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Original Article

Long non-coding RNA *UCA1* Knockdown Assisted by CRISPR/Cas9 in Female Cancer Cell Lines Increases Mir-143 *Tumor-Suppressor*

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Abstract

Background: The lncRNAs has been linked to several malignancies, including breast cancer**.** Our objective was to investigate the impact of urothelial carcinoma associated 1 (*UCA1*) on cellular growth and death by a CRISPR/Cas9 knockdown technique.

Methods: In 2020, the CHOPCHOP program was utilized to design two sgRNAs targeting the *UCA ge*ne. sgRNA1 and sgRNA2 were inserted into two different CRISPR plasmids to produce two recombinant plasmids. These recombinant plasmids were simultaneously transfected into MCF-7 and MDA-MB 231 carcinoma of the breast cells. Proliferation and apoptosis were compared using the MTT test, CCK-8 assay, and flow cytometry evaluation. RNA-hybrid software, quantitative reverse transcription PCR, and luciferase assays were utilized to confirm the relationship between *UCA1* and miR-143.

Results: Proliferated cells were less active in MTT and CCK-8 tests and fellow cytometry analysis. The PX459-sgRNA1,2 group had elevated levels of the cancer biomarker *Caspase-3* gene expression ($P<0.001$). When WT-*UCA1* and miR-143 were co-transfected, the luciferase activity was drastically decreased.

Conclusion: One very effective method of regulating cellular proliferation in vitro is the deletion of *UCA1*, which CRISPR/Cas9 accomplishes.

Keywords: Long non-coding RNA; Urothelial carcinoma associated 1*;* Cancer; Knockdown; miR-143 tumor-suppressor

Introduction

Long noncoding RNAs (lncRNAs) are involved in crucial biological processes, including chromatin rearrangement, histone modification, alternative gene splicing, and gene expression control (1). In addition, they are implicated in genomic imprinting, cell differentiation, organogenesis, and cancer (2). Based on their expression pattern and biological function, lncRNAs may be classed as oncogenes, tumor suppressor genes, or duplex lncRNAs. In a prior study (3), researchers as-

sessed the expression profile of lncRNAs in various cancer cell types using *Reverse transcription polymerase chain reaction (RT-PCR)* and other techniques. They demonstrated that lncRNAs are expressed in cancer cells with varying metastatic capacities.

Urothelial Cancer Associated 1 (*UCA1*) is a kind of lncRNA found in several malignancies (4) as a non-coding RNA. In addition to being a biomarker for predicting malignant and prognosis phenotypes (5), overexpression of *UCA1* enhances the proliferation rate and metastasis of bladder cells. *UCA1*, situated on human chromosome 19 p13.12, was found for the first time in bladder cancer (6). Recent data suggest that *UCA1* significantly controls oncogene activity in cancers such as breast cancer, blood cell carcinoma, colorectal cancer, and ovarian cancer (7, 8). In addition to accelerating the cell cycle, causing cell proliferation, and suppressing the expression of apoptotic genes, excessive *UCA1* expression may result in cancer (9). It has been shown (10) that *UCA1* participates in the activation of the AKT signaling pathway. *UCA1* also functions in breast cancer and suppresses P27 expression, increasing breast cancer tumor development and cell division (11). In breast cancer, *UCA1* is overexpressed, and miR-143 (a tumor suppressor) is down-regulated; these two effects contribute to cancer development. In addition, there are four binding sites between *UCA1* and miR-143, and the ablation of *UCA1* promotes the production of miR-143 (12).

LncRNAs and *UCA1* have a role in developing drug resistance in cancer treatment; *UCA1* overexpression promotes breast cancer cell resistance to some chemotherapy treatments (13). Few studies have demonstrated that inhibiting *UCA1*lncRNA decreases the expression of pro-apoptotic genes (14).

We aimed to execute *UCA1* knockout in breast cancer cells to assess its potential impacts on the expression of apoptosis-related genes, apoptosis, and cell proliferation. We compared treated MCF-7 cells to untreated and vehicle-treated cells.

Materials and Methods

Ethical approval

This study with the approval ID [IR.IAU.SHK.REC.1399.017] was approved by the Ethics and Research Committees of the Islamic Azad University, Shahrekord, Iran.

sgRNA designing and vector construction

Two sgRNAs were synthesized to target specific sites within the *UCA1* gene (3199-3218 and 3942-3961) utilizing the CHOPCHOP webpage (https://chopchop.cbu.uib.no). Table 1 contains the primer sequences for the PX459-sgRNA1 and PX459-sgRNA2 vectors.

Vector Amplification

The CaCl2 technique was used to heat shock converts vectors (1µg) carrying sgRNA1, sgRNA2, and GFP into the E. coli Top10 bacterium. Plasmids were recovered from bacteria using the Favor PrepTM Plasmid Extraction kit (15).

Gene knockout confirmation

PCR Amplification and Detection of Mismatched Duplexes by T7 endonuclease 1 assay

The PCR method was utilized to verify that sgRNAs could effectively eliminate *UCA1*. DNA was extracted using the FavorPrep DNA Extraction Mini Kit. Using a mismatch-sensitive T7 endonuclease 1 test, it was confirmed that DNA cleavage and targeted sequence deletion happened at the proper place. In three petite tubes, 200ng of DNA was combined using 2 µL of 10X NE-Buffer 2 solution and 19 µL of water without nuclease. It took ten minutes to get the samples up to 95°C and then gently cooled to ambient temperature. T7 endonuclease I (5 units/l) was combined with 19 µl of sample and incubated at 37°C for 15min before agarose gel electrophoresis was performed (16).

Table 1: Details of oligonucleotide primers used for PCR and real-time PCR

Target	*Primers Name*	*Sequences*	*Annealing Temperature (°C)*	*Product length (bp)*
UCA1	*UCA1*-F *UCA1*-R	CTGCTCACATATATACTCAGTGACCAC GACTGGACAGGGAGATTGGAG	63	Non Digest:1207 Digest:478
UCA1-qPCR	*UCA1*-qPCR-F *UCA1*-qPCR-R	ACCACCTTTAACTGTAACTTTCC TCCGTATAGAAGACCACCTAAAC	58	165
PX549-sgRAN1	hU6-F *P-UCA1*-sg1	GAGGGCCTATTTCCCATGATT AA-GACCCTGGTATCTCCCTC	62	276
PX549-sgRAN2	hU6-F *P-UCA1*-sg2	GAGGGCCTATTTCCCATGATT AAGACCAAATCTGGGCCAGG	62	276
miR-143	miR-143-F miR-143-R	AGC GTG TGT CGT GGA GTC TCG TGA GAT GAA GCA CTG TAG	59	63
Caspase-3	*Caspase-3*-F *Caspase-3*-R	CAGAACTGGACTGTGGCATTG GCTTGTCGGCATACTGTTTCA	58	192
P53	*P53*-F *P53*-R	TGCGTGTGGAGTATTTGGATGAC CAG-TGTGATGATGGTGAGGATGG	64	170
BCL-2	*BCL2*-F *BCL2*-R	GACGACTTCTCCCGCCGCTAC CGGTTCAGGTACTCAGTCATCACCAC	65	245
FAS	*FAS*-F *FAS*-R	CAATTCTGCCATAAGCCCTGTC GTCCTTCATCACACAATCTACATCTTC	64	163
BAX	*BAX*-F *BAX*-R	AGGTCTTTTTCCGAGTGGCAGC GCGTCCCAAAGTAGGAGAGGAG	65	243
BAK	*BAK*-F *BAK*-R	CGTTTTTTACCGCCATCAGCAG ATAGCGTCGGTTGATGTCGTCC	66	154
SURVIVIN	*SURVIVIN*-F *SURVIVIN*-R	AGAACTGGCCCTTGGAGG CTTTTTATGTTCCTCTATGGGGTC	64	170

