shown in Table 1. To normalize gene expression, the β-*actin* housekeeping gene was used. It was observed that the expression of *CFL1*, *SIX4*, and *MAZ* genes decreased

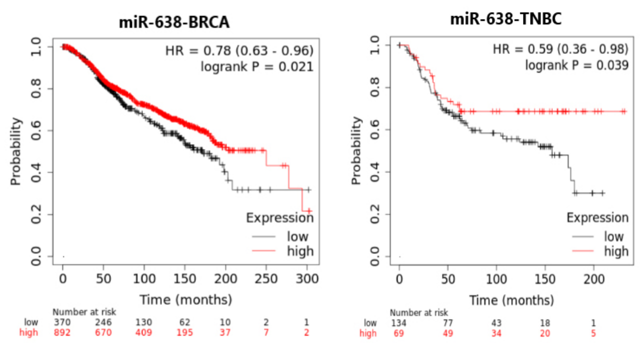

Figure 1. Survival effect of miR-638-5p on BRCA and TNBC. OS analysis was performed via kmplot using METABRIC data (Including 2509 BRCA patients and 300 TNBC patients)

BRCA: Breast cancer; TNBC: Triple negative breast cancer

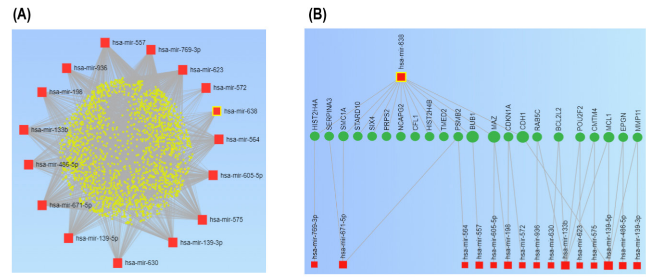

Figure 2. (A) Overlapping miRNAs in the GSE154255 and GSE40525 datasets. Red squares represent 16 overlapping miRNAs, including miR-638-5p, yellow circle shapes indicate probable miRNA targets, and lines illustrate interactions (1856 edge) between miRNAs (16) and genes (1416). **(B)** Relationship between miR-638-5p and 15 other miRNAs and potential more associated genes

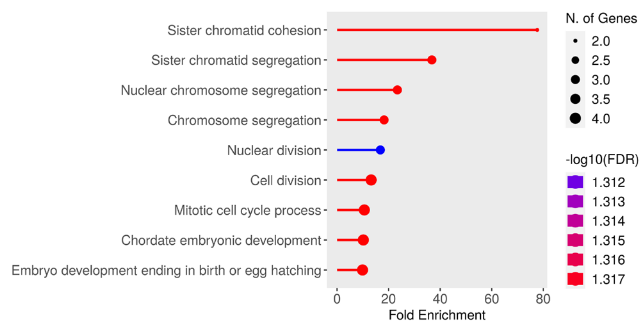

Figure 3. Enrichment analysis of selected possible miR-638-5p 13 targets. Many of these selected genes are related to critical biological events like cell division or mitotic cell cycle process

Table 2. Overlapping miRNAs in the GSE154255 and GSE40525 datasets, which matched the requirements of LogFC >2 and LogFC >1 respectively and *p*<0.05

GSE154255				GSE40525		
Adj. p-value	***p*-value**	**logFC**	**miRNAs**	**logFC**	***p*-value**	**Adj. *p*-value**
0.010161	1.27E-04	-8.61	**hsa-miR-486-5p**	-2.66	3.14e-04	0.018947
0.0012138	6.17E-06	-7.46	**hsa-miR-139-5p**	-2.16	5.21e-04	0.02344
0.0013373	8.00E-06	-7.01	**hsa-miR-557**	-1.78	6.74e-04	0.023942
0.0599677	1.08E-03	-6.66	**hsa-miR-936**	-1.67	2.29e-02	0.1619
0.0148909	2.00E-04	-5.79	**hsa-miR-198**	-1.64	8.49e-03	0.100838
0.0976094	7.36E-02	-5.14	**hsa-miR-564**	-1.55	5.74e-03	0.083076
0.2128058	1.75E-01	-4.97	**hsa-miR-630**	-1.46	1.33e-03	0.034503
0.1205795	9.20E-02	-4.64	**hsa-miR-671-5p**	-1.44	4.21e-02	0.241701
0.0691052	2.08E-02	-4.24	**hsa-miR-572**	-1.32	8.22e-04	0.026259
0.0691052	1.34E-02	-3.73	**hsa-miR-638-5p**	-1.29	1.59e-02	0.139233
0.2189317	1.81E-01	-3.47	**hsa-miR-139-3p**	-1.24	2.24e-02	0.159857
0.0906878	6.80E-02	-3.22	**hsa-miR-575**	-1.22	6.34e-03	0.085311
0.1792597	1.46E-01	-3.19	**hsa-miR-623**	-1.05	6.02e-02	0.285467
0.1457456	1.14E-01	-3.01	**hsa-miR-769-3p**	-1.04	3.87e-03	0.069472
0.4032279	3.70E-01	-2.06	**hsa-miR-133b**	-1.01	7.46e-03	0.091564
0.4032279	3.70E-01	-2.06	**hsa-miR-605**	-1.01	1.05e-01	0.410721

