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Original Research Article

Mediator complex subunit 19 regulates the proliferation, migration and invasion of human breast cancer cells

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Abstract

Purpose: *To investigate the therapeutic implication of mediator complex subunit 19 (Med19) in breast cancer cells.*
Methods: *The mRNA expression of Med19 was assayed using qRT-PCR. Cell viability was determined with 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assay, while 4',6-diamidino-2-phenylindole (DAPI) and annexin V/propidium iodide (PI) assays were used for determination of apoptosis. Wound healing and Transwell assays were used for the determination of cell migration and invasion. Western blotting analysis was used for assay of protein expression levels.*
Results: *The results showed that Med19 was significantly ($p < 0.05$) upregulated in human breast cancer cell lines, relative to normal cells. The up-regulations ranged from 3.7-fold in UACC-2087 cells to 6.4-fold in BT-20 cells. Moreover, Med19 silencing caused significant decrease in the proliferation of BT-20 breast cancer cells ($p < 0.05$). The inhibition of cell proliferation was due to the induction of apoptosis, as was evident in increased Bax/Bcl-2 ratio. Annexin V/PI staining revealed 6 % apoptosis in si-NC-transfected, and about 13.30 % in si-Med19-transfected BT-20 cells. Wound healing and Transwell assays revealed that the invasion of BT-20 breast cancer cells significantly decreased upon Med19 silencing.*
Conclusion: *Med19 regulates the proliferation, migration and invasion of human breast cancer cells. Thus, Med19 may be beneficial in the treatment of breast cancer.*

Keywords: *Breast cancer, Med19, Apoptosis, Migration, Invasion*

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INTRODUCTION

Breast cancer is a lethal disease which accounts for 0.5 million cancer-related deaths out of an estimated 1.7 million cases of breast cancer reported annually [1]. Studies have shown that breast cancer accounts for 14 % of all cancer cases diagnosed in USA annually [1,2]. One out of every 8 women will be diagnosed with breast cancer during her lifetime. Globally, the incidence of breast cancer has been increasing by 3.1 % annually since 1980 [2,3]. The unavailability of reliable molecular markers and drug targets hinders early diagnosis and treatment of breast cancer [4]. It is known that Med19 is a large, ubiquitous and conserved multiprotein complex [5]. It controls the transcription of genes, as well as cellular proliferation and differentiation [6].

Mediator complex subunit 19 (Med19) is a component of a mediator complex which has been shown to be significantly dysregulated in cancer cells [7]. It has been shown to regulate the growth of bladder and hepatocellular carcinoma [8,9]. Studies have reported that silencing of Med19 suppressed the growth of human pancreatic cancer [10]. Moreover, it has been reported that the growth of human breast cancer cells was significantly suppressed upon lentiviral suppression of Me19 in breast cancer [11]. The present study was carried out for the first time, to investigate the expression profile, role and therapeutic implications of Me19 in breast cancer. The effects of Med19 on the migration and invasion of human breast cancer cells were also determined.

EXPERIMENTAL

Cell lines and culture conditions

Human breast cancer cell lines SK-BR-3, UACC-2087, MDA-MB-231 and BT-20, as well as normal MB-157 cell line were acquired from the American Type Culture Collection, USA. The cell lines were cultured using Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Thermo Scientific) in a humidified 5 % CO_2 incubator at 37 ºC.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the breast cancer cell lines and normal cell line using RNeasy kits (Qiagen, Inc., Valencia, CA, USA), while Omniscript RT (Qiagen, Inc.) was used for reverse-transcription of 1 µg of the extracted RNA to cDNA. The cDNA was then used as a template for RT-qPCR with Taq PCR Master Mix kit (Qiagen Inc), according to the manufacturer's protocol. The 20-µl reaction mixture consisted of 1.5 mM MgCl2, 2.5 units Taq DNA Polymerase, 200 µM dNTP, 0.2 µM of each primer, and 0.5 µg DNA. The cycling conditions were as follows: 95 ˚C for 20 sec, followed by 40 cycles of 95 ˚C for 15 sec, and 58 ˚C for 1 min. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control, while 2-ΔΔCq the method was used to calculate the relative expressions amongst samples. The primers used in qRT-PCR are listed in Table 1.