Induction of apoptosis in cells

a) Expression of Apoptosis-Related Genes by Quantitative Real-Time PCR

Apoptosis-related levels of transcription were analyzed utilizing Corbett 5 Plex in real-time PCR. The relative transcription was determined by employing the $2^{-\Delta\Delta Ct}$ technique, with *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* serving as the reference control.

b) Western blot analysis

Rojan Azma Co (Iran) investigated *P53*, *Caspase-3*, and *BCL-2* protein expression patterns in transfected MCF-7 and MDA-MB231 cells utilizing western blot.

c) Flow-Cytometric Analysis

The Annexin-V-FITC Propidium iodide binding experiment was used to measure the amount of apoptosis in cells by the instructions provided by the manufacturer (Invitrogen TM, United Kingdom).

Cell cycle assays

The Multi-Cycle AV program from Phoenix Flow Systems, a biotechnology company in San Diego, California, was employed for the research on cell death.

Cell proliferation

MTT and CCK-8 assay

Proliferative rates in cells implanted with PX459-sgRNA1, PX459-sgRNA2, and PX459, as well as in blank control cells, were assessed utilizing a colorimetric kit I (MTT) (Roche, Switzerland). In addition, the Cell Counting Kit-8 was used to monitor cell proliferation (CCK-8, Beyotime, Shanghai, China).

Migration assay

A cell migration test was carried out using a transwell culture method. The top chamber of the transwell chamber was plated with 5×10^3 cells/ml, and the bottom chamber was filled with growth media. A cotton swab was used to remove non-migratory cells from the top section of the membrane after 24 hours. For ten minutes, formaldehyde was used to fix the invading cells at the filter's bottom. For the next 20 minutes, the samples were treated using a 1% crystal violet mixture. A light microscope (Optika, Italy) was used to count and visualize the number of cells in the sample.

lncRNA-miRNA interaction analysis

In-silico analysis

RNAhybrid, a bioinformatics application, was employed to forecast the interaction between long non-coding RNA (lncRNA) and microRNA (miRNA). We retrieved the protein sequence from the NCBI's GenBank database at ncbi.nlm.nih.gov/genbank. To get a system score over 160, the folding free energy must be below 20. The selected lncRNAs had an alteration prevalence above 3% due to the vast number of lncRNAs exhibiting differential expression in breast cancer. Quantification of miRNA l43 gene expression using quantitative reverse transcription PCR and quantitative real-time PCR.

Quantitative reverse transcription PCR

Quantitative reverse transcription PCR was employed to analyze miRNA expression concentrations. U6 small nuclear RNA was used to normalize the RNA input as an internal control. The primer GGGTCCGAGGTGCACTGGATATGACAAAATATGGAAC-3' was designed for U6 (17).

Assay for luciferase gene reporter

UCA1 wild-type/mutant luciferase reporter plasmids and miR-143 or miR-NC were co-transfected into breast cancer cell lines using Lipofectamine 2000. The luciferase activity was assessed using the dual-luciferase assay technique from Promega. Luciferase activity was examined relative to Renilla's activity. Each experiment was conducted thrice.

Statistical Analysis

The research conducted experiments with biological and technical replicates, evaluated with SPSS version 20 (IBM Corp., Armonk, NY, USA), and compared utilizing independent T-test or ANOVA procedures, with significance levels below 0.05.

Results

The lncRNA UCA1 has a link to the occurrence of the breast cancer

There was a 2.50 odds ratio between having high *UCA1* expression and having low *UCA1* expression, showing that having more *UCA1* expression suggested more LNM. Additionally, each category's assessment findings are shown in Table 2. In both large (n>100, OR = 1, 99) and small (n>100, OR = 2,71) samples sizes, a high *UCA1* expression profile was connected to higher LNM frequencies.

Table 2: Subgroup investigation of *UCA1*'s function in LNM in various cancer types

Subgroup analysis	*No. of studies*	*No. of patients*	*Test of relationship*		*Test of heterogeneity*	
			HR (95% CI)	*P*-value	I^2 (%)	Q-value
Overall	25	2003	2.50 (1.93–3.25)	<0.0001	43	0.01
Sample size						
<100	18	1164	2.71 (2.12–3.47)	<0.00001	46	0.02
≥100	7	839	1.99 (1.50–2.65)	<0.00001	23	0.25
Tumor type						
Respiratory system	2	172	2.54 (0.70–9.23)	0.16	71	0.06
Digestive system	17	1320	2.27 (1.61–3.20)	<0.00001	52	0.006
Reproductive system	3	208	3.65 (1.96–6.81)	<0.0001	0	0.51
Others	3	303	2.90 (1.77–4.77)	<0.0001	0	0.63
Cut off						
Median	15	1077	2.48 (1.63–3.78)	<0.0001	59	0.002
Others	10	926	2.53 (1.92–3.34)	<0.00001	0	0.54
Reference control						
GAPDH	16	1301	2.41 (1.91–3.04)	<0.00001	45	0.03
β-Actin	5	415	2.35 (1.54–3.57)	<0.0001	0	0.5
RNU6B/GUSB	4	287	2.69 (0.95–7.56)	0.06	74	0.009

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Successful Knockout of UCA1 Using CRISPR/Cas9

After utilizing specific primers for *UCA1* (hU6-F/*P*) and UCA2 (hU6-F/*P*)-*UCA1*, the 276-bp band was amplified by PCR for the sgRNA1 and sgRNA2 amplification. The deletion of 729 bp from the *UCA1* gene promoter and exon 1 is seems to be a complete success of the CRISPR/Cas9-mediated knockout.

Fluorescence microscopy after 24 hours of incubation with the GFP-containing plasmid without the sgRNAs was used to construct pSpcas9 (the control cell line), which was analyzed (Fig. 1D).

