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 apoptosis1. On the contrary, stabilization of telomere lengths by expressing telomerase, an RNA-dependent DNA polymerase, may be involved in cellular immortality and carcinogenesis2-4. Changes of telomerase activity and telomere lengths have been found in almost all human cancers4-10, 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 199511, anticancer agents based on inhibition of telomerase RNA have been reported12,13. However, the relationship between telomere 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. An tisense hTR expression construct vector (pBBS212) and hTR recombinant plasmid (pGRN83) were kindly donated by Dr Villeponteall (Geron Corporation, USA).

Telomerase assay

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 TRC3 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 promoter11,14. Cells at density of 2 x 105/well (2 mL) in 6 well plates were transfected with purified plasmids (pBBS-hTR) and a selectable marker, hygromycin, by lipofectin procedure as descri-bed15. 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-32P]UTP (Beijing Huri Co, China). Then a sense hTR or antisens e hTR probe synthesized was used for RNA blot hybri dezation15.

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 bromophenol blue to the bottom of gel at 90V. The gel was dried, denatured, neutralized and hybr idized to a 5’-[32P](TTAGGG)4 probe (T4 polynucleotide kinase, Promega) and autoradiographed. The basal level of telomerase activity was measured by serial dilution of the probe in extracts.

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Cell cycle analysis

Cells in log phase growth were collected, washed twice with phosphate buffered saline (PBS) and fixed in 70 mL/L ethanol at 4°C overnight. Cells were washed with PBS, digested with 20 mg/L RNase A at room temperature for 1 h, and then resuspended in 50 mL/L propidum iodide solution. Cell cycle was analyzed on the FAC/Scan (ELITEESP, Coulter Co) using a computer program 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 gene transfected cells. Antisense hTR expression was high and sense hTR expression was weak in RNA blot hybridization analysis (Figure 1). Sense hTR suppression rate 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 control 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 program 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) Antisense 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 analyzed by TRAP. (A) SGC7901 cells transduced with control vector (pBBS). (B) SGC7901 cells transfected 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).
Cell apoptosis is closely associated with tumourigenesis, tumor growth and tumor metastasis. Changes of telomere lengths may play an important role in maintaining cell division, proliferation, apoptosis and immortalization\(^2,4\). Normal human somatic cells progressively lose their telomeric sequence with replicative senescence till cell "crisis" or apoptosis occurred\(^4,18,19\). In contrast, almost all tumor cells and tissues express telomerase and maintain telomere length through an indefinite number of cell divisions\(^4,10,18-24\). Antisense hTR, hammerhead ribozyme TeloRZ and antisense oligonucleotide against hTR could suppress tumor cell growth by inhibiting telomerase activity or shortening TRF length\(^11-13\). Therefore, it has been proposed that antisense RNA based on telomerase inhibition may potentially reverse uncontrolled proliferation of tumor cells and induce apoptosis of cancer cells\(^25,26\). The present 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 that antisense hTR may mediate apoptosis of human gastric cells. Also, we have found that the antisense hTR expression of SGC7901 cell could down-regulate telomerase 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 induction of cell apoptosis in SGC7901 cells expressing antisense hTR demonstrates the potential of telomerase inhibition as a therapeutic target for human cancer.

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**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).