Effect of antisense oligodeoxynucleotide of telomerase RNA on telomerase activity and cell apoptosis in human colon cancer

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INTRODUCTION
Colorectal cancer is one of the most common malignancies both in the world and in China[1]. More and more patients with early colorectal cancer can now be found due to the improvement in the diagnostic techniques. Although surgery and chemotherapy are effective on patients with localized tumors, the prognosis of patients with advanced or metastatic tumors is not ideal. As a result, it is absolutely necessary to explore a novel treatment modality, namely the gene therapy. Just like other kinds of cancer, colorectal cancer is now recognized as a genetic disease. Colon cancer cells contain many genetic alterations which accumulate as tumor develops. This makes it possible to treat cancer with gene therapy[2,3].

Telomerase is a ribonucleoprotein consisting of two components, RNA and protein. The RNA gene of telomerase is termed as human telomerase RNA (hTR). Two protein subunits have been found, which were named as human telomerase-associated protein (TPEP1) and human telomerase catalytic subunit or human telomerase reverse transcriptase (hTERT)[4,5]. Telomerase activity in humans has been detected in germline and tumor tissues as well as in established cultured cell lines[6]. In normal somatic cells, the absence or low expression of telomerase is thought to result in progressive telomeric shortening with each cell division[7,8,9]. Therefore, it has been suggested that reactivation of telomerase is a critical step in tumorigenesis and that interference with the regulation of telomerase activity may serve as a basis for cancer therapy[9,10]. However, to our knowledge, whether antisense gene therapy directing against hTERT is effective on colon cancer is unknown. We reported here the effect of antisense oligodeoxynucleotide of telomerase RNA on human colon cancer cell line, and investigated the potential value of telomerase as a target for antisense gene therapy of colon cancer.

MATERIALS AND METHODS
Cell culture
SW480 cells, a human colon cancer cell line, were provided by Department of Biology, Wuhan University, China, and maintained in RPMI 1640-10% fetal bovine serum supplemented with 1 mmol/L L-glutamine, 100 U/ml of penicillin plus 100 µg/ml of streptomycin at 37 °C under 5% CO2.

Cell counting
SW480 cells were counted with 5 g/L of trypan blue staining.

Oligodeoxynucleotide synthesis
Two oligodeoxynucleotides were synthesized as described by Feng et al and Norton et al[11,12]. Antisense oligodeoxynucleotides (As-ODN) with the sequence 5’TAGGGTTAGACAA-3’, which can recognize the RNA template region of telomerase, and missense oligodeoxynucleotide (Ms-ODN) with the sequence 5’TGTAAAGAACCTAG 3’ were synthesized by Beijing SBS Biotechnology Engineering Company using the 391 DNA synthesizer. The synthesized oligodeoxynucleotides were subjected to electrophoresis (PAGE) and purified (300V, 1.5 h).

Transfection of oligodeoxynucleotides
Transfection of phosphorothiate oligodeoxynucleotides (ODNs) was carried out with liposomal transfection reagent DOSPER (Roche Diagnostic GmbH) according to the manufacturer’s protocol. Briefly, cells were plated onto 6-well plates and incubated until the cells reached 70-80% confluence. The DOSPER was diluted with serum-free medium the day before transfection. Then, the desired amount of ODNs was added dropwise into 900 µl of serum-free RPMI 1640. After incubated for 6 hours at 37 °C, 1 ml of RPMI 1640 containing 20% FBS was added into each well.
Table 1 Inhibitory effect of telomerase activity by ODNs (mean±SD)

| Groups       | 24 h   | 48 h   | 72 h   | 96 h   | 120 h  |
|--------------|--------|--------|--------|--------|--------|
| As-ODN 10 μmol/L | 0.87±0.194 | 0.40±0.232 | 0.38±0.146 | 0.37±0.203 | 0.29±0.213 |
| Ms-ODN 10 μmol/L | 1.06±0.249 | 1.28±0.179 | 0.95±0.273 | 0.19±0.243 | 1.24±0.178 |
| Positive control | 1.72±0.267 | 1.57±0.418 | 1.24±0.186 | 1.23±0.235 | 1.09±0.347 |
| Negative control | 0.34±0.092 | 0.31±0.076 | 0.28±0.089 | 0.06±0.072 | 0.05±0.023 |

**RESULTS**

**Inhibitory effect of antisense hTR ODNs on telomerase activity**

SW480 cells were transfected with As-ODN (1.0 μmol/L) and Ms-ODN (1.0 μmol/L), and collected at 24, 48, 72, 96 and 120 hours after transfection respectively. Telomerase activities were measured by TRAP-ELISA. Following results were found. The telomerase activity of SW480 cells transfected with As-ODN was greatly inhibited compared with that in the Ms-ODN. The telomerase activity of SW480 cells transfected with As-ODN at 72 and 96 hours after transfection was significantly lower than that both at 24 hours and in positive control as shown in Table 1. These findings suggested that this inhibitory action was sequence specific and in a time-dependent manner.

