MicroRNA-520c-3p negatively regulates EMT by targeting IL-8 to suppress the invasion and migration of breast cancer

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Abstract. Interleukin-8 (IL-8), which is secreted by cancer cells undergoing epithelial-mesenchymal transition (EMT), can promote EMT in adjacent epithelial-like cells. MicroRNAs (miRNAs/miRs) can affect the expression of target genes via binding to their 3'-untranslated regions (3'-UTRs), which may subsequently affect the biological behaviors of cancer cells. In our previous study, miR-520c-3p was predicted to directly target the 3'-UTR of IL-8. Therefore, the present study was carried out to investigate whether miR-520c-3p can interact with the IL-8 gene and regulate the EMT of breast cancer cells. Web-based prediction algorithms were used to identify miRNAs that potentially target the IL-8 transcript. Luciferase reporter assays were used to confirm the targeting of IL-8 by miR-520c-3p. Reverse transcription-quantitative PCR and western blot analyses were used to examine the levels of IL-8 and EMT-related genes in breast cancer cells. The functional impact of miR-520c-3p on EMT phenotype was evaluated using Transwell and wound-healing assays, and rescue experiments were conducted by overexpressing IL-8 to determine its effect on cell properties. miR-520c-3p was predicted by all three databases, which strongly suggested its interaction with the 3'-UTR of IL-8. The relative *Renilla* luciferase activity of luciferase reporter construct containing the wild-type 3'-UTR of IL-8 was markedly decreased by miR-520c-3p transfection when compared with scrambled miRNA control transfection (P<0.001). In addition, compared with the scrambled miRNA control transfection, the overexpression of miR-520c-3p significantly reduced the expression of IL-8, and resulted in increased E-cadherin and decreased vimentin and fibronectin levels in MCF-7 and T47D cells (all P<0.001). Introduction of miR-520c-3p inhibited the invasion and migration of MCF-7 and T47D cells (all P<0.001). By contrast, the rescue of IL-8 expression led to the recovery of EMT-related protein expression patterns and cell motility and invasion capabilities. In conclusion, aberrant miR-520c-3p expression may lead to reduced IL-8 expression and promote the mesenchymal phenotype in breast cancer cells, thereby increasing invasive growth.

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Introduction

Breast cancer is among the leading causes of cancer-related death in women, with an estimated 1.7 million cases and 521,900 mortalities registered in 2012 worldwide, according to the global cancer statistics published in 2015 (1). Despite improvements in therapeutic strategies for the treatment of breast cancer, such as surgery, radiotherapy and chemotherapy, the survival rate of breast cancer is only ~20% for metastatic disease, compared with 90% for localized disease (2). Thus, breast cancer continues to be a major medical issue among women worldwide, and the development of novel therapies for the treatment of breast cancer is urgently required.

MicroRNAs (miRNAs) are a class of small non-coding RNAs of ~22 nucleotides in length, which affects the expression of target genes via binding to their 3'-untranslated regions (3'-UTRs). The interactions between miRNAs and their target genes can affect various biological behaviors, such as cell proliferation, differentiation, apoptosis, invasion and metastasis. In recent years, certain miRNAs have been confirmed as oncogenes or tumor suppressors in the tumorigenesis and metastasis of breast cancer. For example, Zhang *et al* (3) demonstrated that miR-409-3p suppressed the growth and invasive ability of breast cancer cells by targeting Akt1, and Chen *et al* (4) found that miR-340 inhibited cell migration and invasion through the targeting of MYO10 in breast cancer. In addition, Zhang *et al* (5) showed that miR-33a suppressed breast cancer cell proliferation and metastasis by targeting ADAM9 and ROS1, and Song *et al* (6) suggested that miR-200c inhibited breast cancer proliferation by targeting KRAS. Furthermore, a recent study indicated that miR-520d serves a key role in the progression and metastasis of colorectal cancer by targeting

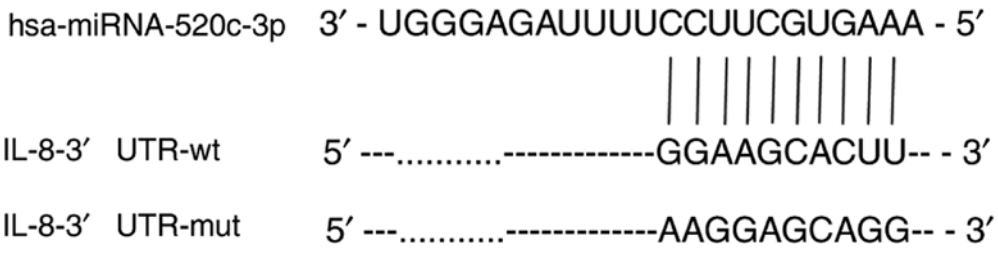

Figure 1. Bioinformatics-based target prediction analysis. Sequence alignment of human miR-520c-3p, predicted binding site in the 3'-UTR of IL-8, and the point-mutated sequence.

CTHRC1 (7). However, the role of miR-520c-3p in breast cancer cells remains unclear.

