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Effect of survivin siRNA on biological behaviour of breast cancer MCF7 cells

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ABSTRACT

Objective: To investigate the expression of survivin in breast cancer cell lines and explore the effect of survivin siRNA on biology behavior of breast cancer cells. **Methods:** Western blot was performed to detect the expression of survivin in breast cancer cell lines. Eukaryotic expression vector pIRES2-EGFP-Survivin siRNA was constructed and transfected in MCF7 cells with liposome, the efficiency of survivin siRNA was measured by Western blot and RT-PCR. Cell proliferation and apoptosis were detected by CCK8 and cell flow respectively. Cell migration and invasion was measured by transwell assay. **Results:** Survivin was highly expressed in MCF-7. Green fluorescence was found in MCF-7 cells tranfected with survivin siRNA and control siRNA by inverted fluorescence microscopy, the protein and mRNA level of survivin was significantly lower in cells tranfected with survivin siRNA compared with control group. Compared with control group, interfering the expression of survivin by siRNA significantly decreased the proliferation, migration and invasion of MCF-7 cells, the percentage of apoptosis cells was greatly promoted. **Conclusions:** Interfering the expression of Survivin can inhibit the cell proliferation, migration and invasion, and promot apoptosis in MCF-7.

1. Introduction

Breast cancer is a malignant tumor occurred in the mammary gland epithelial tissue, its clinical manifestations is associated with the stage of tumor. The incidence of breast cancer is increasing year by year, and the age tends to young women, which brings a serious threat to women's physical and mental health. Breast cancer is characterized with high risk of recurrence and metastasis, despite the surgery, radiotherapy, chemotherapy and endocrine treatment have certain efficiency for early breast cancer, the prognosis remains poor[1–3]. Therefore, it is very urgent to find a new specific target molecule for breast cancer therapy. In recent years, gene therapy has attracted growing attention for breast cancer [4,5], such as survivin. As a member of the inhibitor of apoptosis (IAP) gene family, survivin is high expressed during fetal development and in most tumors, plays an important role in cell proliferation and apoptosis.

In the present study, we constructed a survivin siRNA eukaryotic expression vector and transfected to breast cancer cell line MCF-7 to investigate the interfering efficiency and the influence of survivin expression on the biological characteristics of breast cancer cells.

2. Materials and methods

2.1. Cell culture and transfection

MCF-7 cells were provided by Cancer Hospital, Chinese Academy of Sciences and cultured in RPMI 1640 medium (Sigma, U.S.A.) supplemented with 10% fetal bovine serum (Gibco, U.S.A.), 100 units/mL penicillin G (Sigma, U.S.A.) and 100 μ g/mL streptomycin (Sigma, U.S.A.). Cells were incubated in a humidified atmosphere with 5% CO_2 at 37 ℃. MCF-7 cells were transfected with pIRES2-EGFP-survivin siRNA plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The expression of survivin was detected 48 h after transfection. pIRES2-EGFP-survivin siRNA plasmids was designed and synthesized by Sangon Biotech.

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2.2. Western blot

MCF-7 cells were washed with cold PBS and collected in cell lysate buffer, then centrifuged in a micro centrifuge at 4 ℃ for 30 min to collect the supernatant. Protein concentrations were detected with the NanoDrop (Thermo). The cell lysate was boiled for 5 min in 1×SDS sample buffer and subjected to SDS-polyacrylamide gel electrophoresis (PAGE), then transferred to a polyvinylidene difluoride membrane (Millipore) by a transfer apparatus at 280 mA for 2 h. After blocked with 5 % nonfat milk at room temperature for 3 h, the membranes were incubated with the primary antibody against survivin (anti-rabbit, 1:1 000; Abcam), or glyceraldehyde-3-phosphate dehydrogenase GAPDH (anti-rabbit, 1:1 000; Santa Cruz) at 4 ℃ overnight. After incubating with the horseradish peroxidase-conjugated secondary antibody (HRP Goat to rabbit; Santa Cruz), the protein was visualized using ECL (Thermo Pierce).

