RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.1420664
İst Tıp Fak Derg 2024 / J Ist Faculty Med 2024

EFFECT of CURCUMIN on BREAST CANCER CELLS THROUGH miR-145-5p AND ITS TARGET GENES

KURKUMİNİN miR-145-5p VE HEDEF GENLERİ ÜZERİNDEN MEME KANSERİ HÜCRELERİNE ETKİSİ

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Cite this article as: Abuaisha A, Kaya M, Suer İ, Emiroğlu S, Abanoz F, Palanduz Ş, et al. Effect of curcumin on breast cancer cells through miR-145-5p and its target genes. J Ist Faculty Med 2024;87(3):235-245. doi: 10.26650/IUITFD.1420664

ABSTRACT

Objective: Curcumin is considered an epigenetic regulator with anticancer effects. The micro-RNA (miRNA) miR-145-5p is a tumor suppressor that shows low expression levels in various cancers, including breast cancer (BC). This study aims to investigate whether curcumin inhibits MCF-7 human BC cell line proliferation and migration by regulating miR-145-5p and its possible target genes.

Material and Method: MCF-7 cells were treated with curcumin and its solvent control. Additionally, cells were transfected with an miR-145-5p mimic and a non-targeting miRNA mimic. Cell viability was then evaluated, and the scratch wound assay was used to assess cell migration. The study predicts the miR-145-5p putative target genes by searching for overlapping genes in the miRNet and miRTarBase v8 databases via the overexpressed genes in the BC tissue samples in the Cancer Genome Atlas (TCGA) datasets. Expression levels of miR-145-5p and the selected genes were detected using the quantitative real-time polymerase chain reaction (qRT-PCR). The $2^{-\Delta\Delta Ct}$ method was used for the quantification analysis, with $p<0.05$ being considered statistically significant.

Result: Curcumin treatment and overexpression of miR-145-5p via the transfection of an miR-145-5p mimic significantly decreased the proliferation and migration of MCF-7 cells. Moreover, curcumin treatment significantly increased the

ÖZET

Amaç: Kurkumin anti-kanser etkileri olan bir epigenetik regülatör olarak kabul edilmektedir. miR-145-5p, meme kanseri (MK) dahil olmak üzere bir çok kanserde ekspresyon düzeyi düşük olan bir tümör baskılayıcı mikroRNA'dır (miRNA). Bu çalışmada, kurkuminin miR-145-5p'yi ve olası hedef genlerini regüle ederek MCF-7 insan MK hücre hattının proliferasyonunu ve göçünü inhibe edip etmediğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: MCF-7 hücreleri kurkumin ve onun solvent kontrolü ile muamele edildi. Ayrıca hücreler miR-145-5p mimiği ve negatif kontrol miRNA mimiği ile transfekte edildi. Hücre canlılığı değerlendirildi ve hücre göçü scratch yara (wound) testi kullanılarak değerlendirildi. miR-145-5p'nin potansiyel hedefleri, MK doku örneklerinde aşırı eksprese edilen genlerle miRNet ve miRTarBase v8 veri-tabanları ile örtüşen genlerin Kanser Genom Atlası (TCGA) datasetlerinde araştırılması yoluyla belirlendi. miR-145-5p ve seçilen genlerin ekspresyon düzeyleri, kantitatif gerçek-zamanlı polimeraz zincir reaksiyonu (qRT-PCR) ile tespit edildi. Kantifikasyon analizi için $2^{-\Delta\Delta Ct}$ yöntemi kullanıldı. p-değeri <0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Hem kurkumin ile muamele edilmiş hem de miR-145-5p mimiği ile transfekte edilmiş MCF-7 hücrelerinde, proliferasyonun ve göçün, kontrollere kıyasla azaldığı gözlenmiştir. Ayrıca, kurkumin ile muamele edilen hücrelerde miR-145-5p'nin ekspresyonunun anlamlı düzeyde arttığı görülmüştür. miR-145-5p'nin olası

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Submitted/Başvuru: 16.01.2024 • **Revision Requested/Revizyon Talebi:** 19.01.2024 •
Last Revision Received/Son Revizyon: 06.05.2024 • **Accepted/Kabul:** 07.05.2024 • **Published Online/Online Yayın:** 04.06.2024

expression of miR-145-5p. The possible target genes of miR-145-5p (i.e., *MCM2, MMP1, MMP9, EEF1A2*) were downregulated in curcumin-treated MCF-7 cells. Additionally, miR-145-5p mimic-transfected cells showed low expression levels of the *MCM2, MMP1*, and *MMP9* genes.

Conclusion: Curcumin inhibits the proliferation and migration of MCF-7 cells by acting on miR-145-5p and its possible target genes.

Keywords: Breast cancer, MCF-7 cell line, curcumin, miR-145-5p, target genes

hedef genlerinin, *MCM2, MMP1, MMP9* ve *EEF1A2*, kurkumin ile muamele edilmiş MCF-7 hücrelerinde ekspresyon düzeyinin azaldığı saptanmıştır. Bunun yanı sıra, miR-145-5p mimiği ile transfekte edilmiş hücrelerde, *MCM2, MMP1* ve *MMP9* genlerinin ekspresyon düzeyinin düşük olduğu belirlenmiştir.

Sonuç: Kurkuminin, miR-145-5p ve olası hedef genleri üzerinden etki ederek MCF-7 hücrelerinin hem proliferasyonunu hem de göçünü inhibe ettiği gösterilmiştir.

Anahtar Kelimeler: Meme kanseri, MCF-7 hücre hattı, kurkumin, miR-145-5p, hedef genler

INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed cancer among women, with approximately 2.26 million cases occurring in 2020. BC is the major leading cause of mortality in female cancer patients (1, 2). By 2040, more than three million new cases of BC and one million deaths are predicted to occur each year due to population growth and aging. Global efforts and public health awareness programs aimed at managing the incidence and mortality of BC should include screening, early diagnosis, treatment, and follow-up of BC patients (3). The MCF-7 cell line is a luminal A subtype of BC that expresses both the progesterone receptor (PR) and the estrogen receptor (ER) (4).

Numerous molecular mechanisms have demonstrated the impact of natural nutritional sources such as curcumin on cellular processes (5, 6). Curcumin is a yellow pigment found in *Curcuma longa Linn rhizome* (7). *In vitro* and *in vivo* studies have shown curcumin to have anticancer effects on various tumors by reducing the proliferation and migration of cells and by inducing apoptosis (8). Recent studies have suggested curcumin to be able to act as an epigenetic regulator in several cancers by acting on non-coding RNAs (5, 9).

