Am J Transl Res 2021;13(4):2608-2616
www.ajtr.org /ISSN:1943-8141/AJTR0124459

Original Article

Epidermal growth factor-like domain 7 regulates breast cancer cell proliferation and vascular endothelial growth factor expression via the p38MAPK signaling pathway

Bei Zhou*, Jing Wang*

*Department of Breast Surgery, Hainan Women and Children's Medical Center, Haikou, Hainan Province, China. *Equal contributors and co-first authors.*

Received October 19, 2020; Accepted November 24, 2020; Epub April 15, 2021; Published April 30, 2021

Abstract: Objective: We aimed to investigate the effects of epidermal growth factor-like domain 7 (EGFL7) on breast cancer cell proliferation and angiogenesis and its association with the p38 mitogen-activated protein kinase (p38MAPK) signaling pathway. Methods: The vectors for stable overexpression of EGFL7 and the vectors for EGFL7 knockout were constructed. The breast cancer cell line MDA-MB-231 was selected for this study and the cells were divided into four groups: the control group, the empty vector group (transfected with an empty vector), the EGFL7 overexpression group (transfected with the EGFL7 overexpression vector), and the EGFL7 knockout group (transfected with the EGFL7 knockout vector). After 72 h of transfection, the mRNA and protein levels of EGFL7 in the cells were detected by RT-PCR and Western blot, respectively. The cell proliferation rates at 12 h, 24 h, 48 h and 72 h of culture in each group were detected using the MTT method. An *in vitro* tumor angiogenesis model of tumor-endothelial cells co-culture system was established and the angiogenesis ability at 12 h, 24 h, 48 h and 72 h of culture were compared among the groups using an *in vitro* angiogenesis assay. The cells in the EGFL7 overexpression group were further divided into three groups and were treated with p38MAPK inhibitor SB203580 at a dose of 0 μmol/L, 5 μmol/L, and 10 μmol/L, respectively. Afterward, the cells were co-cultured with endothelial cells for 48 h. Western blot was performed to detect the protein levels of vascular endothelial growth factor (VEGF), p38MAPK, and p-p38MAPK. Results: Compared with the control group, the EGFL7 mRNA level was higher in the EGFL7 overexpression group and lower in the EGFL7 knockout group (both $P<0.05$). Compared with the control group at 12 h, 24 h, 48 h, and 72 h of culture, the cell proliferation rates were lower in the EGFL knockout group and higher in the EGFL overexpression group, respectively (all $P<0.05$). Moreover, compared with the control group at these time points, the number of vascular sprouts and the protein levels of VEGF, p38MAPK, and p-p38MAPK were lower in the EGFL7 knockout group and higher in the EGFL7 overexpression group, respectively (all $P<0.05$). After the cells overexpressing EGFL7 were treated with SB203580, the level of p-p38MAPK was deceased, and the protein expression level of VEGF was inversely related with the SB203580 concentration ($F=44.24$, $P<0.01$). Conclusion: EGFL7 can promote the proliferation of breast cancer cells and angiogenesis, and the mechanism may be associated with the activation of p38MAPK signaling pathway and promotion of VEGF expression.

Keywords: Epidermal growth factor-like domain 7, breast cancer, angiogenesis, p38-MAPK signaling, vascular endothelial growth factor

Introduction

Breast cancer is a common malignant tumor that occurs mostly in women [1]. The local tumor microenvironment can determine the malignant biological properties of the cell, and angiogenesis is an essential element in cancer cell proliferation and metastasis [2, 3]. Vascular endothelial growth factor (VEGF), which has been recognized as a cytokine that can stimulate angiogenesis more significantly than other cytokines, can markedly promote the metastasis of breast cancer cells and plays a key role in cancer cell proliferation [4]. Epidermal growth factor-like domain 7 (EGFL7) is a secretory protein produced by vascular endothelial cells and participates in angiogenesis [5]. Studies have shown that the expression level of EGFL7 in breast cancer tissue is closely related to tumor stage, the depth of invasion, chemotherapy outcome, and prognosis [6]. EGFL7 may also promote the transmission of VEGF signal in the

process of angiogenesis and help maintain vascular integrity, but the specific molecular mechanisms remain unclear [6]. At present, studies have confirmed that p38 mitogen-activated protein kinase (p38MAPK) plays a crucial role in the invasion and metastasis of various malignant tumors and the regulation of VEGF protein expression [4-6]. Based on these previous findings, we conducted an *in vitro* study to further investigate the role of EGFL7 in promoting the proliferation of breast cancer cells and angiogenesis and its association with the p38MAPK signaling pathway, in an effort to provide more data for the diagnosis and treatment of breast cancer.