2.3. Realtime−PCR

The mRNA expression of survivin was determined by real-time PCR by SYBER Green Master Mix (Takara, Dalian, China) and detected with Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, USA). Total RNA was isolated from cells using the Trizol reagent (Life Technologies) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from total RNA with reverse transcription Kit (Fermentas Life Sciences). Primer pairs for survivin: sense, 5'-GCATGGGTCCCCCGACGTTG-3'; antisense, 5'-GCTCCGGCCAGAGGCCTCAA-3'. β-actin was amplified using the following primer: sense, 5'-CGTGAAAAGACCCAGATCA-3'; antisense, 5'-CACAGCCTGGATGGCTACGT-3'. The mRNA expression of survivin was normalized versus β-actin mRNA. The C_t value was quantified with the $2^{-\Delta\Delta Ct}$ method.

2.4. Cell proliferation detection

The MCF-7 cells were diluted into single cell suspensions and seeded in 96-well plates (1×10^6 cells/well) with 100 μL 1% FBS medium, replaced with 10% FBS medium 24 hours later. Then, 10 μL CCK8 solution was added into each well on 0 h, 2 h, 6 h, 12 h, 24 h and 48 h. Air bubbles should be avoided producing during this process. The plates were incubated for 1-4 hours in the incubator. The absorbance was measured at 450 nm using a microplate reader. CCK-8 Kit was purchased from DOJINDO Company (Japan).

2.5. Apoptosis assay

FITC Annexin V Apoptosis Detection Kit (BD Biosciences, USA) was employed to detect apoptosis of MCF-7 cells. The manual of the kit was strictly followed. Briefly, cells were collected and washed twice with cold PBS, and resuspended in binding buffer (1×10^6 cells/mL). Then, 100 μL of MCF-7 cells was transferred to a new tube, 5 μL of FITC-conjugated Annexin V was added in and incubated for 15 min at room temperature in the dark. 5 μL of propidium iodide was added without wash. The stained MCF-7 cells were diluted by the binding buffer and directly analyzed by FACSCalibur (BD Biosciences, USA) and analysis was performed with FlowJo (Treestar, Ashland, OR).Viable cells and apoptotic cells were defined as annexin Ⅴ and PI double-negative and double-positive cells

respectively.

2.6. Cell migration and invasion assays

The potential for migration and invasion of MCF-7 cells transfected with pIRES2-EGFP-survivin siRNA were evaluated by a transwell assay. In the migration assay, 5×10^4 cells were cultured in 200 mL DMEM with 1% bovine serum albumin in the upper chamber of a non-coated transwell insert. In the lower chamber, 600 mL DMEM with 10% FBS was used as a chemo-attractant to encourage cell migration. In the invasion assay, the upper chamber of the transwell inserts were coated with 50 μL of 2.0 mg/mL Matrigel, and 5×10^4 cells were plated in the upper chamber of the Matrigel-coated transwell insert. After 24 h incubation, top (nonmigrated) cells were removed, and bottom (migrated) cells were fixed and stained with crystal violet and counted under an inverted microscope. We selected five random views to count the cells and the independent experiments were repeated three times.

2.7. Statistical analysis

The data were expressed as mean±SD of 3 independent experiments and analyzed with SAS9.1.3 statistical software. The statistical significance of differences between groups was determined by one-way analysis of variance followed by Tukey's *post hoc* multiple comparison tests. $P<0.05$ was considered significant.

3. Results

3.1. Expression of survivin in breast cancer cell lines

Real time PCR was performed to investigate the expression of survivin in different breast cancer cell lines. High expression of survivin was found in MCF-7 cells, while survivin expressed relatively lower in MDA-MB-231, MDA-MB435, MDA-MB-468 and SKBR3 cells (Figure 1).
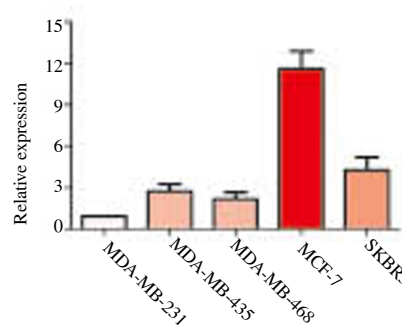

Figure 1. Expression of survivin in different breast cancer cell lines.