Interleukin-8 (IL-8) is a chemokine that serves key roles in numerous biological processes, including cell proliferation, migration, angiogenesis and metastasis (8-12). Studies have identified high levels of serum IL-8 in patients with various types of cancer (13), including pancreatic (14), prostate (15) and breast cancer (16) Furthermore, this was correlated with the survival rate of patients with breast cancer, and was associated with increased rates of metastasis (16). IL-8 is also secreted by cancer cells undergoing epithelial-mesenchymal transition (EMT) and promotes adjacent epithelial-like cells to enter EMT, thus promoting the development and metastasis of carcinoma (17). These findings indicate that interruption of the IL-8 signaling pathway may be a potential treatment target in mesenchymal cells or aggressive malignant cancers. In our previous study, it was predicted that miR-520c-3p directly targeted the 3'-UTR of IL-8, and to the best of our knowledge, no published studies have investigated the relationship between miR-520c-3p and IL-8 in breast cancer. Therefore, the present study was conducted to further investigate whether miR-520c-3p interacts with IL-8 and regulate EMT in breast cancer cells.

Materials and methods

Cell lines and culture. The human breast cancer cell lines MCF-7 and T47D, and 293T cells were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). 293T and MCF-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM), and T47D cells were cultured in RPMI-1640 medium (both from Gibco, Carlsbad, CA, USA). All culture medium was supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA) and 1% penicillin-streptomycin, and cells were cultured in 100% humidity in a cell culture incubator at 37˚C with 5% CO_2, and passaged every 2-3 days.

Bioinformatic analysis and target prediction. The mature mRNA sequence of IL-8 was acquired from the NCBI GenBank (reference sequence: NM_000584.3). The miRNAs predicted to target IL-8 were obtained from the online miRNA target prediction programs TargetScan (human) (http://www.targetscan.org), miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) and miRanda (http://www.microRNA.org). Only miRNAs common to at least two online searches were considered for further investigation in the present study. The miRNA sequences used for analysis were retrieved from miRbase (http://www.mirbase.org).

Plasmid constructs. The wild-type (wt) 3'-UTR of human IL-8 containing the predicted target site of miR-520c-3p was cloned from MCF-7 human genomic DNA, and amplified by PCR with the following primers: 5'-ATCGCCGTGTAAAAGGAAGAGGGCTGAGGAATTCAT-3' (forward) and 5'-CACTGGACTAGTGGAGGAGAGCACATAAAAACATC-3' (reverse). The fragments were inserted into the *Hin*dIII and *Bam*HI sites of a pcDNA3.1-luciferase vector to generate pcDNA3.1-luciferase-IL-8-3'-UTR-wt, according to a previously reported method (18). A mutant (mut)-type pcDNA3.1-luciferase-IL-8-3'-UTR-mut, containing 10 mutations in the 3'-UTR target site of the miR-520c-3p seed sequence, was constructed using the following primers: 5'-GTGATGTTGTGAGGACATGTGGCGTTACAATAAGTTTTTTCATCA-3' (forward) and 5'-TGTTATGATGAAAAAACTTATTGTAACGCCACATGTCCTCACAAC-3' (reverse), using a previously reported mutation method (19). hsa-pri-miR520c-3p microRNA overexpression plasmids was constructed into pdsAAV-CB-EGFP using the following primers: 5'-AATTTCAGGTCCCGGAGGAGGATTGCCCGTTGATGA-3' (forward) and 5'-CACCACCACCGGATCCTACATACTAGTGCTTGGGC-3' (reverse). The sequence alignments of human miR-520c-3p, the predicted binding site in the wt IL-8 3'-UTR, and the point-mutated sequence are shown in Fig. 1. The pcDNA3.1-luciferase plasmid was amplified from a pGL3-basic vector (Promega, Madison, WI, USA) into pcDNA3.1(+) (Invitrogen, Carlsbad, CA, USA), and recombined with insertion of a firefly luciferase gene (20). pRL-SV40, containing wild-type *Renilla* luciferase, was purchased from Promega for fluorescence labeling.

Dual-luciferase reporter assays. The constructs containing the wt or mut 3'-UTR sequences of IL-8, the miR-520c-3p overexpression or control vector, and pRL-SV40 were transfected using a calcium phosphate cell transfection method for the dual-luciferase reporter assays. Cells were cultured in 24-well plates (0.1-0.2 million cells/well) in triplicate for 18-24 h prior to transfection, then 500 ng of pcDNA3.1-luciferase-IL-8-3'-UTR-wt or pcDNA3.1-luciferase-IL-8-3'-UTR-mut and 500 ng of pdsAAV-CB-EGFP-miR-520c-3p or pdsAAV-CB-EGFP were co-transfected into cells, along with 10 ng pRL-SV40 as an internal control. Luciferase activity was detected 24 h after transfection with a Dual-Luciferase Reporter Assay System (Promega), according to the manufacturer's instructions. To control for variations in vector transfection,

the activity of the reporter vector was normalized to *Renilla* luciferase activity; thus, the ratio of firefly luciferase/*Renilla* luciferase was assumed to represent the effects of the pdsAAV-CB-EGFP-miR-520c-3p or pdsAAV-CB-EGFP vector on IL-8.

Transfection. miR-520c-3p RNA mimics (AAAGUGCUUCC UUUUAGAGGGU) were synthesized by RiboBio Co., Ltd. (Guangzhou, China) and stored at -80˚C. The miR-520c-3p mimics (50 nM) or scrambled miRNA controls were transfected into MCF-7 and T47D cells using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's protocol.