MicroRNAs (miRNAs) are noncoding RNAs of about 22 nucleotides (10); miRNAs regulate the expression of genes as oncogenes or tumor suppressors and affect different biological processes (e.g., proliferation and apoptosis) through their involvement in tumor development and progression (2, 11).

miRNA-145-5p (miR-145-5p) is a miRNA with tumor suppressor activity and has low expression in different malignant tissues such as BC compared to normal tissues. miR-145-5p decreases tumor cell proliferation and migration, as well as invasion, and increases cell sensitivity to chemotherapy drugs by acting on a variety of genes. Some studies suggested miR-145-5p to perhaps have value as a target molecule in cancer treatment, apart from its diagnostic and prognostic benefits during the assessment of cancers (12, 13).

By regulating multiple genes, miR-145-5p can serve as a potential biomarker for risk assessment in BC patients (14, 15). miR-145-5p could be a potential predictive biomarker for BC stemness by acting on the SRY-box transcription factor 2 (*SOX2*) gene (14). Moreover, studies have significantly linked decreased miR-145-5p expression to higher Ki-67 expression levels, larger tumors, metastasis, and shorter overall survival (OS) (15). Another study has demonstrated the oncogenic hepatitis B X-interacting protein (*HBXIP*) gene as a target of miR-145 in MCF-7 cells (16). In addition, a recent study has reported that, compared to their controls, both BC cell lines and patient BC tissues significantly showed lower expression levels of miR-145-5p and a negative association with the expression of the programmed death-ligand 1 (*PDL1*) gene (17). The regulatory role of curcumin in miR-145-5p has been investigated in several cancers such as laryngeal squamous cell carcinoma (18). Many studies have also determined low expression levels of miR-145-5p in both BC cell lines and patient tissue samples, thus establishing it as a known tumor suppressor miRNA in BC. However, how curcumin affects BC cells via miR-145-5p and its target genes remains unclear. This study aims to investigate whether curcumin inhibits the proliferation and migration of the MCF-7 human BC cell line by acting on miR-145-5p and its target genes.

MATERIALS and METHODS

Cell culture

The MCF-7 human BC cell line was obtained from the İstanbul University Faculty of Sciences Department of Biology. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose (EcoTech Biotechnology, Türkiye) supplemented with 10% fetal bovine serum (FBS; Gibco, UK) and 1% antibiotic (penicillin-streptomycin; Gibco, NY, USA). Cells were incubated at 37°C, > 90% humidity, and 5% CO_2.

Curcumin treatment

High-purity curcumin (Bio Basic Inc., Canada) was dissolved in dimethyl sulfoxide (DMSO; 1mg/mL; EcoTech Biotechnology, Türkiye). Based on the recommendations of previous literature, the study performed a 10 µM curcumin treatment for 24 h (19-21).

miR-145-5p mimic transfection

A commercial miR-145-5p mimic was used for transfection with the following sequence: 5′-GUCCAGUUUUCCCAGGAAUCCCU-3′ (Thermo Fisher Scientific, USA); a non-targeting miRNA mimic (NT miRNA mimic) with validated random sequences producing no identifiable effects on known miRNA function (Ambion, USA) were used for transfection. Transfection with 30 pmol miR-145-5p mimic and NT mimic miRNA was achieved using the Lipofectamine 2000 Transfection Reagent (Invitrogen, USA) in accordance with the manufacturer's instructions. In short, 1.5 µL transfection reagent and 3 µL 5nmol miR-145-5p mimic were mixed with 125 µL Opti-MEM medium (Gibco, USA) separately and incubated for 5 min at room temperature. Both tubes were then mixed and incubated for 20 min at room temperature. The same was done for the NT mimic miRNA transfection. All transfection processes were performed for 24 h.

Cell proliferation assay

MCF-7 cells were seeded in quadruplicate (5,000 cells per well) in four columns of 96-well plates (Nest Biotechnology Co., China) and incubated under suitable conditions. After 24 h of cell culturing, cells were treated with 10 µM curcumin and its solvent control (DMSO). Additionally, the cells were transfected with 30 pmol miR-145-5p mimic and NT mimic miRNA using the Lipofectamine 2000 Transfection Reagent (Invitrogen, USA). 24 h later, 10 µL of the cell viability detection kit 8 (CVDK8) solution (NutriCulture, Türkiye) were added. After 3 h of plate incubation, absorbance was measured using a microplate reader at 450 nm. The medium was then discarded by flipping the plate. All wells were washed with phosphate-buffered saline (PBS), and cell morphology was evaluated using a phase contrast microscope to capture images (Argenit, Türkiye).

Cell migration

The wound-healing assay was used for evaluating cell migration. MCF-7 cells were seeded in six-well plates (Nest Biotechnology Co., China) and incubated. After the cells became almost completely (90%) confluent, the monolayer was scratched using a sterile 10 µl pipette tip. The wound area was recorded at 0 h and 24 h using a phase contrast microscope. The migration area was calculated as:

$$\text{Migration area (\%)} = [(M_0 - M_{24}) / M_0] \times 100 \quad (1)$$

where M_0 is the wound area at 0 h, and M_{24} is the wound area at 24 h.

Gene expression analysis

Analyzing the miR-145-5p expression level

MCF-7 cells were cultured and treated with curcumin and its solvent control. Cells were also transfected with the miR-145-5p mimic and NT miRNA mimic. 24 h later, the cells were harvested and resuspended in 1 mL TRIzol Reagent (Invitrogen, USA) for total RNA isolation. The concentration and purity of the isolated RNAs were evaluated utilizing a NanoDrop ND-2000c spectrophotometer (Thermo Fisher Scientific Inc.). Complementary DNA (cDNA) synthesis for miRNAs was performed to validate the transfection and to evaluate the relative expression levels of miR-145-5p using miRNA reverse transcription primers (Thermo Fisher Scientific Inc.) and Taqman miRNA Reverse Transcriptase Kit (Applied Biosystems, Thermo Fisher Scientific Baltics UAB, Lithuania).

The LightCycler® 480 instrument (Roche, Germany) was used for transfection validation and for evaluating the alterations in miR-145-5p expression. All reactions were conducted in duplicate using the TaqMan miRNA probes (Thermo Fisher Scientific Inc.) and TaqMan Universal Master Mix Kit (Applied Biosystems, Thermo Fisher Scientific Baltics UAB, Lithuania). RNU43 expression was used to normalize miRNA expression. The quantitative real-time polymerase chain reaction (qRT-PCR) protocol was conducted as presented in Table 1.

