



*Research article*

## **The regulation of miR-139-5p on the biological characteristics of breast cancer cells by targeting COL11A1**

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**Abstract:** *Objective:* This study aimed to explore the regulatory mechanism of miR-139-5p on the biological characteristics of breast cancer cells by targeting Collagen type XI alpha 1 chain (COL11A1). *Method:* GEO2R was used to identify the differentially expressed genes (DEGs) of breast cancer in GEO database. miRDB, miRanda and TargetScan databases were used to predict the miRNAs that regulate COL11A1. qRT-PCR was used to detect the expressions of COL11A1 and miR-139-5p in breast cancer cells, and western blot was used to detect the protein level of COL11A1. RNA binding protein immunoprecipitation assay was employed to test the targeted relationship between miR-139-5p and COL11A1, which was further verified by dual-luciferase reporter gene assay. CCK-8 assay was performed to detect the cell proliferation, and flow cytometry was carried out to examine the cell apoptosis. Moreover, western blot was used for the detection of caspase-3, Bax and Bcl-2 protein levels. *Results:* A total of five DEGs were screened from the GEO database, of which COL11A1 was the only highly expressed in breast cancer. According to the database analysis, we predicted that miR-139-5p was much likely to target the expression of COL11A1. MiR-139-5p was poorly expressed in breast cancer cells and targeted inhibited COL11A1. Silencing COL11A1 or overexpressing miR-139-5p both could inhibit the proliferation and promote the apoptosis, while overexpressing the two factors simultaneously could reverse such effect. *Conclusion:* Overexpression of miR-139-5p inhibits the proliferation and promotes the apoptosis of breast cancer

cells by inhibiting the expression of COL11A1.

**Keywords:** miR-139-5p; COL11A1; breast cancer; proliferation; apoptosis; GEO2R

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## 1. Introduction

Breast cancer is a major threat to women's health, with an annual morbidity accounting for nearly 11.6% of the world. In China, the morbidity of middle-aged women continuously increases, and more and more young women are involved in [1]. At present, the study on the breast cancer-related molecular mechanism has been highly focused, especially on the role of miRNA in breast cancer [2]. Previous studies have shown that miRNAs play an important role in the invasion and metastasis of various malignant tumors [3]. For example, the overexpression or knockout of miR-503 partially regulates apoptotic activity and alters the resistance of ovarian cancer cells to cisplatin [4]. MiR-1266 promotes the progression of pancreatic cancer and chemotherapy resistance via STAT3/NF- $\kappa$ B axis [5]. Moreover, miR-139-5p has been reported to be associated with lung cancer [6], breast cancer [7], prostate cancer [8] and other diseases.

Collagen is the main component of interstitial extracellular matrix. It is well known that extracellular matrix plays an active role in various biological processes, such as cell morphology, proliferation, migration, differentiation, apoptosis and carcinogenesis [9]. Collagen I, for instance, promotes cell metastasis in pancreatic cancer by activating terminal kinase 1 of c-Jun NH2 and up-regulating the expression of N-cadherin [10]. In addition, specific collagens like collagen type I and type III can promote metastasis of pancreatic cancer cells by down-regulating the expression of E-cadherin gene in a Src kinase dependent fashion [11]. COL11A1, a member of small fibre collagen, is encoded by COL11A1 gene located on chromosome 1p21, and can be produced by cartilage and various non-cartilage tissues like bone, vitreous, skin and heart [12]. In recent years, studies have found that COL11A1 is associated with the tumorigenesis and development, and plays an important role in biological processes like cell proliferation and invasion [13]. However, whether miR-139-5p can affect the biological characteristics of breast cancer cells by targeted regulating COL11A1 has not been reported.

In this study, we found that COL11A1 was highly expressed in breast cancer through the bioinformatics analysis. The targeted relationship between COL11A1 and miR-139-5p was predicted by prediction websites, which provided a target for clinical research on the biological characteristics of breast cancer. Our research may provide new insight into the pathogenesis of breast cancer and theoretical basis for a new direction of targeted therapy.

## 2. Materials and methods

### 2.1. Bioinformatics analysis

Three gene expression chips of breast cancer were obtained from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>), including GSE65194 (178 arrays) [14], GSE42568 (104 breast cancer and 17 normal breast biopsies) [15] and GSE29431 (54 primary breast carcinomas samples).

GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2R/>) was employed to screen differentially expressed genes (DEGs) with the criterion of  $|\text{LogFC}| > 1$  and adj P-value  $< 0.01$ . miRDB (<http://www.mirdb.org/microDB/policy.html>), miRanda (<http://www.microna.org/microna/home.do>) and TargetScan databases ([http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)) were employed to predict the regulatory miRNAs of COL11A1.

## 2.2. Cell culture and transfection

Human normal breast epithelial cell MCF 10A (CBP60419) and human breast cancer cell lines T47D (CBP60397), MCF-7 (CBP60380), ZR-75-1 (CBP60402) and HCC1954 (CBP60374) were purchased from Nanjing CoBioer Co., Ltd. Cancer cells in logarithmic phase were grouped as follows: si-NC group, si-COL11A1 group, mimic NC group, miR-139-5p mimic group, mimic NC+oe-NC group, miR-139-5p mimic+oe-NC group, and miR-139-5p mimic+oe-COL11A1 group. Plasmids such as si-NC, si-COL11A1, mimic NC, miR-139-5p mimic, oe-NC and oe-COL11A1 were all purchased from Shanghai GenePharma Co., Ltd.

## 2.3. qRT-PCR

Total RNA was extracted by Trizol (No. 16096020, ThermoFisher Scientific, New York, USA), 5  $\mu\text{g}$  among which was reversely transcribed to cDNA with the cDNA kit (K1622; Fermentas Inc., Ontario, CA, USA). qRT-PCR was carried out using TaqMan MicroRNA Assay and TaqMan® Universal PCR Master Mix: 95 °C for 2 min, followed by 45 cycles at 95 °C for 15 s, 60 °C for 45 s and 72 °C for 45 s. U6 was used as internal reference.