RNA extraction and reverse transcription (RT)-quantitative PCR (qPCR). At 48 h after transfection with miR-520c-3p mimics or scrambled miRNA controls, total RNA was harvested from MCF-7 and T47D cells with TRIzol reagent (Invitrogen), following the supplier's protocol. The primers used for IL-8 and EMT-related marker genes are shown in Table I and were purchased from Invitrogen. All PCR amplifications were performed using a SYBR-Green RT-qPCR kit (GenePharma, Shanghai, China), and relative gene expression was analyzed based on the comparative quantification cycle (Cq) method (21).

Western blotting. The breast cancer cell lines MCF-7 and T47D were transfected with miR-520c-3p mimics or scrambled miRNA controls using Lipofectamine 2000, following the manufacturer's instructions. Cells were collected 48 h post-transfection in RIPA lysis buffer (Boster, Wuhan, China), and were boiled at 100˚C for 10 min to extract proteins with 5X SDS loading buffer. A BCA protein assay kit (KeyGen Biotech Co., Ltd., Nanjing, China) was used to measure the protein concentration. The extracted proteins were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking in 5% fat-free milk in Tris-buffered saline with Tween-20 (TBST) at room temperature, the membranes were incubated at 4˚C overnight (>12 h) with the following primary antibodies: anti-IL-8 (1:2,000; Abcam, Shanghai, China), anti-E-cadherin (1:1,000), anti-vimentin (1:1,000), anti-fibronectin (1:1,000; all from Bioworld, Shanghai, China), and anti-GAPDH (1:1,000; KangChen Bio-tech, Shanghai, China). Horseradish peroxidase-conjugated secondary antibodies were subsequently incubated with the membranes for 2 h at room temperature. The protein bands were visualized using a Pierce ECL Plus Western Blotting Substrate (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and GAPDH was used as an internal control to normalize the expression levels of the aforementioned proteins.

Invasion and migration assays. The invasive abilities of MCF-7 and T47D cells were assessed with Transwell assays. At 24 h post-transfection, $4x10^4$ cells suspended in serum-free culture medium were plated in the upper chambers of 24-well plates containing Transwell inserts pre-coated with Matrigel (BD Biosciences, San Jose, CA, USA), following the manufacturer's protocol, and the lower chambers were filled with culture medium containing 10% FBS. After 48 h, the cells that had invaded to the bottom of the chambers were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. All the assays were performed in triplicate.

Table I. The primers of IL-8 and EMT-related marker genes.

Gene names		Forward primer 5'-3' Reverse primer 5'-3'
IL-8	F	5'-GGTGCAGTTTTGCCAAGGAG-3'
	R	5'-TTCCTTGGGGTCCAGACAGA-3'
E-cadherin	F	5'-TCCAGTGAACAACGATGGCA-3'
	R	5'-CCTGGGCAGTGTAGGATGTG-3'
Vimentin	F	5'-GGACCAGCTAACCAACGACA-3'
	R	5'-AAGGTCAAGACGTGCCAGAG-3'
Fibronectin	F	5'-AGCCTGGGAGCTCTATTCCA-3'
	R	5'-CTTGGTCGTACACCCAGCTT-3'
GAPDH	F	5'-CAGCGACACCCACTCCTC-3'
	R	5'-TGAGGTCCACCACCCTGT-3'

IL-8, interleukin-8; EMT, epithelial-mesenchymal transition.

The post-transfection migratory ability of cells was assessed with a wound-healing assay. MCF-7 and T47D cells were seeded into 6-well plates. When cells reached 80% confluency 24 h after transfection, a 200-μl plastic pipette tip was used to draw across the center of the cultured cells to generate a 1-mm '+' wound area. The migration of MCF-7 and T47D cells into the wound area was examined by microscopy.

Statistical analysis. All assays were performed in triplicate, and all steps were executed independently at least three times. Data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $P<0.05$.

Results

Bioinformatic analysis of IL-8 3'-UTR. The whole 3'-UTR of IL-8 is 1,192 bp and is conserved in *Homo sapiens*, as shown in Fig. 2. To identify the potential miRNAs that interact with the 3'-UTR of IL-8, three publically available online databases (miRWalk, TargetScan and miRanda) were used. As illustrated in Table II, the miR-520 family (miR-520a, b, c, d and e) was commonly identified in the three databases, and miR-520c-3p was predicted by all three databases to regulate the 3'-UTR of IL-8.

miR-520c-3p regulates IL-8 expression by directly targeting its 3'-UTR. The plasmids pcDNA3.1(+)-luciferase-IL-8-3'-UTR-wt and pcDNA3.1(+)-luciferase-IL-8-3'-UTR-mut were cloned for use in dual-luciferase reporter assays to validate the 3'-UTR of IL-8 as a direct target of miR-520c-3p. The relative effect of miR-520c-3p was determined as the ratio of firefly/*Renilla* luciferase activities in the 293T cells in reference to the researches of Wang *et al* and Shi *et al* (22,23). As shown in Fig. 3, the results verified that the relative luciferase activity of pcDNA3.1(+)-luciferase-IL-8-3'-UTR-wt in the 293T cells co-transfected