Analyzing the expression levels of the identified genes

The cDNA synthesis was conducted utilizing the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Baltics UAB, Lithuania). The LightCycler® 480 instrument (Roche, Germany) was used to determine the expression levels of the identified genes. All reactions were conducted in duplicate using the 5X HOT FIREPol EvaGreen qPCR Supermix (Solis Biodyne, Estonia). The β-actin (*ACTB*) gene was used for expression normalization. The sequences of the primers are presented in Table 2 (22-27). The qRT-PCR protocol was conducted as presented in Table 3.

Table 1: The qRT-PCR cycles protocol for TaqMan Universal Master Mix regarding miRNA detection

Cycle step		Temperature	Time	Cycles
Enzyme Activation		95°C	15 min	1
Quantification	Denaturation	95°C	15 s	40
	Annealing	60°C	60 s	
	Elongation	60°C	1 s	
Cooling		40°C	30 s	1

min: Minutes, s: Seconds

Table 2: Forward and reverse primer sequences of the studied genes

Gene	Forward Primer	Reverse Primer	Ref.
MCM2	5′-CTACCAGCGTATCCGAATCCA-3′	5′-CCTACAGCAACCTTGTTGTCCT-3′	(22)
MMP1	5′-ATTTGCCGACAGAGATGAAGTCC-3′	5′-GGGTATCCGTGTAGCACATTCTG-3′	(23)
MMP9	5′-TCCCTGGAGACCTGAGAACC-3′	5′-CGGCAAGTCTTCCGAGTAGTT-3′	(24)
EEF1A2	5′-GGACCATTGAGAAGTTCGAGA-3′	5′-AGCACCCAGGCATACTTGAA-3′	(25)
FN1	5′-GTGTGACCCTCATGAGGCAAC-3′	5′-CTGGCCTCCAAAGCATGTG-3′	(26)
ACTB	5′-GCCTCGCCTTTGCCGATC-3′	5′-CCCACGATGGAGGGGAAG-3′	(27)

Ref.: Reference, *MCM2*: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1, *ACTB*: actin beta

Table 3: The qRT-PCR cycles protocol for Hot FirePol EvaGreen qPCR Super mix regarding gene expression detection

Cycle step		Temperature	Time	Cycles
Enzyme activation		95°C	12 min	1
Quantification	Denaturation	95°C	15 s	40
	Annealing	60°C	25 s	
	Elongation	72°C	25 s	
Melting curve		95°C	5 s	1
		62°C	60 s	
Cooling		40°C	20 s	1

min: Minutes, s: Seconds

Bioinformatic analyses

Predicting the target genes of miR-145-5p

Different *in silico* analyses were conducted to find the possible target genes of miR-145-5p. Overexpressed genes that met the logFC>+2 and p<0.001 criteria were determined in the Cancer Genome Atlas (TCGA) BC datasets (1,135 tumor tissue samples against 114 normal tissue samples). The expression analyses were performed using GEPIA2 (28, 29), an interactive web tool for gene expression evaluation based on thousands of cancers and normal specimens from the TCGA and GTEx databases that are processed through a standard RNA sequencing workflow. The miRNet 2.0 and miRTarBase v8 databases were also used to identify the target genes of miR-145-5p (30, 31). Subsequently, the genes that overlapped between those overexpressed in TCGA BC and the miR-145-5p probable candidate targets in the miRNet and miRTarBase databases were identified.

The identified genes were searched in PubMed using words such as "gene name", "breast cancer", "BC", "overexpression", "downregulation", "oncogene", and "tumor suppressor". Other databases were used to search for these genes, including Ensembl and Genecards (32, 33). Additionally, enrichment analyses for the identified genes were performed using the Metascape and the DisGeNET databases (34, 35). Moreover, the OS rates for BC associated with the selected genes were investigated utilizing the Kaplan-Meier plotter tool (36). While gene co-expression analysis involves a number of complex processes, it is an effective approach for identifying gene partners and predicting gene function (37). The current study used the Correlation AnalyzeR tool (Bishop Lab, UT Health San Antonio), with Pearson correlation being preferred in the analysis, for performing a genome-wide co-expression correlation analysis in order to determine the genes that correlate with those studied in BC (38).

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Student's t-test was used to compare the means of the two groups. The $2^{-\Delta\Delta Ct}$ method was performed for the relative quantitation analysis. All study results were presented as mean ± SD, with p<0.05 being considered statistically significant. The figures were drawn using GraphPad Prism 5.

RESULTS

Curcumin treatment and overexpression of miR-145-5p decrease cell proliferation

Microscopic images of MCF-7 cells at 24 h after curcumin treatment and miR-145-5p mimic transfection were cap-

tured and compared to their controls (Figure 1a). Both cell viability and morphology results indicate the curcumin treatment to have statistically significantly reduced the proliferation of MCF-7 cells compared to the control group at 24 h ($p<0.01$). Additionally, the miR-145-5p mimic transfection had statistically significantly decreased the proliferation of MCF-7 cells compared to the NT miRNA mimic transfected group at 24 h ($p<0.001$; Figure 1b).

Curcumin treatment and overexpression of miR-145-5p reduce cell migration

According to the wound healing assay results, curcumin treatment statistically significantly decreased MCF-7 cell migration compared to the control cells at 24 h (Figure 1a). Moreover, miR-145-5p mimic transfection significantly reduced the migration compared to NT mimic miRNA-transfected cells at 24 h (Figure 2b).

Curcumin increases the expression level of miR-145-5p

The MCF-7 cells transfected with mimic miR-145-5p expressed significantly higher levels of miR-145-5p than the NT mimic miRNA control ($p<0.001$). This finding indicates the miR-145-5p mimic transfection into cells to have been successfully achieved. The MCF-7 cells treated with curcumin had higher levels of miR-145-5p expression than the control group ($p<0.01$; Figure 3a).