Cell transfection

At 80 % confluence, BT-20 cells were transfected with negative control (si-NC) and si-Med19 (10 pmol each) (Shanghai GenePharma, Shanghai, China) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc) as per the manufacturer's protocol.

Determination of cell viability using MTT assay

The BT-20 cells were cultured in 96-well plates. The cells were thereafter transfected with si-NC or si-Med19, and cultured for 24 h at 37 ºC. The culture medium was replaced with 10 µL of MTT (5 mg/mL), followed by incubation at 37 ºC for 4 h. Finally, 100 µL of DMSO was added to dissolve the resultant formazan crystals. The absorbance of the formazan solution was read at 570 nm at different time intervals, and the values were used to determine cell viability.

Apoptosis assays

The BT-20 cancer cells transfected with si-NC and si-Med19 were cultured for 24 h. Following centrifugation, the cell pellets were washed with PBS and fixed using 70 % ethanol. Thereafter, the cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) and analyzed for morphological changes under a fluorescent microscope.

Annexin V/PI double staining assay

Annexin V-FITC/PI staining assay was used to determine the level of cell apoptosis in transfected BT-20 cells. The transfected BT-20 cancer cells were fixed with methanol, stained with dual Annexin V-FITC/PI, and subjected to flow cytometric analysis.

Wound healing assay

The BT-20 cancer cells transfected with si-NC or si-Med19 were cultured to 80 % confluence.

Table 1: Primers used in qRT-PCR

Primer	Direction	Sequence
Med19	Forward	5′- TGACAGGCAGCACGAATC -3′
	Reverse	5′-CAGGTCAGGCAGGAAGTTAC-3′
GADPH	Forward	5′-TCTCTGCTCCTCCTGTTC-3′
	Reverse	5′-GGTTGAGCACAGGGTACTTTATTGA-3′

A wound was scratched on each plate with help of sterile pipette tip, and photographs were taken. The plates were then incubated at 37 ˚C for 24 h, and photographs were taken again.

Cell invasion assay

Transwell chambers containing polycarbonate membranes with a pore size of 8 µm were put into six-well plates. The lower Transwell compartment was coated with an I-type collagen (10 µg/mL) and subsequently dried. About 200 mL transfected BT-20 cells were inoculated in the Matrigel-coated upper compartment at a density of 1.5 × 10^5 cells/mL. The lower compartment was filled with 800 µL of media containing FBS (20 %). The entire system was subjected to incubation at 37 ºC for 24 h. Cells that invaded through the membrane with Matrigel were stained with crystal violet and photographed under an inverted microscope.

Western blot assay

Total protein was extracted from BT-20 cells via lysing with RIPA buffer (Thermo Scientific). The protein content of the cell lysate was determined using Bradford method. Equal amounts of protein were separated on SDS-PAGE and blotted onto PVDF membranes. The membranes were incubated with primary antibodies for Bax, Bcl-2 and β-actin, followed by incubation with horse radish peroxidase-conjugated secondary antibody. The bands of specific proteins were detected using enhanced chemiluminescence substrate (ECS), with human β-actin as the internal control.

Statistical analysis

All experiments were performed in triplicate. Data are presented as mean ± SD. Student's t-test and one-way ANOVA were used for statistical analyses. Values of $p < 0.05$ were taken as indicative of statistically significant difference.

RESULTS

Med19 was significantly upregulated in breast cancer cells

The expression of Med19 was determined in normal MB-157 cells and in SK-BR-3, UACC2087, MDA-MB-231 and BT-20 breast cancer cell lines. The results in Figure 1 show that Med19 was significantly upregulated in all the breast cancer cell lines (SK-BR-3, UACC2087, MDA-MB-231 and BT-20), relative to the normal MB-157 cells ($p < 0.05$). The up-regulation ranged from 3.7-fold in UACC-2087 cells, to 6.4-fold in BT-20 cells. The highest expression of Med19 was seen in BT-20 cells. Thus, BT-20 cells were selected for use in subsequent experimentation.