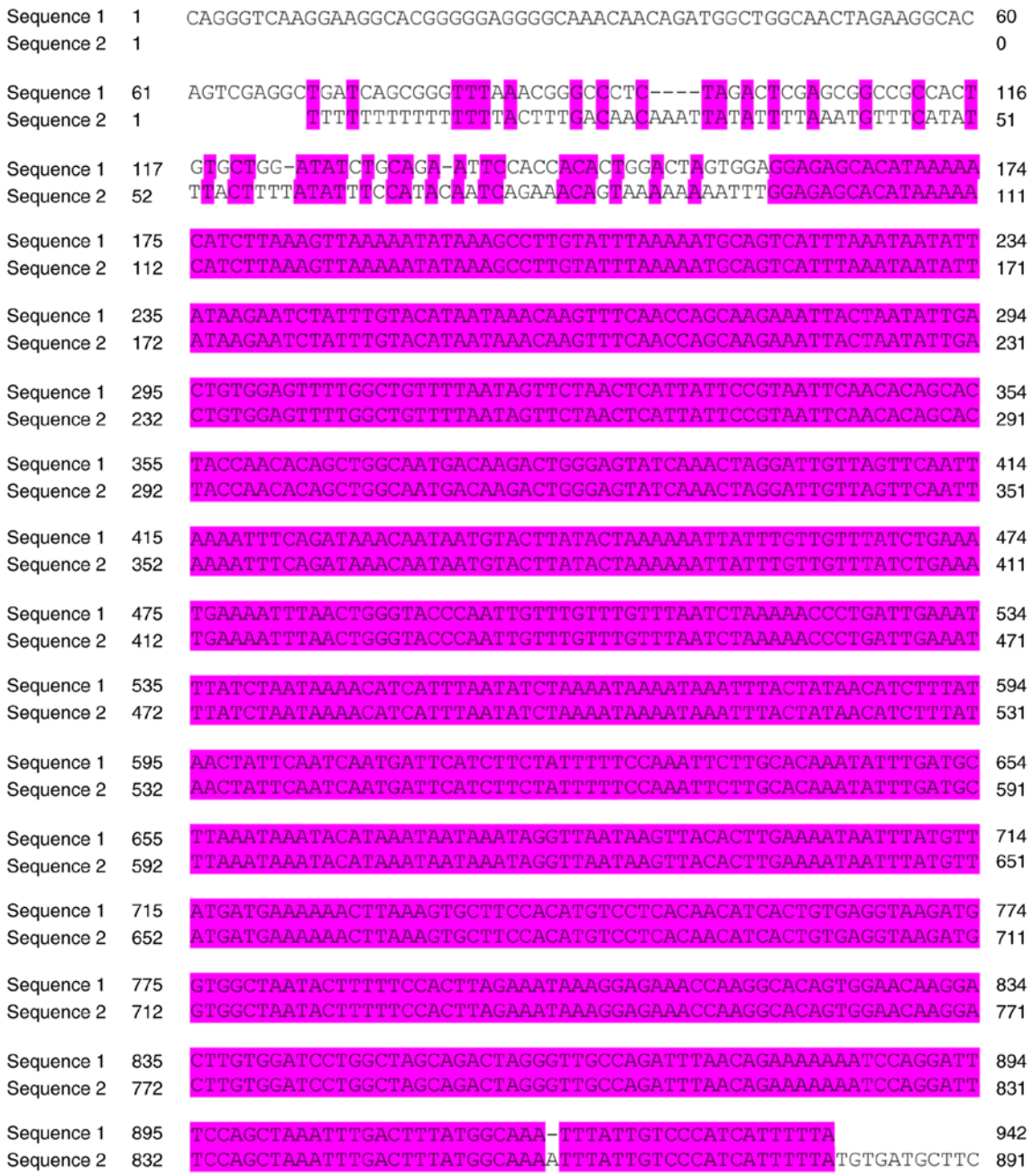

Figure 2. Bioinformatic analysis of the 3'-UTR in the human IL-8 gene using Genbank. The 3'-UTR of human IL-8 is 1,192 bp in length and is highly conserved throughout evolution. In the display tracks, conservation is shown in pink.

with miR-520c-3p was markedly decreased compared with that in cells transfected with scrambled miRNA control (P<0.001). However, no significant inhibitory effect of miR-520c-3p was detected when the 3'-UTR of IL-8 was mutated, indicating that miR-520c-3p inhibited luciferase reporter activity by direct interaction with the wt 3'-UTR of IL-8. These results were consistent with bioinformatic predictions, and indicated that IL-8 may be directly targeted and functionally regulated by miR-520c-3p in MCF-7 and T47D cells. RT-qPCR, western blotting, Transwell and wound-healing assays were subsequently performed to further verify the effects of miR-520c-3p on IL-8 expression in MCF-7 and T47D cells.

miR-520c downregulates the expression of IL-8 and alters the expression of EMT-related factors in breast cancer cell lines. To further investigate whether miR-520c-3p effectively regulates the expression of IL-8 and EMT-related genes, including E-cadherin, vimentin and fibronectin, in MCF-7 and T47D cells, RT-qPCR and western blot analyses were performed. The results confirmed that overexpression of miR-520c-3p significantly reduced the expression of IL-8 and resulted in increased E-cadherin and decreased vimentin and fibronectin levels in MCF-7 and T47D cells, as compared with scrambled miRNA control transfectants (Figs. 4 and 5; P<0.001). Conversely, overexpression of IL-8 led to a decrease in E-cadherin expression and increases in vimentin and fibronectin levels (P<0.001) (Figs. 4 and 5).

miR-520c-3p inhibits the invasion and migration of MCF-7 and T47D cells. IL-8 has been reported to regulate tumor

Table II. The potential miRNAs occupy the putative site on the 3'-UTR of IL-8.

