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Suppression of Gastric Cancer Cell Growth by Suppressing Survivin Gene Expression Using RNAi Technology

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Abstract: Survivin gene plays an important role in the growth of cancer cells. To investigate its effects on the proliferation of MGC-803 cells, the expression of survivin gene in MGC-803 cells was silenced by the siRNA expression vector-based RNA interference (RNAi) technique. The survivin siRNA expression plasmid was transfected into MGC-803 cells by lipofectamine. The expression of survivin mRNA was determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). The changes of cell cycle distribution and the cell proliferation were analyzed using flow cytometry and MTT assay, respectively. The survivin siRNA expression plasmid significantly down-regulated the expression of survivin gene in MGC-803 cells with a percentage of 48.2% (vs vector controls) and it arrested cell at G0/G1 phase (77.4%). The cell proliferation was significantly inhibited and the optical density in siRNA-transfected cells was markedly lower than that in the vector controls ($p<0.01$). The growth inhibitory rates were 53.4, 66.7 and 86.3% after 24, 48 and 72 h of the transfection, respectively. Down-regulation the expression of survivin can inhibit the cell proliferation of gastric cancer cells *in vitro*.

Key words: Survivin, RNA interference, cell cycle, cell proliferation, gastric cancer

INTRODUCTION

The abnormal in apoptosis and cell cycle are the major reasons of un-control growth of cancer cells. Recently studies showed that survivin played a key role in the regulations of apoptosis and cell cycle (Altieri, 2003; Johnson, 2004). Survivin is an anti-apoptosis gene. In many types of tumor tissues, survivin gene is over expressed. Gastric cancer is one of the most common cancers in China and other orient countries. And several studies have shown that survivin gene over expressed in gastric cancer tissues (Wang *et al*., 2004; Zhu *et al*., 2003; Yu *et al*., 2002; Krieg *et al*., 2002). Other studies found that the expression level of survivin was associated with the prognosis of cancers (Okada *et al*., 2001; Ikegucki *et al*., 2002).

RNA interference (RNAi) is a sequence-specific posttranscriptional gene silencing phenomemon, which is triggered by double-stranded RNA (dsRNA) and leads to degradation of mRNAs sharing sequence homology with the dsRNA. RNAi has been shown to be a novel powerful gene knockdown technology. Because any protein can essentially be suppressed by introducing dsRNA corresponding to the target mRNA, RNAi has many experimental applications and tremendous therapeutic potential.

In the present study, in order to establish a novel method based on the suppressing the expression of survivin gene, we used RNAi technology to suppress the expression of suvivin gene and then observed the cell growth of gastric cancer MGC-803 cells and its mechanisms.

MATERIALS AND METHODS

This study was conducted from January, 2005 to December, 2006 in Department of Biochemistry and Molecular Biology at Ningbo University School of Medicine, China.

MGC-803 gastric cancer cell line was purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. The plasmid expressing survivin siRNA was kindly obtained from Guangdong Medical College (Li *et al*., 2004). Lipofectamine 2000 and DMEM medium were purchased from Invitrogen and Gibco BRL, respectively.

Transfection: Cells were cultured in multi-well plates at 37°C in a humidified atmosphere of 5% CO_2 with DMEM medium for 24 h. Cells were transfected with plasmid using Lipofectamine 2000 according to the use manual and then incubated for 24, 48 and 72 h.

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MTT assay: When the cell treated time reached, 30 μL of MTT solution (2 mg mL^{-1}, in phosphate buffered saline) was added to each well of 96-well plates. After cells were incubated at 37°C for 4 h, the medium was removed and 150 μL of Dimethylsulfoxide (DMSO) was added to each well to solubilize the formazan. Then, the microplate was shaken on a rotary platform for 10 min. Finally, the Absorbance (A) was measured at 492 nm using a Wellscan (Labsystems, USA).

Cell cycle analysis and apoptosis measurement: Cells harvested with 0.25% trypsin were sedimented by centrifugation at 3000 rpm for 5 min at room temperature. After the supernatant was removed, ice-cold 70% ethanol was added. Finally, cell cycle was analyzed using a Coulter Flow Cytometer (Beckman Coulter, Inc., Miami, Florida). The percentages of cells in different cell cycle phases (G0/G1, S, or G2/M phase) were calculated using Coulter Epicx XL-MCL DNA Analysis Software.

Detection of suvivin mRNA using reverse transcription-polymerase chain reaction (RT-PCR): Total RNA was isolated using Trizol reagent. One-step RT-PCR was performed using the mRNA Selective PCR Ver 1.1 (Takara Biotechnology, Dalian, China). Gene-specific primers for RT-PCR were synthesized by Sangon, Shanghai, China. The primers of survivin were as follows: upstream 5'-GGC ATG GGT GCC CCG ACG TT-3', downstream 5'-AGA GGC CTC AAT CCA TGG CA-3'. β-actin mRNA was used as an internal control and its primers were as follows: 5'-ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3' and 5'-CGT CAT ACT CCT GCT TCC TGA TCC ACA TCT GC-3'. The molecular size of amplified products was 439 bp for survivin mRNA and 838 bp for â-actin mRNA. The reaction mixture was incubated at 50°C for 30 min, following by 30 cycles at 94°C for 1 min, 62°C for 1 min and 72°C for 1 min and after that, it was incubated at 72°C for 8 min. RT-PCR products were electrophoresed on 1.5% agarose gels and visualized with ethidium bromide staining. According to the amount of DNA marker, the electrophoresis band's intensity was quantified using the Discovery Series Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Co., Hercules, USA).

Statistical analysis: Results were expressed as mean±S.D. Unpaired Student's *t*-test was used to analyze variables between groups using SPSS10.0 software.

