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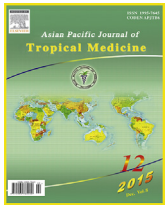

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TPX2 promotes migration and invasion of human breast cancer cells

Yong Yang[1], Da-Peng Li[2], Na Shen[3], Xiao-Cheng Yu[1], Jie-Bao Li[1], Qi Song[1], Jia-Heng Zhang[1*]

[1]*Department of Breast and Thyroid Surgery, Wuhan No. 1 Hospital, Wuhan, Hubei 430022, China*

[2]*Department of Head and Neck Surgery, Thyroid and Neck Oncology, Tianjin Medical University Cancer Institute and Hospital, Tianjin 300000, China*

[3]*Department of Breast and Thyroid Surgery, Union Hospital Affiliated to Huazhong University of Science and Technology, Wuhan, Hubei 430000, China*

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ABSTRACT

Objective: To investigate the expression of targeting protein for Xenopus kinesin-like protein 2 (TPX2) in breast cancer tissue and to explore its role in proliferation, migration and invasion of breast cancer cells.

Methods: The mRNA and protein expressions of TPX2 in breast cancer tissue and cell lines were assessed by quantitative RT-PCR and Western blot. The effect of TPX2 with RNA interference on proliferation, invasion and migration of breast cancer cells was observed by MTT and Transwell assays.

Results: Both mRNA and protein expressions of TPX2 were upregulated in breast cancer tissues compared to tumor-adjacent tissue. TPX2 expression was also upregulated in breast cancer cell lines, and the TPX2 interfered by small interfering RNA could inhibit the proliferation, invasion and migration of breast cancer cells by inhibiting matrix metalloproteinase-2 and matrix metalloproteinase-9.

Conclusions: Significantly upregulated TPX2 expression is observed in breast cancer tissue and cells, and contributes to promote the proliferation, migration and invasion of breast cancer cells.

1. Introduction

Breast cancer is the most common tumor in females [1]. Despite of development in sorts of advanced diagnostic equipment and standardized treatment guideline including surgery, radiotherapy, chemotherapy and hormonotherapy, the prognosis of breast cancer is still not ideal [2]. The main reason of lethality is the metasta of cancer cells [3,4]. Metasta is a multi-step process, including proliferation, migration, invasion, and clonal expansion by adherence to attachment point [5]. However, the specific regulation mechanism still remains unclear. Therefore, it is the key point in both discovering new therapeutic target and improving prognosis of breast cancer to search for new transferred molecular markers [6,7].

Targeting protein for Xenopus kinesin-like protein 2 (TPX2) is a prohibitin strictly regulated by cell cycle, with involvement in microtubule-associated proteins formed by spindle apparatus in mitosis, and was first found by Heidebrecht in 1997 [8]. TPX2 is a microtubule-associated protein strictly regulated by cell cycle which appears in G_1-S phase of the cell cycle and disappears after completement of mitosis [9]. In the phase of mitosis [10], TPX2 is released through Ras-related nuclear protein GTPase pathway and activated by phosphorylation of Aurora A; TPX2 and Aurora A are mutually combined during the spindle apparatus assembly. The structure destruction will result in error in spindle apparatus assembly and abnormality of chromosome will lead to incidence of tumor. There are also researches showing that TPX2 plays an important role in DNA damage stress and regulating γ-H2AX signaling pathway. In recent years, connection between TPX2 and incidence as well as development of malignant tumor is becoming the focus of researches [11]. More and more evidences show that the abnormal expression of TPX2 is related to the invasion and development of tumors. In many tumor tissues, abnormally high expression of TPX2 has been confirmed [12,13], such as

*Corresponding author: Jia-Heng Zhang, Bachelor Degree Physician, Department of Breast and Thyroid Surgery, Wuhan No. 1 Hospital, Wuhan, Hubei 430022, China.
Tel: +86 13995699198
E-mail: zjh2396@163.com

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colon cancer [14], esophagus cancer [15,16], bladder cancer [17], and liver cancer [18]. In addition, TPX2 is also confirmed to be a molecular marker for unfavorable prognosis in patients. Meanwhile, as a pro-cancer gene, TPX2 can upregulate expression of matrix metalloproteinases (MMP) family through activating PI3K/Akt pathway in colon cancer. The lately research shows that inhibiting the TPX2 expression through downregulating expressions of MMP2 and MMP9 can inhibit the invasion of liver cancer cells. However, the function of TPX2 in breast cancer is still unclear and its specific regulation mechanism in tumor development has not been well studied. The present study aims to investigate the expression of TPX2 in breast cancer tissue and cells and its function in the cell invasion and migration.

