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Antisense telomerase RNA induced human gastric cancer cell apoptosis

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INTRODUCTION

Human tissue homeostasis is precisely regulated by cellular division, differentiation and death. Normal human somatic cells progressively lose telomere restriction fragment (TRF) length with each successive cell division, eventually leading to cellular quiescence, chromosomal end-degradation and apoptosis[1]. On the contrary, stabilization of telomere lengths by expressing telomerase, an RNA-dependent DNA polymerase, may be involved in cellular immortality and carcinogenesis[2-4]. Changes of telomerase activity and telomere lengths have been found in almost all human cancers[4-10], but the evidence of their relationship with immortalization of cancers cell remain to be directly demonstrated. Since shortened telomere was first discovered in Hela cells with antisense RNA techniques in 1995[11], anticancer agents based on inhibition of telomerase RNA have been reported[12,13]. However, the relationship between telomerase inhibition and cell apoptosis has not been fully understood. In this study, we investigated the effect of blocking telomerase activity on apoptosis of human gastric cancer cells *in vitro* using an antisense vector for human telomerase RNA component (hTR) into human gastric cancer cells.

MATERIALS AND METHODS

Reagents

EcoR I, *BaL* I, *SaL* I, SP6 or T7 polymerases (Promega), hygromycin (Boehringer Mannheim) and lipofectamine (Gibco BRL) were commercially obtained. Antisense hTR expression construct vector (pBBS212) and hTR recombinant plasmid (pGRN83) were kindly donated by Dr Villeponteall (Geron Corporation, USA).

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Cell line

The human gastric cancer cell line SGC7901 (Fourth Military Medical University, China) was cultured in RPMI1640 (Gibco BRL) containing 100 mL/L fetal bovine serum.

Transfection of antisense hTR expression vector

The TRC_3 piece (hTR) was flanked by 2 *EcoR* I sites and was inserted into *EcoR* I site of pBBS212 to make the plasmids pBBS-hTR, which can express the antisense of hTR under the MPSV promoter[11,14]. Cells at density of 2×10^5/well (2 mL) in 6 well plates were transfected with purified plasmids (pBBS-hTR) and a selectable marker, hygromycin, by lipofectin procedure as described[15]. As a control, cells were transfected either with an hTR-free plasmid (pBBS212) containing hygromycin marker or with hygromycin alone four clonal cells from each group were used for a series of experiments.

hTR expression by blot hybridization

Total RNA was prepared from each group cells by RNA isolation kit (Promega). PGR N83 was lined with *BaL* I or *SaL* I and transcribed *in vitro* using T7 RNA polymerase and SP6 RNA polymerase respectively. The transcription was carried out according to the manufacturer's instructions in the presence of 370GBg [α-^{32}P]UTP (Beijing Huri Co, China). Then a sense hTR or antisense hTR probe synthesized was used for RNA blot hybrization[15].

Telomerase assay

Cell extracts were prepared by detergent CHAPS (Pierce), and telomerase activity was measured by telomeric repeat amplification protocol (TRAP) methods as described by Kim *et al*[5,16]. The TRAP reaction products were separated by 100 g/L polyacrylamide gel electrophoresis and autoradiographed. The basal level of telomerase activity was measured by serial dilution of the protein extracts.

Determination of TRF length

Genomic DNA extracted from the gene transfected cells and the control cells were digested with *Hinf* I and *Rsa* I. DNA samples (8 μg each) were loaded onto a 8 g/L agarose gel and electrophoresed for bromphenol blue to the bottom of gel at 90V. The gel was dried, denatured, neutralized and hybridized to a 5'-[^{32}P](TTAGGG)$_4$ probe (T_4 polynucleotide kinase, Promega) and *autoradi-*

ographed 48 h at -20℃[2,17].

Cell cycle analysis

Cells in log phase growth were collected, washed twice with phosphate buffered s aline (PBS) and fixed in 70 mL/L ethanol at 4℃ overnight. Cells were wa shed with PBS, digested with 20 mg/L RNase A at room temperature for 1 h, and then resuspended in 50 mg/L propidum iodied solution. Cell cyc le was analyzed on the FAC/ Scan (ELITEESP, Coulter Co) using a computer prog ram interfaced with the integrator.

Ultrastructural observation

Ultrastructure in the gene transfected cells was observed under a transmissive electron microscope (JEM-2000EX, Japan).