hsa-miR-638-5p and hsa-miR-139-3p that may be more closely associated with BRCA in GSE154255 and GSE40525 are highlighted in red. Adj. *p*-value: Adjusted *p*-value

Table 3. Overlapping 13 genes between miRNet and TCGA BRCA data

Gene symbol	Gene name	LogFC	Adj. *p*-value
BUB1	BUB1 mitotic checkpoint serine/threonine kinase	2.650	1.11e-163
STARD10	StAR related lipid transfer domain containing 10	2.481	2.21e-84
HIST2H4A	H4 clustered histone 14	1.743	1.11e-50
CDH1	Cadherin 1	1.729	1.42e-27
MAZ	MYC associated zinc finger protein	1.611	7.77e-197
HIST2H4B	H4 clustered histone 15	1.560	2.98e-54
SIX4	SIX homeobox 4	1.542	3.97e-65
SERPINA3	Serpin family a member 3	1.391	8.62e-9
TMED2	Transmembrane P24 trafficking protein 2	1.302	7.54e-73
PRPS2	Phosphoribosyl pyrophosphate synthetase 2	1.266	7.59e-60
NCAPG2	Non-SMC condensin II complex subunit G2	1.224	7.85e-68
CFL1	Cofilin 1	1.046	7.84e-143
SMC1A	Structural maintenance of chromosomes 1A	1.046	9.27e-19

13 overlapping genes were identified with logFC >1 and *p*<0.05 values. TCGA: The Cancer Genome Atlas Program; BRCA: Breast cancer; Adj. *p*-value: Adjusted *p*-value

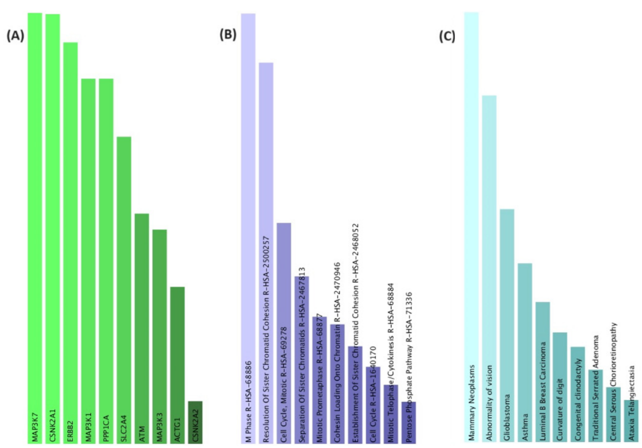

Figure 4. Enrichment analysis of the selected 13 potential miR-638-5p targets in TCGA BRCA samples. **(A)** The hub proteins of the 13 miR-638-5p targets according to huMAP (*p*<0.05). **(B)** Pathways associated with the 13 target genes according to Reactome 22. **(C)** Most related diseases of the 13 target genes in DisGeNet database