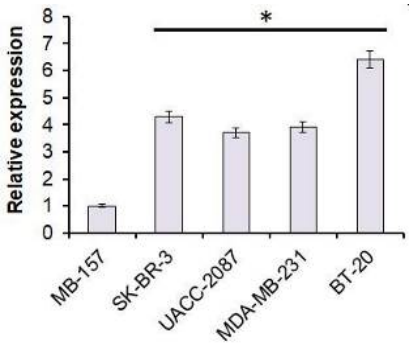

Figure 1: Expression of Med19 in normal MB-157 and breast cancer cell lines. The experiments were performed in triplicate. Data are expressed as mean ± SD; *$p < 0.05$

Med19 silencing inhibited proliferation of breast cancer cells

The effect of Med19 on the proliferation of BT-20 cells was determined by silencing its expression. It was found that silencing of Med19 in BT-20 resulted in significant ($p < 0.05$) decline in the viability of BT-20 cells (Figure 2B). These results point towards the role of Med19 in breast cancer cell proliferation.

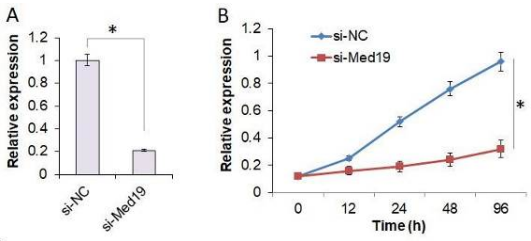

Figure 2: Effect of si-NC and si-Med19 transfections on Med19 expression. (A) Relative expressions of Med19 in si-NC and si-Med19-transfected BT-20 cells (B) Time-course profiles of expressions of Med19 in si-NC and si-Med19-transfected BT-20 cells. The experiments were performed in triplicate. Data are expressed as mean ± SD; *$p < 0.05$

Med19 silencing promoted apoptosis of BT-20 cells

The results showed that silencing of Med19 caused significant apoptotic alterations in the nuclear morphology of BT-20 cells (Figure 3 A). Annexin V/PI staining revealed 6 % apoptosis in si-NC-transfected BT-20 cells, and around 13.30 % apoptosis in si-Med19-transfected BT-20 cells (Figure 3 B). The Med19 silencing also caused suppression of Bcl-2 and upregulation of Bax,

further confirming apoptosis in BT-20 cells (Figure 4). Taken together, these results suggest that Med19 silencing promotes apoptosis in breast cancer cells.

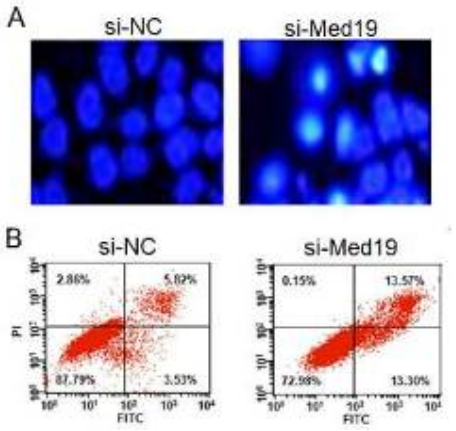

Figure 3: Effect of si-NC and si-Med19 transfections on apoptosis in BT-20 cells. (A) DAPI staining and (B) Annexin V/PI staining showing that silencing of Med19 induced apoptosis in BT-20 cells. The experiments were performed in triplicate

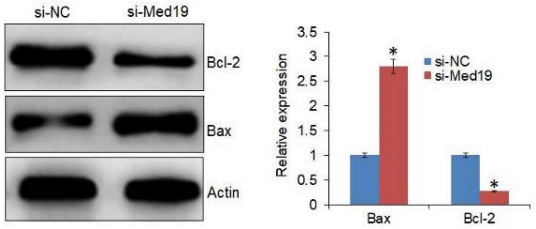

Figure 4: Effect of Med19 silencing on the expressions of Bax and Bcl-2 in BT-20 cells. The experiments were performed in triplicate. The results are expressed as mean ± SD; *p < 0.05