RESULTS

Antisense telomerase RNA inhibited the sense telomerase RNA expression in the ge ne transfected cells. Antisense hTR expression was high and sense hTR expression was weak in RNA blot hybridization analysis (Figure 1). Sense hTR suppression r ate analyzed by thin layer scanning reached 60% (Figure 1). Antisense RNA to hTR mediating telomerase activity was down-regulated in the gene transfected cells but not in the control plasmids transfected cells (Figure 2). Mean TRF length of the gene transfected cells (11.0 ± 5.6) was also shorter than that of the cont rol plasmids transfected cells (Figure 3) . Compared with the control plasmids transfected cells, G1 phase decreased by 25% in the gene transfected cells and its apoptotic peak reached in 46.2% of cell cycle in a computer apoptotic progr am analysis (Figure 4). Either denaturation and necrosis or chromatin compaction and apoptotic body appeared in the gene transfected cells while the none-gene transfected cells showed no changes of this kind (Figure 5).

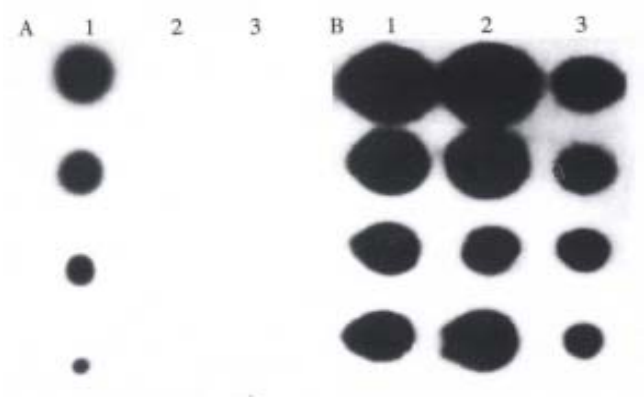

Figure 1 Blotting hybridization of hTR. (A) Antisens e hTR expression. (B) Sense hTR expression. (1) SGC7901 cells. (2) SGC7901 cells transduced with control vector (pBBS). (3) SGC7901 cells transduced with vector expressing antisense hTR (pBBS-hTR).

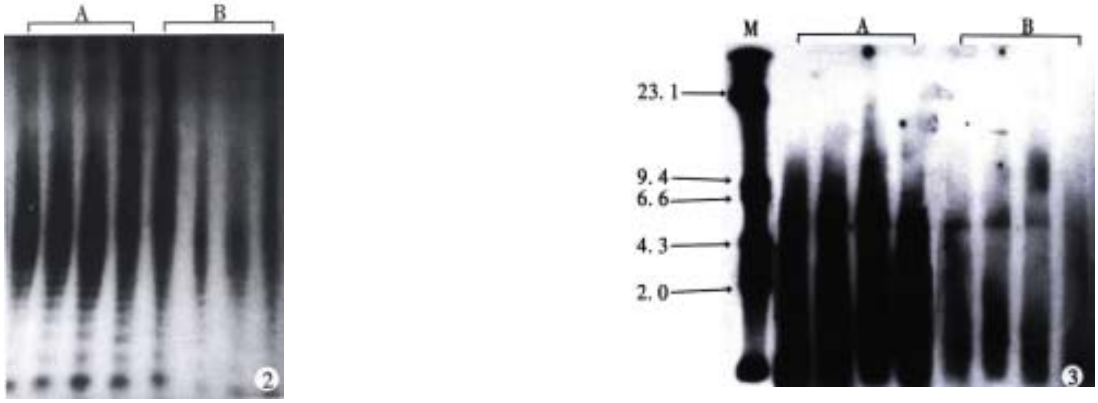

Figure 2 Telomerase activity of SGC7901 cells analyz ed by TRAP. (A) SGC7901 cells transduced with control vector (pBBS). (B) SGC7901 cells transduced with vector expressing antisense hTR (pBBS-hTR).

Figure 3 Telomeric lengths of SGC7901 cells analyzed by hybridization of nucleic acids directly in agarose gels. (M) λ -DNA / Hind III Molecular Markers. (A) SGC7901 cells transduced with control vector (pBBS). (B) SGC7901 cells transduced with antisense hTR expressing vector (pBBS-hTR).