Materials and methods

Cell culture

The breast cancer cell line MDA-MB-231 (Sangon Biotech, Shanghai, China) was cultured in RPMI-1640 medium (Sigma-Aldrich, USA) containing 10% fetal bovine serum, 100,000 U/L penicillin, and 100 mg/L streptomycin at 37°C in 5% CO_2 and saturated humidity. After 24 hours, the medium was changed, and the cells were sub-cultured after digestion. The cells of the third generation were used for the subsequent experiments.

Epidermal growth factor-like domain 7 (EGFL7) overexpression and EGFL7 knockout

MDA-MB-231 cell line overexpressing EGFL7: The cDNA of EGFL7 was cloned by RT-PCR, and the pcDNA3.1-EGFL7 vector was constructed. The liposome-based vectors were transfected into MDA-MB-231 cell line followed by G418 screening and RT-PCR verification.

MDA-MB-231 cell line with EGFL7 knockout: The siRNA fragment was designed and the pcDNA3.1-EGFL7-siRNA vector was constructed. The liposome-based vectors were transfected into MDA-MB-231 followed by G418 screening and RT-PCR verification.

Grouping

The cells were divided into four groups: the control group (blank control, no transfection), the empty vector group (transfected with empty vectors), EGFL7 overexpression group (transfected with EGFL7 overexpression vector), and EGFL7 knockout group (transfected with EGFL7 knockout vector). After 72 h of transfection, the mRNA and protein expression levels of EGFL7 in the cells were detected by RT-PCR and Western blot. The cell proliferation rate was detected using the MTT method at 12 h, 24 h, 48 h and 72 h of culture. The *in vitro* model of tumor angiogenesis of tumor-endothelial cells co-culture was established. The angiogenesis ability was detected by the *in vitro* angiogenesis assay. The protein levels of VEGF, p-p38MAPK, and p38MAPK were detected by western blot. Five replicate wells were set at each time point in each group, and the average value was calculated.

The cells in EGFL7 overexpression group were divided into three groups which were treated with p38MAPK inhibitor SB203580 at a dose of 0 μmol/L, 5 μmol/L, and 10 μmol/L, respectively. Afterward, the cells were co-cultured with endothelial cells for 48 h. Subsequently, the protein expression levels of VEGF, p-p38MAPK, and p38MAPK were measured.

Test methods

RT-PCR: RNA was isolated from the cells according to the manufacturer's instructions of the Trizol kit (Boster Biological Technology, China) using the single-step method. After measuring the RNA concentration and purity, the RNA was reversely transcribed into cDNA. The total PCR reaction volume was 25 μL and the mixture contained 12.5 μL of SYBR® Premix Ex Taq™ (2×), 1 μL of 10 μM forward primer, and 1 μL of 10 μM reverse primer. The reaction parameters were set as follows: predenaturation at 92°C for 20 s, denaturation at 96°C for 2 s, annealing at 85°C for 20 s, and extension at 80°C for 6 s. The cycle was repeated 40 times (PCR instrument: ABI PCR System 9700, USA). The relative expression level of the target mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the internal reference. The primer sequences are listed in **Table 1**.

MTT assay: The cells in each group were seeded into 96-well plates at a cell density of $5*10^3$ cells/well. The MTT solution (Boster Biological Technology, China) was added into each well (20 μL of MTT/well) at 12 h, 24 h, 48 h, and 72 h, respectively, followed by another 4 h culture. Afterward, the optical density (OD) was measured with a microplate reader.

Table 1. Primer sequence

	Forward primer	Reverse primer	Size (bp)
EGFL7	5'-AACGCTGGCTCCGAAAACTT-3'	5'-CCTAAGGCGGTAAAATGCC-3'	256
GAPDH	5'-GGCTACAGCAACAGGGTG-3'	5'-TTTGGTTGAGCACAGGGT-3'	148

Note: EGFL7: epidermal growth factor-like domain 7.

Table 2. The mRNA and protein expression levels of EGFL7 in each group ($\bar{x}$ ± sd)

	EGFL7 mRNA	EGFL7/β-actin
EGFL7 overexpression group	0.64±0.11*,#,※	0.56±0.12*,#,※
EGFL7 knockout group	0.27±0.13*,※	0.21±0.03*,※
Control group	0.46±0.09	0.38±0.05
Empty vector group	0.45±0.07	0.36±0.04
F	12.684	10.573
P	0.000	0.000

Note: *P<0.05 vs. the control group; #P<0.05 vs. the EGFL7 knockout group; ※P<0.05 vs. the empty vector group. EGFL7: epidermal growth factor-like domain 7.