**Table 1.** Primer sequence.

Gene	Primer sequence
GAPDH[1]	F:5'-AGGTCGGAGTCAACGGATTTG-3'
	R:5'-GTGATGGCATGGACTGTGGT-3'
Hsa-miR-139-5p[2]	F:5'-TCTACAGTGCACGTGTC-3'
	R:5'-GAATACCTCGGACCCTGC -3'
U6	F:5'-CTCGCTTCGGCAGCACA-3'
	R:5'-AACGCTT CACGAATTTGCGT-3'
COL11A1[3]	F:5'-TGGTGAT CAGAATCAGAAGTTTCG -3'
	R:5'-AGGAGAGTTGAGAATTGGGAATC-3'

qRT-PCR was carried out based on the instruction of TaqMan Gene Expression Assays protocol (Applied Biosystems, Foster City, CA, USA). GAPDH was taken as internal reference. The programming of PCR was as below: 95 °C for 10 min, followed by 35 cycles at 95 °C for 15 s, 60 °C

for 30 s and 72 °C for 45 s. 3 compounds were set for both qRT-PCR reactions. Primers designed were showed in Table 1. Relatively quantitative method ( $2^{-\Delta\Delta CT}$ ) was used to calculate the relative transcription level of target gene:  $\Delta\Delta Ct = \Delta Ct$  (experimental group) -  $\Delta Ct$  (control group),  $\Delta Ct = Ct$  (target gene) -  $Ct$  (internal reference), relative transcription level of target gene mRNA =  $2^{-\Delta\Delta Ct}$  [16].

#### 2.4. Western blot

Total protein was extracted from cells, and the protein concentration was determined by BCA kit (Thermo, USA). 30  $\mu$ g of the total protein was separated by polyacrylamide gel electrophoresis with a constant voltage of 80 V for 35 min, followed by 120 V for 45 min, and then transferred onto the PVDF membrane (Amersham, USA). The membrane was blocked with 5% skim milk at room temperature for 1h. COL11A1 rabbit polyclonal antibodies (1:1000, abcam, Cambridge, UK), Caspase-3 mouse monoclonal antibodies (1:500, abcam, Cambridge, UK), Bax mouse monoclonal antibodies (1:1000, abcam, Cambridge, UK), Bcl-2 mouse monoclonal antibodies (1:500, abcam, Cambridge, UK) and GAPDH rabbit monoclonal antibodies (1:10000, abcam, Cambridge, UK) were added onto the membrane for incubation overnight at 4 °C. PBST buffer (containing 0.1% Tween-20 of PBS buffer) was used to wash the membrane three times. Then, the membrane was incubated with the secondary antibody horseradish peroxidase-labeled goat anti-rabbit IgG H&L (HRP) (1:3000, abcam, Cambridge, UK) or goat anti-mouse IgG H&L (HRP) (1:3000, abcam, Cambridge, UK) at room temperature for 1 hour. Optical Luminiferous Instrument (GE, USA) was used to visualize the protein bands, and Image Pro Plus 6.0 (Media Cybernetics, USA) software was used to analyze the relative expression of protein by gray-scale scanning.

#### 2.5. CCK-8

The transfected breast cancer cells were digested and resuspended to a concentration of  $1 \times 10^5$  cells/mL and cultured in 96-well plates (100  $\mu$ L/well) overnight. 10  $\mu$ L of CCK-8 (Beyotime, Shanghai, China) was added into the wells at 24, 48, 72 and 96 hours, respectively. The absorbance at 450 nm was measured by enzyme label and the growth curve was drawn.

#### 2.6. Flow cytometry

After 48 hours of transfection, cells were digested with 0.25% trypsin (EDTA-free) (YB15050057, Yubo, Shanghai, China) and collected in flow tubes. Then the cells were centrifuged and the supernatant was discarded. Cold PBS was used to wash cells 3 times. The cells were subsequently centrifuged for the second time. According to the instruction of Annexin-V-FITC cell apoptosis detection kit (K201-100, Biovision, USA), Annexin-V-FITC, PI and HEPES buffer solution were prepared into Annexin-V-FITC/PI dye solution at the ratio of 1:2:50.  $1 \times 10^6$  of cells were resuspended in 100 $\mu$ L of the dye solution. After incubation at room temperature for 15 min, 1mL of HEPES buffer (PB180325, Procell, Wuhan, China) was added to the cells. FITC and PI fluorescence were detected by 525 and 620 nm of band-pass filters that were excited by 488 nm excitation light. The experiment was repeated three times [17].

### 2.7. RNA binding protein immunoprecipitation (RIP)

RIP kit (Millipore, USA) was used to detect the binding relationship between miR-139-5p and COL11A1. Cells were firstly washed with pre-cooled PBS and the supernatant was discarded. Pyrolysis solution (P0013B, Bi Yun Tian) of equal volume was used to lyse cells on ice for 5 min, and proteins obtained were centrifuged at 14,000 rpm and 4 °C for 10 min. One part of the cell extract was taken out as input and the other part was taken out to incubate and co-precipitate with antibodies. The specific steps were as follows: 50 µL of the co-precipitation was washed with magnetic beads and then resuspended in 100 µL of RIP Wash Buffer. According to the experimental grouping, 5 µg of antibodies was added into the tubes to form the magnetic beads-antibody complex, which was then resuspended in 900 µL of RIP Wash Buffer after washed, and incubated with 100 µL cell extract at 4 °C overnight. The complex was collected. The antibodies used in RIP were Ago2 (ab32381, 1:50, Abcam, UK) and IgG (1:100, ab109489, Abcam, UK). The samples Input were digested by protease K, then RNA was extracted for subsequent PCR detection.

### 2.8. Dual-luciferase reporter gene assay

The full length of COL11A1 3'UTR region was amplified. The PCR products were cloned into the luciferase pmirGLO (Promega, WI, USA) vector with endonuclease sites, Spe I and Hind III, and the COL11A1-WT was constructed. The target gene database was used to predict the binding sites of miR-139-5p on the target gene, and the COL11A1-MUT vector was constructed by site-directed mutagenesis through PCR. Then luciferase reporter vector was co-transfected with each plasmid into HEK-293T cells, respectively, with Renilla luciferase expression vector pRL-TK (TaKaRa, Dalian, China) as internal reference. The dual-luciferase activity was examined using Promega's Digital-Luciferase Reporter Assay System (Promega, Madison, WI, USA). The experiment was repeated three times.