2. Materials and methods

2.1. Pathological tissues and cell lines

The female cases with no access to radiotherapy and chemotherapy before surgery were included in this study. The age range was between 29 and 73 years with the median age of 54.8 years. Without the knowledge of clinical data about pathologic specimens, two pathologists confirmed the specimens to be breast cancer ones through independent double-blind method. All the tissues were the fresh specimen tissues acquired after surgery, which were transferred to liquid nitrogen for further experiment. The consent was acquired from all the patients in the study.

Human breast cancer cell lines MCF7 and SKBR3 were cultured in dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco). Cell line MDA-MB-231 was cultured in L15 medium (Invitrogen) containing 10% FBS in an incubator with 5% CO_2 and kept at 37 °C. Cells at logarithmic phase were used for further experiment.

2.2. Quantitative RT-PCR

The total RNA in breast cancer tissue and cells was extracted by using TRIZOL kit (Invitrogen). The synthesis of cDNA was operated according to the specification of revertid first strand cDNA synthesis kit, namely, reverse transcription at 37 °C for 30 min, inactivation of reverse transcriptase at 94 °C for 2 min, with *β-actin* gene as the internal reference. The amplified reaction was conducted based on the specification of SYBR Premix Ex Taq™ II. The reaction conditions were as follows: 94 °C for 2 min, 94 °C for 30 s, 72 °C for 45 s, with 30 circulation of amplification; extension at 72 °C for 10 min at last. The results were analyzed with $2^{-\Delta\Delta Ct}$ to reduce deviation. Three repeated holes were set for each sample and the experiment was performed in triplicate. The primer sequence was as follows: *TPX2*: sense 5′-ACCTTGCCCTACTAAGATT-3′, antisense 5′-ATGTGGCACAGGTTGAGC-3′; *β-actin*: Sense 5′-GAGCTACGAGCTGCCTGACG-3′, Antisense 5′-CCTAGAAGCATTTGCGGTGG-3′.

2.3. Western blot assay

Total protein in liver cancer tissue and cells was extracted by using RIPA lysis buffer (high intensity) (Beyotime Biotechnology, Beijing, China). The concentration of protein sample was determined by bicinchoninic acid assay. A total of 60 μg protein was added to each sample and transferred to PVDF membrane after electrophoretic separation with 10% sodium dodecyl sulfate-polyacrylamide gelelectrophoresis. The 5% skimmed milk powder was used to seal the sample for 2 h, after which the 5% bovine serum albumin/phosphate buffered saline tween was added to dilute the TPX2 polyclonal antibody (Abcam, ab134309, 1:1000) and β-actin polyclonal antibody (Santa Cruz, sc-7210, 1:2000), and rabbit anti-MMP2 polyclonal antibody (sc-10736), rabbit anti-MMP9 polyclonal antibody (sc-10737), and kept at 4 °C overnight. Tris buffered saline (TBS) with tween (TBS, 0.1% Tween-20) was used to wash membrane for 3 times with 5 min for each time. The horse radish peroxidase marked second antibody (goat anti rabbit) was added for incubation at room temperature for 2 h. TBS with tween (TBS, 0.1% Tween-20) was used to wash membrane for 3 times with 5 min for each time and ECL chemiluminescence reagent was used for scotography.

2.4. Small interfering RNA (siRNA) transfection

The siRNA specific to TPX2 and control RNA were purchased from Santa Cruz Biotechnology (USA). Cells were inoculated in a 6-well plate (2×10^6/well). According to specification of lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), the siRNA transfected cells were filtrated to make the optimum concentration of 100 nmol/L. Cells at logarithmic phase were added into synthesized specific TPX2 siRNA and control siRNA respectively. After 48 h of culture, total RNA and protein were extracted and silencing effect was detected by using RT-PCR and Western blot assays.

2.5. MTT assay

Effect of TPX2 after siRNA interference on proliferation of breast cancer cells was detected by MTT assay (Sigma, St. Louis, MO, USA). The total volume of 200 μL cells were inoculated in a 96-well plate with 0.2×10^4 cells in each well. At each time point, 20 μL 5 mg/mL MTT was added to wells for incubation at 37 °C for 4 h, after which 150 μL dimethyl sulfoxide was added to vibrate for 10 min. The optical density was determined at 490 nm. All the experiments were conducted in triplicate.