**Effect of antisense hTR ODNs on induction of SW480 cell apoptosis**

**Cytologic morphological changes**

SW480, transferred with 1 μmol/L As-ODN for 3 days, cytologic morphology was observed under Olympus optical microscope and Hitach transmission electron microscope. It was found that cells rounded up off the plasids, exhibiting cytoplasmic blebbing, fragmentation and chromatin condensation, features of apoptosis. No apoptotic features (normal morphology) were observed in SW480, transfected with 1 μmol/L As-ODN for 3 days, cytologic morphology was observed under Olympus optical microscope and Hitach transmission electron microscope. It was found that cells rounded up off the plasids, exhibiting cytoplasmic blebbing, fragmentation and chromatin condensation, features of apoptosis.

**Table 2 Effect of ODNs on induction of SW480 cell apoptosis (mean±SD)**

| Groups       | 48 h   | 72 h   | 96 h   |
|--------------|--------|--------|--------|
| As-ODN 1 μmol/L | 4.99±0.54 | 8.63±0.59 | 9.96±0.41 |
| Ms-ODN 1 μmol/L | 3.86±0.39 | 4.88±0.57 | 4.92±0.67 |
| HRT blank     | 1.57±0.18 | 1.79±0.21 | 1.71±0.32 |

**Detection of apoptotic cells**

To determine the apoptotic rate, SW480 cells were transfected with 1 μmol/L As-ODN and Ms-ODN for 2 days. After permeablization, the cells were stained with propidium iodide and analysed by flow cytometry. The apoptotic rate of SW480 cells transfected with As-ODN increased (4.99±0.54, 8.63±0.59,
and 9.96±0.41 at 48 h, 72 h and 96 h, respectively, *P*<0.001), but no significant changes of apoptosis were observed in SW480 cells transfected with 1 μmol/L Ms-ODN as shown in Table 2, indicating that this apoptotic induction was sequence specific and in a time-dependent manner.

**DISCUSSION**

Compared with normal somatic cells, cancer cells have an unlimited replicating capacity. This important characteristic of cancer, named immortality, has been gaining more and more attention, seeing that cancer cells might achieve cellular immortality through only a major pathway, the activation of telomerase[15]. Telomerase has been found to play an important role in carcinogenesis, thus becoming the basis of the widely held view of telomerase as a highly selective target for antisense gene therapy of cancer[14].

The RNA component of telomerase (hTR) was crucial to the telomerase activity[16-17]. Human cell lines expressing hTR mutated in the template region could generate the predictive mutant telomerase activity. Recent experiments have shown that antisense gene therapy directing against telomerase RNA component could effectively inhibit telomerase activity and induce apoptotic cell death in ovarian cancer, prostate cancer, bladder cancer, malignant gliomas and human breast epithelial cells[18-22]. However, whether such an anti-cancer effect can be obtained in human colon cancer is still unknown. Therefore, we examined the effect of antisense hTR oligodeoxynucleotide on human colon cancer cell line. As the results showed, our experiment clearly demonstrated that antisense-hTR oligodeoxynucleotide could significantly inhibit telomerase activity and induce apoptosis of human colon cancer cells, which was supported by the results obtained in our previous experiment[23]. All these findings provide the strong evidence that telomerase may be an ideal target for antisense gene therapy of human colon cancer.

Recently, it has been showed that telomerase activity was the dominant mechanism providing telomere maintenance to human immortalized cells. However, the exact mechanisms of how telomerase activity is regulated in tumour cells remain poorly understood. Some researchers have shown that telomerase activity correlated with the growth rate of immortal cells[24-26], whereas others found no significant association between telomerase activity and proliferation index in tissue specimens from breast carcinoma[27], gastric carcinoma[28], and Wilms’ tumour[29].

Inhibition of telomerase activity has been proposed as a potential method for the treatment of human malignancies. It is suggested that telomerase inhibition may serve as an effective tool for eliminating tumour cells that have short telomeres. Such tumours may provide reasonable targets for agents that inhibit telomerase. These experiments await the development of specific inhibitors for the components of telomerase complex.

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