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MED27 promotes malignant behavior of cells by affecting Sp1 in breast cancer

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Abstract. – OBJECTIVE: The aim of this study was to investigate the expression of mediator complex subunit 27 (MED27) in breast cancer (BC) and explore its effects on the proliferation and apoptosis of BC cells.

PATIENTS AND METHODS: The expression of MED27 in 60 BC tissues and para-cancer tissues was detected. Based on the significantly high expression level of MED27 in tumors, the tumor samples were divided into high-expression group and low-expression group according to the median standard, with 30 samples in each group. Then, the association between MED27 expression and clinicopathological features of patients was analyzed. The correlation between MED27 expression and survival time of patients was estimated using the Kaplan-Meier method. Next, the expression level of MED27 in cells was also measured using qRT-PCR assay. *In vitro* study, si-MED27 was designed to interfere with the expression of MED27 in MDA-MB-231 cells. To further explore the mechanism of MED27 in BC, the expression level of SP1 in cells was examined after different treatments.

RESULTS: In quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay, MED27 was found to be highly expressed in both BC tissues and cells. Then, the relationship between MED27 expression and clinical pathological data was statistically analyzed, and it was found that MED27 expression was correlated with tumor size and grade. In the 60-month follow-up and Kaplan-Meier analysis, patients with high expression of MED27 had a poor prognosis. In in vitro study, MED27 expression in cells was down-regulated by transfection with si-MED27. Western blot (WB) analysis suggested that si-MED27 could effectively reduce the protein expression level of MED27 in cells, and the specificity protein 1 (Sp1) expression was also limited. In CCK-8, clone formation and flow cytometry experiments, the proliferation of cells with low MED27/Sp1 expression was suppressed, while cell apoptosis was promoted.

CONCLUSIONS: MED27 acted as an oncogene in BC. By affecting Sp1, MED27 could be a new therapeutic target for the treatment of BC.

Key Words:
Breast cancer (BC), Mediator complex subunit 27 (MED27), Specificity protein 1 (Sp1).

Introduction

Breast cancer (BC) is a tumor with the highest morbidity rate in women around the world[1]. According to the latest statistics from the International Agency for Research on Cancer (IARC), the morbidity rate of BC is high up to 22.9%, and its mortality rate is 13.7% among all the female malignant tumors in the world. What is worse, approximately 34% patients already have suffered from distant metastases when diagnosed, while the therapeutic effects of surgery, radiotherapy, chemotherapy, hormone therapy and targeted therapy on patients in the intermediate and advanced stage were far from satisfactory. Therefore, the 5-year survival rate of BC patients remained relatively low. Hence, further investigating the occurrence and development mechanisms of BC was of important significance to explore new therapeutic methods.

Mediator complex subunit (MED), one of the research hotspots in recent years, was widely distributed in eukaryotes and highly conserved in multiple species. It plays a critical bridging role in transcription process[2,3], has an extremely close correlation with ribonucleic acid (RNA) polymerase II (Pol II) and participates in almost all the transcription processes mediated by Pol II[4,5]. It could be observed under a high-resolution electron microscope that Pol II is tightly enclosed by MED[6]. In addition, MED is directly or indirectly associated with a majority of transcription factors, which may be either protein-protein interaction or functional interaction[7,8]. The roles of MED in the occurrence and development of tumors has gradually attracted attention over the past few years. It had been proven that some protein subunits of MED are im-

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portant players in the occurrence and development of malignant tumors. Under normal conditions, MED12 can exert regulatory effects on cell growth and differentiation, and it is abnormally expressed in intestinal cancer and prostate cancer[9,10]. Moreover, the deletion of MED12 can induce the resistance of liver cancer cells and melanoma cells to chemotherapy drugs[11]. Besides, investigations[12,13] have also been conducted for the functions of MED19 in several tumors, including gastric cancer and lung cancer. Research on the effects of MED27 on the occurrence and development of tumors is still in the infancy stage, and a recent investigation has manifested that MED27 is able to promote the proliferation and migration and inhibit the apoptosis of melanoma cells by acting on the PI3K/AKT/Bcl-2/Caspase-3 and C-raf/MEK/ERK signaling pathways[14]. However, there is still a lack of studies on the expression and relevant action mechanism of MED27 in BC, so this research was carried out to investigate the expression of MED27 in BC and explore its effects on cell proliferation and apoptosis.

