Effect of RNAi-Mediated Gene Silencing of Livin on Apoptosis of Human Breast Cancer Cell Line ZR-75-30

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ABSTRACT

Objective: To explore the effects of Livin gene knock down using sequence-specific siRNA on apoptosis of human breast cancer cell ZR-75-30. **Methods:** Chemically synthesized double stranded RNA(dsRNA) targeting Livin was transfected into human breast cancer cell ZR-75-30 with lipofectamine^{TM}2000. The transfection efficiency was observed under a fluorescence confocal microscope. Expression of Livin at both mRNA and protein levels were detected by reverse transcription-polymerase chain reaction(RT-PCR) and immunohistochemical analysis. The effects on apoptosis of ZR-75-30 cells were assessed by FCAS. **Results:** The Livin siRNA can effectively and specifically inhibited the expression of Livin gene in ZR-75-30. The inhibition rate was 53.66% at mRNA level and 58.32% at protein level. After 24h, (8.36±0.20)%cells transfected with siRNA were induced to apoptosis. **Conclusion:** Chemically synthesized short Livin-siRNA can effectively inhibit Livin over expression and remarkably induce apoptosis in human breast cancer cell line ZR-75-30. Livin RNAi has a potential value in gene therapy of breast cancer.

Keywords: Livin; Breast cancer; RNA interference; Apoptosis

Breast cancer is the leading cause of cancer death in women worldwide. Despite advances in diagnosis and chemotherapy of breast cancer, still many women with breast cancer die of this malignancy. In recent years, it has become increasingly clear that apoptosis deficiency is a major cause of the therapeutic resistance of solid tumors, since many of the anticancer agents used in the clinic act through induction of apoptosis[1]. Livin is a novel member of the inhibitors of apoptosis protein(IAP) gene family, which encodes negative regulatory proteins that prevent cell apoptosis[2-5].

Livin is selectively expressed in most human neoplasms and possibly involved in tumor cell resistance to chemotherapeutic agents and radiotherapy. There is evidence that Livin gene expression was typically detected in breast cancerous tissues, but not, or at substantially lower levels, in the adjacent normal tissues[6]. Furthermore, a substantial fraction of patients with breast cancer develop auto-antibodies to Livin[7].

RNA interference(RNAi) is an evolutionarily conserved, post-transcriptional gene silencing mechanism in which small interfering (21-25 mucleotides) double stranded RNA(siRNA) mediates sequence-specific degradation of mRNA[8,9]. Since it was demonstrated that siRNA can trigger RNAi to silence target genes in mammalian cells, there has been intensive interest to develop the technique into therapeutics. In the present study, we employed siRNA targeting Livin to silence Livin gene expression in breast cancer cell ZR-75-30, and explored the effects on apoptosis of human breast cancer cell ZR-75-30, in order to provide a theoretical basis for breast cancer gene therapy.

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MATERIALS AND METHODS

siRNA

Stealth™ siRNA duplex oligoribonucleotides were synthesized by Invitrogen. (AF 311388_stealth_649). Cells were treated in parallel with negative control siRNA, and blank control. Negative control FAM-siRNA and negative control siRNA (sence, 5'-UUCUCCGAACGUGUCACGUTT-3'; antisense, 5'-ACGUGACACGUUCGGAGAATT-3') were obtained from Shanghai GenePharma Co.

Cell Lines and Culture

The human breast cancer cell line ZR-75-30 was obtained from Cell Bank of Shanghai Biological Institute, Chinese Academy of Science. The cells were routinely maintained in RPMI1640 (Cat. No. SH30809. 01B, Hyclone) with 10% fetal bovine serum in a well humidified atmosphere of 5%CO_2 at 37℃. The medium was changed once every three days and subcultured when confluence was reached.

Transfection of Cells

One day before transfection, human breast cancer cells ZR-75-30 were seeded into a 6-well plate at 4×10^5 cells per well. When they were 40%-60% confluent, transfection was performed with Lipofectamine™2000 (Invitrogen) in serum-free OPTI- MEMI (Invitrogen) according to the instruction of the manual. In short, 12.5μl Lipofectamine™2000 and 250pmol of siRNAs were diluted in OPTI-MEMI and mixed in a final volume of 500μl transfection solution. Cells were transfected in medium without fetal bovine serum and the medium was changed 4h later.

RT-PCR for Livin mRNA

Twenty-four hours after transfection, total RNA was isolated from the cultured cells by Trizol Reagent (Invitrogen) according to the manufacturer' instructions. RNA concentration and quality were assessed spectrophotometrically at wavelengths 260 and 280nm. Reverse transcription was performed using one step RT-PCR kit (Promega, USA). Equal amount of RNA was used as templates in each reaction. The primers of human Livin were 5'-GTTCCCCAGCTGT CAGTTC-3' (forward primer) and 5'-CGTCTTCCGG TTCTTCCCA-3' (reverse primer). As a housekeeping gene, the primers used for GAPDH amplification were 5'-GAAGGTGAAGG TCGGAGTC-3' (Forward primer) and 5'-GAAGATG GTGATGGGATTTC-3' (reverse primer). Thermal cycle conditions were as follows: 48℃ for 45 min, 94℃ for 2 min, followed by 35 cycles of 94℃ 30 s, 52℃ 1 min (56℃ 1 min for GAPDH), 68℃ 1 min, with a final extension at 68℃ for 7 min. The PCR products were separated by 2% agarose gel electrophoresis. For the semi-quantification, an image of the gel was captured, and the intensity of the bands was quantitated using Chemi Imager 5500 (USA). The inhibition ratio of Livin expression was calculated according to the following formula: inhibition ratio of Livin expression= (1-the expression intensity of Livin in the observation group/the expression intensity of Livin in the blank control group)/transfection efficiency ×100%.

Immunohistochemical Analysis of Livin Protein

The cover slips were put into a 6-well plate. ZR-75-30 cells were seeded at 4×10^5 cells per well. When they were 40%-60% confluent, transfection was performed with Lipofectamine™2000, negative control siRNA and Livin-siRNA. Twenty-four hours after transfection, the cover slips were taken out from the culture plate and fixed with 4% Paraformaldehyde for 90 min at room temperature. SABC routine immuno- histochemical method was used to detect the expression of Livin protein. A petroline section of breast cancer expressing Livin was used as a positive control. PBS was used to replace the Livin antibody as a negative control. Cells with brown- yellow granules in endochylema or nuclei were considered as positive cells. According to the coloration, the cells were scored as following: 0. no staining; 1. faint-yellow; 2. brown-yellow; 3. dark- brown. Five fields of vision of each cover slip were calculated and scored. The sum of the scores divided by the number of cells was considered as the expression intensity of Livin. The inhibition ratio of Livin expression was calculated according to the following formula: inhibition ratio of Livin expression = (1-the expression intensity of Livin in the observation group/the expression intensity of Livin in the blank control group)/transfection efficiency×100%.

Apoptosis Analysis by FCAS