BRCA: Breast cancer; TCGA: The Cancer Genome Atlas Program

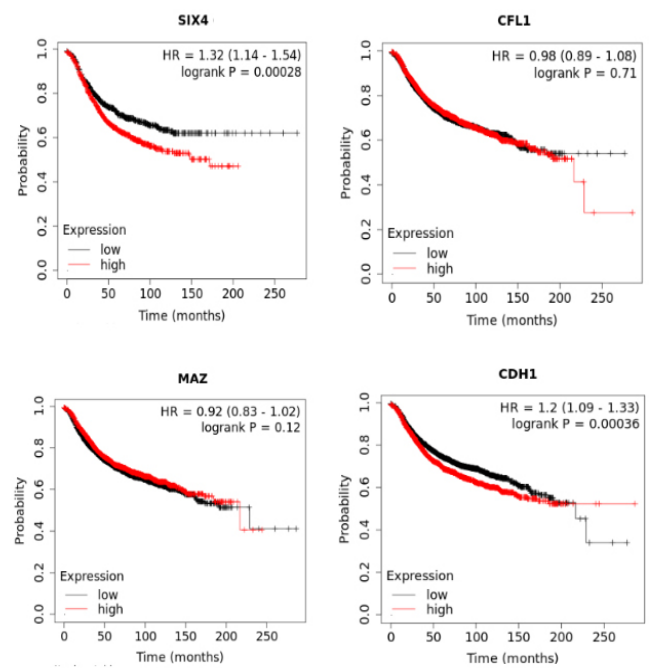

Figure 5. The effect of selected genes' overexpression on BRCA patients OS. Overexpression of the *SIX4* and *CDH1* genes was associated with OS, but not the *CFL1* and *MAZ* genes

HR: Hazard ratio; BRCA: Breast cancer; OS: Overall survival

significantly in both MDA-MAB-231 cells administered curcumin and MDA-MB-231 cells transfected with the miR-638-5p mimic (Figure 9).

Discussion and Conclusion

Numerous studies have shown that curcumin inhibits cancer cell proliferation, increases apoptosis, and disrupts migration via its effect on miRNAs. It has been reported that curcumin inhibits the progression of colorectal cancer cells by regulating the *CDCA3*/*CDK1* pathway via miR-134-5p (15). Curcumin has been shown to inhibit cell growth in BRCA through the miR-21/*PTEN*/Akt pathway. Liang et al. (16) reported that curcumin suppressed the survival, migration, and invasion of papillary thyroid cancer cells by modulating the miR-301a-3p/*STAT3* axis.

In the bioinformatics section of the present study, we found that only miR-638 and miR-139-3p had a significant effect on the OS of both BRCA, and specifically TNBC, patients among the overlapping miRNAs in the GSE154255 and GSE40525 datasets (Figure 1).

MiR-638-5p was chosen for the *in vitro* study based on the data obtained from the literature review, as miR-638-5p may be more closely associated with BRCA. However, taking the current study's bioinformatics data and literature results into account, we would like to emphasize that miR-139-3p may also be closely related to BRCA and that more comprehensive studies on the relationship between this miRNA and BRCA and specifically TNBC, are required.

To the best of our knowledge, there is no previous study into the effects of curcumin on cancer processes in BRCA cells specifically via miR-638 and its target genes. Using TCGA data and *in silico* tools, 13 potential miR-638-5p target genes were identified. Five of these 13 genes (*CFL1*, *SIX4*, *MAZ*, *CDH1*, and *SMC1A*) were chosen for *in vitro* examination. In the *in vitro* study, MDA-MB-231 cells were treated with curcumin, and it was found that the expression of miR-638-5p increased in the curcumin-supplemented group compared to the control group, while the expression of *CFL1*, *SIX4*, *MAZ*, and *CDH1* genes decreased. Subsequently, MDA-MB-231 cells were transfected with a miR-638-5p mimic. The expression of *CFL1*, *SIX4*, and *MAZ* genes was found to be reduced in the transfected group (Figure 9B).

miR-638-5p is a tumor suppressor miRNA that has been linked to a variety of cancers (17, 18). Zheng et al. (19) showed that miR-638-5p acts as a tumor suppressor in glioma by regulating *HOXA9*. Another study found that miR-638-5p inhibited cell proliferation in human osteosarcoma by repressing *PIM1* expression (20).

On November 28, 2023, a Pubmed search with the keywords "miR-638, breast cancer" yielded 19 results. Studies into BRCA, specifically investigating the role of miR-638-5p, have revealed that miR-638-5p expression was reduced in BRCA, and that miR-638-5p may be connected with resistance to various chemotherapeutics, radiotherapy, and ultraviolet (UV) sensitivity. He et al. (21) showed that CircNCOR1 regulates the efficacy of radiotherapy in BRCA through the miR-638-5p/*CDK2* axis. Wang et al. (22) reported that miR-638-5p could be used as a biomarker for 5-fluorouracil sensitivity in BRCA treatment. Another study revealed that miR-638-5p/BRCA1 regulation affects DNA repair, as well as sensitivity to UV and cisplatin in TNBC.