In vitro angiogenesis assay: Matrigel matrix was prepared and added onto the 24-well plates (150 μL of Matrigel matrix/well) followed by ultraviolet disinfection overnight. The breast cancer cells were cultured for 3 days and then centrifuged at 2,500 rpm for 30 min. The supernatant was collected and mixed with the medium at a ratio of 1:1. Next, the mixture was added into the 24 well plates pre-coated with Matrigel matrix (3 mL of mixture/well). The isolated culture of human umbilical vein endothelial cells ($5*10^5$ cell, Sangon Biotech, Shanghai) were then inoculated to establish *in vitro* tumor angiogenesis model of tumor-endothelial cells co-culture. At 12 h, 24 h, 48 h, and 72 h of culture, the results were photographed using an inverted fluorescence microscope (Olympus IX71, Olympus, Japan), respectively. Five fields were selected under high magnification, and the number of vascular sprouts was counted to calculate the mean value.

Western blot: The samples were treated with RIPA lysis buffer (2 mL), homogenized, and placed in an ice bath for 30 min. Afterward, the samples were centrifuged at 40°C 500×g for 5 min. After removing the supernatant and ultrasonic cell disintegration, the samples were centrifuged again, and 10 μL of the supernatant was collected. The samples were loaded on the gel for electrophoresis. Afterward, the proteins were transferred from the gel to the membrane. The membrane was cut and blocked with the blocking solution for 1 h and was then added with the monoclonal antibodies in the following order: rat anti-human VEGF (1:100, Beyotime, China), p38MAPK (1:200, Boster Biological Technology, China), p-p38MAPK (1:200, Boster Biological Technology, China), EGFL7 (1:100, Boster Biological Technology, China), and β-actin (1:100, Boster Biological Technology, China). Next, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (1:100, Boster Biological Technology, China). The membrane was exposed with a Bio-Rad imaging system (VersaDoc 3000, Bio-Rad, USA), and the OD value was measured using the Image Lab Software (VersaDoc 3000, Bio-Rad, USA). The relative expression levels of VEGF, p38MAPK, and EGFL7 were calculated as the ratio of the gray value of these proteins to the gray value of β-actin. The relative expression of p-p38MAPK was calculated as the ratio of the gray value of p-p38 MAPK to the gray value of p38 MAPK.

Statistical analysis

SPSS 20.0 software was used for statistical analysis. The sample size per group was 3. The measurement data in the three groups are presented as mean ± standard deviation ($\bar{x}$ ± sd). One-way analysis of variance was conducted for comparison among the three groups, and LSD-t method was conducted for pairwise comparison. P<0.05 indicated a statistically significant difference.

Results

The mRNA and protein expression levels of EGFL7 in each group

Compared with the control group, the mRNA and protein expression levels of EGFL7 were higher in the EGFL7 overexpression group and lower in the EGFL7 knockout group (both P< 0.05). See **Table 2** and **Figure 1**.

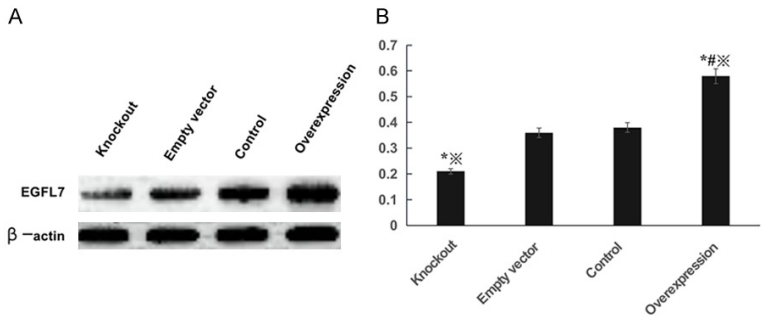

Figure 1. EGFL7 protein expression levels in each group. A: Western blot results; B: Histogram of the EGFL7 protein expression levels in each group. *P<0.05 vs. the control group; #P<0.05 vs. the EGFL7 knockout group; ※P<0.05 vs. the empty vector group. EGFL7: epidermal growth factor-like domain 7.

Breast cancer cell proliferation rates at different time points in each group

MTT assay showed that compared with the control group at 12 h, 24 h, 48 h and 72 h of culture, the cell proliferation rates were higher in the EGFL7 overexpression group and lower in the EGFL7 knockout group, respectively (all P<0.05). See **Table 3**.

Angiogenesis at different time points in each group

An *in vitro* angiogenesis assay showed that compared with the control group at 12 h, 24 h, 48 h and 72 h of culture, the numbers of vascular sprouts were higher in the EGFL7 overexpression group and lower in the EGFL7 knockout group, respectively (all P<0.05). See **Table 4** and **Figure 2**.

The protein levels of VEGF, p-p38MAPK, and p38MAPK at different time points in each group

Western blot analysis showed that compared with the control group at 12 h, 24 h, 48 h, and 72 h of culture, the protein levels of VEGF, p38MAPK, and p-p38MAPK were higher in the EGFL7 overexpression group and lower in the EGFL7 knockout group, respectively (all P< 0.05). See **Tables 5-7** and **Figure 3**.