### 2.9. Statistical analysis

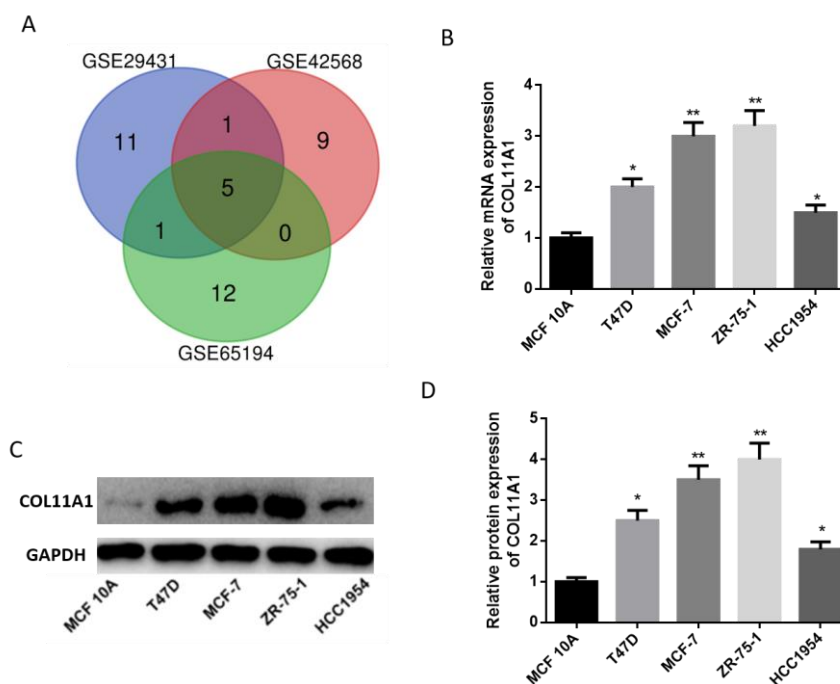
All data were processed by SPSS 21.0 statistical software (SPSS, Inc., Chicago, IL, USA). Measurement data were expressed in the form of mean value  $\pm$  standard deviation (SD). Comparison between two groups was performed by t test, and comparison among multiple groups was performed by one-way ANOVA. The counting data was analyzed by chi-square test.  $P < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. High expression of COL11A1 in breast cancer cells

The first 10 significantly up-regulated DEGs and 10 down-regulated DEGs of each chip (GSE65194, GSE42568, GSE29431) were selected to make a Venn diagram. Five DEGs were obtained, containing FABP4, RBP4, ADH1B, COL11A1 and LEP, of which COL11A1 was the only significantly highly-expressed gene (Figure 1A). The expression of COL11A1 in human normal

breast epithelial cell, MCF 10A, and human breast cancer cell lines, T47D, MCF-7, ZR-75-1 and HCC1954, were detected by qRT-PCR. As shown in Figure 1B, compared with MCF 10A, the expression of COL11A1 mRNA were significantly up-regulated in breast cancer cell lines. Meanwhile, the protein levels of COL11A1 in all cell lines were tested by Western Blot. As shown in Figure 1C and 1D, COL11A1 protein level in breast cancer cell lines were significantly up-regulated by the comparison with normal cells. These results indicated that COL11A1 was highly expressed in breast cancer cell lines, and ZR-75-1, the cell line with the highest expression of COL11A1, was selected for subsequent experiments.

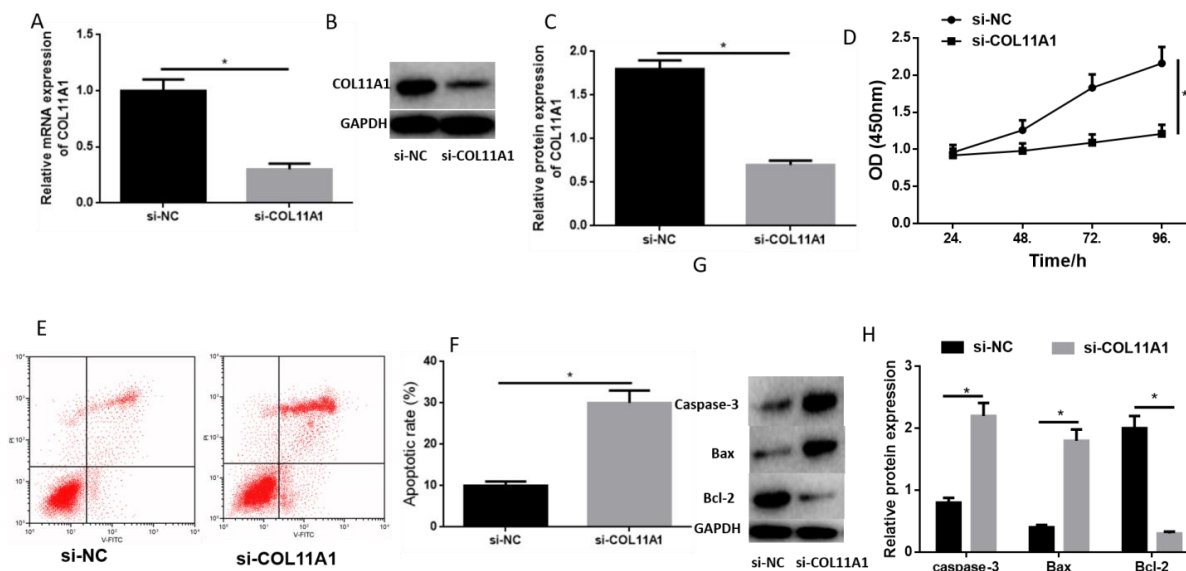


**Figure 1.** High Expression of COL11A1 in Breast Cancer Cell lines. (A) DEGs of breast cancer in three chips and Venn diagram for target genes exploration (online tool: <http://bioinformatics.psb.ugent.be/webtools/Venn/>); (B) The expression of COL11A1 mRNA in human normal breast epithelial cell, MCF 10A, and human breast cancer cell lines, T47D, MCF-7, ZR-75-1 and HCC1954 by qRT-PCR; (C-D) Protein levels of COL11A1 in all cell lines by WB. “\*” means  $P < 0.05$ , the experiment was repeated 3 times. Values in the figure were measurement data expressed in the form of mean value  $\pm$  standard deviation (SD). Comparison between two groups were performed by t test.