miRWalk			microRNA.org			TargetScan		
Potential miRNAs	Seed sequences	Located sites	Potential miRNAs	Seed sequences	Located sites	Potential miRNAs	Seed sequences	Located sites
miR-302a	UGGAAGCACUU	588-598	miR-124	GUGCCUU	508-514	miR-302c-3p	AAGCACU	591-597
miR-302b	UGGAAGCACUU	588-598	miR-506	GUGCCUU	508-514	**miR-520f-3p**	AAGCACU	591-597
miR-302c	UGGAAGCACUU	588-598	miR-128	CACUGUG	504-510	miR-302d-3p	AGCACUU	592-598
miR-302d	UGGAAGCACUU	588-598	miR-93	AGCACUUU	592-599	miR-302a-3p	AGCACUU	592-598
miR-520e	GGAAGCACUUU	589-599	miR-519d	GCACUUU	593-599	miR-302b-3p	AGCACUU	592-598
miR-526b	GGAAGCACUUU	589-599	miR-20b	AGCACUUU	592-599	miR-302c-3p	AGCACUU	592-598
miR-520b	GGAAGCACUUU	589-599	miR-20a	AAGCACUUU	591-599	miR-302e-3p	AGCACUU	592-598
miR-302e	UGGAAGCACUU	588-598	miR-17	AAGCACUUU	591-599	miR-372-3p	AGCACUU	592-598
miR-520f	GGAAGCACUU	589-598	miR-106b	AGCACUUU	592-599	miR-373-3p	AGCACUU	592-598
miR-520a-3p	GGAAGCACUU	589-598	miR-106a	AAGCACUUU	591-600	**miR-520e**	AGCACUU	592-598
miR-520c-3p	GGAAGCACUU	589-598	miR-372	AGCACUU	592-598	**miR-520d-3p**	AGCACUU	592-598
miR-520d-3p	GAAGCACUUU	590-599	**miR-520e**	GGAAGCACUU	589-598	**miR-520a-3p**	AGCACUU	592-598
miR-567	AGAACAUACU	991-1000	**miR-520d-3p**	GAAGCACUU	590-598	**miR-520b**	AGCACUU	592-598
miR-17	AAGCACUUU	588-596	**miR-520a-3p**	GGAAGCACUU	589-598	**miR-520c-3p**	AGCACUU	592-598
miR-20a	AAGCACUUU	588-596	miR-373	GAAGCACUU	590-598	miR-20a-5p	GCACUUUA	593-600
miR-106a	AAGCACUUU	588-596	miR-302d	UGGAAGCACUU	588-598	miR-93-5p	GCACUUUA	593-600
miR-106b	AGCACUUUA	587-595	miR-302c	UGGAAGCACUU	588-598	miR-526b-3p	GCACUUUA	593-600
miR-373	GAAGCACUU	589-596	miR-302b	UGGAAGCACUU	588-598	miR-106a-5p	GCACUUUA	593-600
miR-656	UAUAAUAUU	87-95	miR-302a	UGGAAGCACUU	588-598	miR-106b-5p	GCACUUUA	593-600
miR-23a	AAUGUGAU	817-824	miR-302e	UGGAAGCACUU	588-598	miR-519d-3p	GCACUUUA	593-600
miR-93	AGCACUUU	588-595	**miR-520c-3p**	GGAAGCACUU	589-598	miR-20b-5p	GCACUUUA	593-600
miR-23b	AAUGUGAU	817-824	**miR-520b**	GGAAGCACUU	589-598	miR-17-5p	GCACUUUA	593-600
miR-106b	AGCACUUU	588-595	miR-154	UAACCU	644-649			
miR-372	AGCACUUU	588-595						
miR-340	GCUUUAUA	50-57						
miR-20b	AGCACUUU	588-595						
miR-621	UGCUAGCC	705-712						
miR-634	UGCUGGUU	127-134						
miR-653	UUCAACAC	1113-1120						
miR-656	UAUAAUAU	88-95						

Table II. Continued.

	miRwalk		microRNA.org		TargetScan	
Potential miRNAs	Seed sequences	Located sites	Potential miRNAs	Seed sequences	Potential miRNAs	Seed sequences	Located sites
miR-889	GAUAUUAA	412-419					
miR-944	AAUAAUUU	941-948					
miR-1294	ACCUCACA	619-626					

According to the bioinformatic websites: miRWalk (http://www.microwalk.org), TargetScan release 6.2 (http://www.targetscan.org), miRanda (http://www.microRNA.org). 3'-UTR, 3'-untranslated regions; IL-8, interleukin-8.

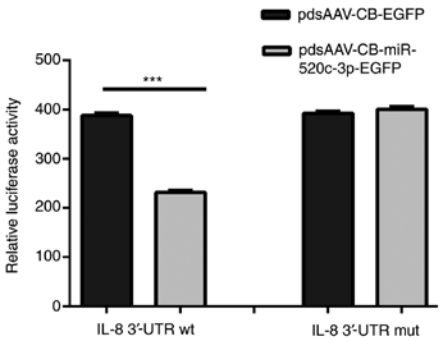

Figure 3. Dual-luciferase reporter assay results. 293T cells were transfected with constructs containing IL-8-3'-UTR-wt or -mut, and a dual-luciferase reporter assay was performed after 24 h; ***P<0.001.