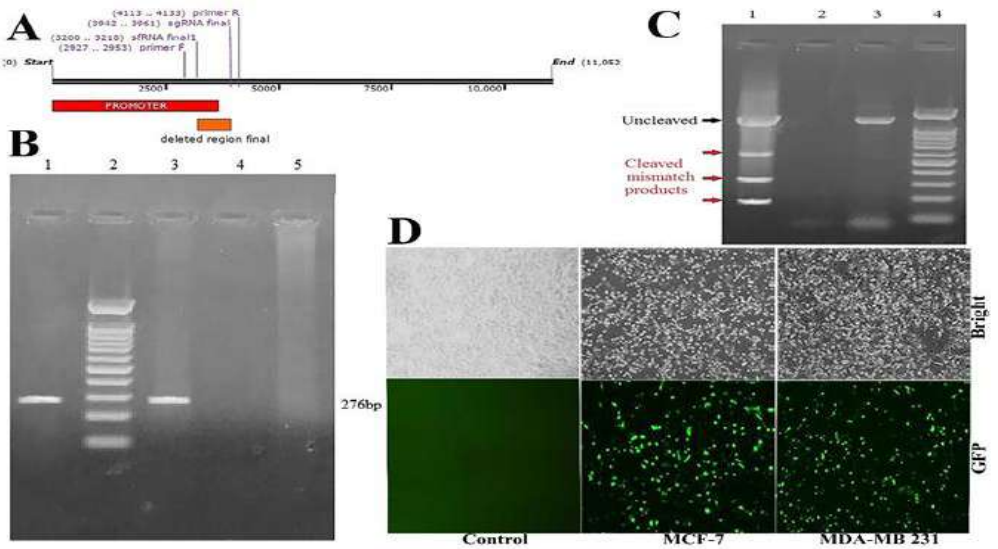

Fig. 1: **(A)** Schematic image of the sgRNA and primer binding sites. **(B)** Validation of vector construction by PCR. Lane 1: PX459-sgRNA1, Lane 2:100 bp ladder, Lane 3: PX459-sgRNA2, Lane 4: Negative control (no DNA), Lane 5: PX459. **(C)** T7 Endonuclease 1 assay. Lane 1: CRISPR/Cas9 knocked out *UCA1* DNA digested by T7E1, Lane 2: Negative control for the PCR reaction, Lane 3: PX459-group (Negative control group for CRISPR/Cas9 knockout), Lane 4: 100bp DNA Ladder. **(D)** GFP visibility was used to assess pSpcas9 transfection efficiency

CRISPR/Cas9-Mediated NOX4 Knockout Validated at single clone analysis and mRNA Levels

The two *UCA1* genes have been altered to produce the homozygous genotype, seen in Fig. 2A as the single 478bp band. In contrast to the unmanipulated cells (PX459 and the blank control), which had *UCA1* knocked out (Fig. 2B, 2C), the manipulated cells (i.e., PX459 –sgRNA1, 2) had no expression of UAC1.

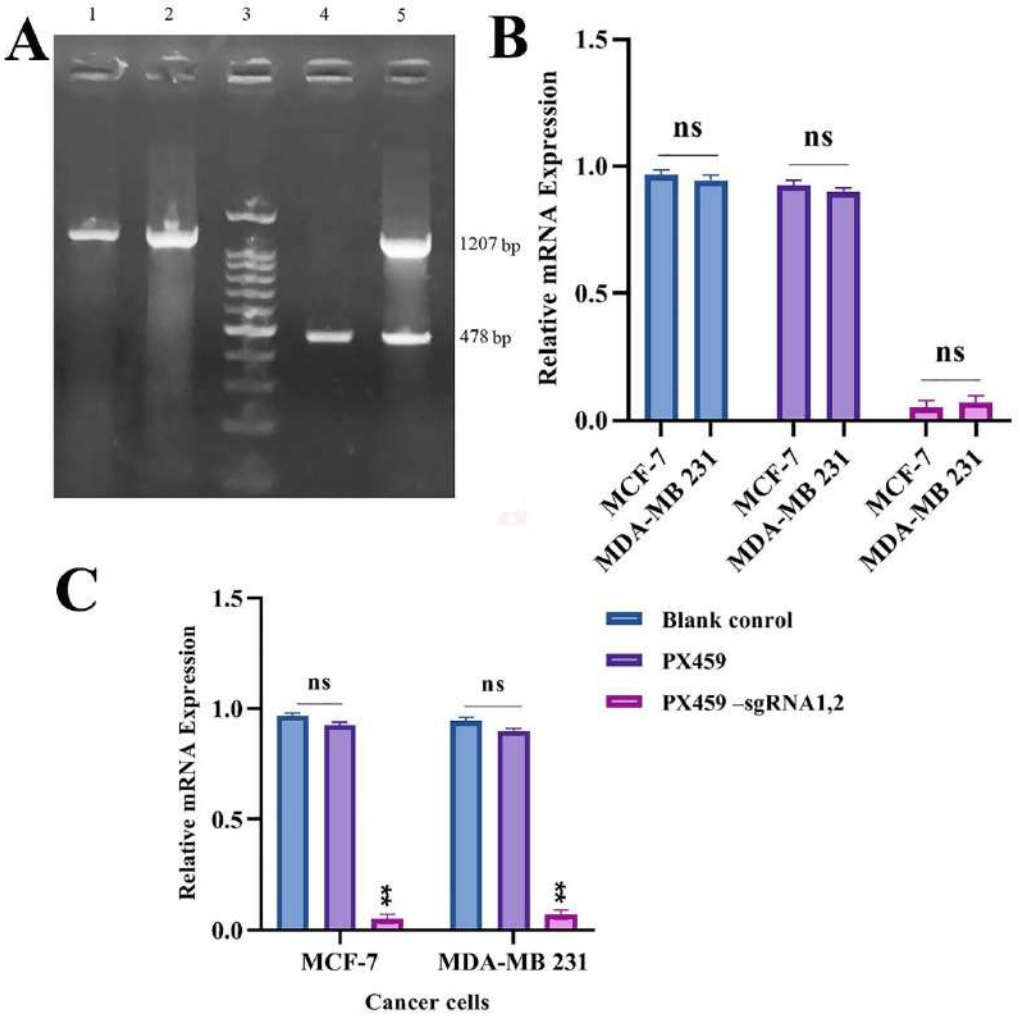

Fig. 2: (**A)** Gel electrophoresis of PCR fragments for *UCA1* gene in cancer cells.
Lane 1: non digest *UCA1* gene with 1207bp fragment in non-transfected cells (*UCA1*$^{+}$ / *UCA1*$^{+}$)
Lane 2: non-digest *UCA1* gene with 1207bp fragment in control group (*UCA1*$^{+}$ / *UCA1*$^{+}$)
Lane 3: 100bp DNA Ladder
Lane 4: digested *UCA1* gene with 478bp fragment in PX495-sgRNA1, 2 groups with homozygous genotype (*UCA1*$^{-}$/*UCA1*$^{-}$)
Lane 5: digested *UCA1* gene with 478bp and 1207bp fragments in PX495-sgRNA1/2, group with heterozygous (*UCA1*$^{+}$/*UCA1*$^{-}$).
(B) *UCA1* RNA expression in MCF-7 cells in comparison with MDA-MB231.
(C) *UCA1* expression in MCF-7, and MDA-MB231cells compared to control groups. After knocking out *UCA1* (PX459-sgRNA1,2) mRNA expression was decreased which shows the proper function of the CRISPR system and a significant difference (**P<0.001) with the control cell lines. ns: non-significant

CRISPR/Cas9-mediated Knockout of UCA1 Increased Apoptosis

Pro-apoptosis genes *P53* (*P*=0.0355) and *BAX* (*P*=0.363) are significantly reduced in MCF-7 cells, whereas *P53* (*P*=0.045) and *BAX* (*P*=0.23) are significantly decreased in MDA-MB231 cells, and *FAS* (*P*=0.0021) is significantly decreased in MDA-MB231 cells. *UCA1* deletion treatment significantly decreases the expression of two anti-apoptosis genes, BCL2 and *SURVIVIN*, in both MCF-7 and MDA-MB-231 cell lines, compared to the control cells (*P*=0.0081 and *P*=0.0032, respectively) (Fig. 3).