### 3.2. Silencing COL11A1 inhibits cell proliferation and promotes apoptosis in breast cancer

To investigate the effect of COL11A1 on the biological characteristics of breast cancer cells, we firstly silenced COL11A1 in breast cancer cell line, ZR-75-1. Results of qRT-PCR and WB (Figure 2A-C) showed that, the mRNA and protein expression of COL11A1 in the si-COL11A1 group was significantly lower than that in the si-NC group. Then CCK-8 method was used to detect the proliferation of ZR-75-1. As shown in Figure 2D, compared with si-NC group, the proliferation ability of ZR-75-1 cells in si-COL11A1 group was significantly decreased. Meanwhile, flow

cytometry (Figure 2E,F) suggested that cells in si-COL11A1 group had significantly higher apoptotic rate. WB assay was performed to detect the expression of apoptosis-related proteins Caspase-3, Bax and Bcl-2. As shown in Figure 2G,H, compared with si-NC group, the protein levels of Caspase-3 and Bax in the si-COL11A1 group were significantly up-regulated, while the level of anti-apoptosis protein Bcl-2 was significantly down-regulated. These results suggested that silencing COL11A1 could inhibit the proliferation and promote the apoptosis of breast cancer cells.

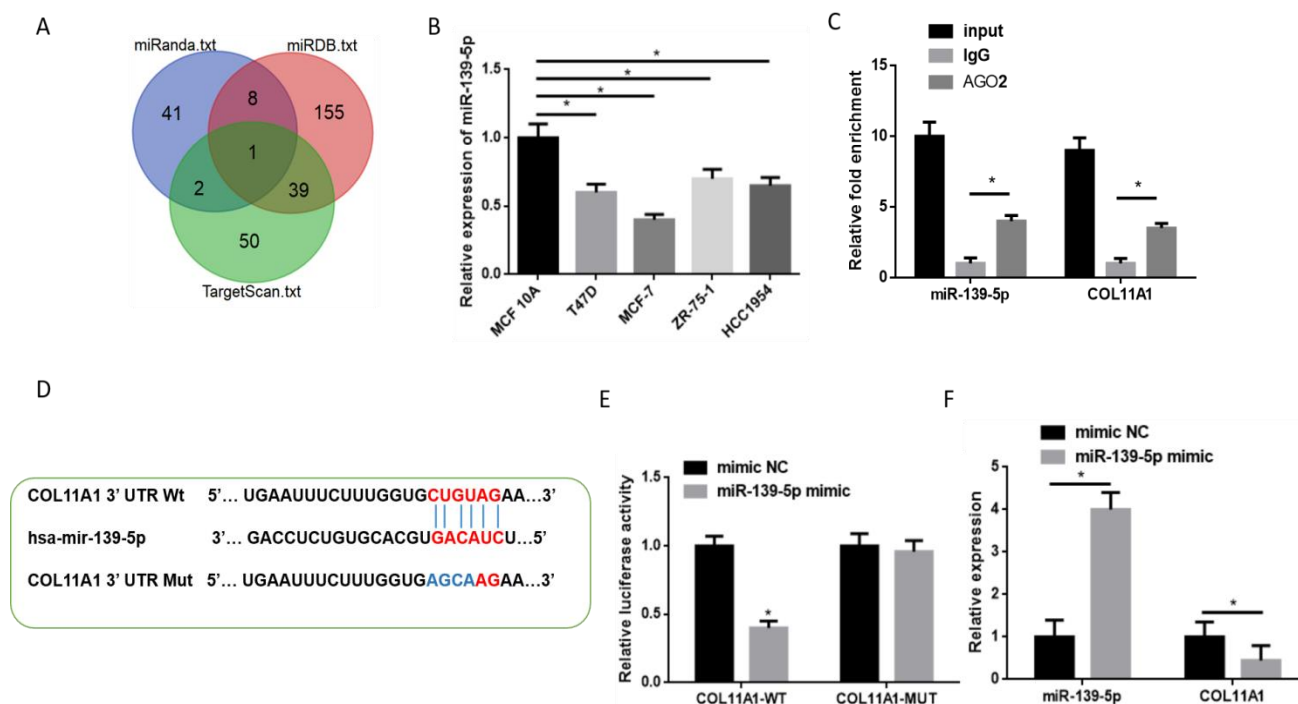


**Figure 2.** Silencing COL11A1 Inhibits the Proliferation and Promotes the Apoptosis of Breast Cancer Cells. (A) The expression of COL11A1 mRNA by qRT-PCR after COL11A1 was silenced in ZR-75-1; (B-C) The protein level of COL11A1 by WB after silencing COL11A1; (D) The proliferation ability of ZR-75-1 cells by CCK-8 after silencing COL11A1; (E-F) The apoptotic rate of ZR-75-1 cells by flow cytometry after silencing COL11A1; (G-H) The protein levels of Caspase-3, Bax and Bcl-2 by WB after silencing COL11A1. “\*” means  $P < 0.05$ , the experiment was repeated 3 times. Values in the figure were measurement data expressed in the form of mean value  $\pm$  standard deviation (SD). Comparison between two groups were performed by t test.

### 3.3. miR-139-5p targets the expression of COL11A1

In order to further study the regulatory mechanism of COL11A1 in breast cancer cells, three databases miRDB, miRanda and TargetScan databases were employed to predict the regulatory miRNAs of COL11A1. Venn diagram was made, and miR-139-5p was finally obtained (Figure 3A). Therefore, we hypothesized that miR-139-5p may regulate the biological characteristics of breast cancer cells by targeted inhibiting the expression of COL11A1. To verify our hypothesis, qRT-PCR was performed to test the expression of miR-139-5p in human normal breast epithelial cell and breast cancer cell lines. As shown in Figure 3B, compared with MCF-10A, the expression of miR-139-5p was much lower in breast cancer cell lines. Furthermore, RIP assay was carried out to verify that miR-139-5p could bind with COL11A1 (Figure 3C), and TargetScan website ([http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)) was applied to predict the binding sites of the two genes (Figure 3D). Dual-luciferase reporter gene assay

was then carried out, and the results shown in Figure 3E suggested that miR-139-5p overexpression could significantly inhibit the luciferase activity of effect on COL11A1-MUT. Meanwhile, qRT-PCR was used to detect the expression of miR-139-5p and COL11A1 after miR-139-5p overexpressed in ZR-75-1. As shown in Figure 3F, compared with mimic NC group, the expression of miR-139-5p in miR-139-5p mimic group was significantly up-regulated, while COL11A1 was significantly down-regulated. These findings suggested that miR-139-5p could targeted inhibit the expression of COL11A1.