2.6. Cell invasion and migration by transwell assay

According to specification of Costar Transwell, the Matrigel which was frozen at −20 °C was placed at 4 °C overnight to melt into liquid. The serum-free DMEM was added to dilute Matrigel into 1 mg/mL on ice. After complete blending, it was added to Transwell chamber with 100 μL for each well and was coated at 37 °C for 1 h. Serum-free culture solution was used to wash for 3 times and then kept at 37 °C for standby application. Cells at logarithmic phase were used to transfect siRNA, and after 24 h of culture, cells were collected and serum-free DMEM was used to resuspend cells to make the cell concentration of 2.5×10^5/mL. For the upper embedded culture chambers in the previously coated Matrigel (invasion experiment) or uncoated Matrigel (migration experiment), 100 μL cell suspension was added. For the bottom culture chamber, 600 μL DMEM with 10% FBS was added. Three repeated holes were set for each group. The

experiment was conducted in 5% CO_2 at 37 °C for 48 h. The liquid in chambers was ejected and cells on the internal surface of chamber bottom were wiped with cotton swab, fixed with paraformaldehyde, stained with crystal violet, and rinsed with phosphate buffered saline. The random 4 views were chosen for each culture well under the light microscope, and the number of cells in each view was counted.

2.7. Statistical analysis

Data were analyzed by using SPSS 18.0 software. Measurement data were expressed as mean ± SD, and differences with $P < 0.05$ were considered to be significantly statistical.

3. Results

3.1. Upregulated expression of TPX2 both in breast cancer tissue and cells

To study the expression of TPX2 in breast cancer tissue and cells, the mRNA and protein expressions of TPX2 were analyzed by using quantitative RT-PCR and Western blot assays. It was found that the mRNA transcription level and protein expression of TPX2 in breast cancer tissue were significantly higher than those in tumor-adjacent tissue (Figure 1). The mRNA and protein expressions of TPX2 were found to be significantly higher in breast cancer cell lines MDA-MB-231 and MCF7 than SKBR3 cells. All the results suggested that TPX2 may have the carcinogenesis in breast cancer.

3.2. Downregulation in TPX2 expression in breast cancer cells by siRNA

Previous researches showed that TPX2 could promote the formation and metasta of tumors. To study the role of TPX2 in breast cancer, specific TPX2 siRNA was used to transfect MDA-MB-231 and MCF7 cells, and interference efficiency was determined by quantitative RT-PCR and Western blot. As it can be seen in Figure 2, specific siRNA could successfully downregulate the expression of TPX2 in both MDA-MB-231 and MCF7 cells.

3.3. Inhibition of breast cancer cell proliferation by downregulated TPX2

To verify the role of TPX2 in breast cancer, the effect of downregulated TPX2 on proliferation of breast cancer cells was determined by MTT assay. It was found that the proliferation of breast cancer cells was significantly inhibited by TPX2 which was downregulated by siRNA (Figure 3).

3.4. Inhibition of breast cancer cell migration by downregulated TPX2

To verify the effect of TPX2 in breast cancer cell migration, the role of TPX2 downregulated by siRNA in breast cancer cell migration was investigated in Transwell migration experiment. It was found that downregulated TPX2 could inhibit the migration of tumor cells (Figure 4), which suggested that TPX2 may play an important role in migration of breast cancer cells. Staining of crystal violet showed the migration of MDA-MB-231 and MCF7 cells cultured for 48 h in Transwell chambers without Matrigel (100×).