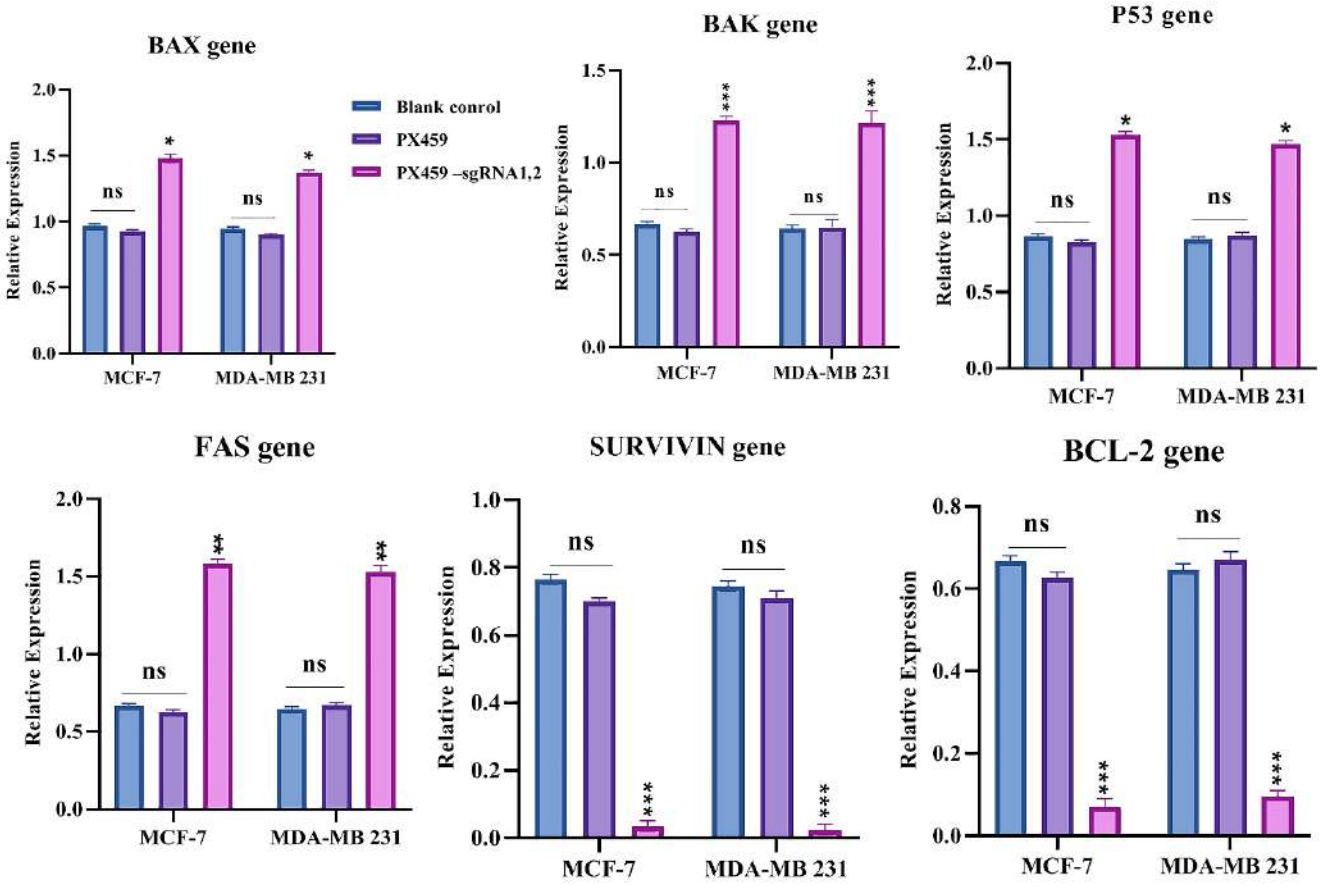

Fig. 3: The mRNA levels of the *P53, BCL2*, *FAS, BAK, SURVIVIN,* and *BAX* genes were evaluated in the PX459-sgRNA1,2, blank control, and PX459 groups. The data obtained from the qRT-PCR assay were normalized versus the GAPDH (reference gene). * *P*<0.05, ** *P*<0.01, *** *P*<0.001, ns: non-significant

CRISPR/Cas9-mediated Knockout of UCA1 Increased Caspase-3

Fig. 4A shows that *Caspase-3* cancer biomarker gene expression was higher in the treated cancer cells group (*UCA1*-/UCA-) than in the control group cells (*UCA1*+/*UCA1*+). Also, *BCL-2* protein expression was reduced in *UCA1* knockout cells (Fig. 4B). However, *BCL-2* protein expression was not reduced in the control group (Fig. 4A). *Caspase-3* and *P53* antibodies levels in the control groups were much lower (Fig. 4B, 4C). Early, late, and necrotic cells were all less than 10% (Fig. 4C), but the percentage of alive cells was more significant than 90%. The early apoptosis, late apoptosis, necrosis, and viable MCF-7 cells of the *UCA1*Knockout cells were 29.44 %,

34.24 %, 2.50 %, and 33.82 %, respectively. Overall, 14.22%, 47.90%, 14.24%, and 23.64 % of MDA-MB231 cells were in early, late, or necrotic apoptosis (Fig. 4D).