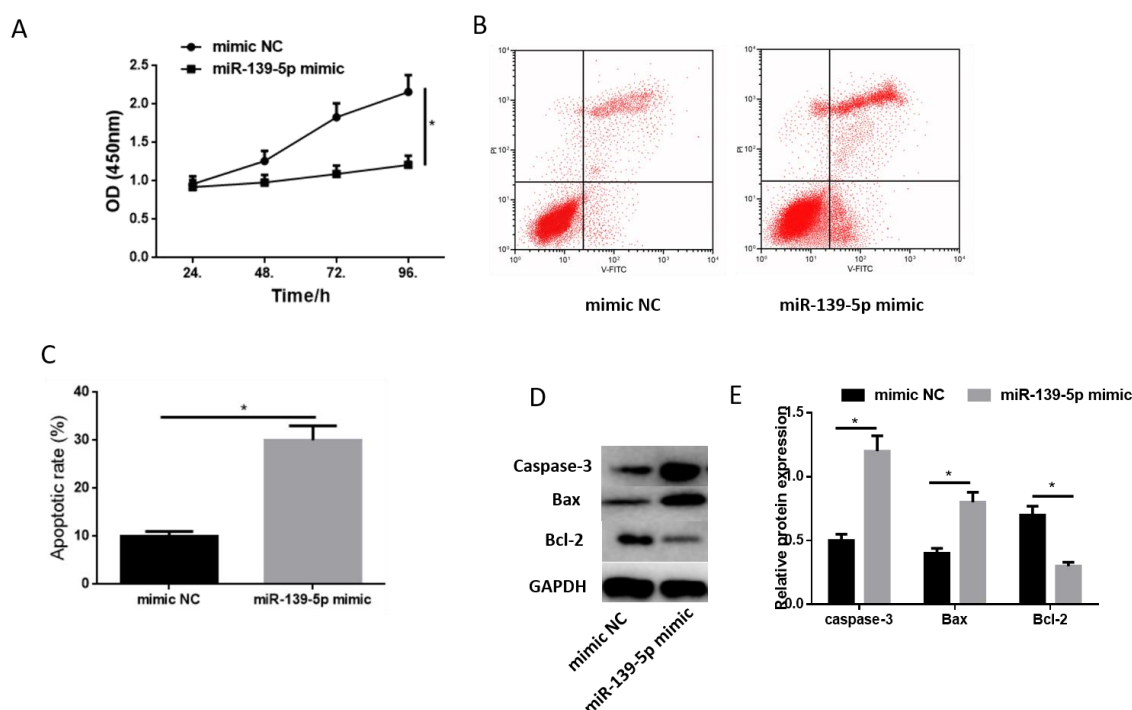
**Figure 3.** miR-139-5p Targeted Inhibits COL11A1. (A) The prediction of regulatory miRNAs of COL11A1 by miRDB, miRanda and TargetScan databases and Venn diagram for target one; (B) The expression of miR-139-5p in human normal breast epithelial cell, MCF 10A, and human breast cancer cell lines, T47D, MCF-7, ZR-75-1 and HCC1954 by qRT-PCR; (C) Verification of the relationship between miR-139-5p and COL11A1 by RIP assay; (D) Prediction of the binding sites between miR-139-5p and COL11A1 with TargetScan website ([http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)); (E) The luciferase activity of cells by dual-luciferase reporter gene assay; (F) The expression of miR-139-5p and COL11A1 by qRT-PCR after overexpressing miR-139-5p in ZR-75-1 cells. “\*” means  $P < 0.05$ , the experiment was repeated 3 times. Values in the figure were measurement data expressed in the form of mean value  $\pm$  standard deviation (SD). Comparison between two groups were performed by t test.