Bioinformatic analyses results

miR-145-5p candidate genes

In total, 261 overexpressed genes with logFC values greater than +2 and $p<0.001$ were chosen from the TCGA BC dataset. The miRNet and miRTarbase v8 databases were used to identify 881 potential target genes of miR-145-5p (Figure 1). The minichromosome maintenance com-

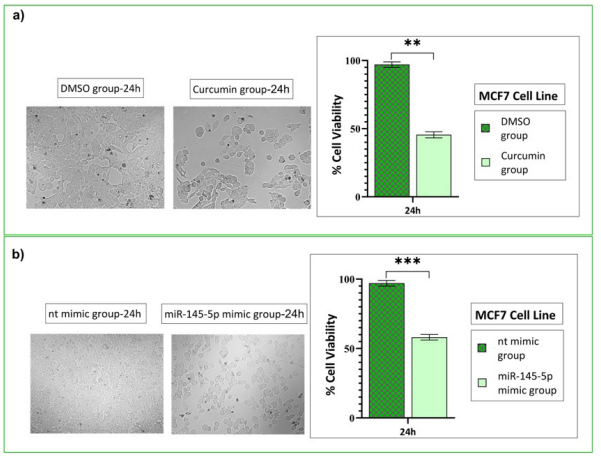

Figure 1: The effects of curcumin and miR-145-5p on MCF-7 cell proliferation. a) microscopic images for MCF-7 cells 24 h after curcumin treatment and miR-145-5p mimic transfection compared to their controls. b) Cell proliferation (%cell viability) of cells was evaluated by the CVDK8 assay 24 h after curcumin treatment and mimic miR-145-5p transfection compared to their controls (NT mimic = non-targeting mimic).
**p<0.01, ***p<0.001

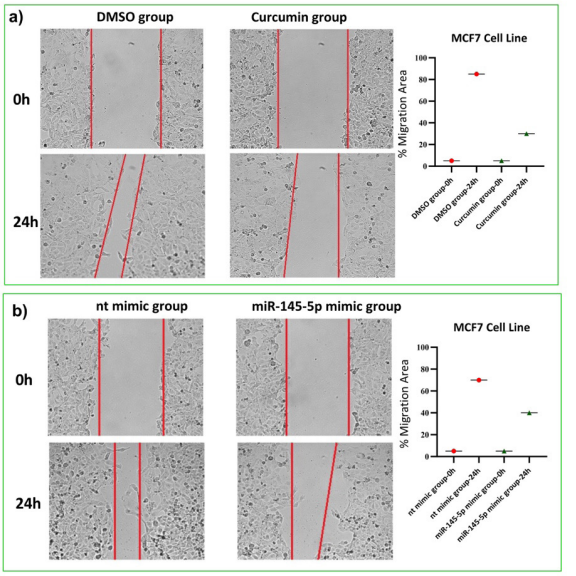

Figure 2: Scratch wound assay for evaluating the cell migration of MCF-7 cells. a) The effects on cell migration at 24 h post-curcumin treatment. b) The effects on cell migration at 24 h after miR-145-5p mimic transfection.

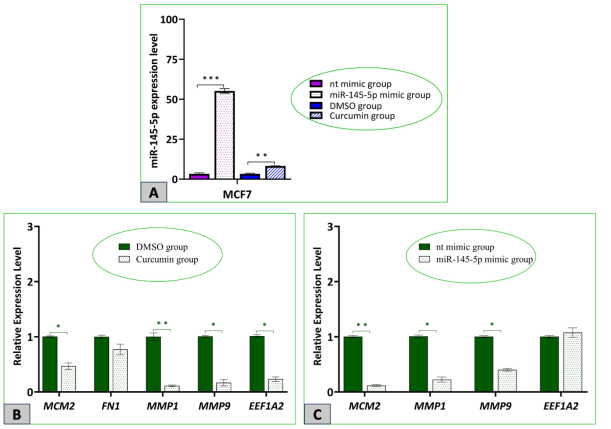

Figure 3: a) Expression level of miR-145-5p in MCF-7 cells after 24 h of curcumin treatment and miR-145-5p mimic transfection compared to their controls. b) Expression levels of the studied genes in MCF-7 cells after 24 h of curcumin treatment compared to the solvent control (DMSO) group. c) Expression levels of the studied genes in MCF-7 cells after 24 h of miR-145-5p mimic transfection compared to the NT mimic miRNA control group.
*p<0.05, **p<0.01, ***p<0.001, *MCM2*: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1

plex component 2 (*MCM2*), matrix metalloproteinase 1 (*MMP1*), matrix metalloproteinase 9 (*MMP9*), eukaryotic translation elongation factor 1 alpha 2 (*EEF1A2*), and fibronectin 1 (*FN1*) genes were found to overlap among the TCGA BC data and probable targets of miR-145-5p in the miRNet and miRTarbase v8 databases (Figure 4).

Significance of the selected genes

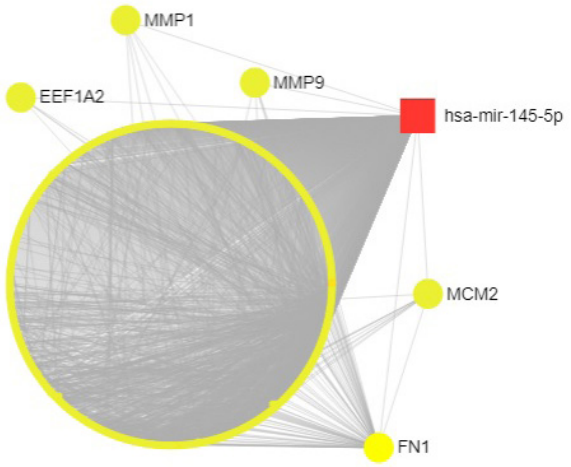

Figure 4: The miR-145-5p *in silico* target genes. Yellow nodes involve 881 genes, including *MCM2*, *FN1*, *EEF1A2*, *MMP1* and *MMP9*. The red square is miR-145-5p. Interaction edges number 2,723 (Created using miRNet).

Because all five genes are oncogenes and associated with BC, they are considered good candidates for the *in vitro* investigations, as shown in Figure 5a. In the enrichment analysis, the identified possible target genes of miR-145-5p were associated with many malignancies, with BC being one of these (Figure 5b). Upregulation of *MCM2*, *MMP1*, and *MMP9* reduced OS for BC. In contrast, expression changes in the *FN1* and *EEF1A2* genes had no influence on the OS (Figure 6). In the correlation analysis, the top genes likely to be correlated with the studied genes (i.e., *MCM2*, *MMP1*, and *MMP9*) were selected and are presented in Table 4. These genes were shown to be associated with BC through both *in silico* and *in vitro* methods.