EGFL7 regulates VEGF expression in breast cancer cell line MDA-MB-231 via p38MAPK signaling pathway

In order to investigate whether EGFL7 regulates VEGF expression through p38MAPK signaling pathway, we chose SB203580, a specific small-molecule inhibitor for p38MAPK signaling pathway, at a dose of 0, 5, and 10 μmol/L to block the activated p38MAPK signaling pathway in MDA-MB-231 cells overexpressing EGFL7 to observe whether the expression level of VEGF protein was reversed. Western blot results showed that at 48 h after treatment with SB203580 at a dose of 0, 5, and 10 μmol/L, the protein level of p38MAPK did not change, but the level of activated p-p38MAPK/p38MAPK decreased with the increase of SB203580 concentration (**Figures 4**, **5**). These results indicate that SB203580 can effectively inhibit the activation of p38MAPK signaling pathway induced by EGFL7 protein upregulation in MDA-MB-231 cell line.

In addition, the VEGF protein expression level gradually decreased with the increase of SB203580 concentration (**Figures 4**, **5**). The analysis of variance test showed significant differences among the three groups (F=44.24, P<0.01) and suggested that the inhibition of VEGF protein expression was dose-dependent on the SB203580 concentration.

Discussion

EGFL7 is mainly secreted by vascular endothelial cells and is highly expressed during angiogenesis during early embryonic development [7]. As the human body develops, the structure and function of blood vessels becomes mature, and the demand for angiogenesis is decreased markedly. With the decrease of angiogenesis, the level of EGFL7 is also decreased and the expression of EGFL7 is only high in heart, lung, kidney, tumor tissues (including benign and malignant tumors), and inflammatory tissues [8]. Angiogenesis is a way of repairing the human body, which can provide sufficient nutrition for the locally proliferated cells. When the body repair is finished, the angiogenesis process ends. Angiogenesis is not a process that occurs from scratch, rather, it is a process of sprouting new and incomplete micro-vessels from existing vascular structures. This complex process is regulated by various cell growth factors, such as VEGF/

Table 3. Breast cancer cell proliferation rates in each group ($\bar{x}$ ± sd)

	12 h	24 h	48 h	72 h
EGFL7 overexpression group	48.37±5.31*,#,※	71.26±8.27*,#,※	92.64±11.91*,#,※	93.11±12.16*,#,※
EGFL7 knockout group	13.09±3.27*,※	16.38±5.11*,※	28.55±6.45*,※	31.09±3.85*,※
Control group	24.02±5.82	41.19±9.02	51.05±10.25	52.92±11.50
Empty vector group	31.24±6.01	57.86±7.35	72.28±9.05	73.25±8.57
F	12.356	10.056	10.856	11.002
P	0.005	0.011	0.008	0.007

Note: *P<0.05 vs. the control group; #P<0.05 vs. the EGFL7 knockout group; ※P<0.05 vs. the empty vector group. EGFL7: epidermal growth factor-like domain 7.

Table 4. Number of vascular sprouts in each group ($\bar{x}$ ± sd)

	12 h	24 h	48 h	72 h
EGFL7 overexpression group	6.59±0.64*,#,※	8.13±0.82*,#,※	10.05±1.23*,#,※	11.11±2.08*,#,※
EGFL7 knockout group	1.34±0.11*,※	1.64±0.13*,※	1.99±0.16*,※	2.08±0.21*,※
Control group	2.05±0.75	3.96±0.13	4.15±0.25	4.98±0.59
Empty vector group	3.01±0.31	4.66±0.15	5.81±0.08	5.87±0.34
F	15.324	14.367	10.568	11.256
P	0.000	0.000	0.000	0.000

Note: *P<0.05 vs. the control group; #P<0.05 vs. the EGFL7 knockout group; ※P<0.05 vs. the empty vector group. EGFL7: epidermal growth factor-like domain 7.

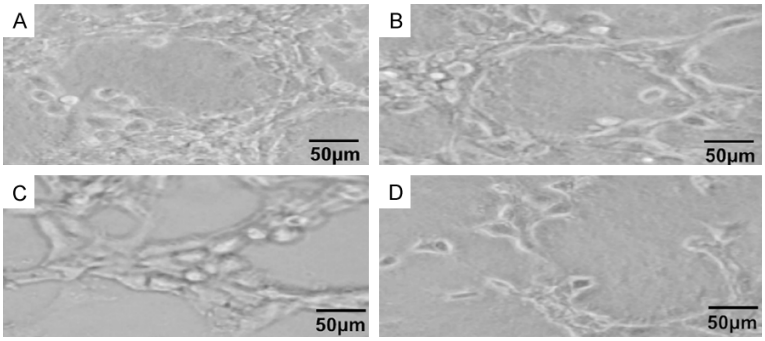

Figure 2. Number of vascular sprouts in each group at 72 h (40×). A: EGFL7 overexpression group; B: Empty vector group; C: Control group; D: EGFL7 knockout group. EGFL7: epidermal growth factor-like domain 7.