3.2. Efficiency of survivin siRNA in MCF−7 cells

MCF-7 cells were transfected by survivin siRNA and control siRNA, and green fluorescence protein marker was observed by inverted fluorescence microscope at 48 h after transfection (Figure

3A, B). Real time PCR and Western blot results revealed that the mRNA level (Figure 3C) and protein level of survivin in MCF-7 cells transfected with survivin siRNA (Figure 3D) was dropped greatly, when compared with the control group. The expression of survivin in MCF-7 cells was effectively interfered by specific siRNA.
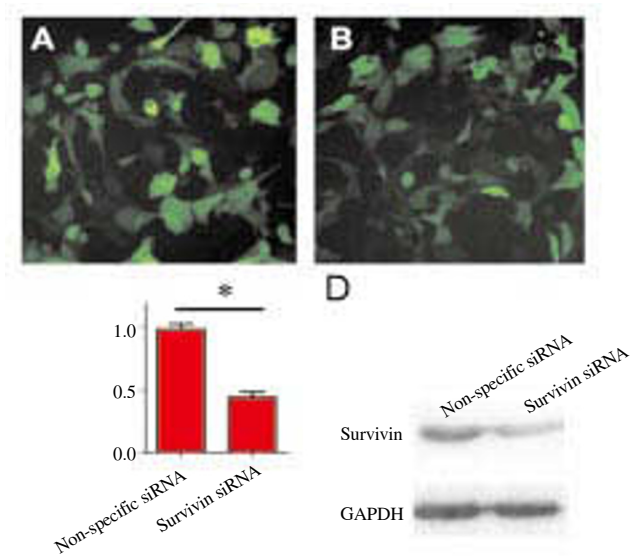

Figure 2. Transfection efficiency of survivin siRNA in MCF-7 cells.
Green fluorescence was found in MCF-7 cells 48 h after transfected with Non-specific siRNA (A) and survivin siRNA (B); the mRNA (C) and protein (D) expression of survivin was detected by real time PCR and Western blot respectively.

3.3. Change of MCF−7 cells proliferation and apoptosis after transfected with survivin siRNA

After transfected with non-specific siRNA and survivin siRNA, CCK8 and flow cytometry were adopted to detect proliferation and apoptosis of MCF-7 cells respectively. Results showed that cell proliferation had no significant difference between the non-specific siRNA transfected MCF-7 cell and control group. However, proliferation of survivin siRNA transfected cells was significantly suppressed (Figure 3). Annexin and PI staining was detected by flow cytometry, we found that the proportion of both early apoptosis (Annexin positive and PI negative) and late apoptosis (Annexin and PI double positive) in survivin siRNA transfected MCF-7 cells was significantly higher than control group (Figure 4).
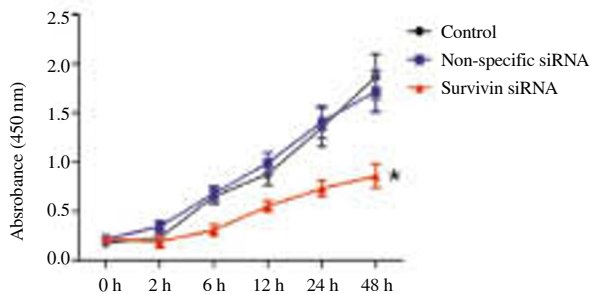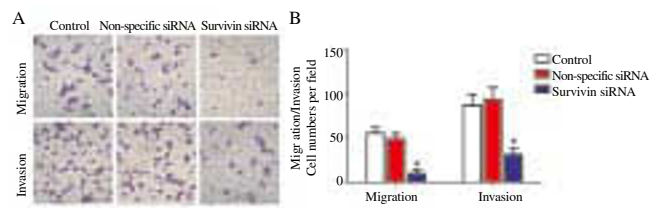