Curcumin modulates the expression of MCM2, MMP1 and MMP9 genes by regulating miR-145-5p

Relative quantification analysis of the *MCM2*, *MMP1*, *MMP9*, *EEF1A2*, and *FN1* genes was performed using the primers described in Table 2. The *MCM2*, *MMP1*, *MMP9*, and *EEF1A2* genes were significantly downregulated in curcumin-treated MCF-7 cells compared to the controls ($p<0.05$; Figure 3b). Moreover, the *MCM2*, *MMP1*, and *MMP9* genes were significantly downregulated in the mimic miR-145-5p-transfected cells compared to the NT miRNA mimic control cells ($p<0.05$; Figure 3c). The *MCM2*, *MMP1*, and *MMP9* genes were the commonly less-expressed genes in both the curcumin-treated and miR-145-5p mimic-transfected MCF-7 cells. These findings suggest that curcumin may be a negative regulator

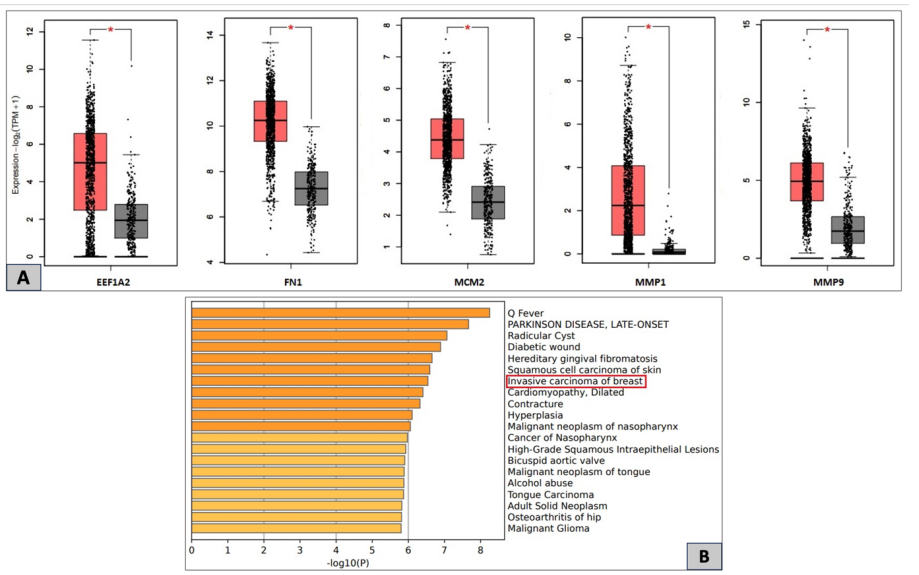

Figure 5: a) GEPIA2 relative expression analysis of the studied miR-145-5p target genes in BC samples. All five genes are overexpressed in the BC tumor tissue samples. GEPIA2 TCGA. (log2FC Cutoff = 1; p_{Cutoff}=0.01; 1085 tumor samples vs. 291 normal samples). b) The associations the five identified miR-145-5p potential targets (i.e., *MCM2, FN1, EEF1A2, MMP1*, and *MMP9*) have with different diseases. Invasive BC is one of the diseases related to these genes, as seen in the red box. According to the figure, these genes are also linked to other cancers such as squamous cell skin carcinoma, nasopharynx cancer, and tongue cancer.
MCM2: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1

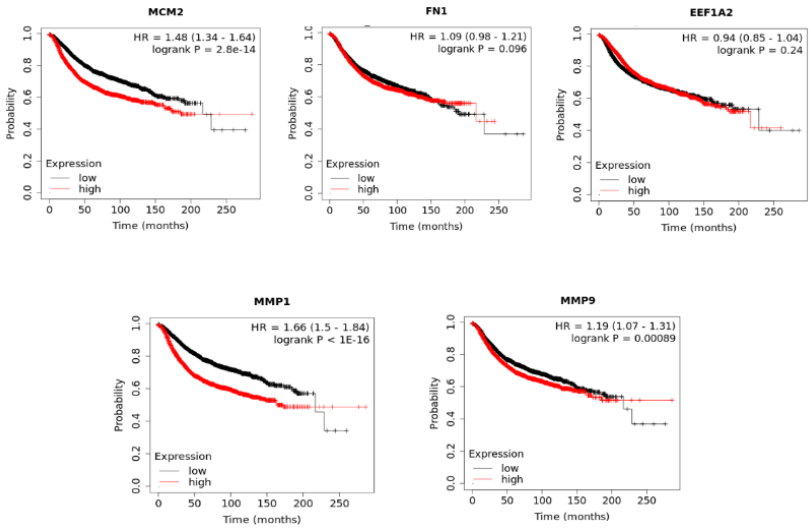

Figure 6: The effect of the selected genes on BC survival using the Kaplan-Meier plotter. Overexpression of *MCM2, MMP1* and *MMP9* decreases OS for BC. However, alterations in the expression of *FN1* and *EEF1A2* genes have no effect on OS for BC.
MCM2: minichromosome maintenance complex component 2, *MMP1*: matrix metalloproteinase 1, *MMP9*: matrix metalloproteinase 9, *EEF1A2*: eukaryotic translation elongation factor 1 alpha 2, *FN1*: fibronectin 1

that modulates the expression of *MCM2, MMP1*, and *MMP9* genes by acting on miR-145-5p in MCF-7 cells.

DISCUSSION

Turmeric (Curcuma longa) is a famous Indian spice. Polyphenol curcumin is the primary constituent of turmeric and the one accountable for the majority of its biological effects (9). Curcumin has been utilized for an extensive period to address issues regarding the gastrointestinal tract, liver, respiratory system, and sinusitis; to promote wound healing; and to alleviate pain and burning sensations. Studies have demonstrated the pharmacological properties of curcumin, including its anti-oxidant, anti-inflammatory, cell death-inducing, and anticarcinogenic effects in various diseases (5). The potential of curcumin to alter miRNA expression and its target genes has created new opportunities for cancer-targeted treatment (39). The present study has investigated the effects of curcumin on the proliferation and migration of the MCF-7 human BC cell line and whether its anticancer effects are mediated by acting on miR-145-5p and its possible target genes. The findings have shown curcumin treatment and the overexpression of miR-145-5p to decrease the proliferation of MCF-7 cells as well their migration. Additionally, the relative expression level of the tumor suppressor miRNA miR-145-5p was significantly elevated in the curcumin-treated cells. Curcumin treatment reduced the relative expression of the putative miR-145-5p target genes *MCM2*, *MMP1*, *MMP9*, and *EEF1A2*. Furthermore, the overexpression of miR-145-5p decreased the expression levels of the *MCM2*, *MMP1*, and *MMP9* genes.

Previous studies have demonstrated the expression of miR-145-5p to be lower in BC tissues compared to normal breast tissues, with this being linked to large tumors, metastasis, and high Ki-67 expression levels (14, 15, 17, 40). By modifying the expression of different signaling proteins and molecular pathways, curcumin suppresses the proliferation of BC cells. Consequently, one of the best ways to treat cancer is to target oncogenes and tumor suppressor genes (41). Studies have shown curcumin to be able to successfully inhibit the proliferation of different cancerous cells by acting on miR-145-5p, such as in laryngeal squamous cell carcinoma and in human prostate cancer stem cells (18, 42). When considered together, no study has been conducted evaluating the regulatory effect of curcumin on miR-145-5p in BC. The current results suggest curcumin treatment and miR-145-5p overexpression to both markedly suppress MCF-7 cell proliferation and migration by modulating the expression of the *MCM2*, *MMP1*, and *MMP9* genes.