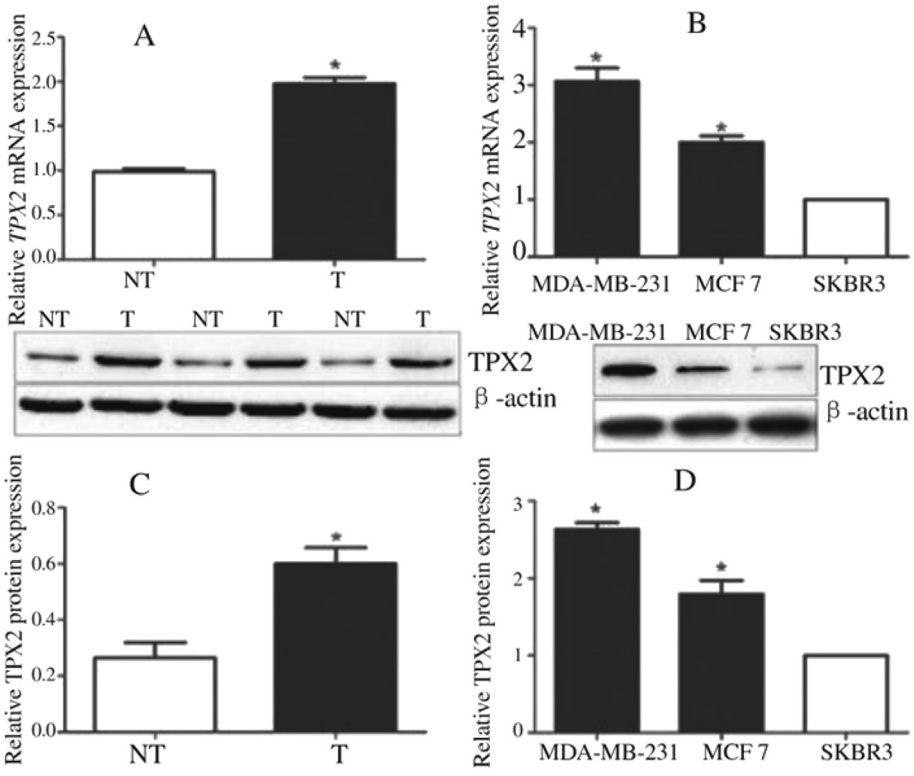

Figure 1. mRNA and protein expressions of TPX2 in breast cancer tissue and cell lines.
A: Relative *TPX2* mRNA expression in breast cancer tissue and tumor-adjacent tissue by RT-PCR; B: Relative *TPX2* mRNA expression in different breast cancer cell lines by RT-PCR; C: Relative TPX2 protein expression in breast cancer tissue and tumor-adjacent tissue by Western blot; D: Relative TPX2 protein expression in different breast cancer cell lines by Western blot; NT: Tumor-adjacent tissue; T: Breast cancer tissue; *: $P < 0.05$.

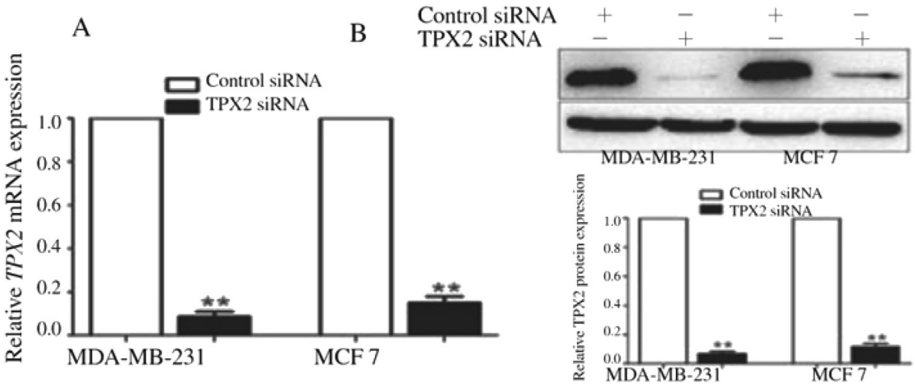

Figure 2. Downregulation in expression of TPX2 in MDA-MB-231 and MCF7 cells by specific TPX2 siRNA.
A: Interference effect of siRNA on *TPX2* in MDA-MB-231 and MCF7 cells by RT-PCR; B: Interference effect of siRNA on TPX2 in MDA-MB-231 and MCF7 cells by Western blot; **: $P < 0.05$.