Patients and Methods

A total of 60 cases of cancer tissue specimens and corresponding para-carcinoma normal tissue specimens were collected from patients with triple negative breast cancer (ER, PR, TNBC) in our hospital from December 2017 to July 2019, and the personal information and detailed clinical data of patients, including patient's gender, age, smoking history, tumor size, lymph node metastasis and pathological type, were also collected. All specimens were collected from diagnosed patients who received no radiotherapy and chemotherapy before operation. All tissue specimens were quickly cryopreserved in liquid nitrogen after resection. All of the 60 patients were followed up for general conditions, clinical symptoms and imaging by telephone and review after discharge from December 2017 to July 2019. This investigation was approved by the Ethics Committee of The Central Hospital of Lishui City. Signed written informed consents were obtained from all participants before the study. The patients were included based on the Guidelines proposed by the Union for International Cancer Control (UICC).

Cell Culture and Transfection

MDA-MB-231 cell line and normal breast epithelial MCF-10A cell line were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in the Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS, HyClone, South Logan, UT, USA) under 5% CO_2 at 37°C. Cells in the logarithmic growth period were used for transfection.

MDA-MB-231 cells were inoculated into a 6-well plate and the cell density was adjusted to 2×10^5/well, transfection was carried out according to the instructions of Lipofectamine™ 3000 reagent (Invitrogen, Carlsbad, CA, USA) when the fusion of cells reached about 70%.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted using TRIzol reagent method (Invitrogen, Carlsbad, CA, USA) and reversely transcribed into complementary DNA (cDNA). Then, quantitative polymerase chain reaction (qPCR) was performed to detect the mRNA expression level of MED27 with the cDNA obtained as the template. The data of qPCR were recorded as cycle threshold (Ct), and the experimental results were calculated by $2^{-\Delta\Delta Ct}$ method. Finally, the relative expression of MED27 in the samples in experimental group was quantified with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference. The sequences were shown in Table I.

Western Blot (WB) Analysis

At 48 h after transfection, the cells were lysed to extract proteins, which were added with loading buffer, boiled at 100°C for 10 min and separated *via* 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto a polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland). Later, the membrane was sealed in Tris-Buffered Saline and Tween-20 (TBST) containing 50 g/L skim milk at room temperature for 1 h. Subsequently, corresponding rabbit anti-human MED27, Specificity protein 1 (Sp1) and GAPDH antibodies were added for incubation at 4°C overnight. The next day, the membrane was washed with TBST for 3 times, incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG antibody at room temperature for 1 h and washed again in TBST for 3 times, followed by image fixation and color development using enhanced chemiluminescence (ECL) color development system. Finally, the expression of target proteins

Table I. Sequences of primers.

Gene	Primers sequences	
MED27	Forward primer	5'-ACCTCTTCCCCTAGAATCTG-3'
	Reverse primer	5'-TAGACCTCTTAATGGATGTT-3'
GAPDH	Forward primer	5'-AGTACCAGTCTGTTGCTGG-3'
	Reverse primer	5'-TAATAGACCCGGATGTCTGGT-3'

was determined with GAPDH as the internal reference.

Cell Proliferation

The cells in each group were seeded into a 96-well plate (4×10^3 cells/well). The day when the cells adhered to the wall was recorded as the 1st day, and the proliferative capacity of the cells was examined at 24, 48 72 and 96 h. Specifically, the cells in each well were added with 15 μL of cell counting kit-8 (CCK-8) solution (Dojindo Molecular Technologies, Kumamoto, Japan) at each time point and then cultured in an incubator for 4 h. Finally, the absorbance in each well was measured at 450 nm, and the growth curves were plotted.

Clone Formation

The cells were harvested at 48 h after transfection and then inoculated in a new 6-well plate (1,000 cells/well), with 4 replicate wells set for every group. 10 d later, the cells were fixed in absolute alcohol for 15 min, stained with 0.1% crystal violet for 10 min and washed in phosphate-buffered saline (PBS) for 5 min × 3 times, followed by photography and counting.

Cell Apoptosis

After transfection for 48 h, the cells were digested by 0.25% trypsin without EDTA (ethylenediaminetetraacetic acid), then collected and washed with phosphate-buffered saline (PBS) twice at 4°C. Next, the cells were resuspended in 100 μL of 1× binding buffer, added with 5 μL of fluorescein isothiocyanate (FITC) and 5 μL of propidium iodide (PI) in sequence and incubated in the dark at 4°C for 20 min. Finally, the cells were mixed with 400 μL of 1× binding buffer, so as to detect the apoptosis rate in each group using a flow cytometer (FACSCalibur, BD Biosciences, Detroit, MI, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The association of MED27 with clinical pathological data was analyzed by logistic regression analysis, Kaplan-Meier curve in survival analysis. $p<0.05$ for the difference was statistically significant.