MCM2 is a promising proliferative marker in numerous different cancer types (e.g., thyroid, rectal, and BC). MCM2 is also a more effective proliferative and prognostic marker in BC than Ki-67 (39). Samad et al.'s study demonstrated *MCM2* gene expression levels to be higher in BC patients, suggesting *MCM2* as a useable biomarker for the prognosis of BC (43).

The genes studied herein have been shown to be correlated with many genes previously linked to BC (Table 4), with the flap structure-specific endonuclease 1 (*FEN1*) being one example of a gene correlated with the *MCM2* gene. *FEN1* overexpression has been shown to promote BC.

Table 4: The top 10 co-expression correlations of *MCM2, MMP1*, and *MMP9* in BC according to the Correlation AnalyzeR results

Studied gene	Correlated genes	Gene name	r	p
MCM2	*MCM5*	Minichromosome Maintenance Complex Component 5	0.861	4.235e-08
	FEN1	Flap Structure-Specific Endonuclease 1	0.859	4.35e-08
	MCM7	Minichromosome Maintenance Complex Component 7	0.848	5.016e-08
	MCM6	Minichromosome Maintenance Complex Component 6	0.845	5.207e-08
	CDCA5	Cell Division Cycle Associated 5	0.843	5.338e-08
	CDK2	Cyclin Dependent Kinase 2	0.837	5.741e-08
	RACGAP1	Rac Gtpase Activating Protein 1	0.835	5.879e-08
	MYBL2	MYB Proto-Oncogene Like 2	0.833	6.02e-08
	PRC1	Protein Regulator of Cytokinesis 1	0.832	6.091e-08
	MCM3	Minichromosome Maintenance Complex Component 3	0.829	6.307e-08
MMP1	*PRDM8*	PR/SET Domain 8	0.756	1.334e-07
	LAYN	Layilin	0.741	1.529e-07
	G0S2	G0/G1 Switch 2	0.739	1.557e-07
	PTX3	Pentraxin 3	0.734	1.627e-07
	COL13A1	Collagen Type XIII Alpha 1 Chain	0.732	1.656e-07
	ROBO4	Roundabout Guidance Receptor 4	0.718	1.872e-07
	PRSS3	Serine Protease 3	0.715	1.921e-07
	ANTXR2	ANTXR Cell Adhesion Molecule 2	0.712	1.971e-07
	HBEGF	Heparin Binding EGF-Like Growth Factor	0.711	1.987e-07
	SERPINE1	Serpin Family E Member 1	0.71	2.004e-07
MMP9	*IL10RA*	Interleukin 10 Receptor Subunit Alpha	0.667	2.863e-07
	C1QA	Complement C1q A Chain	0.664	2.934e-07
	PIK3R5	Phosphoinositide-3-Kinase Regulatory Subunit 5	0.649	3.309e-07
	CD86	CD86 Molecule	0.633	3.758e-07
	SPI1	Spi-1 Proto-Oncogene	0.625	4.003e-07
	CD2	CD2 Molecule	0.621	4.131e-07
	C1QB	Complement C1q B Chain	0.619	4.196e-07
	C1QC	Complement C1q C Chain	0.618	4.229e-07
	IGHG1	Immunoglobulin Heavy Constant Gamma 1	0.611	4.468e-07
	IGHG1	Immunoglobulin Heavy Constant Gamma 1	0.611	4.468e-07

r: Pearson correlation coefficient. The p values are presented by the scientific mathematical method. All p values indicate p<0.001.

Zou et al. showed a negative association between *FEN1* expression and cisplatin sensitivity in BC cells. Moreover, curcumin treatment (dose-dependent) downregulated the expression of *FEN1*. In addition, they showed an *in vitro* and *in vivo* combination of curcumin with cisplatin to increase the sensitivity to cisplatin, which was found to occur through the downregulation of *FEN1* in BC. Interestingly, their study concluded that curcumin sensitizes cells to cisplatin via the downregulation of *FEN1* (44). As a result, this opens up a new approach to treatment by increasing cancer cells' sensitivity to chemotherapy treatments.

MMP1 is associated with many physiological processes via the modification of the tumor microenvironment and the extracellular matrix (45). *MMP1* is upregulated in BC tissues and has been shown to be associated with invasion and metastasis (46). The current study has demonstrated curcumin's ability to alter the expression of oncogenic genes such as MMP1 by down-regulating their expression.

MMP9 is also a member of the MMP family that promotes the invasion and migration of BC cells. *MMP9* gene was found to be linked to the signal transducer and activator of transcription 3 (*STAT3*) gene activation, which is involved in cellular proliferation, apoptosis, tumorigenesis, and angiogenesis. Furthermore, the knockdown of *STAT3* suppressed *MMP9* expression and metastasis in BC cells (47). These findings suggest the inhibition of *MMP1* and *MMP9* expression by curcumin and miRNA miR-145-5p to be possible novel strategies for controlling BC.

The present study has investigated the prognostic significance of the five hub genes with regard to the OS of BC patients and revealed the overexpression of the *MCM2*, *MMP1*, and *MMP9* genes to decrease the OS of BC patients. However, no influence was found regarding alterations in the expression of the *FN1* and *EEF1A2* genes (Figure 6). A comprehensive bioinformatics analysis study assessing the significant value of MCMs in BC demonstrated the *MCM2-7* genes to be significantly overexpressed in BC, particularly in tumor subtypes that proliferate and spread quickly. This predicted a worse prognosis with shorter OS and relapse-free survival (RFS) for BC patients, providing insight into the prognostic value of MCMs regarding the various molecular subtypes of BC (48). Consequently, the current and other findings have suggested *MCM2* to be a possible good prognostic biomarker for BC and to perhaps be a potential therapeutic target. One study showed the Y-box binding protein-1 (*YB-1*) to mediate the invasion and metastasis of BC by acting on *MMP1* and beta-catenin (*CTNNB1*). That study's analysis of breast tumor samples from the Gene Expression-Based Outcome for Breast Cancer Online (GOBO) database showed a markedly reduced 10-year distant metastasis free survival to be predicted with the overexpression of the *YB-1*, *MMP1*, and *CTNNB1* genes (49). *MMP1* may be a good marker of the prognosis for BC metastases (49). Moreover, the elevated expression level of the *MMP9* gene in both blood and tumor tissue samples of 108 BC patients has indicated worse prognoses (50). Accordingly, MMPs are being extensively investigated as potential therapeutic targets for cancer progression.