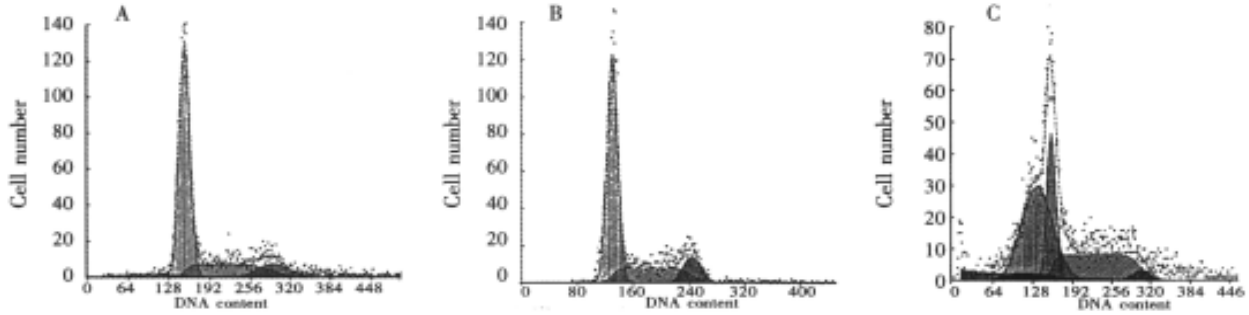

Figure 4 Cell cycle analyzed by flow cytometry. (A) SGC7901 cells. (B) SGC7901 cells transduced with control vector (pBBS). (C) SGC7901 cells transduced with vector expressing antisense hTR (pBBS-hTR).

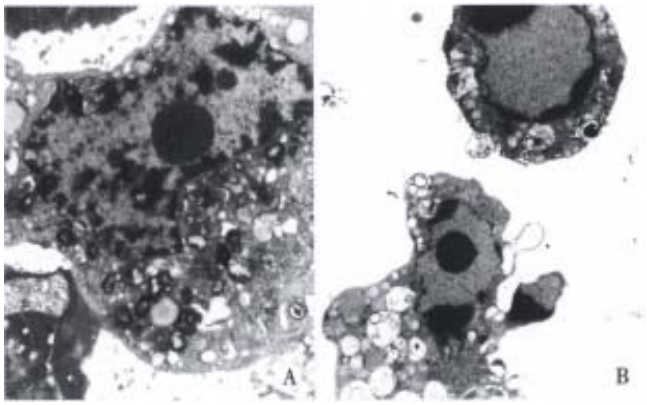

Figure 5 Ultrastructure observed under transmissive electron microscope. (A) SGC7901 cells transduced with control vector (pBBS). (B) SGC7901 cells transduced with antisense hTR expressing vector (pBBS-hTR).

DISCUSSION

Cell apoptosis is closely associated with tumourigenesis, tumor growth and tumor metastasis. Changes of telomere lengths may play an important role in maintai ning cell division, proliferation, apoptosis and immortalization[2,4]. N ormal human somatic cells progressively lose their telomeric sequence with repli cative senscence till cell "crisis" or apoptosis occurred[4,18,19]. I n contrast, almost all tumor cells and tissues express telomerase and maintain telomere length through an indefinite number of cell divisions[4-10,19-24]. Antisense hTR, hammerhead ribozyme TeloRZ and antisense oligonucleode agains t hTR could suppress tumor cell growth by inhibiting telomerase activity or shor tening TRF length[11-13]. Therefore, it has been proposed that antisense RNA based on telomerase inhibition may potentially reverse uncontroled prolifer ation of tumor cells and induce apoptosis of cancer cells[25,26]. The pr esent study is undertaken to investigate the possible relationship between the antisense hTR expression and apoptosis of human gastric cancer cells.

Our study has demonstrated that the antisense hTR expression level of SGC7901 cells was high. Based on the histogram of flow cytometry, G1 phase was arrested and there was a typical apoptotic peak in the antisense hTR expression vector transfected cells. Meanwhile, compaction of nuclear chromatin and apoptotic body were observed under a transmissive electron microscope. These results suggest tha t antisense hTR may mediate apoptosis of human gastric cells. Also, we have found that the antisense hTR expression of SGC7901 cell could down-regulate te lomerase activity and shorten telomere length. This supports the changes of telo mere length and telomerase activity in antisense hTR transfected tumor cells[11,12], indicating that telomerase can mediate telomeric sequence replicati on in gastric cancer cells and that shortening of telomere length by the antisense hTR may be associated with apoptosis of human gastric cancer. Thus, the induc tion of cell apoptosis in SGC7901 cells expressing antisense hTR demonserates the potential of telomerase inhibition as a therapeutic target for human cancer.

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