Results

High MED27 Was Expressed in BC Tissues

The expression of MED27 in 60 BC tissues and para-normal tissues was detected, and it was found that the expression of MED27 in cancer tissues was much higher than that of para-cancer tissues, and the difference was statistically significant ($p<0.05$) (Figure 1A). Based on the significantly high expression level of MED27 in tumors, the tumor samples were divided into high expression group and low expression group according to the median standard, with 30 samples in each group. Then, the association between MED27 expression and clinicopathological features of patients was analyzed. As shown in Table II, the clinical features including patient's age, menopausal status, tumor's pathological type, size, grade, lymph node metastasis, distant metastasis and TMN stage were examined, and the MED27 expression was found to be associated with the tumor's size and grade, displaying statistically significant differences ($p<0.05$).

Expression of MED27 in BC Cells

The expression level of MED27 in cells was also measured using qRT-PCR assay, and it was found that MED27 showed an increased expression in MDA-MB-231 cells (Figure 1B). Together with clinical results, these data suggested that MED27 seemed to act as an oncogenic gene in BC, which was consistent with other reports.

Effect of MED27 on the Prognosis of Patients with BC

The correlation between MED27 expression and survival time of patients was estimated

using the Kaplan-Meier method. The results revealed that the patients with a high level of MED27 had a poor prognosis, their overall survival time was much shorter than that of patients with a low level of MED27, displaying statistically significant differences ($p<0.05$) (Figure 1C).

Detection of Transfection Efficiency

In *in vitro* study, si-MED27 was designed to interfere with the expression of MED27 in MDA-MB-231 cells. As shown in Figure 1D-1E, si-MED27 significantly decreased the protein expression of MED27 in cells.

MED27 on SP1 Expression

To further explore the mechanism of MED27 in BC, the expression level of SP1 in cells was examined after different treatments. According to the results of WB, the intracellular inhibition of MED27 could limit the protein expression of SP1. Therefore, it was considered that MED27 might participate in the occurrence and development of BC by affecting the expression of SP1 (Figure 1F).

MED27 on Cell Function

The acquisition of non-inhibitory proliferation is an important feature of cancer cells. In the experiments of this study, the proliferative capacity of MDA-MB-231 cells after transfection was detected by CCK8 and clone formation assay. As shown in Figure 2A-2D, when MED27 expression was decreased in cells, cell proliferation including OD value at 96 h and clone number were significantly attenuated. However, the proliferative capacity of MDA-MB-231 cells was not affected in si-NC group. The cell apoptotic abilities were detected by a flow cytometer. The apoptotic rate was significantly increased after the decrease of intracellular MED27 expression. (Figure 2E-2F).

Discussion

BC is not only the most common malignant tumor in women but also one of the most ubiquitous causes

Table II. Clinicopathological variables and expression of miR-301a in the study cohort.

Variables	No.	High MED27	Low MED27	*p*-value
Total	60	30	30	-
Age (year)				0.2668
>50	41	18	23	
≤50	19	12	7	
Menopausal status				0.1923
Pre	26	16	10	
Post	34	14	20	
Pathological type				0.5623
Non-invasive	11	4	7	
Early-invasive	27	15	12	
Invasive	22	11	11	
Tumor size (cm)				0.0149*
>5	21	15	6	
≤5	39	15	24	
Grade				0.0420*
1	24	9	15	
2	27	14	13	
3	9	7	2	
Lymph node metastasis				0.0668
Positive	25	16	9	
Negative	35	14	21	
Distant metastasis				0.1967
Positive	12	8	4	
Negative	48	22	26	
TNM stage				0.7596
I	22	9	13	
II	24	12	12	
III	14	9	5	