Figure 3. Effect of survivin siRNA on cell proliferation.
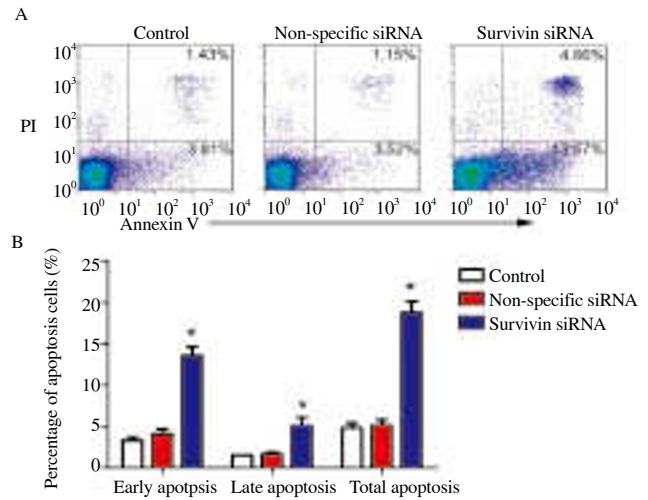
Figure 4. Effect of survivin siRNA on cell apoptosis.
(A)Cell apoptosis was detected by Annexin and PI staining after transfection;
(B)Cells in different stage of apoptosis was counted, $*P < 0.05$.

3.4. Effect of survivin siRNA on MCF−7 cells migration and invasion

Compared with control group, cells migration and invasion did not change after tranfected with non-specific siRNA, but greatly inhibited after the expression of survivin was interfered (Figure 5A). The number of cells got through membrane were significantly decreased in non-coated and matrigel-coated tranwell assays (Figure 5B).

Figure 5. Effect of survivin siRNA on cell migration and invasion of MCF-7.
(A) Cell migration and invasion was measured by transwell assay. (B) The number of migration and invasion cells, compared with control group, $*P < 0.05$.

4. Discussion

Eventually clearance of tumor cells is depends on the specific immune response, however, the immunogenicity of tumor antigen on tumor cells is always weak, which cannot effectively induce antitumor immune response[6]. Apoptosis is a tightly regulated programmed cell death which involves a series of biochemical events leading to specific cell morphology characteristics and ultimately death of cells and always caused by the change of internal and external environment, cell death signals also could be triggered by genetic regulation. It is widely believed that apoptosis plays a negative regulatory role in the occurrence and development process

of tumor, suppress the growth of tumor which was strictly regulated by pro-apoptosis and anti-apoptosis factors.The occurrence of breast cancer is closely related to abnormal cellular proliferation and apoptosis. Abnormal expression of proliferation and apoptosis related gene is one of the crucial factors in the process of tumor development[7,8].

Survivin is a powerful apoptosis inhibitor in the IAP family, performing its function by inhibiting the activity of caspase3 and caspase7 or block the inhibition of SMAC/Biablo to XIAP. Over-expression of survivin can suppress the process of cell apoptosis, resulting in abnormal proliferation. Survivin almost has no expression in normal tissues, but highly expressed in a variety of tumor cells, and was positively correlated[9–11] with prognosis of cancer. This study also found that survivin was expressed in breast cancer cell line MDA-MB-231, MDA-MB435, MDA-MB-468, MCF-7 and SKBR3 were expressed, especially in MCF-7, which was consistent with domestic and foreign researches[12].

Small interfering RNA (siRNA) interferes with the expression of specific genes by targeting homologous mRNA intracellular and causing degradation of mRNA before translation. RNAi interference is a simple and effective alternative gene knockout technology with the advantages of high stability, strong inhibition, and has been an ideal tool for enzyme gene therapy better than antisense nucleic acid and ribozyme gene therapy[13,14]. Xu[15] established survivin adenoviral siRNA vectors and transfected in hepatoma cells HepG2, survivin mRNA and protein expression was significantly inhibited, cellular apoptosis was found to be strongly promoted.

Survivin siRNA1 and survivin siRNA2 was applied in therapy of osteosarcoma cells MG-63 by Zhao QH and his colleagues[16], they found apoptosis of tumor cells was significantly increased. Similar results were observed in the present study, survivin siRNA can inhibit cell proliferation and induce apoptosis in the breast cancer cell line MCF-7. In addition, we also found that survivin siRNA resulted in the inhibition of migration and invasion of MCF-7 cells. These findings indicated that inhibition the expression of survivin might be a potential target for cancer genetic therapy.

Above all, the basically cause of tumor is the losing of normal precise control of cell-cycle at the gene level. In the present study, we found survivin was highly expressed in breast cancer cell line MCF-7, and the silencing of survivin promoted tumor cell apoptosis, inhibited tumor cell migration and invasion. Therefore, we speculate gene therapy combined with traditional surgery might get better therapeutic effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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