RESULTS

Cell growth was inhibited by survivin siRNA expressing plasmid: After transfected with survivin siRNA expressing plasmid, cell growth of MGC-803 was significantly inhibited ($p<0.01$, Fig. 1). Their inhibitory rates were 53.4, 66.7 and 86.3% for the treatment of 24, 48 and 72 h, respectively. No inhibitory effects were found in three control groups (medium, Lipofectamine and vector controls).

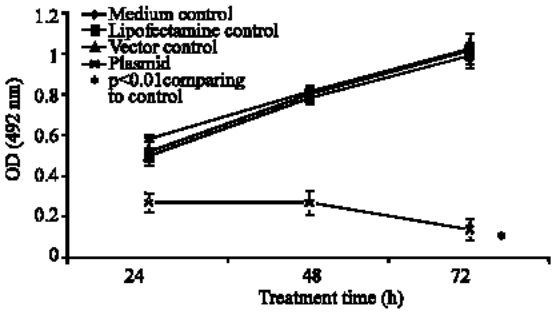

Fig. 1: The cell growth inhibitory effect of survivin siRNA expressing plasmid on gastric cancer cells

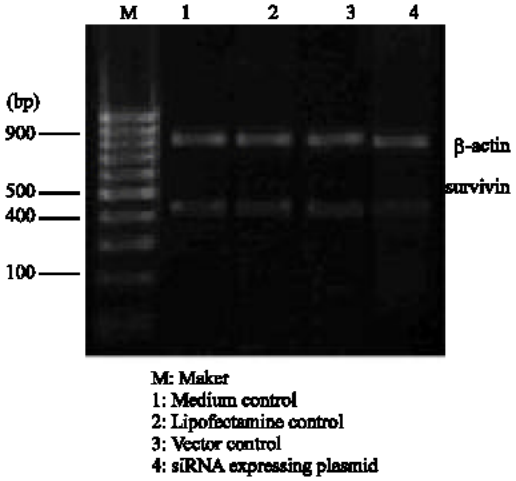

Fig. 2: RT-PCR results of survivin mRNA

Cells were arrested at G0/G1 phase by survivin siRNA expressing plasmid: In order to decipher the suppressive mechanisms of survivin siRNA expressing plasmid on MGC-803 cells, we monitored changes of cell cycle distribution by flow cytometry. Treatment for 48h resulted in G0/G1 arrest from 60.1-77.4%.

Survivin gene was suppressed by siRNA expressing plasmid: As shows in Fig. 2, the expression of survivin was suppressed by siRNA expressing plasmid. This indicates that the cell growth inhibition and cell cycle interruption are associated with down-regulation of survivin gene.

DISCUSSION

Survivin was initially identified as a member of the Inhibitor of Apoptosis Protein (IAP) family. By inhibiting apoptosis and promoting mitosis, survivin facilitates cancer cell survival and growth. Survivin is a key molecular of balancing cell growth. The highly specific expression of survivin was found in many human cancers, including gastric cancer. In gastric cancer, increased and sustained survivin expression provides survival advantage and facilitates tumor progression and resistance to anti-cancer drugs. Based on its specific over-expression in cancer cells and its anti-apoptotic function, survivin represents a suitable target for antitumor approaches. Previous studies have shown that the expression suppression of survivn gene by using antisense oligonucleotides would inhibit cancer cell growth and induce apoptosis (Tong *et al*., 2005; Dai *et al*., 2000; Yang *et al*., 2004; Ma *et al*., 2005). As the RNAi is more powerful than antisene in the silencing gene expression (Aoki *et al*., 2003; Cioca *et al*., 2003), we used this new method to investigate the growth inhibitory effect of survivin expressing plasmid. The results showed that this plasmid decreased survivin gene expression (Fig. 2) and more important inhibited cell growth in a time-dependent manner (Fig. 1). At the same time, cells were arrested at G0/G1 phase by the transfection of survivin siRNA expressing plasmid. This indicates that survivin gene plays an important role in the acceleration of gastric cancer cell growth.

Studies have shown that survivin over-expressed in several kinds of tumor tissues (Falleni *et al*., 2003; Chen *et al*., 2004; Ning *et al*., 2004). As the RT-PCR results in this study showed that the survivin gene expression in untreated gastric cancer cells was higher than that in treated cells (Fig. 2). Survivin gene selectively expresses at G2/M and high level of survivin will accelerate cell growth (Suzuki *et al*., 2000). Besides, Giodini et al thought that survivin affected cell division (Giodini *et al*., 2002). Over-expression of survivin lead cells to escape the checkpoints of cell cycle and have the anti-apoptosis property (Grosmann *et al*., 2001). For these reasons, by means of suppressed the expression of survivin gene will suppress the cancer cell growth.

RNAi is a newly discovered cellular pathway for the silencing of sequence-specific genes at mRNA level by the introduction of the cognate double-stranded RNA. RNAi as a protecting mechanism against invasion by foreign genes was first described in *C. elegans* and has subsequently been demonstrated in diverse eukaryotes such as insects, plants, fungi and vertebrates. The basic mechanism behind RNAi is the breaking of a dsRNA matching a specific gene sequence into short pieces called short interfering RNA, which trigger the degradation of mRNA that matches its sequence. In mammalian cells siRNA molecules are capable of specifically silencing gene expression without induction of unspecific interferon response pathway. As the results showed in our study, using chemical transfection is one of the effective strategies to deliver siRNA to target cells in cell culture. RNAi provides a potential intracellular defense system against cancer and the use of survivin siRNA expression plasmid deserves further investigations as a novel approach to cancer therapy for the survivin-expression cancers.

CONCLUSION

Survivin gene can be regarded as a very good target gene in gene therapy for gastric cancer and even other survivin-expression cancers.

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