VEGF receptor, angiotensin/Tie signaling pathway, transforming growth factor β, fibroblast growth factor, and Notch signaling pathway [9-13]. EGFL7, as one of these factors, plays a critical role in regulating endothelial cell adhesion and migration and in inhibiting vascular smooth muscle cell migration. Endothelial cell adhesion and migration are key elements in angiogenesis, and EGFL7 regulates the migration direction and spatial structure of the endothelial cell [14]. EGFL7 induces angiogenesis by mainly promoting the migration of endothelial cells and the formation of the vascular bed structure, whereas VEGF, fibroblast growth factor, and angiotensin induce angiogenesis by directly stimulating endothelial cell proliferation, whereas [15]. EGFL7 can inhibit the migration of vascular smooth muscle cell but cannot inhibit the proliferation of the smooth muscle cell [16].

Studies have found that when a tumor diameter exceeds 2 mm, the tumor will heavily rely on the local newly formed vessels to supply energy and nutrition for proliferation and invasion; meanwhile, high level of EGFL7 protein can be detected in these neovascular endothelial cells [17]. Due to large intercellular space, loose cell junctions, and incomplete basement membrane in newly formed vessels, the tumor cells can pass through the vascular barrier easily to enter the blood system [18]. EGFL7 can promote the migration, infiltration and metastasis of tumor cells through autocrine and paracrine mechanisms [19]. Meanwhile, EGFL7 can increase the number of cells entering the blood system [20]. The surface of distant organs and tissues can

Table 5. Protein expression levels of VEGF in each group ($\bar{x}$ ± sd)

	12 h	24 h	48 h	72 h
EGFL7 overexpression group	0.43±0.07*,#,※	0.57±0.04*,#,※	0.79±0.04*,#,※	0.89±0.11*,#,※
EGFL7 knockout group	0.22±0.02*,※	0.27±0.03*,※	0.31±0.03*,※	0.36±0.02*,※
Control group	0.33±0.04	0.39±0.02	0.54±0.07	0.57±0.02
Empty vector group	0.37±0.01	0.42±0.03	0.55±0.05	0.59±0.07
F	9.048	7.284	7.182	7.582
P	0.006	0.013	0.009	0.007

Note: *P<0.05 vs. the control group; #P<0.05 vs. the EGFL7 knockout group; ※P<0.05 vs. the empty vector group. EGFL7: epidermal growth factor-like domain 7; VEGF: vascular endothelial growth factor.

Table 6. Protein expression levels of p38MAPK in each group ($\bar{x}$ ± sd)

	12 h	24 h	48 h	72 h
EGFL7 overexpression group	0.37±0.03*,#,※	0.41±0.05*,#,※	0.45±0.04*,#,※	0.57±0.07*,#,※
EGFL7 knockout group	0.11±0.02*,※	0.14±0.02*,※	0.16±0.03*,※	0.19±0.02*,※
Control group	0.16±0.02	0.19±0.02	0.21±0.05	0.23±0.02
Empty vector group	0.18±0.01	0.23±0.03	0.25±0.06	0.29±0.05
F	11.086	10.643	9.573	9.338
P	0.000	0.000	0.003	0.002

Note: *P<0.05 vs. the control group; #P<0.05 vs. the EGFL7 knockout group; ※P<0.05 vs. the empty vector group. EGFL7: epidermal growth factor-like domain 7; MAPK: mitogen-activated protein kinase.

Table 7. Protein levels of p-p38MAPK in each group ($\bar{x}$ ± sd)

	12 h	24 h	48 h	72 h
EGFL7 overexpression group	0.36±0.04*,#,※	0.39±0.05*,#,※	0.45±0.04*,#,※	0.58±0.08*,#,※
EGFL7 knockout group	0.09±0.01*,※	0.14±0.02*,※	0.17±0.04*,※	0.24±0.02*,※
Control group	0.16±0.04	0.19±0.05	0.23±0.02	0.31±0.02
Empty vector group	0.19±0.04	0.21±0.04	0.25±0.04	0.33±0.06
F	10.283	8.107	8.234	7.382
P	0.001	0.003	0.003	0.005

Note: *P<0.05 vs. the control group; #P<0.05 vs. the EGFL7 knockout group; ※P<0.05 vs. the empty vector group. EGFL7: epidermal growth factor-like domain 7; MAPK: mitogen-activated protein kinase.