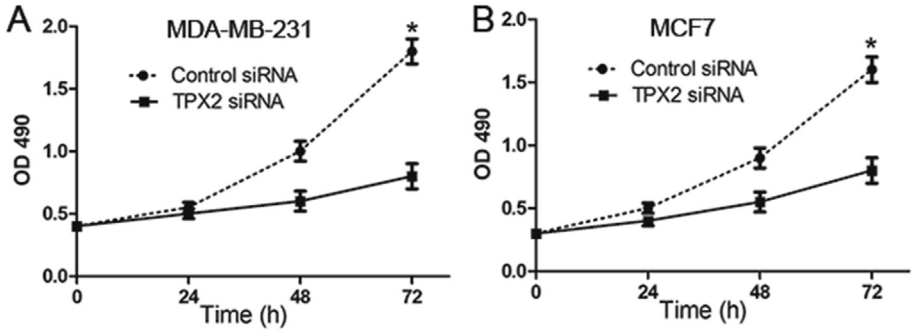

Figure 3. Effect of TPX2 downregulated by siRNA on proliferation of breast cancer cells.
A: Effects of downregulated TPX2 and control siRNA on proliferation of MDA-MB-231 cell by MTT assay; B: Effects of downregulated TPX2 and control siRNA on proliferation of MCF7 cell by MTT assay; *: $P < 0.05$.

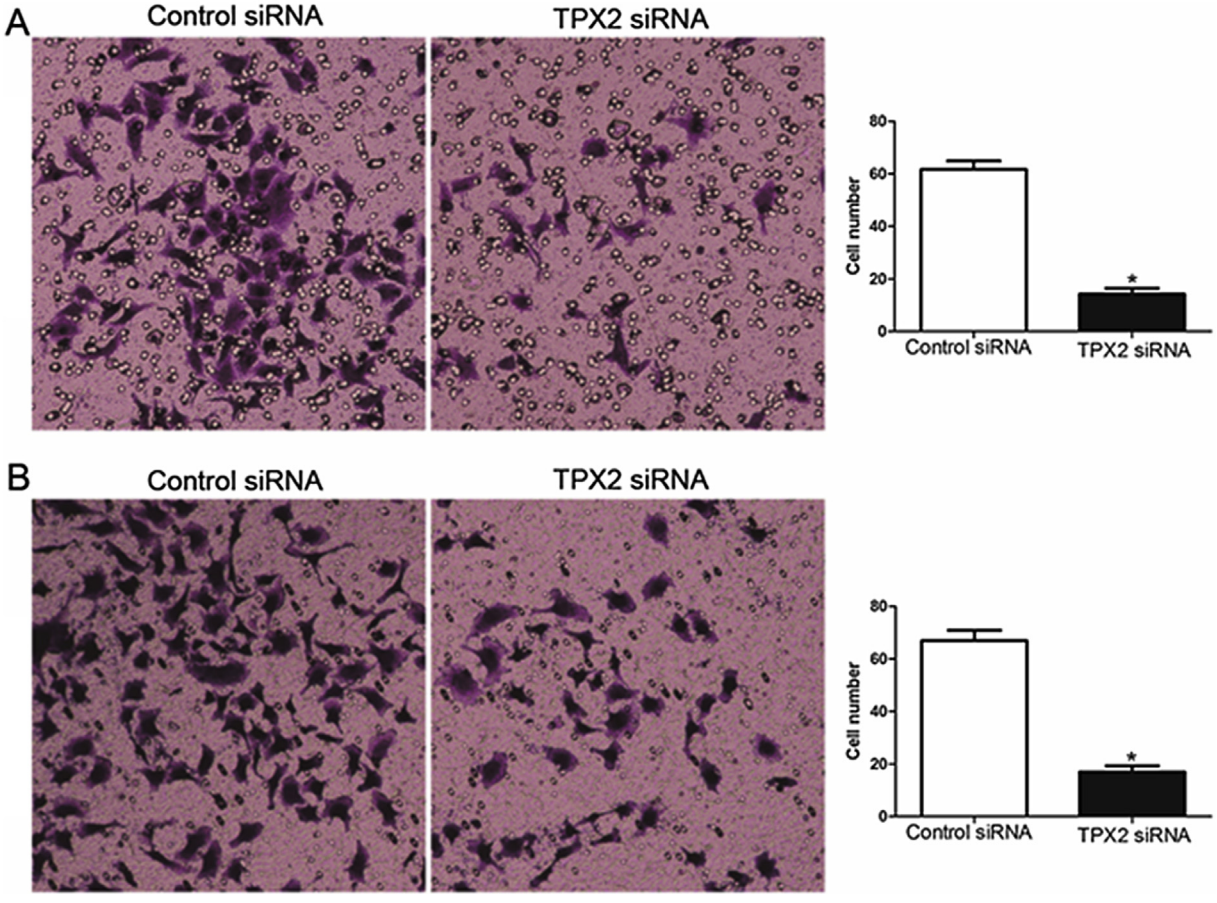

Figure 4. Effect of TPX2 downregulated by siRNA on breast cancer cell migration by Transwell assay.
A: MDA-MB-231 cell; B: MCF7 cell; *: $P < 0.05$.