This study has combined a bioinformatic analysis with *in vitro* experiments, including cell proliferation assays, scratch wound assays, and expression studies, to investigate the effect of curcumin in combating cancer through miR-145-5p and its target genes. One limitation of the study is that it only examined the expression of the selected genes at the mRNA level. It did not evaluate changes in protein levels to verify the relationship between miR-145-5p and its target genes. Additional research is required to analyze the collective influence of miR-145-5p and curcumin on cell proliferation and migration, as well as their effects on the expression of target genes.

CONCLUSION

Curcumin exerts a strong anticancer impact on BC MCF-7 cells by suppressing cellular growth. This effect is likely mediated by the upregulation of miR-145-5p and the downregulation of the *MCM2*, *MMP1* and *MMP9* genes. The upregulation of *MCM2*, *MMP1* and *MMP9* genes is negatively associated with the OS in BC. Obviously, different studies should validate the findings of the *in silico* and *in vitro* studies at the protein levels. These will be valuable for evaluating the potential therapeutic role and clinical utility of curcumin and the miR-145-5p mimic prior to clinical trials and therapeutic usage when considering a tumor's pathological results. Additionally, due to the study's bioinformatic analysis providing information about the potential the target genes of miR-145-5p have regarding OS, further studies are recommended on patient-derived BC samples based on their clinical, biological and pathological characteristics for a better understanding of the impact these genes have on OS.

Ethics Committee Approval: No ethics committee approval was need for this study, since the study was conducted on human breast cancer cell line and public available datasets.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.A., M.K.; Data Acquisition- A.A., M.K., İ.S., S.E.; Data Analysis/Interpretation- A.A., M.K., İ.S., S.E., Ş.Ö.; Drafting Manuscript- A.A., M.K., İ.S., S.E., F.A.; Critical Revision of Manuscript- A.A., M.K., Ş.P., K.Ç., Ş.Ö.; Final Approval and Accountability- A.A., M.K., İ.S., S.E., F.A., Ş.P., K.Ç., Ş.Ö.

Acknowledgment: The authors would like to thank Assoc. Prof. Dr. İdil Çetin from the Istanbul University Science Faculty Department of Biology for supplying them with the MCF-7 cell line.

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

Financial Disclosure: Financial support was provided by Istanbul University- Scientific Research Projects Coordination Unit (BAP), Project No: TDK-2023-39955.

REFERENCES

1. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. Br J Radiol 2022;95(1130):20211033. [CrossRef]
2. Erdogan C, Suer I, Kaya M, Ozturk S, Aydin N, Kurt Z. Bioinformatics analysis of the potentially functional circRNA-miRNA-mRNA network in breast cancer. PLoS One 2024;19(4):e0301995. [CrossRef]
3. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast 2022;66:15-23. [CrossRef]
4. Guofeng Q, Wei W, Wei D, Fan Z, Sinclair AJ, Chatwin CR. Bioimpedance analysis for the characterization of breast cancer cells in suspension. IEEE Trans Biomed Eng 2012;59(8):2321-9. [CrossRef]
5. Liu Y, Sun H, Makabel B, Cui Q, Li J, Su C, et al. The targeting of non-coding RNAs by curcumin: Facts and hopes for cancer therapy (Review). Oncol Rep 2019;42(1):20-34. [CrossRef]
6. Bagatir G, Kaya M, Suer I, Cefle K, Palanduz A, Palanduz S, et al. The effect of Anzer honey on X-ray induced genotoxicity in human lymphocytes: An in vitro study. Microsc Res Tech 2022;85(6):2241-50. [CrossRef]
7. Zhang JY, Lin MT, Zhou MJ, Yi T, Tang YN, Tang SL, et al. Combinational Treatment of Curcumin and Quercetin against Gastric Cancer MGC-803 Cells in Vitro. Molecules 2015;20(6):11524-34. [CrossRef]
8. Su J, Zhou X, Wang L, Yin X, Wang Z. Curcumin inhibits cell growth and invasion and induces apoptosis through down-regulation of Skp2 in pancreatic cancer cells. Am J Cancer Res 2016;6(9):1949-62.
9. Kaya M, Abuaisha A, Suer I, Emiroglu S, Abanoz F, Palanduz S, et al. Turmeric Inhibits MDA-MB-231 Cancer Cell Proliferation, Altering miR-638-5p and Its Potential Targets. Eur J Breast Health 2024;20(2):102-9. [CrossRef]
10. 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]
11. 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]
12. 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]
13. Kaya M, Suer I, Ozgur E, Capik O, Karatas OF, Ozturk S, et al. miR-145-5p suppresses cell proliferation by targeting IGF1R and NRAS genes in multiple myeloma cells. Turkish Journal of Biochemistry. 2023;48(5):563-9. [CrossRef]
14. Rajarajan D, Kaur B, Penta D, Natesh J, Meeran SM. miR-145-5p as a predictive biomarker for breast cancer stemness by computational clinical investigation. Comput Biol Med 2021;135:104601. [CrossRef]
15. Tang W, Zhang X, Tan W, Gao J, Pan L, Ye X, et al. miR-145-5p Suppresses Breast Cancer Progression by Inhibiting SOX2. J Surg Res 2019;236:278-87. [CrossRef]
16. Jiang Y, Wang D, Ren H, Shi Y, Gao Y. MiR-145-targeted HBXIP modulates human breast cancer cell proliferation. Thorac Cancer 2019;10(1):71-7. [CrossRef]
17. Hajibabaei S, Sotoodehnejadnematalahi F, Nafissi N, Zeinali S, Azizi M. Aberrant promoter hypermethylation of miR-335 and miR-145 is involved in breast cancer PD-L1 overexpression. Sci Rep 2023;13(1):1003. [CrossRef]
18. Zhu X, Zhu R. Curcumin suppresses the progression of laryngeal squamous cell carcinoma through the upregulation of miR-145 and inhibition of the PI3K/Akt/mTOR pathway. Onco Targets Ther 2018;11:3521-31. [CrossRef]
19. Shen Y, Han Z, Liu S, Jiao Y, Li Y, Yuan H. Curcumin Inhibits the Tumorigenesis of Breast Cancer by Blocking Tafazzin/Yes-Associated Protein Axis. Cancer Manag Res 2020;12:1493-502. [CrossRef]
20. Chatterjee B, Ghosh K, Suresh L, Kanade SR. Curcumin ameliorates PRMT5-MEP50 arginine methyltransferase expression by decreasing the Sp1 and NF-YA transcription factors in the A549 and MCF-7 cells. Mol Cell Biochem 2019;455(1-2):73-90. [CrossRef]
21. Liu JL, Pan YY, Chen O, Luan Y, Xue X, Zhao JJ, et al. Curcumin inhibits MCF-7 cells by modulating the NF-κB signaling pathway. Oncol Lett 2017;14(5):5581-4. [CrossRef]
22. Tang Z, Yang Y, Chen W, Li E, Liang T. Demethylation at enhancer upregulates MCM2 and NUP37 expression predicting poor survival in hepatocellular carcinoma patients. J Transl Med 2022;20(1):49. [CrossRef]
23. Ezure T, Sugahara M, Amano S. Senescent dermal fibroblasts negatively influence fibroblast extracellular matrix-related gene expression partly via secretion of complement factor D. Biofactors 2019;45(4):556-62. [CrossRef]
24. Kang H, Jang SW, Pak JH, Shim S. Glaucine inhibits breast cancer cell migration and invasion by inhibiting MMP-9 gene expression through the suppression of NF-κB activation. Mol Cell Biochem 2015;403(1-2):85-94. [CrossRef]
25. Worst TS, Waldbillig F, Abdelhadi A, Weis CA, Gottschalt M, Steidler A, et al. The EEF1A2 gene expression as risk predictor in localized prostate cancer. BMC Urol 2017;17(1):86. [CrossRef]
26. Ifon ET, Pang AL, Johnson W, Cashman K, Zimmerman S, Muralidhar S, et al. U94 alters FN1 and ANGPTL4 gene expression and inhibits tumorigenesis of prostate cancer cell line PC3. Cancer Cell Int 2005;5:19. [CrossRef]
27. Suer I, Karatas OF, Yuceturk B, Yilmaz M, Guven G, Buge O, et al. 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. [CrossRef]
28. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019;47(W1):W556-w60. [CrossRef]
29. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015;19(1a):A68-77. [CrossRef]
30. Chang L, Zhou G, Soufan O, Xia J. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. Nucleic Acids Res 2020;48(W1):W244-51. [CrossRef]
31. Huang HY, Lin YC, Cui S, Huang Y, Tang Y, Xu J, et al. miRTarBase update 2022: an informative resource for experimentally validated miRNA-target interactions. Nucleic Acids Res 2022;50(D1):D222-d30. [CrossRef]
32. Martin FJ, Amode MR, Aneja A, Austine-Orimoloye O, Azov AG, Barnes I, et al. Ensembl 2023. Nucleic Acids Res 2023;51(D1):D933-d41.

33. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics 2016;54:1.30.1-33. [CrossRef]
34. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 2019;10(1):1523. [CrossRef]
35. Piñero J, Ramírez-Anguita JM, Saüch-Pitarch J, Ronzano F, Centeno E, Sanz F, Furlong LI. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res 2020;48(D1):D845-55. [CrossRef]
36. Lánczky A, Győrffy B. Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. J Med Internet Res 2021;23(7):e27633. [CrossRef]
37. Zogopoulos VL, Saxami G, Malatras A, Papadopoulos K, Tsotra I, Iconomidou VA, Michalopoulos I. Approaches in Gene Coexpression Analysis in Eukaryotes. Biology (Basel) 2022;11(7):1019. [CrossRef]
38. Miller HE, Bishop AJR. Correlation AnalyzeR: functional predictions from gene co-expression correlations. BMC Bioinformatics 2021;22(1):206. [CrossRef]
39. Sadoughi F, Maleki Dana P, Asemi Z, Yousefi B. Targeting microRNAs by curcumin: implication for cancer therapy. Crit Rev Food Sci Nutr 2022;62(28):7718-29. [CrossRef]
40. Ding Y, Zhang C, Zhang J, Zhang N, Li T, Fang J, et al. miR-145 inhibits proliferation and migration of breast cancer cells by directly or indirectly regulating TGF-β1 expression. Int J Oncol 2017;50(5):1701-10. [CrossRef]
41. Song X, Zhang M, Dai E, Luo Y. Molecular targets of curcumin in breast cancer (Review). Mol Med Rep 2019;19(1):23-9. [CrossRef]
42. Liu T, Chi H, Chen J, Chen C, Huang Y, Xi H, et al. Curcumin suppresses proliferation and in vitro invasion of human prostate cancer stem cells by ceRNA effect of miR-145 and lncRNA-ROR. Gene 2017;631:29-38. [CrossRef]
43. Samad A, Haque F, Nain Z, Alam R, Al Noman MA, Rahman Molla MH, et al. Computational assessment of MCM2 transcriptional expression and identification of the prognostic biomarker for human breast cancer. Heliyon 2020;6(10):e05087. [CrossRef]
44. Zou J, Zhu L, Jiang X, Wang Y, Wang Y, Wang X, Chen B. Curcumin increases breast cancer cell sensitivity to cisplatin by decreasing FEN1 expression. Oncotarget 2018;9(13):11268-78. [CrossRef]
45. Chabottaux V, Noel A. Breast cancer progression: insights into multifaceted matrix metalloproteinases. Clin Exp Metastasis 2007;24(8):647-56. [CrossRef]
46. Akter T, Aziz MA, Islam MS, Sarwar MS. Association of MMP1 gene polymorphisms with breast cancer risk: A narrative review. Health Sci Rep 2023;6(10):e1607. [CrossRef]
47. Qu J, Zhao X, Liu X, Sun Y, Wang J, Liu L, et al. Natriuretic peptide receptor a promotes breast cancer development by upregulating MMP9. Am J Cancer Res 2019;9(7):1415-28.
48. Cheng L, Tan Z, Huang Z, Pan Y, Zhang W, Wang J. Expression Profile and Prognostic Values of Mini-Chromosome Maintenance Families (MCMs) in Breast Cancer. Med Sci Monit 2020;26:e923673. [CrossRef]
49. Lim JP, Nair S, Shyamasundar S, Chua PJ, Muniasamy U, Matsumoto K, et al. Silencing Y-box binding protein-1 inhibits triple-negative breast cancer cell invasiveness via regulation of MMP1 and beta-catenin expression. Cancer Lett 2019;452:119-31. [CrossRef]
50. Schveigert D, Cicenas S, Bruzas S, Samalavicius NE, Gudleviciene Z, Didziapetriene J. The value of MMP-9 for breast and non-small cell lung cancer patients' survival. Adv Med Sci 2013;58(1):73-82. [CrossRef]