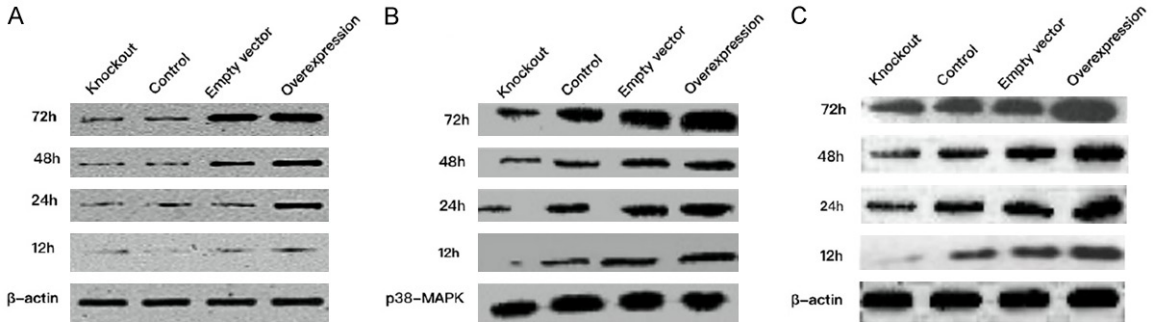

Figure 3. Protein levels of VEGF, p-p38MAPK, and p38MAPK at different time points in each group detected by Western blot. A: VEGF protein; B: p-p38MAPK protein; C: p38MAPK. VEGF: vascular endothelial growth factor; MAPK: mitogen-activated protein kinase.

bind to EGFL7 protein in the circulating blood through relative receptors and capture tumor cells from blood to complete tumor colonization [21]. Recent studies have confirmed that

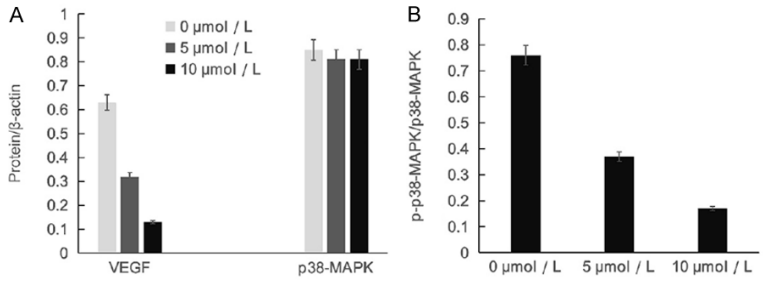

Figure 4. Protein levels of VEGF, p-p38MAPK, and p38MAPK at different time points in each group after treatment with SB203580. A: Protein levels of VEGF and p38MAPK; B: Protein levels of p-p38MAPK. VEGF: vascular endothelial growth factor; MAPK: mitogen-activated protein kinase.

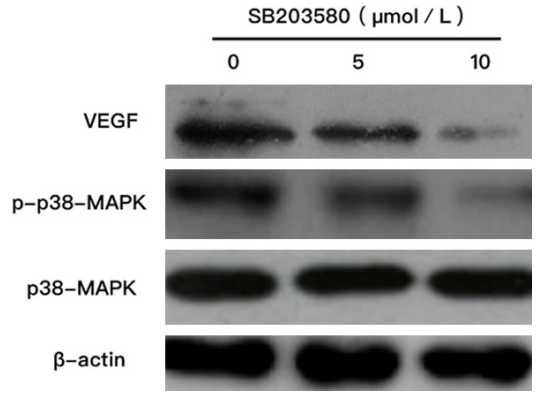

Figure 5. Western blot results of the protein levels of VEGF, p-p38MAPK, and p38MAPK at different time points in each group after treatment with SB203580. VEGF: vascular endothelial growth factor; MAPK: mitogen-activated protein kinase.

EGFL7 can be used as a chemoattractant to enhance the ability of cell migration. Chemoattractant can not only mediate the chemotaxis of cells, but also enhance the cell proliferation ability by activating downstream signaling pathways through G protein-coupled receptors, activating the RAS/MAPK pathway, and regulating morphological changes of cells to mediate actin polymerization and formation of pseudopodia-like processes [22]. The results in our study showed that the cell proliferation rate in the EGFL7 overexpression group was much higher than that in the control and the EGFL7 knockout groups, suggesting that EGFL7 can promote the proliferation of breast cancer cells.

Previous studies have found that EGFL7 protein can promote epithelial-to-mesenchymal transition of breast cancer cells by regulating Wnt-1/β-catenin signaling pathway, thereby enhancing the invasion ability of tumor cells [23]. VEGF can induce the proliferation and migration of lymphatic endothelial cells, increase the permeability of blood vessels, and promote the formation and metastasis of tumor lymphatic vessels [24]. During this process, the p38MAPK signaling pathway serves an essential regulatory role. It has been reported that the p38MAPK inhibitor, SB203580m can significantly reduce the expression level of VEGF in lung cancer, oral cancer, gastric cancer, and other malignant tumors [25, 26]. Various growth factors and other extracellular signals can act on tumor cell membrane receptors. The signals then enter into the cell and cause phosphorylation cascades of p38MAPK and aberrant expression of many downstream target genes and proteins [27, 28].