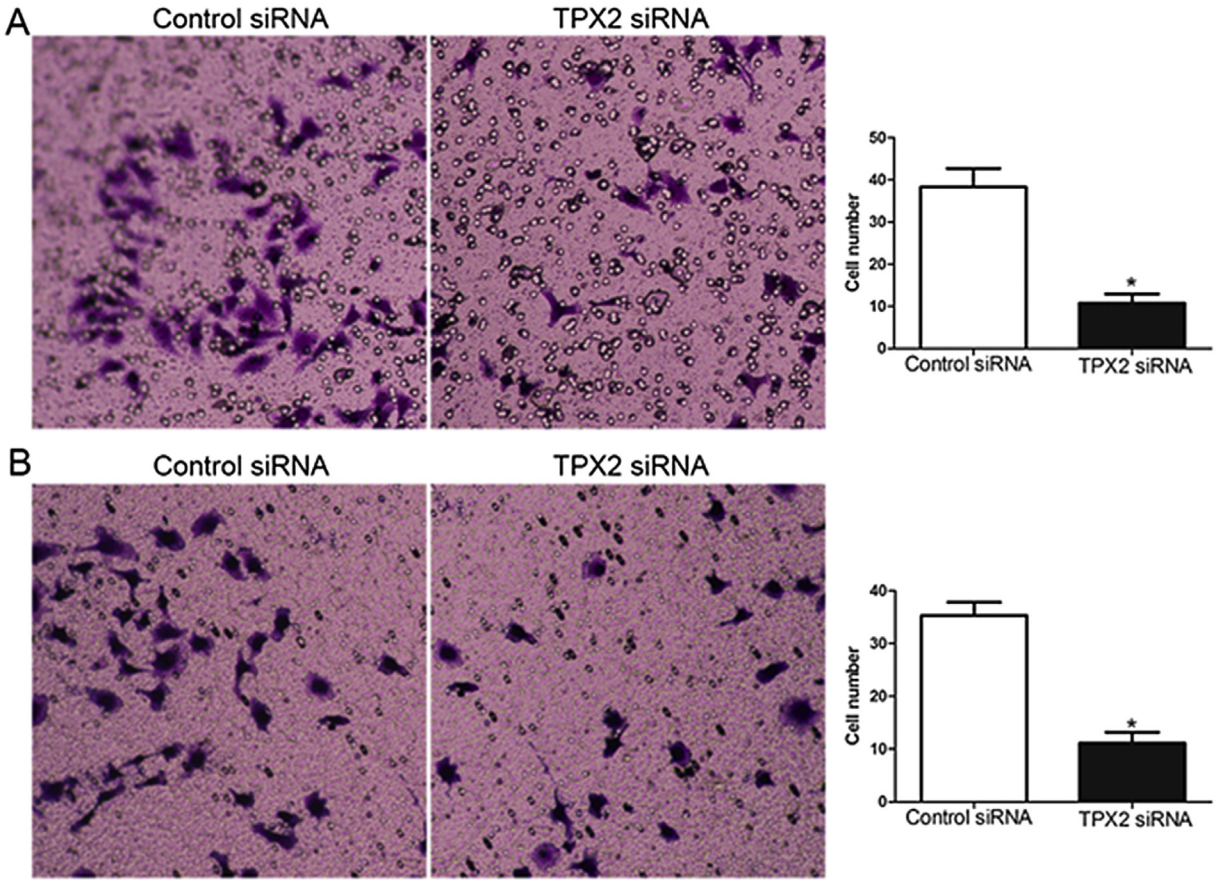

Figure 5. Effect of TPX2 downregulated by siRNA on breast cancer cell invasion by Transwell assay. A: MDA-MB-231 cell; B: MCF7 cell; *: $P < 0.05$.

3.5. Inhibition of breast cancer cell invasion by downregulated TPX2

To verify the role of TPX2 in the breast cancer cell invasion, the effect of TPX2 downregulated by siRNA on invasion of breast cancer cells was determined by using Transwell migration experiment. It was found that downregulated TPX2 could significantly inhibit the migration of breast cancer cells (Figure 5), suggesting that TPX2 may play a key role in invasion of breast cancer cells. Staining of crystal violet showed the invasion of MDA-MB-231 and MCF7 cells cultured for 48 h in Transwell chambers with Matrigel (100×).