### 3.4. miR-139-5p overexpression inhibits the proliferation and promotes the apoptosis of breast cancer cells

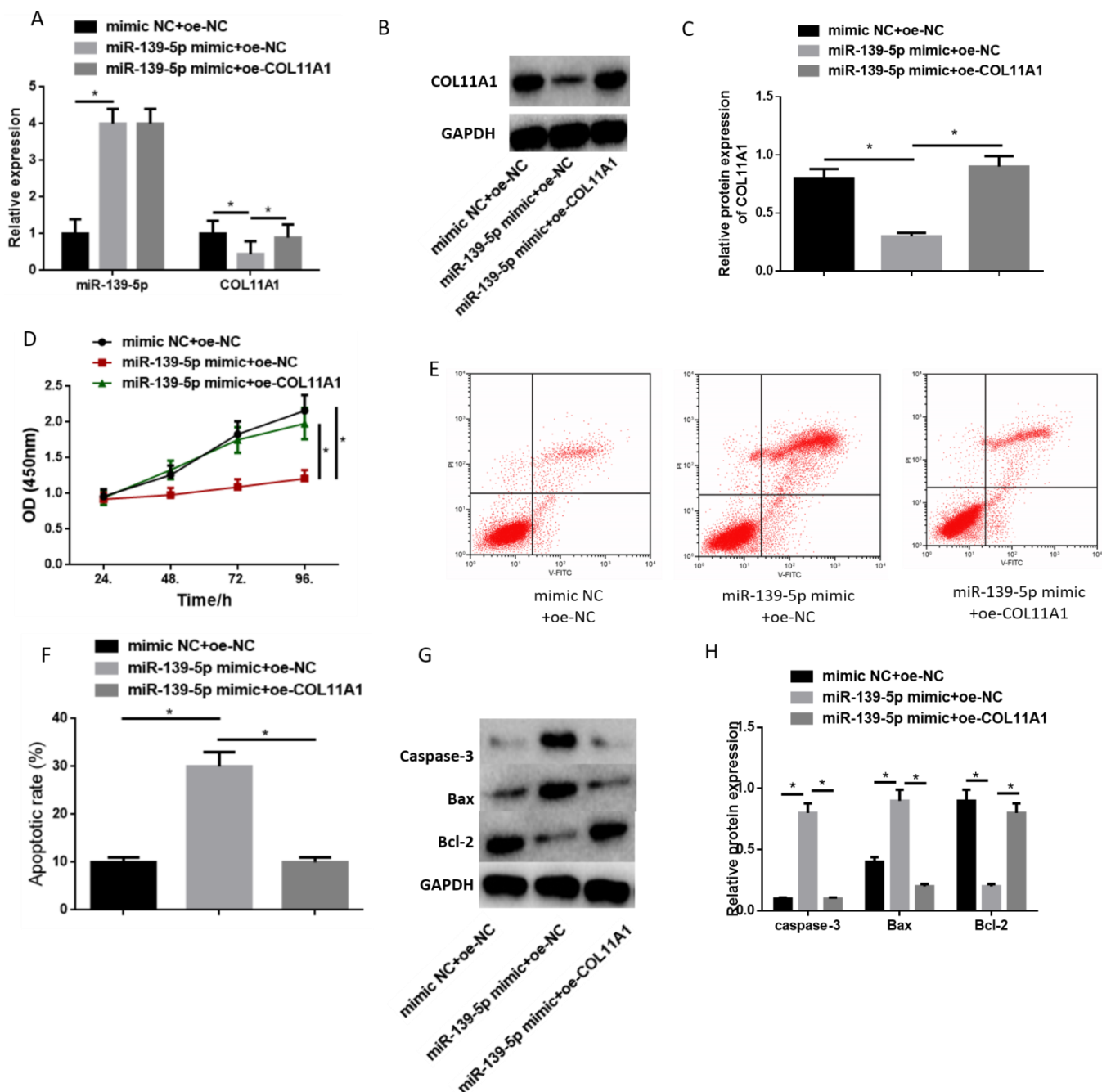


In order to investigate the effect of miR-139-5p on the biological characteristics of breast cancer cells, miR-139-5p was firstly overexpressed in ZR-75-1 cells. Results of CCK-8 (Figure 4A) indicated that cells in the miR-139-5p mimic group had significant lower proliferation in comparison with that in the mimic NC group. At the same time, flow cytometry was used to detect the apoptotic rate of ZR-75-1 cells. As shown in Figure 4B,C, compared with mimic NC group, the apoptotic rate was significantly increased in the miR-139-5p mimic group. WB (Figure 4D,E) was applied to examine the protein levels of Caspase-3, Bax and Bcl-2, finding that the expression of Caspase-3 and Bax in miR-139-5p mimic group were significantly up-regulated, while that of Bcl-2 was significantly down-regulated. These results suggested that overexpressing miR-139-5p could inhibit the proliferation and promote the apoptosis of breast cancer cells.

### 3.5. miR-139-5p Overexpression inhibits the proliferation and promotes the apoptosis of breast cancer cells through inhibiting COL11A1



**Figure 4.** Overexpression of miR-139-5p Inhibits the Proliferation and Promotes the Apoptosis of Breast Cancer Cells. (A) The proliferation ability of ZR-75-1 cells by CCK-8 after overexpressing miR-139-5p; (B-C) The apoptotic rate of ZR-75-1 cells by flow cytometry after overexpressing miR-139-5p; (D-E) The protein levels of Caspase-3, Bax and Bcl-2 in ZR-75-1 cells by WB after overexpressing miR-139-5p. “\*” means  $P < 0.05$ , the experiment was repeated 3 times. Values in the figure were measurement data expressed in the form of mean value  $\pm$  standard deviation (SD). Comparison between two groups were performed by t test.



**Figure 5.** Overexpression of miR-139-5p inhibits the proliferation and promotes the apoptosis of breast cancer cells by inhibiting COL11A1. (A) The expression of COL11A1 mRNA in ZR-75-1 cells by qRT-PCR; (B-C) The protein level of COL11A1 in ZR-75-1 cells by WB; D: The proliferation ability of ZR-75-1 cells by CCK-8; (E-F) The apoptosis rate of ZR-75-1 cells by flow cytometry; (G-H) The protein levels of Caspase-3, Bax and Bcl-2 in ZR-75-1 cells by WB. “\*” means  $P < 0.05$ , the experiment was repeated 3 times. Values in the figure were measurement data expressed in the form of mean value  $\pm$  standard deviation (SD). Comparison between two groups were performed by t test.

To investigate the role of miR-139-5p/COL11A1 axis in breast cancer, miR-139-5p and COL11A1 were simultaneously overexpressed in ZR-75-1 cells. qRT-PCR was performed to detect the expression of miR-139-5p and COL11A1, and WB was carried out for the examination of COL11A1 protein level. As shown in Figure 5A-C, compared with mimic NC+oe-NC group, the expression of miR-139-5p in the miR-139-5p mimic+oe-NC group was significantly up-regulated, while the mRNA expression and protein level of COL11A1 were both down-regulated. In addition, in the miR-139-5p mimic+oe-COL11A1 group, the down-regulation of COL11A1 caused by miR-139-5p was reversed. Subsequently, CCK-8 was performed to test the cell proliferation, finding that cells in the miR-139-5p mimic+oe-NC group had poorer proliferation ability than those in the mimic NC+oe-NC group, and such effect could be reversed by simultaneously overexpressing miR-139-5p and COL11A1 (Figure 5D). Furthermore, WB and flow cytometry were employed for cell apoptosis detection as shown in Figure 5E-H. Results suggested that miR-139-5p overexpression could promote cell apoptosis, and overexpressing COL11A1 at the same time could make the apoptosis rate back to the level near that in the control group. All these findings indicated that overexpression of miR-139-5P could inhibit cell proliferation and promote cell apoptosis in breast cancer, and such effect could be reversed through overexpressing COL11A1.

#### 4. Discussion

Breast cancer is an aggressive disease that seriously threatens human health. Despite the continuous improvement of the treatment, the morbidity of breast cancer is still high. Studies have shown that invasion and metastasis are the key factors leading to death in breast cancer patients [18]. Recently, research on the mechanism of breast cancer has attracted much attention, especially on the way of controlling the invasion and metastasis, cancer prevention, therapeutic methods and the improvement of the survival rate and life quality of patients [19]. Therefore, it is very important to study the molecular mechanism of breast cancer.