In the present study, we found that compared with the control group at different time points, the numbers of vascular sprouts and protein levels of VEGF and p38MAPK were higher in the EGFL7 overexpression group and lower in the EGFL7 knockout group. The activity of p-p38MAPK in the EGFL7 overexpression group also increased significantly, suggesting that p38MAPK signaling pathway is activated in this process. EGFL7 may mediate the activation of p38MAPK signaling pathway to complete angiogenesis. SB203580 is a common selective inhibitor of p38MAPK signaling pathway, it can penetrate the cells and inhibit the subsequent signaling of p38MAPK. We used SB203580 with a concentration gradient to inhibit the activated p38MAPK signaling pathway in the breast cancer-endothelial cells overexpressing EGFL7. After the pathway was blocked by SB203580, the upregulation of VEGF protein expression in endothelial cells mediated by EGFL7 was successfully reversed. The reversal effect and the blocking of p38MAPK pathway was dose-dependent on the concentration of the inhibitor. These findings suggest that p38MAPK signaling pathway can participate in the regulation of VEGF protein mediated by EGFL7.

In conclusion, EGFL7 may promote *in vitro* proliferation of breast cancer cells and angiogen-

esis by activating p38MAPK signaling pathway. In the future, we will conduct studies to inhibit EGFL7, VEGF, and p38MAPK expressions in animal models to explore whether these inhibitions can reduce tumor growth and angiogenesis, in an effort to provide more evidence for the clinical treatment of breast cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Bei Zhou, Department of Breast Surgery, Hainan Women and Children's Medical Center, Changbin Road, Xiuying District, Haikou 570312, Hainan Province, China. Tel: +86-18689856209; E-mail: zhoubei2tqa@163.com

References

[1] Shao ZM, Jiang ZF, Li JJ, Chen S and Wang H. Expert consensus on neoadjuvant therapy for breast cancer treatment in China (2019 Edition). Chin Oncol 2019; 29: 390-400.

[2] Zhang ST, Wang SZ, Yu QX and Yuan SH. Analysis of clinicopathologic features of postoperative breast cancer recurring and metastasizing beyond 5 years. Zhong Liu Fang Zhi Za Zhi 2019; 26: 406-409.

[3] Zhang JS, Zhu ZH, Wang GS and Qu SH. Expressions of ING4, HIF-1α and HIF-2α in breast cancer tissues andtheir relationship with tumor angiogenesis. Anhui Med Pharm J 2017; 21: 847-851.

[4] Su JL, Wang JM, Luo JM and Li HL. Ultrasound-mediated destruction of vascular endothelial growth factor (VEGF) targeted and paclitaxel loaded microbubbles for inhibition of human breast cancer cell MCF-7 proliferation. Mol Cell Probes 2019; 46: 101415.

[5] Hansen TF, Andersen RF, Olsen DA, Sørensen FB and Jakobsen A. Prognostic importance of circulating epidermal growth factor-like domain 7 in patients with metastatic colorectal cancer treated with chemotherapy and bevacizumab. Sci Rep 2017; 7: 2388.

[6] Huang YJ, Fang JS, Lu WQ, Wang ZH, Wang Q, Hou Y, Jiang XW, Reizes O, Lathia J, Nussinov R, Eng C and Cheng FX. A systems pharmacology approach uncovers wogonoside as an angiogenesis inhibitor of triple-negative breast cancer by targeting hedgehog signaling. Cell Chem Biol 2019; 26: 1143-1158, e1146.

[7] Reustle A and Torzewski M. Role of p38 MAPK in atherosclerosis and aortic valve sclerosis. Int J Mol Sci 2018; 19: 3761.

[8] Jiang HJ, Jiang GQ, Sun JX and Zhang B. Advances in research on the relationship between GDF-15 and breast cancer. Intern Med and Health Guid News 2019; 25: 3503-3506.

[9] Fukui Y, Kawashima M, Kawaguchi K, Takeuchi M, Hirata M, Kataoka TR, Sakurai T, Kataoka M, Kanao S, Nakamoto Y, Hirata K, Yoshimura M, Yoshikawa K, Ishiguro H and Toi M. Granulocyte-colony-stimulating factor-producing metaplastic carcinoma of the breast with significant elevation of serum interleukin-17 and vascular endothelial growth factor levels. Int Cancer Conf J 2018; 7: 107-113.

[10] Cao LS, Shen W and Cao XR. Angiotensin II promotes the migration of breast cancer cells. J Nanjing Normal Univ (Natu Sci Edi) 2018; 41: 83-87.