3.6. Inhibition of MMP2 and MMP9 by downregulated TPX2

MMP plays a significant role in the migration and invasion of tumors, especially MMP2 and MMP9. To get to know more about the role of TPX2 in downstream signaling pathway of breast cancer cells, whether or not the expressions of MMP2 and MMP9 were affected by TPX2 downregulated by siRNA was determined. The results of Western blot showed that downregulated TPX2 could significantly decrease the expressions of MMP2 and MMP9 (Figure 6). In all, in the breast cancer cells, TPX2 can play a role in promoting proliferation, migration and invasion through MMP2 and MMP9.

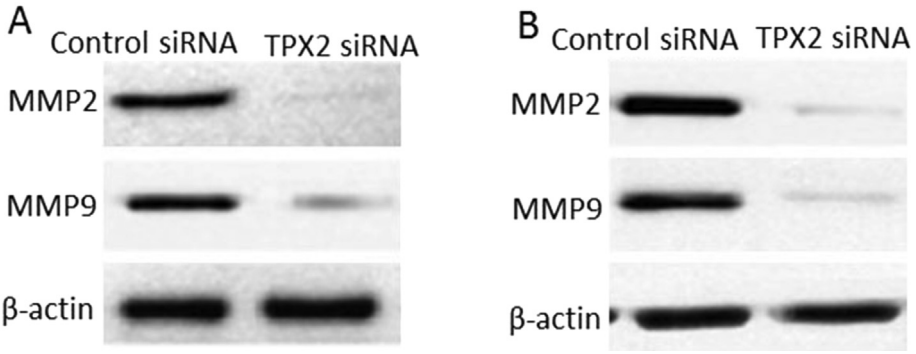

Figure 6. Expressions of MMP2 and MMP9 by Western blot. A: MDA-MB-231 cell; B: MCF7 cell; *: $P < 0.05$.

4. Discussion

Breast cancer is the major reason for death in female patients with cancer [19], and its relapse and migration are the major factors in affecting its prognosis. The main reason for unfavorable prognosis in breast cancer patients is due to the remote metasta of tumor [20,21]. The knowledge about molecular mechanism of incidence, development and remote metasta of breast cancer is the preferential choice in all kinds of treatments [22]. More and more evidences are showing that microtubule-associated protein TPX2 plays an important role in nuclear proliferation, cell cycle and formation of spindle apparatus in mitosis [23–25]. Through mitosis, TPX2 and its downstream molecules interact with each other and locate in microtubule-associated proteins formed by spindle apparatus of Aurora A in mitosis so that to activate the Aurora A kinase [26,27]. Therefore, TPX2 has been regarded as the oncogene in many human tumors. However, role of TPX2 in breast cancer has not yet been reported.

Researches show that TPX2 can promote the formation, incidence and development of tumors as well as the growth and remote metasta [28]. Liu *et al*. reported that downregulated TPX2 can inhibit the expressions of MMP2 and MMP9 through inhibiting the activation of Akt so as to inhibit the metasta and invasion of liver cancer cells [29]. The present study was the first to explore the expression of TPX2 in the breast cancer tissue and tumor-adjacent tissue with results showing that mRNA and protein expressions of TPX2 were significantly higher in the breast cancer tissue than those in tumor-adjacent tissue, and that the expressions in different breast cancer cell lines were also obviously elevated. Downregulated TPX2 can significantly inhibit the proliferation, migration and invasion of breast cancer cells. Furthermore, it is found in the present study that downregulation of TPX2 can lead to the decrease in expressions of MMP2 and MMP9. In all, these data show that upregulation of TPX2 in breast cancer tissue and its effect in promoting proliferation, migration and invasion of breast cancer cells through upregulating the MMP2 and MMP9 expressions [30].

In conclusion, the results of present study show that TPX2 expression is obviously upregulated in breast cancer and that TPX2 downregulated by siRNA can inhibit the proliferation, invasion and migration of breast cancer cells through inhibiting expressions of MMP2 and MMP9. Nevertheless, the connection between TPX2 and prognosis, and specific molecular mechanism still need further research.

Conflict of interest statement

We declare that we have no conflict of interest.

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