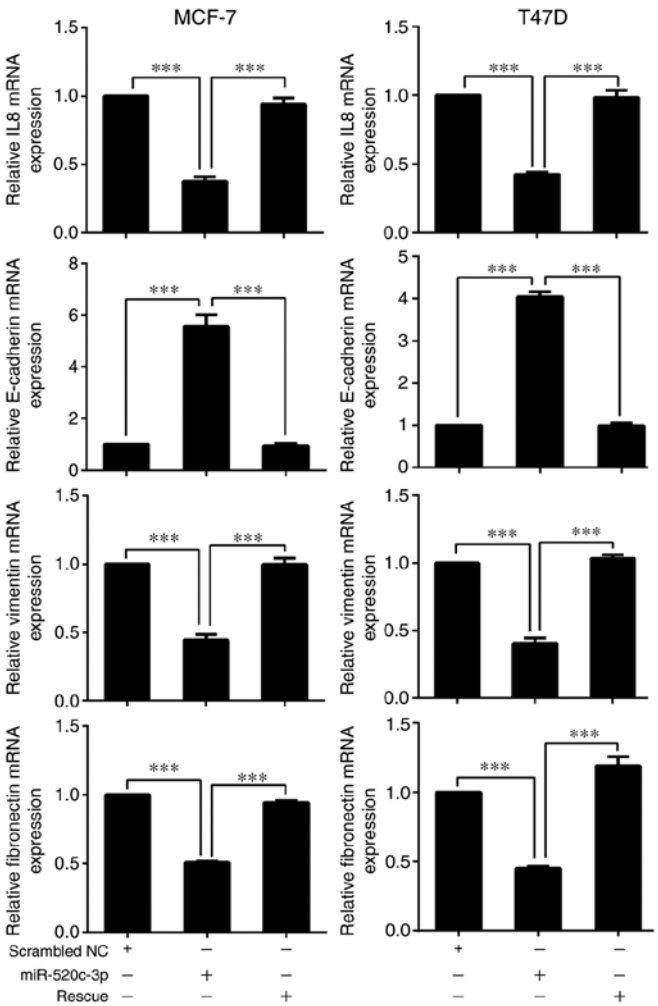

Figure 4. The mRNA level of IL-8, and protein levels of the epithelial marker E-cadherin and mesenchymal markers vimentin and fibronectin were detected in RNA and protein extracts from MCF-7 and T47D cells by RT-qPCR; ***P<0.001.

invasion and metastasis (9-16), and the present study verified that miR-520c-3p regulates the expression of IL-8 by directly targeting its 3'-UTR. Transwell and wound-healing assays were subsequently performed to evaluate whether miR-520c-3p co-transfected with pcDNA3.1(+)-luciferase-IL-8-3'-UTR affected the invasive and migratory abilities of the MCF-7 and T47D breast cancer cell lines. As shown in Fig. 6, the results