[11] Cui GZ, Ding MJ, Bai Y, Yang WH, Yang M and Dai DL. Correlation between transforming growth factor beta 1 and three negative breast carcinoma. Hebei Med J 2018; 40: 1207-1210, 1214.

[12] Cheng ZY, Wang JN, Sun FY, Xu Z and Li HY. Advances in fibroblast growth factors receptor-targeted drugs for breast cancer. World Clin Drugs 2019; 40: 161-169.

[13] Tang H, Xiao WR, Liao YY, Li L, Xiao X, Xu XP and Feng H. EGFL7 silencing inactivates the notch signaling pathway; enhancing cell apoptosis and suppressing cell proliferation in human cutaneous melanoma. Neoplasma 2019; 66: 187-196.

[14] Thakker S, Strahan RC, Scurry AN, Uppal T and Verma SC. KSHV LANA upregulates the expression of epidermal growth factor like domain 7 to promote angiogenesis. Oncotarget 2018; 9: 1210-1228.

[15] Shen XC, Han Y, Xue XF, Li W, Guo XB, Li P, Wang YL, Li DC, Zhou J and Zhi QM. Epidermal growth factor-like domain 7 promotes cell invasion and angiogenesis in pancreatic carcinoma. Biomed Pharmacother 2016; 77: 167-175.

[16] Richter A, Alexdottir MS, Magnus SH, Richter TR, Morikawa M, Zwijsen A and Valdimarsdottir G. EGFL7 mediates BMP9-induced sprouting angiogenesis of endothelial cells derived from human embryonic stem cells. Stem Cell Reports 2019; 12: 1250-1259.

[17] Usuba R, Pauty J, Soncin F and Matsunaga YT. EGFL7 regulates sprouting angiogenesis and endothelial integrity in a human blood vessel model. Biomaterials 2019; 197: 305-316.

[18] Gong C, Fang J, Li G, Liu HH and Liu ZS. Effects of microRNA-126 on cell proliferation, apoptosis and tumor angiogenesis via the down-regulating ERK signaling pathway by targeting EGFL7 in hepatocellular carcinoma. Oncotarget 2017; 8: 52527-52542.

[19] Que HT, Cao B and Tai ZG. Research progress of EGFL7 in breast cancer. Med Info 2018; 31: 32-36.

[20] Jiang QM, Zhou WW, Li GX and Xiao J. MiR-126 targeting EGFL7 inhibits epithelial mesenchymal transition in breast cancer cell line MDA-MB-231. Intern J Pathol Clin Med 2018; 38: 2055-2062.
[21] Jiang QM, Zhou WW and Xiao J. Clinical significance of EGFL7 and B-catenin protein detection in breast cancer. Intern J Pathol Clin Med 2018; 38: 472-479.
[22] Zhang YH, Qin XB, Jiang J and Zhao WJ. MicroRNA-126 exerts antitumor functions in ovarian cancer by targeting EGFL7 and affecting epithelial-to-mesenchymal transition and ERK/MAPK signaling pathway. Oncol Lett 2020; 20: 1327-1335.
[23] Wei S, Dai MM, Liu ZT, Ma YQ, Shang HQ, Cao Y and Wang Q. The guanine nucleotide exchange factor Net1 facilitates the specification of dorsal cell fates in zebrafish embryos by promoting maternal B-catenin activation. Cell Res 2017; 27: 202-225.
[24] Rusak A, Jablonska K, Piotrowska A, Grzegrzolka J, Wojnar A and Dziegiel P. Correlation of expression of CHI3L1 and Nogo-A and their role in angiogenesis in invasive ductal breast carcinoma. Anticancer Res 2019; 39: 2341-2350.
[25] Fu LX, Xi L, Chen YL, Yang D and Liu MR. Cancer-associated fibroblast (CAF) promotes migration of human breast cancer cells via activating the P38/MAPK signaling pathway. J Chongqing Med Univ 2019; 44: 163-168.
[26] Xiong QY, Zhu JH, Sun HX, Guo HJ, Hu LX and Ye HP. Effect of Qingzao Jiufei Tang on TGF-β1, Smad2 and p38MAPK in mice with lung cancer induced by urethane. Pharmacol Clin Chin Mater Med 2018; 34: 14-18.
[27] Guo M, Zhai XJ, Zhang H, Li WH and Liu M. Effect of p38 mitogen-activated protein kinase signaling pathway on autophagy and apoptosis activity of triple negative breast cancer cell mediated by osteopontin. J Xinxiang Med Coll 2018; 35: 1058-1063.
[28] Xin J, Chen HB and Shang YT. Effect of p38MAPK singaling pathway inhibitor on TNF-A-induced apoptosis of breast cancer cell. Matern Child Health Care China 2019; 34: 1375-1379.