Med19 silencing suppressed the migration and invasion of BT-20 cells

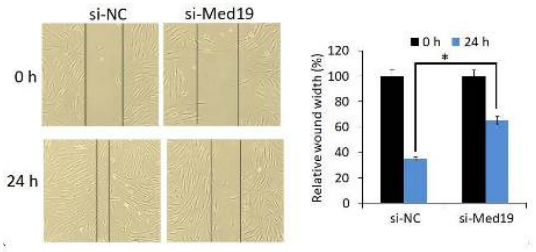

Figure 5: Effect of Med19 silencing on migration of BT-20 cells. Wound healing assay showed that Med19 silencing inhibited the migration of BT-20 cells. The experiments were performed in triplicate. The results are expressed as mean ± SD; *p < 0.05

The migration of the si-Med19-transfected cells was significantly inhibited, relative to si-NC transfected cells (Figure 5). The effect of Med19 silencing was also investigated on the invasion of BT-20 cells. The results showed that the invasion of the BT-20 cells was inhibited by 65 %, relative to control cells (Figure 6).

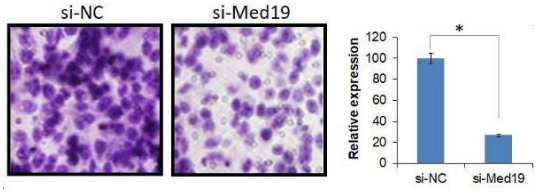

Figure 6: Transwell assay showing that Med19 silencing inhibited the invasion of BT-20 cells. The experiments were performed in triplicate. Results are expressed as mean ± SD (*p < 0.05)

DISCUSSION

Breast cancer results in high level of human mortality across the globe, and its incidence is increasing at an alarming rate [12]. Thus, there is need for development of efficient strategies for the management of breast cancer [12]. This study was undertaken to determine the role and therapeutic implications of Med19 in breast cancer. Studies have shown the involvement of Med19 in different cancer-related processes [7]. For instance, it has been shown to regulate the proliferation and migration of bladder cancer cells [13]. It has also been reported that Med19 is involved in tumorigenesis of lung cancer [14]. In yet another study, it was demonstrated that Med19 promoted the cellular growth of gastric cancer [15].

Furthermore, the role of Med19 in bone metastasis of bladder urothelial carcinoma cells has also been established [16]. In another study, silencing of Med19 caused significant decreases in the proliferation of human osteosarcoma cells via induction of cell cycle arrest [17]. It has been reported that Med19 regulated the chemosensitivity of the lung cancer cells to cisplatin [18].

The results obtained in the present study showed that the expression of Med19 was significantly downregulated in human breast cancer cells. This is consistent with previous findings where Med19 was shown to be significantly downregulated in breast cancer [19]. To gain insight into the role of Med19 in human breast cancer, the expression of Med19 was silenced in human breast cancer cells. The silencing of Med19 suppressed the viability of human breast cancer cells via activation of the apoptotic cell death. Apoptosis is an important process that allows for the elimination of cancer cells. It is

known that Bax and Bcl-2 proteins are important markers of apoptosis [20].

The results obtained in this study are consistent with those reported in a previous study where silencing of Med19 was shown to trigger apoptotic cell death in human laryngo-carcinoma cells [21]. Cell migration and invasion are critical steps in the metastasis of cancer cells [22]. This is in agreement with a previous study wherein Med19 were shown to suppress the migration and invasion of prostate cancer cells [23]. Taken together, the results of the present study point towards the therapeutic implications of Med19 in breast cancer.

CONCLUSION

The findings of the present study reveal that Med19 is significantly upregulated in human breast cancer cells. Silencing of Med19 suppresses the proliferation of breast cancer cells via induction of apoptosis. Moreover, Med19 silencing inhibits the metastasis of human breast cancer cells. Thus, Med19 may exhibit therapeutic implications for breast cancer. However, further studies are required to validate this conclusion.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The whole study was designed by Yanyan Dong. All of experiments are performed by Yanyan Dong and Yunxiao Liu. Liping Zhao, Chongxiao Qu collected data, and gave suggestions in designing this paper.

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