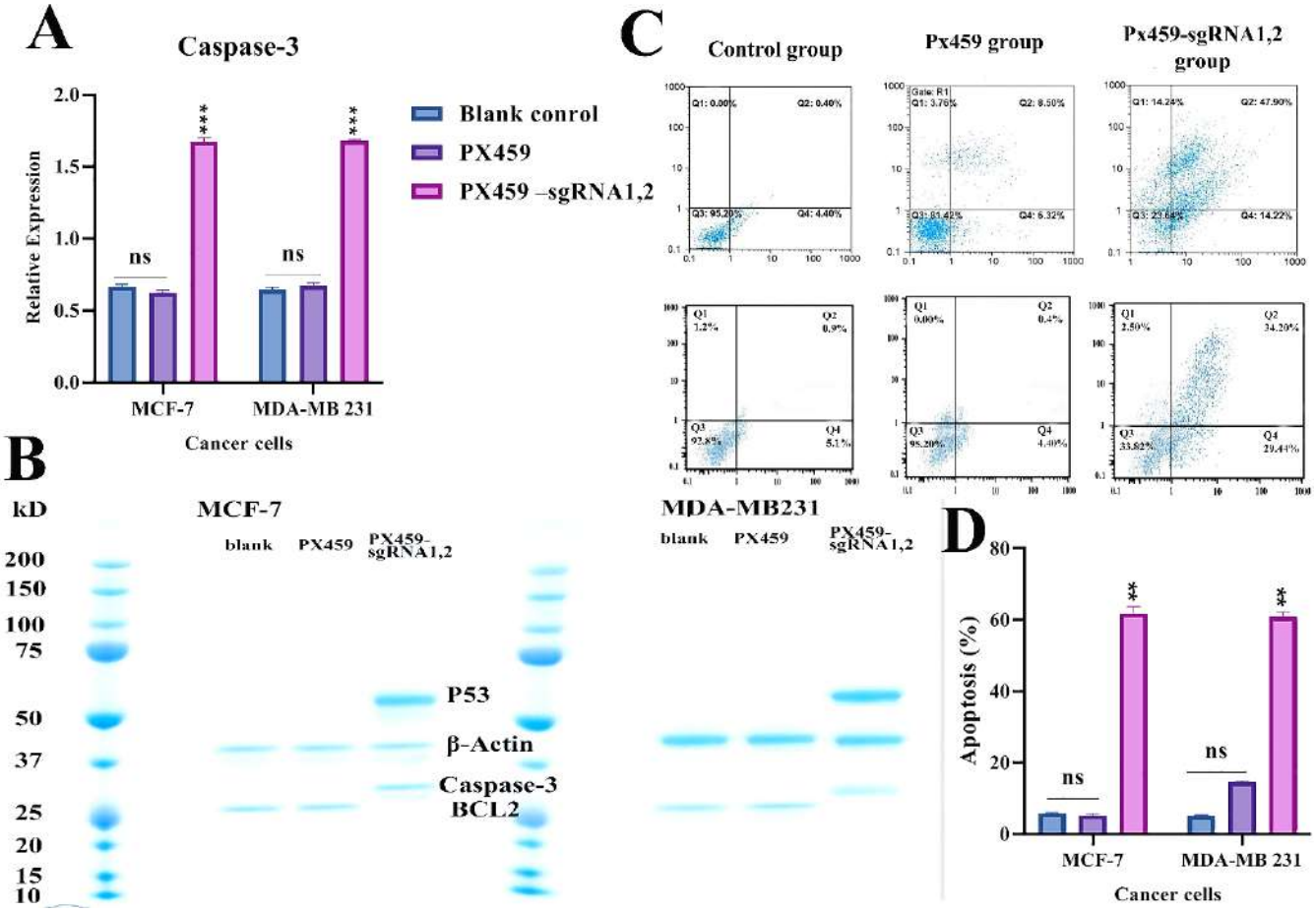

Fig. 4: Evaluation of the cell apoptosis. (**A)** *Caspase-3* cancer biomarker gene expression was increased in PX459-sgRNA1,2 group (***P<0.001). (**B)** The results of western blots using *Caspase-3*, *P53* antibodies revealed that these proteins had a strong expression in *UCA1* knockout cells (PX459-sgRNA1,2 groups) but BCL2 had not expression in *UCA1* knockout cells. (**C)** Flow cytometer analysis of the apoptotic and necrotic cells. Q1: Necrotic %age, Q2: late apoptotic %age, Q3: Live %age, and Q4: early apoptotic percentage. (**D)** lncRNA*UCA1* Knockout enhances apoptosis (63.68%) in Px459-sgRNA1,2 group of MCF-7 cells and (62.12%) in Px459-sgRNA1,2 group of MDA-MB231 cells. ns: non-significant

CRISPR/Cas9-mediated Knockout of UCA1 Decreased cell proliferation

As shown in Fig. 5, the proliferation rate of breast cancer cells was also lowered in the PX459-sgRNA1 group (P<0.05). There was no significant difference in cell proliferation between the PX459 group and the blank control breast cancer cells. Flow cytometry was used to analyze cell cycle progression. Sub-G1 levels were higher in the pX459-sgRNA1,2 cell line than in the pX459 and blank control cell lines, which delayed the entrance of the cells into the G2 phase and reduced cell proliferation. *UCA1* deletion primarily slowed down the cell cycle in the G0/G1 and S phases of the cell cycle (P<0.001).