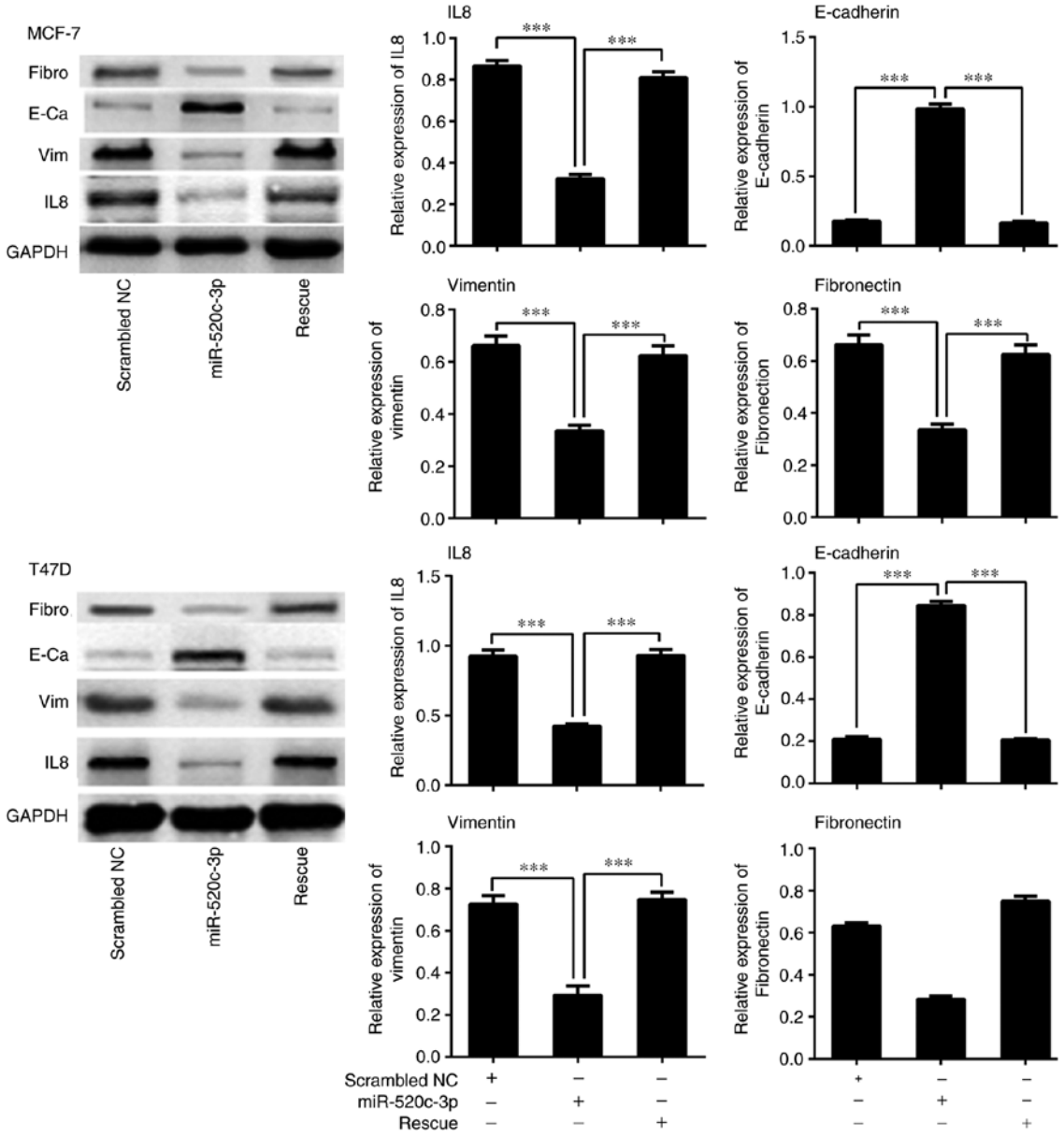

Figure 5. Protein was extracted from the MCF-7 and T47D cell lines after transfection with scrambled NC/miR520c-3p mimics/miR520c-3p mimics plus an IL-8 overexpression plasmid. The protein expression levels of IL-8, E-cadherin, vimentin and fibronectin in the different groups of cells were detected by western blotting; ***P<0.001.

demonstrated that overexpression of miR-520c-3p significantly inhibited the invasion and migration of MCF-7 and T47D cells, while rescue experiments attenuated this inhibition, restoring the migratory and invasive abilities. This indicated that the metastatic capacities of cells in the rescue group were promoted by IL-8 expression.

Discussion

Breast cancer is a malignant cancer that is highly prevalent in females, and has a particularly high incidence rate and mortality rate in China (24). Over the past several decades, significant advances have been made in our understanding of breast cancer-related mechanisms and in the treatment of breast cancer. Tumor grade, stage and lymph node status are known to be important factors that affect the prognosis of patients with breast cancer, and metastasis is a leading contributor to poor prognosis and a high rate of mortality. Many patients with breast cancer develop metastases prior to diagnosis, which markedly influences therapeutic efficacy and prognosis. It is well established that EMT in tumor cells promotes migration and invasion (25,26). Therefore, EMT is closely associated with the metastasis of solid cancers, and suppressing EMT in tumor cells is an important therapeutic strategy.

Numerous studies have confirmed that inflammation serves crucial roles in the development and progression of cancer, and many types of cancer originate from a background of infection and inflammation. Inflammatory cells in the tumor microenvironment, along with their secreted chemokines and cytokines, can affect the progression of various types of tumors by regulating the development, transformation and differentiation

