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• BRIEF REPORTS •

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

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Abstract

AIM: To explore the effect of antisense oligodeoxynucleotide (As-ODN) of telomerase RNA on telomerase activity and cell apoptosis in human colon cancer.

METHODS: As-ODN was transfected into SW480 cells by liposomal transfection reagent. Telomerase activity of SW480 cells was examined by telomeric repeat amplification protocol (TRAP) and enzyme-linked immunosorbent assay (ELISA). Apoptosis was analyzed by morphology and flow cytometry.

RESULTS: The telomerase activity in SW480 cells transfected with 1.0 μmol/L of As-ODN for 2-5 days, was significantly decreased in a time-dependent manner, and the cells underwent apoptosis. The missense ODN (Ms-ODN) and the control group transfected with SW480 cells did not show these changes.

CONCLUSION: As-ODN can specifically inhibit the telomerase activity of SW480 cells and induce apoptosis.

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INTRODUCTION

Colon cancer is one of the most common malignancies both in the world and in China[1]. More and more patients with early colon 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, colon 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 (TEP1) 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]. 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 hTR 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 ℃ under 5% CO_2.

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' TGTAAGGAACTAG 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 incubated for 15 minutes with diluted DOSPER. The ODNs/ DOSPER mixture (100 μl) was added dropwise into 900 μl of serum-free RPMI 1640. After incubated for 6 hours at 37 ℃, 1 ml of RPMI 1640 containing 20% FBS was added into each

Table 1 Inhibitory effect of telomerase activity by ODNs (mean±SD)

Groups	Active duration				
	24 h	48 h	72 h	96 h	120 h
As-ODN 10 μmol/L	0.872±0.194	0.406±0.232	0.386±0.146	0.307±0.203	0.289±0.213
Ms-ODN 10 μmol/L	1.063±0.249	1.285±0.179	0.959±0.273	0.109±0.243	1.247±0.178
Positive control	1.725±0.267	1.571±0.418	1.243±0.186	1.236±0.235	1.098±0.347
Negative control	0.349±0.092	0.312±0.076	0.283±0.089	0.063±0.072	0.057±0.023

well. Cells were harvested and analysed after 48, 72, 96 and 120 hours, respectively.

Telomerase activity assay

Telomerase activity was measured by polymerase chain reaction and enzyme linked immunosorbent assay (PCR-ELISA). Briefly, 2×10^6 cells were isolated, mixed with 200 μl of protein extraction buffer by vortes, and left on ice for 30 minutes. One hundred and seventy-five μl of supernatant was collected after centrifugation (16 000×g, 20 minutes, 4 ℃). PCR was performed in a system of 50 μl containing 25 μl of transfer reaction mixture, 2 μl protein extract and 2 μl primers, and 23 μl of nuclease-free water was added. The PCR condition was as the follows: at 25 ℃ for 30 minutes for primer elongation, at 94 ℃ for 5 minutes for telomerase inactivation. Thirty cycles of amplification were performed, each cycle was performed at 94 ℃ for 30 s for denaturation, at 50 ℃ for 30 s for annealing, and at 72 ℃ for 90 s for polymerization. Five μl of the amplified product and 20 μl of the denatured reagent were incubated at room temperature, 225 μl of hybridization buffer was then added and mixed, and 100 μl of them was distributed in the wells of a microtitering plate. After 2 hours of incubation (37 ℃, 300 rpm), 100 μl of anti-DIG-POD working solution was added and incubated for another 30 minutes, followed by the addition of 100 μl of TMB substrate solution, and 100 μl of stop reagent was added at last. The OD value in each well was read at the wave lengths 450 nm and 655 nm on a microtiter plate reader (Bio-RAD Model 550 microplate reader). The result of OD_{450} minus OD_{655} greater than 1.5 unit was judged as a positive control using a protein extract from immortalized telomerase-positive human embryonic kidney cells (293 cells). The negative control was considered as OD_{450} minus OD_{655} less than 0.2 unit by reading the protein extract pretreated with RNase A at 65 ℃ for 10 minutes. Telomerase activity was considered positive when the value of OD_{450} minus OD_{655} of a sample was at least 0.2 unit higher than that of the negative control, otherwise it was considered negative. Each sample was examined more than twice. The final value was presented as mean±SD after a statistical treatment by using *t* test.

Apoptotic features

To determine whether SW480 transfected with AS-ODN displayed an apoptotic morphology, the tranfected cells were observed under Olympus optical microscope and Hitach transmission electron microscope.

To determine the apoptotic rate and cell cycle distribution, the cells were fixed and stained by propidium iodide (PI, Sigma product), and analyzed by a FACSORT flow cytometer (Becton Dickinson). Briefly, cells were trypsinized, washed once in ice-cold PBS, and incubated with annexin-V-fluoroescein/PI, and then analyzed immediately by FACSORT flow cytometry. All data were analyzed using the Cell Quest software.

Statistical analysis

Results were expressed as mean±SD. Statistical analyses were carried out with the software package SPSS10.0. A *P* value less than 0.05 was considered statistically significant.

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 plastids, exhibiting cytoplasmic blebbing, fragmentation and chromatin condensation, features of apoptosis. No apoptotic features (normal morphology) were observed in SW480 transfected with 10 μmol/L Ms-ODN (Figure 1).

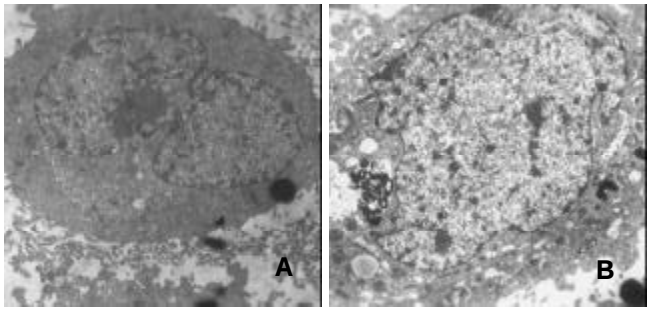

Figure 1 Morphplogic observation under transmission electron microscope. A: cells had no apoptotic features. B: cell rounded up off the plastic, exhibiting cytoplasmic blebbing, fragmentation and chromatin condensation, features of apoptosis.

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

Groups	Active duration		
	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 permeabilization, 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[13]. 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[15-17]. 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 Wilm' s 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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