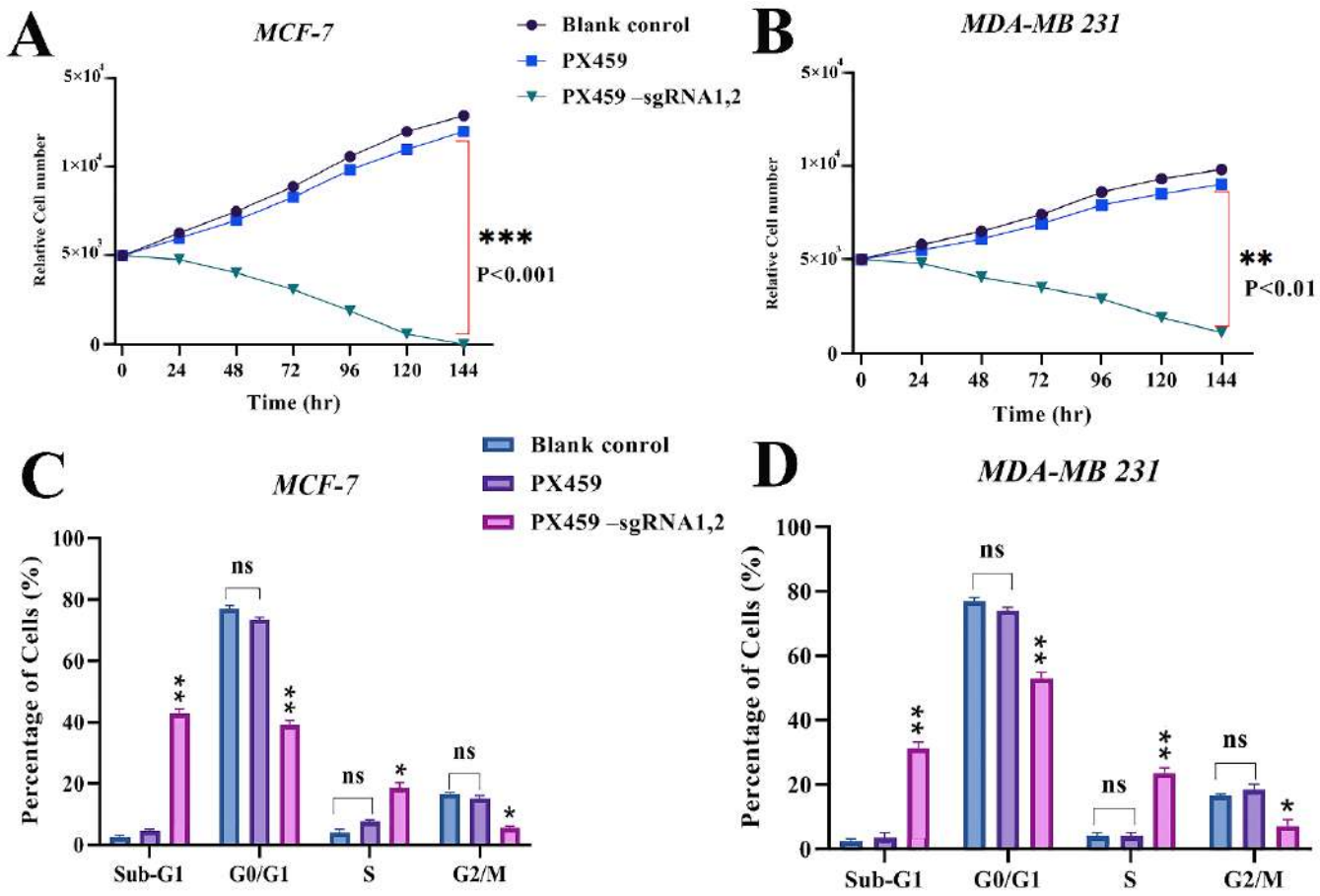

Fig. 5: The effect of *UCA1* knockout on MCF-7 **(A)** and MDA-MB231 **(B)** cancer cell proliferation was evaluated by MTT. Analysis of the results showed a significant difference between the growth rate of the control groups and the PX459-sgRNA1, 2 groups after 48hr ($P<0.05$).

UCA1 knockout arrested cell cycle at S phase in MCF-7 **(C)** and MDA-MB231 **(D)** cancer cell ($P<0.001$). Column diagram analysis was performed for cells %age at each phase in 4 different groups: Sub-G1, G0/G1 phase, S phase, and G2/M phase. At G0/G1 phase, there was a significant difference in cells %age in the PX459-sgRNA1,2 groups compared with the blank control group and PX459 group, respectively. Cell cycle analysis showed that *UCA1* knockout mainly arrested the cell cycle at S phase ($P<0.001$). $^{**}P<0.01$, $^{***}P<0.001$, ns: non-significant

Knocking UCA1 lncRNA out inhibits the migration of breast cancer cells

As shown in Fig. 6, the number of breast cancer cells that moved to the bottom chamber reduced considerably after transfection with PX459-sgRNA1 and PX459-sgRNA2 vectors compared to the PX459 and Blank control groups ($P<0.001$). The observations indicate that*UCA1*knockout limits the migration of breast cancer cells. As shown by the CCK-8 test, *UCA1* deletion lowered the proliferation of MCF-7 and MDA-MB231 cells in the PX459-sgRNA1,2 group (Fig. 6C).

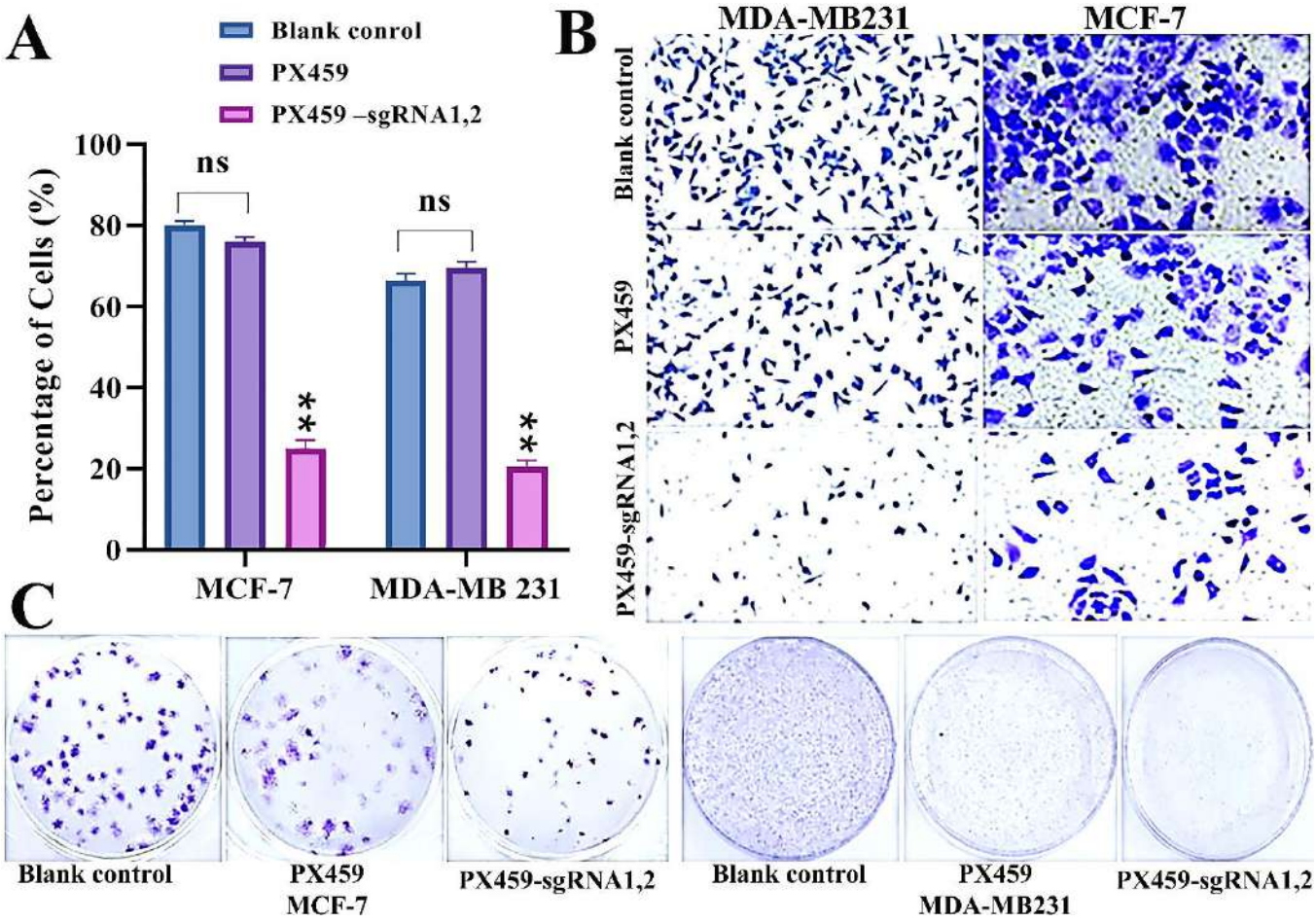

Fig. 6: (**A**) *UCA1*lncRNA knockout inhibits the migration of breast cancer cells. (**B)** Representative images show the migration of MCF-7 and MDA-MB231 cells in the PX459-sgRNA1,2 group. ***P* <0.01
(C) PX459-sgRNA1,2, PX459, and blank control of breast cancer cells expressing were cultured in 96-well plates, and cell proliferation was measured using CCK-8 assay. In the PX459-sgRNA1,2 group, *UCA1* knockout decreased the proliferation of MCF-7 and MDA-MB231 cells. ***P* <0.01, ns: non-significant

LncRNA UCA1 inhibits miR-143 expression in breast cancer cells

This research hypothesized an interaction between MiR-143 and *UCA1*. The potential binding sites are shown in Fig. 7A. MiR-143 was not expressed in the control groups (Fig. 7B). However, miR-143 expression was revealed by the band produced on the gel. In control groups of breast cancer cells, the expression of miR-143 was decreased, but the expression of *UCA1* was enhanced in PX459-sgRNA1,2 cancer cells. In addition, real-time PCR research revealed that when *UCA1* was inhibited, the expression of miR-143 in breast cancer cells increased (Fig. 7C). Co-transfection of WT-*UCA1* and miR-143 mimics dramatically decreased luciferase activity, according to the findings. The luciferase activity in the subgroup of MUT-*UCA1* and miR-143 mimics co-transfection did not change substantially (Fig. 7D).