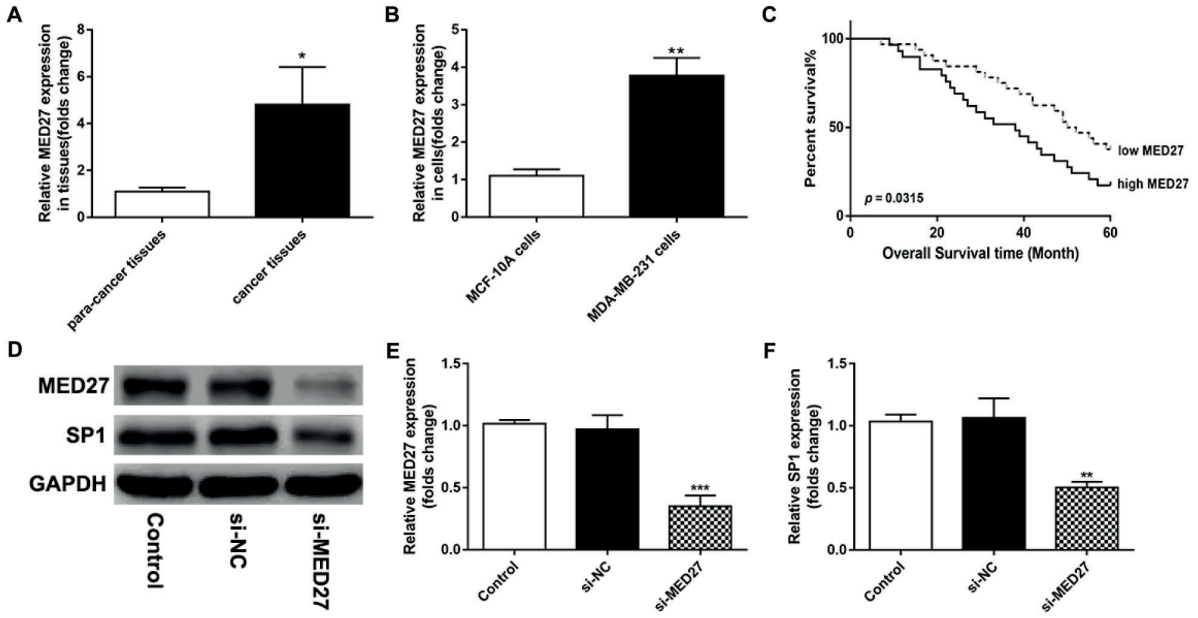

Figure 1. The expressions of MED27 in BC tissue samples (**A**) and BC cells (**B**). (*p<0.05, **p<0.01). **C,** The relationship of MED27 expression with overall survival of BC patients. **D-F,** The protein expressions of MED27 and Sp1 in BC cells after different treatment. (**p<0.01, ***p<0.001). Data were presented as means ± standard deviations.

of death from female malignant tumors. Besides, the mortality of BC remains persistently high in spite of constant improvement in treatment techniques.

Gene-targeted therapy was a novel treatment method developed in recent years, and aberrant amplification of oncogenes and inhibition of anti-oncogenes were generally present in various tumor cells including BC cells[15,16]. Meanwhile, abnormalities in the signaling pathways where such abnormal genes are located often lead to malignant behaviors, such as excessive proliferation and metastasis[17-19]. Therefore, suppressing the occurrence and development of tumors through intervening in these abnormal gene targets may become a new direction of BC treatment.

Human MED27 gene, a subunit of MED located in chromosome 9q34.13 with 219,802 bp in length, is extensively distributed in the cytoplasm and nucleus. As a co-activator in the nucleus, it was involved in the transcriptional regulation of nearly all the Pol II-dependent genes. Scholars[20-22] have demonstrated that MED27 plays vital roles in Pol II recruitment and activation, as well as assembly of preinitiation complex by interacting with a variety of trans-activators binding to deoxyribonucleic acid (DNA), revealing that MED27 occupies an important position in the transcriptional regulation of eukaryotic genes. Sp1 belongs to the sequence-specific DNA binding proteins and extensively exists in the nucleus of diverse tissues. The abnormal expression and activation of Sp1 in tumor tissues enable the tumor cells to obtain growth advantage, create an appropriate micro-environment for tumor growth and enhance the tumor proliferative and angiogenic capability, finally inducing the emergence and metastasis of tumor[23]. Therefore, MED27 can serve as a subunit of cofactor required for Sp1 activation complex to transcribe and activate Sp1, together with TFIID[24].

The results of this study were similar to those of other reports. MED27 was significantly higher expressed in tumor tissues than surrounding normal tissues. Further, BC patients with a high expression of MED27 had a worse prognosis than those with a low expression of MED27. In mechanism experiments, it was found that inhibition of MED27 in cells could reduce the malignancy behavior of BC cells, promote cell apoptosis and limit cell proliferation. Also, the expression of SP1 was affected with the decrease of MED27 expression.