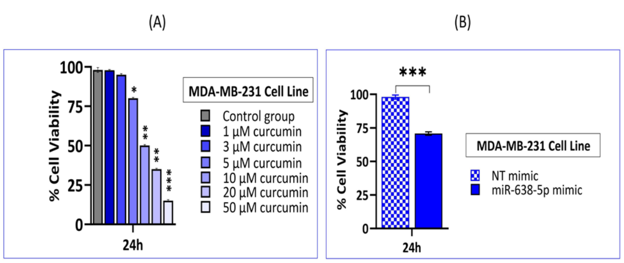

Figure 6. Effect of curcumin treatment and miR-638-5p mimic transfection on MDA-MB-231 cell viability. **(A)** It was observed that the viability of cells treated with different concentrations of curcumin decreased significantly depending on increasing doses. **(B)** Ectopic expression of miR-638-5p was observed to significantly reduce cell viability

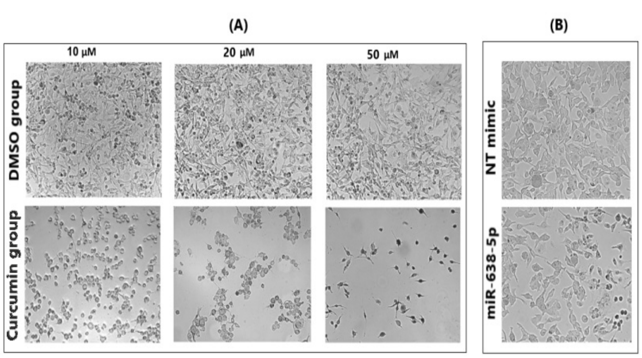

Figure 7. (A) Images of MDA-MB-231 cells treated with 10 μM, 30 μM, and 50 μM curcumin under a 10X light microscope. It has been determined that curcumin significantly reduces cell viability due to increasing concentrations. **(B)** The viability of miR-638-5p mimic transfected cells was significantly reduced compared to NT mimic transfected cells

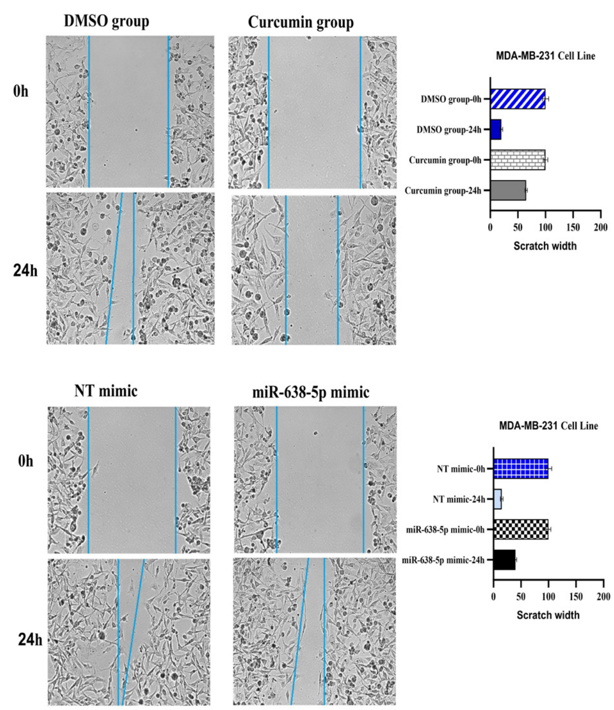

Figure 8. Effect of 10 μM curcumin and miR-638-5p mimic on MDA-MB-231 cell migration. Curcumin treatment and miR-638-5p overexpression inhibited cell migration