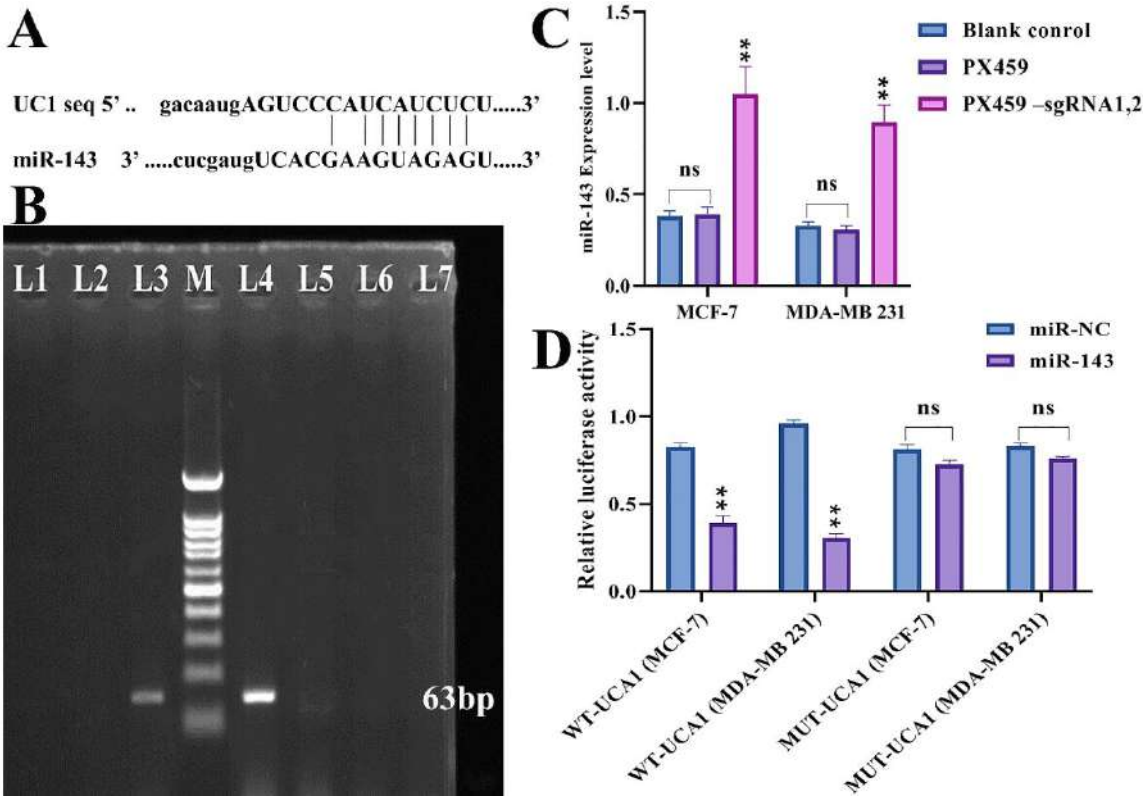

Fig. 7: In breast cancer, *UCA1* functions as a sponge for miR-143. (**A)** Potential miR-143 interaction location on *UCA1*. (**B)** Expression of miR-143 in breast cancer cells. L1: MDA-MB231 blank control, L2: MDA-MB231 PX459, L3: MDA-MB231 PX459-sgRNA1,2, M: 50bp marker, L4: MCF-7 blank control, L5: MCF-7 PX459, L6: MCF-7 PX459-sgRNA1,2. **(C)** In MDA-MB231 and MCF-7 cell lines, knocking down *UCA1* boosted miR-143 expression. **(D)** Breast tumor cells co-transfected with WT/MUT *UCA1* and miR-143 mimics or miR-NC in dual-luciferase reporter experiments. **P*<0.05, ***P*<0.01, ns: non-significant

Discussion

UCA1 is a long non-coding RNA known for its oncogenic properties in several types of cancers such as the gastrointestinal tract, and ovarian tumors (18). Numerous investigations have shown an increase in the expression of this long non-coding RNA, indicating its crucial involvement in breast cancer (19, 20). This oncogene promotes tumor growth and advancement by engaging with many cellular systems (21). The research discovered that increased UCA1 expression decreases miR143 stages, a tumor suppressor that controls BCL2 and hinders cell proliferation, maintaining the standard cell cycle (18, 20). Our strategy included using CRISPR/Cas9 to target the promoter and exon one domain of the UCA1 gene in MCF-7 breast carcinoma cells. As a result, pro-apoptotic genes like FAS, BAK, BAX, and P53 were upregulated. Furthermore, cells lacking UCA1 have reduced anti-apoptotic genes like BCL2 and SURVIVIN levels.

Recent research has linked *UCA1* to cellular proliferation, invasion, and metastasis in hepatocellular carcinoma, bladder cancer, and oral squamous cell carcinoma (OSCC) (22). Research on breast cancer cells shows that overexpression of *UCA1* can enhance invasiveness, affecting invasion rate and mesenchymal characteristics through Wnt/-catenin signaling (14-16). *UCA1* controls PTP1B, enhancing breast cancer proliferation and invasion rates. *UCA1*'s strong affinity for miR206 inhibits its activity, indirectly promoting cellular proliferation and disease development by regulating PTP1B mRNA degradation (17-20). In the

present study, we found a substantial reduction in cellular proliferation following *UCA1* deletion, consistent with findings from prior research (16-18) indicating that *UCA1* suppression decreases proliferation and invasion rates. *UCA1* and hnRNP play a role in inhibiting tumor suppressor miR143, influencing breast cancer growth and migratory capacity, and competing with P27 protein levels (19-22).

lncRNAs have a substantial impact on breast cancer, with 790 lncRNAs being overexpressed and 637 being downregulated in comparison to normal breast tissues (23-25). We silenced the *UCA1* gene in MCF-7 mammary cancer cells using CRISPR/Cas9. Two sgRNAs were directed into the promoter and exon one areas, resulting in the upregulation of pro-apoptotic genes and the downregulation of anti-apoptotic genes in knockdown cells. Studies have connected *UCA1* to cell growth, spread, and spread to other parts of the body in different types of cancer, such as hepatocellular carcinoma and OSCC (26-29). *UCA1* also affects the rate of invasion and features of mesenchymal cells. *UCA1*'s regulation of PTP1B promotes the growth and spread of breast cancer (27-30). *UCA1*'s high attraction to miR206 hinders its function, encouraging cell growth and disease progression (29, 30). Our flow cytometry analysis showed significant alterations in apoptosis in *UCA1* mutated cells, suggesting cell cycle arrest, modified gene expression, and triggering of death.