Conclusions

The novelty of this study was that MED27 was firstly found to act as a cancer-promoting factor in BC. By targeting SP1, MED27 enhanced cell proliferation and inhibited cell apoptosis. Therefore,

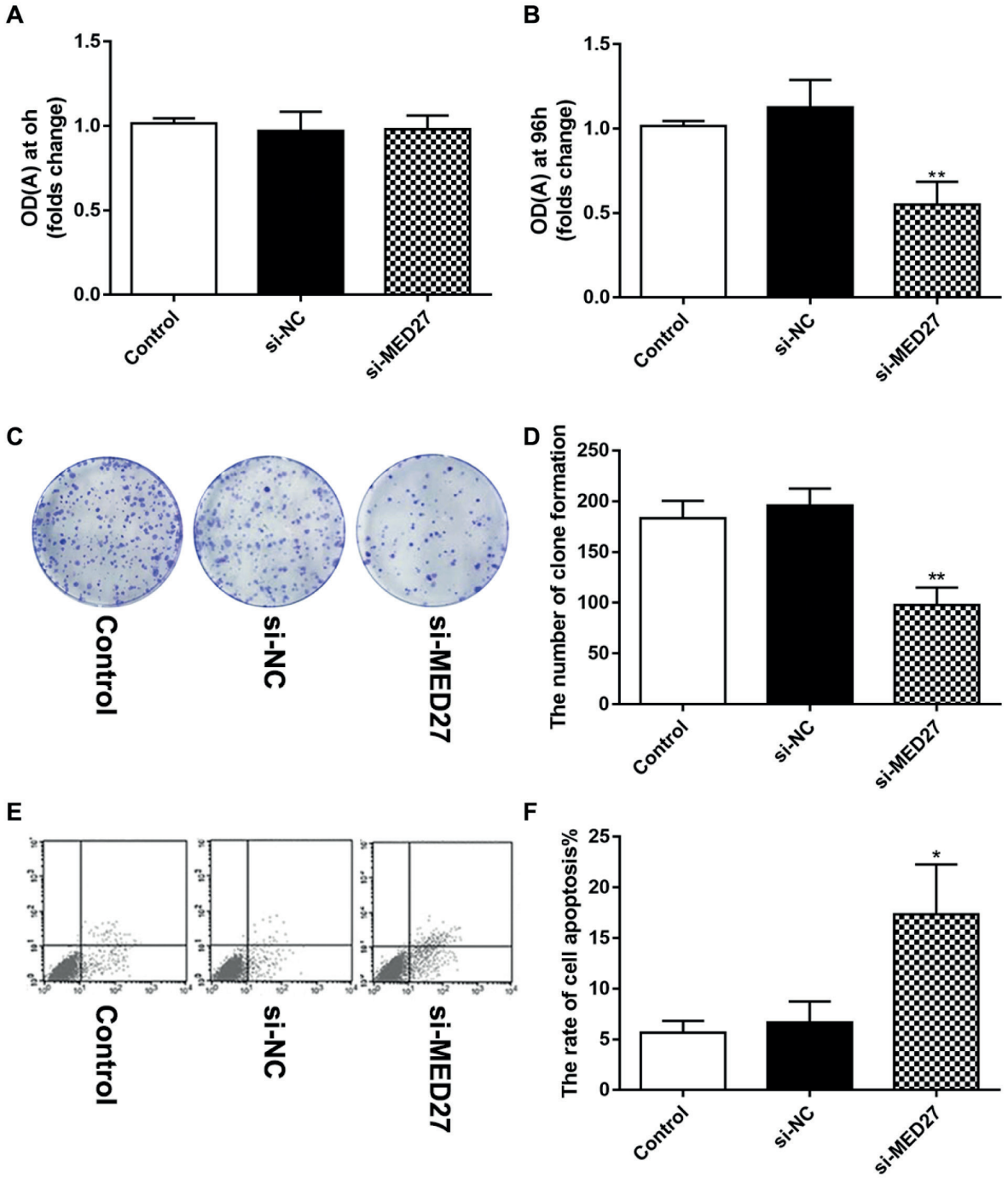

Figure 2. si-MED27 limited the cell proliferation from CCK8 (**A-B**) and clone formation (**C-D**) assay and promoted the apoptosis from flow cytometer (magnification: 40×) (**E-F**). Data were presented as means ± standard deviations. (*p<0.05, **p<0.01).

it was believed that MED27/SP1 could be a new target for BC treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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