In this study, we found that COL11A1 was highly expressed in breast cancer cell lines, and silencing COL11A1 could inhibit cell proliferation and promote apoptosis. It is reported that COL11A1 is up-regulated in various cancers, such as colorectal cancer [9], pancreatic cancer [20], and non-small cell lung cancer [21]. Moreover, high expression of COL11A1 has been found to be closely related with various malignancies like ovarian cancer [22] and lung cancer [23]. For example, COL11A1 is over expressed in gastric cancer and regulates the proliferation, migration and invasion of HGC-27 gastric cancer cells *in vitro* [24]. While in ovarian cancer, COL11A1 can activate TWIST1 to regulate the expression of NF- $\kappa$ B-mediated IKK  $\beta$ , thereby promoting cell chemotherapy resistance and inhibiting apoptosis [25].

miRNAs are small non-coding RNAs with a length of about 19-24 nucleotides, which work in cells by scavenging mRNAs or inhibiting the translation process [26]. For example, miRNA-188-5p inhibits cell proliferation and promotes apoptosis of breast cancer cells via the MAPK signaling pathway by targeting Rap2c [27]; miR-483-3p suppresses the proliferation and progression of human triple negative breast cancer cells by targeting the HDAC8 oncogene [28]; miRNA-326 is decreased in breast cancer tissues, and plays an anti-tumor role in breast cancer by targeting ErbB/PI3K Signaling Pathway [29]. In this study, we predicted that miR-139-5p could target COL11A1, and be involved in regulating the biological characteristics of breast cancer cells. Several studies have shown that miR-139-5p is closely related to many diseases, such as colon cancer [30], uterine cancer

[31], bladder cancer [32]. In addition, miR-139-5p is significantly down-regulated in breast cancer cells, and inhibits the cell proliferation, metastasis as well as induces apoptosis, leading to cell cycle arrested in S phase. In breast cancer, miR-139-5p not only attenuates the development of breast cancer cells but also mediates drug-resistance by regulating the expression of the target gene Notch1 in the downstream [33]. Moreover, another study has shown that miR-139-5p plays an important role in the migration and invasion of breast cancer cells, and may serve as a prognostic marker for invasive breast cancer [34].

In this study, miR-139-5p was found to be poorly expressed in breast cancer cells, and targeted inhibit the expression of COL11A1. Silencing COL11A1 or overexpressing miR-139-5p could inhibit the proliferation and promote the apoptosis of breast cancer cells, while overexpressing miR-139-5p and COL11A1 simultaneously could reverse such effect. The findings could provide a new theoretical basis for further research on the mechanism of the occurrence and development of breast cancer cells.

### Acknowledgements

None

### Conflict of interest

The authors declare that they have no conflict of interest.

### References

1. F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA. Cancer J. Clin.*, **68** (2018), 394–424.
2. H. Fang, J. Xie, M. Zhang, Z. Zhao, Y. Wan, Y. Yao, miRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN, *Am. J. Transl. Res.*, **9** (2017), 953–961.
3. Y. Xie, P. Zong, W. Wang, D. Liu, B. Li, Y. Wang, Hypermethylation of potential tumor suppressor miR-34b/c is correlated with late clinical stage in patients with soft tissue sarcomas, *Exp. Mol. Pathol.*, **98** (2015), 446–454.
4. D. Wu, P. Lu, X. Mi, J. Miao, Downregulation of miR-503 contributes to the development of drug resistance in ovarian cancer by targeting PI3K p85, *Arch. Gynecol. Obstet.*, **297** (2018), 699–707.
5. X. Zhang, D. Ren, X. Wu, X. Lin, L. Ye, C. Lin, miR-1266 Contributes to Pancreatic Cancer Progression and Chemoresistance by the STAT3 and NF-kappaB Signaling Pathways, *Mol. Ther. Nucleic Acids.*, **11** (2018), 142–158.
6. Y. You, X. Wang, M. Xu, J. Zhao, Expression and clinical significance of miR-139-5p in non-small cell lung cancer, *J. Int. Med. Res.*, **47** (2019), 867–874.
7. J. Tu, Z. Zhao, M. Xu, X. Lu, L. Chang, J. Ji, NEAT1 upregulates TGF-beta1 to induce hepatocellular carcinoma progression by sponging hsa-mir-139-5p, *J. Cell. Physiol.*, **233** (2018), 8578–8587.