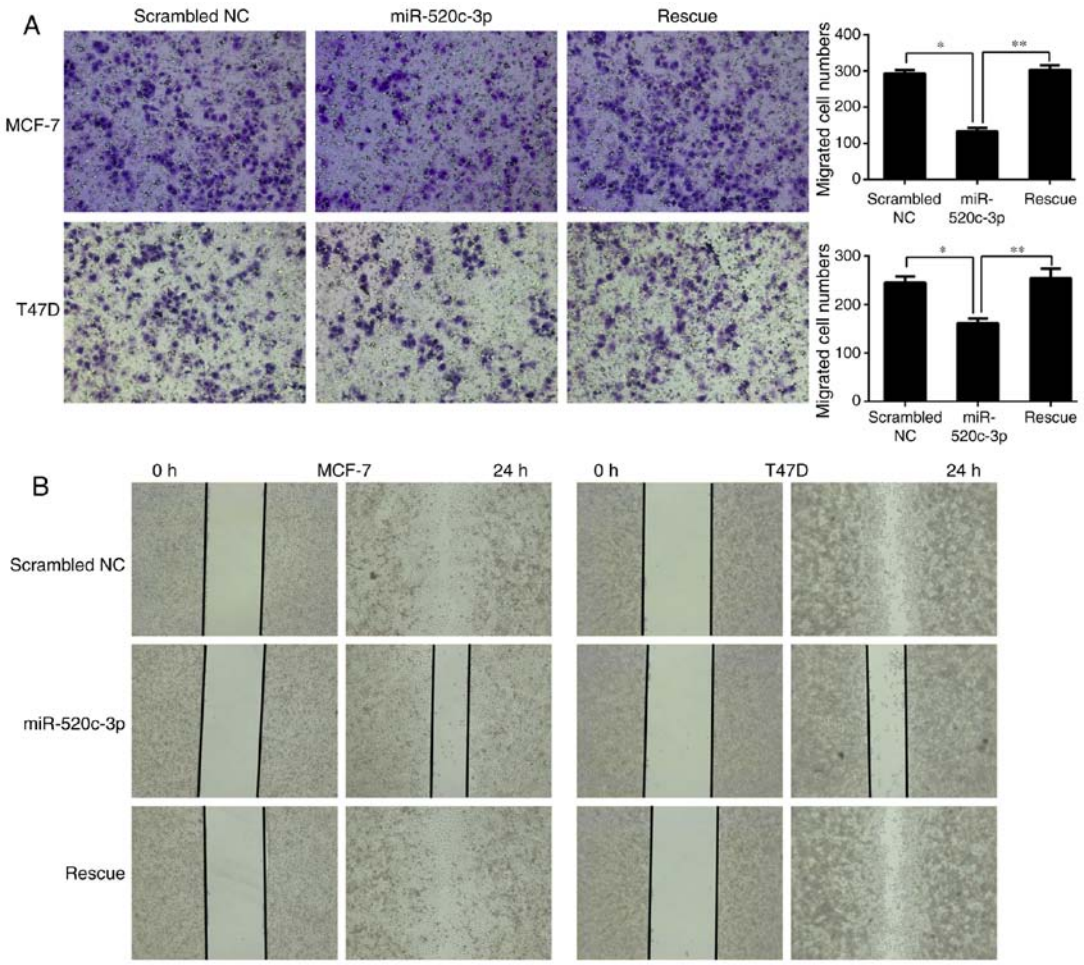

Figure 6. Overexpression of IL-8 in MCF-7 and T47D cells rescues the inhibition of EMT. Cells were transfected with scrambled NC/miR520c-3p mimics/miR520c-3p mimics plus an IL-8 overexpression plasmid. (A) A Transwell invasion assay demonstrated the inhibitory effect of miR-520c-3p overexpression on the invasion of MCF-7 and T47D cells 48 h after transfection. (B) A wound-healing assay demonstrated the inhibitory effect of miR-520c-3p on the migration of MCF-7 and T47D cells; the migration distance was recorded after 24 h.

of tumor cells (27,28). For example, growth factors and inflammatory factors in the microenvironment of pancreatic cancer can sustain and promote the growth of cancer cells, and have the ability to inhibit immune responses (29). Therefore, cytokines and their functional regulation have become topics of great interest in the context of cancer research and treatment.

IL-8 is a cytokine that induces the chemotaxis of lymphocytes and neutrophils, and may promote tumor angiogenesis; the antisense nucleotide of IL-8 suppresses macrophage-induced angiogenesis, which suggests a function of IL-8 in mediating angiogenesis in inflammation and tumor progression of various types of cancer (11,30,31). A large number of clinical studies and experimental research have demonstrated that IL-8 serves critical roles in the development of breast cancer (32). Research has shown that EMT in cancer depends on comprehensive stimulation by soluble growth factors, cytokines and extracellular matrix components in the tumor microenvironment; TGF-β, FGF, EGF, IL-8 and IL-6 promotes the occurrence of EMT in various kinds of tumors. The metastasis of breast cancer is closely associated with the occurrence of EMT; however, the relationship between IL-8 and EMT in breast cancer and its molecular mechanisms remain unclear. Thus, the present study aimed to investigate the effect of miR-520c-3p overexpression on IL-8 and EMT in the breast cancer cell lines MCF-7 and T47D, to explore its potential molecular mechanisms and provide an experimental basis for the treatment of breast cancer.

Accumulating studies have demonstrated that many different miRNAs serve critical roles in the diagnosis and treatment of breast cancer by influencing tumorigenesis, cell cycle regulation and differentiation, or by regulating the expression of target genes involved in the development and prognosis of breast cancer (3-6). Previous research has indicated that IL-8 regulates EMT and contributes to cell proliferation, migration and the regulation of angiogenesis in different types of tumor, including breast cancer. However, to the best of our knowledge, no study has confirmed the association between miR-520c-3p and IL-8 in breast cancer. The data from the present study demonstrated that overexpression of miR-520c-3p negatively regulated the expression of IL-8 in the MCF-7 and T47D cells. Therefore, we hypothesized that miR-520c-3p may inhibit EMT in breast cancer cells *in vitro* by directly targeting IL-8.

Dual-luciferase reporter assays, western blotting and RT-qPCR verified that overexpression of miR-520c-3p could directly suppress the expression of endogenous IL-8 in MCF-7 and T47D cells at a post-transcriptional level, confirming IL-8 to be a target gene of miR-520c-3p, as predicted by the bioinformatic analysis.

The effects of miR-520c-3p expression on MCF-7 and T47D cells were also investigated *in vitro*. Transwell and wound-healing assays showed that miR-520c-3p mimics effectively suppressed the invasion and migration of breast cancer cells at a post-transcriptional level, whereas the rescue of IL-8 expression attenuated the effects of the miR-520c-3p mimics, increasing the invasion and migration of MCF-7 and T47D cells. Thus, miR-520c-3p appears to act as a suppressor of invasion and migration in breast cancer cells. In addition, western blot analysis indicated a regulatory effect of miR-520c-3p on the expression of the EMT-related proteins E-cadherin, vimentin and fibronectin, indicating that EMT may be significantly suppressed by miR-520c-3p in breast cancer cells. Therefore, we concluded that overexpression of miR-520c-3p inhibits IL-8 protein expression and suppresses EMT in the breast cancer cell lines MCF-7 and T47D.

In conclusion, to the best of our knowledge, the present study was the first to identify IL-8 as a target gene of miR-520c-3p. The invasion, migration, and EMT of MCF-7 and T47D cells was negatively regulated by miR-520c-3p overexpression, and this result was likely associated with the expression of IL-8. The mechanism regarding the regulation of IL-8 expression by miR-520c-3p remains to be established.

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