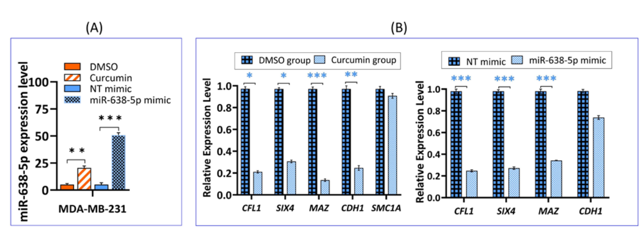

Figure 9. (A) Curcumin treatment and miR-638-5p mimic transfection significantly increased miR-638-5p expression. **(B)** When curcumin was introduced to MDA-MB-231 cells, the expression of *CFL1*, *SIX4*, *MAZ*, and *CDH1* genes decreased significantly, whereas *SMC1A* gene expression did not change. *CFL1*, *SIX4*, and *MAZ* gene expression decreased significantly in MDA-MB-231 cells transfected with miR-638-5p mimic, while there was no significant change in *CDH1* gene expression

The enrichment analysis performed in the present study on the potential target genes of miR-638-5p revealed that the disease was most likely to be associated with the identified genes is mammary neoplasms (Figure 4). Cell cycle and chromosome segregation are two biological events in which these genes may play important roles that are closely related to the cancer process. All of these suggest that further research into the relationship between miR-638-5p and the candidate genes may assist in understanding BRCA, and specifically TNBC, biology.

The decreased expression of all three selected miR-638-5p potential target genes (*CFL1*, *SIX4,* and *MAZ)* in both the curcumin-added and miR-638-5p transfected groups is an important clue about the functioning of the curcumin/miR-638/target gene axis. Despite the fact that no studies have been conducted to explain the relationship between miR-638-5p and *CFL1* in BRCA, it has been demonstrated that *CFL1* may contribute to the BRCA process by changing its expression via different miRNAs. miR-342 has been shown to inhibit the growth, migration, and invasion of BRCA cells by targeting *CFL1* (23). Another study demonstrated that miR-200b-3p and miR-429-5p inhibit the growth and motility of BRCA cells by targeting the *LIMK1*/*CFL1* pathway (24).

Although *SIX4* is a gene linked to some cancers, including BRCA, there are fewer details about it compared to other selected miR-638-5p targeted genes. *SIX4* promotes metastasis in BRCA via *STAT3* induction, according to one of the few studies (25). Wu et al. (26) reported elevated *SIX4* expression in BRCA that serves an oncogenic role by reducing the immune response, particularly in luminal subtypes, and is associated with diminished promoter methylation levels.

MAZ represents one of the genes involved in gene expression regulation and development of tumors. *MAZ* dysregulation has been related to the progression of many tumors, involving BRCA (27). *MAZ*-regulated *SIPL1* has been shown to promote tumor progression in TNBC, and dysregulation of this *MAZ* expression may be associated with a poor prognosis in TNBC (28).

In the present study, using *in silico* and *in vitro* methods, curcumin was shown to affect cancer processes in MDA-MB-231 cells by altering the expressions of miR-638-5p and its potential target genes. Numerous studies have suggested that miRNAs may be potential therapeutic molecules in cancer in the future (29). However, research into the complicated interactions between miRNAs and their target genes is currently incomplete. The findings of the present study will contribute to the existing literature. It should be noted, however, that the expression of the selected genes was determined at the mRNA level. Therefore it is recommended that in future studies, the findings obtained using *in silico* and *in vitro* approaches should be validated in BRCA tissue samples and with other *in vivo* methods.

The findings of this study showed that curcumin appears to inhibit MDA-MB-231 cancer cell proliferation and migration by altering the expression of miR-638-5p and its potential target genes *CFL1*, *SIX4*, and *MAZ*. It is suggested that miR-638-5p and its target gene axis in BRCA, should be investigated further in future studies.

Ethics Committee Approval: Because of this study was prepared using publicly available bioinformatics data and *in vitro* study utilizing MDA-MB-231 cell line it does not require ethical approval.

Informed Consent: Because of this study was prepared using publicly available bioinformatics data and in vitro study utilizing MDA-MB-231 cell line it does not require informed consent.

Authorship Contributions

Concept: M.K., A.A., S.O.; Design: M.K., A.A., S.P.; Data Collection and/or Processing: M.K., A.A., I.S., S.P., Analysis and/or Interpretation: M.K., S.P., K.C., S.O.; Literature Search: M.K., I.S., S.E., F.A.; Writing: M.K., A.A., S.E., F.A., S.P., S.O.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: Council of Higher Education Research Universities Support Program project, ADEP-Istanbul University, TSA-2023-39483.

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