8. B. Yang, W. Zhang, D. Sun, X. Wei, Y. Ding, Y. Ma, Downregulation of miR-139-5p promotes prostate cancer progression through regulation of SOX5, *Biomed. Pharmacother.*, **109** (2019), 2128–2135.
9. H. Fischer, R. Stenling, C. Rubio, A. Lindblom, Colorectal carcinogenesis is associated with stromal expression of COL11A1 and COL5A2, *Carcinogenesis*, **22** (2001), 875–878.
10. Y. Shintani, M. A. Hollingsworth, M. J. Wheelock, K. R. Johnson, Collagen I promotes metastasis in pancreatic cancer by activating c-Jun NH(2)-terminal kinase 1 and up-regulating N-cadherin expression, *Cancer Res.*, **66** (2006), 11745–11753.
11. A. Menke, C. Philippi, R. Vogelmann, B. Seidel, M. P. Lutz, G. Adler, Down-regulation of E-cadherin gene expression by collagen type I and type III in pancreatic cancer cell lines, *Cancer Res.*, **61** (2001), 3508–3517.
12. H. Yoshioka, P. Greenwel, K. Inoguchi, S. Truter, Y. Inagaki, Y. Ninomiya, Structural and functional analysis of the promoter of the human alpha 1(XI) collagen gene, *J. Biol. Chem.*, **270** (1995), 418–424.
13. D. Sun, H. Jin, J. Zhang, X. Tan, Integrated whole genome microarray analysis and immunohistochemical assay identifies COL11A1, GJB2 and CTRL as predictive biomarkers for pancreatic cancer, *Cancer Cell Int.*, **18** (2018), 174.
14. V. Maire, F. Nemati, M. Richardson, A. V. Salomon, B. Tesson, G. Rigaiil, Polo-like kinase 1: A potential therapeutic option in combination with conventional chemotherapy for the management of patients with triple-negative breast cancer, *Cancer Res.*, **73** (2013), 813–823.
15. C. Clarke, S. F. Madden, P. Doolan, S. T. Aherne, H. Joyce, L. O’Driscoll, Correlating transcriptional networks to breast cancer survival: a large-scale coexpression analysis, *Carcinogenesis*, **34** (2013), 2300–2308.
16. M. Xie, T. Ma, J. Xue, H. Ma, M. Sun, Z. Zhang, The long intergenic non-protein coding RNA 707 promotes proliferation and metastasis of gastric cancer by interacting with mRNA stabilizing protein HuR, *Cancer Lett.*, **443** (2019), 67–79.
17. D. Diaz, A. Prieto, E. Reyes, H. BarcenillaJorge, M. Melchor, A. Mon, Flow cytometry enumeration of apoptotic cancer cells by apoptotic rate, *Methods Mol. Biol.*, **1219** (2015), 11–20.
18. Z. L. Sun, S. C. Zhang, H. L. Li, Y. C. Gao, EBP50 inhibits the invasion and metastasis of breast cancer cells through Wnt3a/ $\beta$ -catenin signaling pathway, *Chin. J. Biochem. Mol. Bio.* (2016), 1027–1032.
19. J. Li, J. Zhang, Y. Wang, X. Liang, Z. Wusiman, Y. Yin, Synergistic inhibition of migration and invasion of breast cancer cells by dual docetaxel/quercetin-loaded nanoparticles via Akt/MMP-9 pathway, *Int. J. Pharm.*, **523** (2017), 300–309.
20. L. Badea, V. Herlea, S. O. Dima, T. Dumitrascu, I. Popescu, Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia, *Hepatogastroenterology*, **55** (2008), 2016–2027.
21. I. W. Chong, M. Y. Chang, H. C. Chang, Y. P. Yu, C. C. Sheu, J. R. Tsai, Great potential of a panel of multiple hMTH1, SPD, ITGA11 and COL11A1 markers for diagnosis of patients with non-small cell lung cancer, *Oncol. Rep.*, **16** (2006), 981–988.
22. Y. H. Wu, Y. F. Huang, C. C. Chen, C. Y. Chou, Akt inhibitor SC66 promotes cell sensitivity to cisplatin in chemoresistant ovarian cancer cells through inhibition of COL11A1 expression, *Cell. Death. Dis.*, **10** (2019), 322.

23. T. Rizou, F. Perlikos, M. Lagiou, M. Karaglani, S. Nikolopoulos, I. Toumpoulis, Development of novel real-time RT-qPCR methodologies for quantification of the COL11A1 mRNA general and C transcripts and evaluation in non-small cell lung cancer specimens, *J. BU ON.*, **23** (2018), 1699–1710.
24. A. Li, J. Li, J. Lin, W. Zhuo, J. Si, COL11A1 is overexpressed in gastric cancer tissues and regulates proliferation, migration and invasion of HGC-27 gastric cancer cells *in vitro*, *Oncol. Rep.*, **37** (2017), 333–340.
25. Y. H. Wu, Y. F. Huang, T. H. Chang, C. Y. Chou, Activation of TWIST1 by COL11A1 promotes chemoresistance and inhibits apoptosis in ovarian cancer cells by modulating NF-kappaB-mediated IKKbeta expression, *Int. J. Cancer*, **141** (2017), 2305–2317.
26. K. Tu, X. Zheng, C. Dou, C. Li, W. Yang, Y. Yao, MicroRNA-130b promotes cell aggressiveness by inhibiting peroxisome proliferator-activated receptor gamma in human hepatocellular carcinoma, *Int. J. Mol. Sci.*, **15** (2014), 20486–20499.
27. X. Zhu, J. Qiu, T. Zhang, Y. Yang, S. Guo, T. Li, MicroRNA-188-5p promotes apoptosis and inhibits cell proliferation of breast cancer cells via the MAPK signaling pathway by targeting Rap2c, *J. Cell Physiol.* **2019** (2019).
28. M. N. Menbari, K. Rahimi, A. Ahmadi, S. M. Yeganeh, A. Elyasi, N. Darvishi, MiR-483-3p suppresses the proliferation and progression of human triple negative breast cancer cells by targeting the HDAC8 oncogene, *J. Cell Physiol.* **2019** (2019).
29. Z. Ghaemi, B. M. Soltani, S. J. Mowla, MicroRNA-326 Functions as a Tumor Suppressor in Breast Cancer by Targeting ErbB/PI3K Signaling Pathway, *Front. Oncol.*, **9** (2019), 653.
30. Q. Li, X. Liang, Y. Wang, X. Meng, Y. Xu, S. Cai, MiR-139-5p Inhibits the Epithelial-Mesenchymal Transition and Enhances the Chemotherapeutic Sensitivity of Colorectal Cancer Cells by Downregulating BCL2, *Sci. Rep.*, **6** (2016), 27157.
31. J. Liu, C. Li, Y. Jiang, Y. Wan, S. Zhou, Wen. Cheng, Tumor-suppressor role of miR-139-5p in endometrial cancer, *Cancer Cell Int.*, **18** (2018), 51.
32. M. Yonemori, N. Seki, H. Yoshino, R. Matsushita, K. Miyamoto, M. Nakagawa, Dual tumor-suppressors miR-139-5p and miR-139-3p targeting matrix metalloprotease 11 in bladder cancer, *Cancer Sci.*, **107** (2016), 1233–1242.
33. H. D. Zhang, D. W. Sun, L. Mao, J. Zhang, L. Jiang, MiR-139-5p inhibits the biological function of breast cancer cells by targeting Notch1 and mediates chemosensitivity to docetaxel, *Biochem. Biophys. Res. Commun.*, **465** (2015), 702–713.
34. K. Krishnan, A. L. Steptoe, H. C. Martin, D. R. Pattabiraman, K. Nones, N. Waddell, MiR-139-5p is a regulator of metastatic pathways in breast cancer, *RNA*, **19** (2013), 1767–1780.



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