Conclusion

This research focuses on the *UCA1* gene in breast carcinoma, demonstrating its role in suppressing the proliferation and death of MCF-7 cells. Deleting *UCA1* with CRISPR/Cas9 effectively limits cellular proliferation in vitro.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

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Conflict of Interest

The authors declare that there is no conflict of interests.

References

1. Hashemzehi R, Doosti A, Kargar M, et al (2018). Cloning and expression of *nlpA* gene as DNA vaccine candidate against Acinetobacter baumannii. *Mol Biol Rep*, 45: 395-401.
2. Zhuo Li 1, Dehai Yu 2, Haijun Li, et al (2019). Long non-coding RNA *UCA1* confers tamoxifen resistance in breast cancer endocrinotherapy through regulation of the EZH2/p21 axis and the PI3K/AKT signaling pathway. *Int J Oncol*, 54 (3): 1033-1042.
3. Choudhry H (2021). *UCA1* Overexpression Promotes Hypoxic Breast Cancer Cell Proliferation and Inhibits Apoptosis via HIF-1α Activation. *J Oncol*, 2021: 5512156.
4. Yang G, Shi R, Zhang Q (2020). Hypoxia and oxygen-sensing signaling in gene regulation and cancer progression. *Int J Mol Sci*, 21 (21): 8162.
5. Moezi P, Kargar M, Doosti A, et al (2019). Multiplex touchdown PCR assay to enhance specificity and sensitivity for concurrent detection of four foodborne pathogens in raw milk. *J Appl Microbiol*, 127 (1): 262-273.
6. Wang J, Zhang X, Chen W, et al (2020). Regulatory roles of long noncoding RNAs implicated in cancer hallmarks. *Int J Cancer*, 146 (4): 906-916.
7. Hosseini NF, Manoochehri H, Khoei SG, et al (2021). The functional role of long noncoding RNA *UCA1* in human multiple cancers: a review study. *Curr Mol Med*, 21 (2): 96-110.
8. Neve B, Jonckheere N, Vincent A, et al (2018). Epigenetic regulation by lncRNAs: an over-

view focused on *UCA1* in colorectal cancer. *Cancers (Basel)*, 10 (11): 440.
9. Doosti A, Amini-Bavil-Olyaee S, Tajbakhsh E, et al (2009). Prevalence of viral hepatitis and molecular analysis of HBV among voluntary blood donors in west Iran. *New Microbiol*, 32 (2): 193-8.
10. Wang ZQ, Cai Q, Hu L, et al (2017). Long noncoding RNA *UCA1* induced by SP1 promotes cell proliferation via recruiting EZH2 and activating AKT pathway in gastric cancer. *Cell Death Dis*, 8 (6): e2839.
11. Kargar M, Rashidi A, Doosti A, et al (2012). Prevalence of *Coxiella burnetii* in bovine bulk milk samples in southern Iran. *Comp Clin Pathol*, 22 (3): 331-334.
12. Zhou Y, Meng X, Chen S, et al (2018). IMP1 regulates *UCA1*-mediated cell invasion through facilitating *UCA1* decay and decreasing the sponge effect of *UCA1* for miR-122-5p. *Breast Cancer Res,* 20: 32.
13. Latgé G, Poulet C, Bours V, et al (2018). Natural antisense transcripts: molecular mechanisms and implications in breast cancers. *Int J Mol Sci*, 19 (1): 123.
14. Huang J, Zhou N, Watabe K, et al (2014). Long non-coding RNA *UCA1* promotes breast tumor growth by suppression of p27 (Kip1). *Cell Death Dis*, 5 (1): e1008.
15. Nekoei S, Hafshejani TT, Doosti A, et al (2015). Molecular detection of bovine leukemia virus in peripheral blood of Iranian cattle, camel and sheep. *Pol J Vet Sci*, 18(4):703-7.
16. Safarpour-Dehkordi M, Doosti A, Jami MS (2020). Impacts of the Staphylococcal Enterotoxin H on the Apoptosis and lncRNAs in PC3 and ACHN. *Mol Gen Microbiol Virol*, 35: 180-188.
17. Safarpour-Dehkordi M, Doosti A, Jami MS (2020). Integrative analysis of lncRNAs in kidney cancer to discover a new lncRNA (*LINC00847*) as a therapeutic target for staphylococcal enterotoxin *tst* gene. *Cell J*, 22(Suppl 1):101-109.
18. Liu R, Liao J, Yang M, et al (2012). The cluster of miR-143 and miR-145 affects the risk for esophageal squamous cell carcinoma through co-regulating *FAS*cin homolog 1. *PLoS One*, 7 (3): e33987.
19. Miao Y, Fan R, Chen L, et al (2016). Clinical significance of long non-coding RNA MALAT1 expression in tissue and serum of breast cancer. *Ann Clin Lab Sci*, 46 (4): 418-424.
20. Soudyab M, Iranpour M, Ghafouri-Fard S (2016). The role of long non-coding RNAs in breast cancer. *Arch Iran Med*, 19 (7): 508-17.
21. Dhanasekaran R, Deutzmann A, Mahauad-Fernandez, et al (2022). The MYC oncogene—the grand orchestrator of cancer growth and immune evasion. *Nat Rev Clin Oncol*, 19 (1): 23-36.
22. Piri-Gharaghie T, Doosti A, Mirzaei SA (2023). Novel adjuvant nano-vaccine induced immune response against Acinetobacter baumannii. *AMB Express*, 13 (1): 31.
23. Xu L, Zhu H, Gao F, et al (2019). Upregulation of the long non-coding RNA CBR3-AS1 predicts tumor prognosis and contributes to breast cancer progression. *Gene*, 721S:100014..
24. Wang H, Guan Z, He K, et al (2017). LncRNA *UCA1* in anti-cancer drug resistance. *Oncotarget*, 8 (38): 64638- 64650.
25. Wang F, Li X, Xie X, et al (2008). *UCA1*, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. *FEBS Lett*, 582 (13): 1919-1927.
26. Xiao C, Wu CH, Hu HZ (2016). LncRNA *UCA1* promotes epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt/beta-catenin signaling pathway. *Eur Rev Med Pharmacol Sci*, 20 (13): 2819-2824.
27. Li Y, Zeng Q, Qiu J, et al (2019). Long noncoding RNA *UCA1* promotes breast cancer by upregulating PTP1B expression via inhibiting miR-206. *Cancer Cell Int*, 19:275.
28. Xin H, Liu N, Xu X, et al (2019). Knockdown of lncRNA-*UCA1* inhibits cell viability and migration of human glioma cells by miR-193a-mediated downregulation of CDK6. *J Cell Biochem*, 120 (9): 15157-15169.
29. Liu C, Jin J, Shi J, et al (2019). Long noncoding RNA *UCA1* as a novel biomarker of lymph node metastasis and prognosis in human cancer: a meta-analysis. *Biosci Rep*, 39 (4): BSR20180995
30. Piri-Gharaghie T (2021). Polycystic ovary syndrome and genetic factors influencing its development: A review article. *Pers M J*, 6 (23): 25-29.