Attenuation of Telomerase Activity by siRNA
Targeted Telomerase RNA Leads to Apoptosis
and Inhibition of Proliferation in Human Renal
Carcinoma Cells

OBJECTIVE Telomerase is an attractive molecular target for cancer therapy because the activation of telomerase is one of the key steps in cell immortalization and carcinogenesis. RNA interference using small-interfering RNA (siRNA) has been demonstrated to be an effective method for inhibiting the expression of a given gene in human cells. The aim of the present study was to investigate whether inhibition of telomerase activity by siRNA targeted against human telomerase RNA (hTR) can inhibit proliferation and induce apoptotic cell death in human renal carcinoma cells (HRCCs).

METHODS The siRNA duplexes for hTR were synthesized and 786-0 HRCCs were transfected with different concentrations of hTR-siRNA. The influence on the hTR mRNA level, telomerase activity, as well as the effect on cell proliferation and apoptosis was examined.

RESULTS Anti-hTR siRNA treatment of HRCCs resulted in specific reduction of hTR mRNA and inhibition of telomerase activity. Additionally, significant inhibition of proliferation and induction of apoptosis were observed.

CONCLUSION siRNA against the hTR gene can inhibit proliferation and induce apoptosis by blocking telomerase activity of HRCCs. Specific hTR inhibition by siRNA represents a promising new option for renal cancer treatment.

KEYWORDS: human telomerase RNA, telomerase, small-interfering RNA, renal cell carcinoma, proliferation.

The incidence of renal cell carcinoma (RCC), the most common malignant disease of the adult kidney, has steadily increased. A lack of effective treatment for RCC is largely due to the resistance of the cancer cells to conventional modes of treatment, such as radiotherapy and chemotherapy. Therefore, new therapeutic approaches are required for RCC.

It has been shown that the introduction of double-stranded oligoribonucleotides, also called small-interfering RNA (siRNA) into mammalian cells, triggers the degradation of the endogenous mRNA to which the siRNA hybridizes. Many studies have demonstrated that the transfer of siRNA into cells is an effective method for inhibiting the expression of oncogenes. siRNA cell transfer is currently being evaluated as a potentially useful method for genetic-based cancer therapy.

Human telomerase is a specialized reverse transcriptase that cataly-
ses telomeric repeat addition at the ends of chromosomes.[8] Telomerase consists of a protein component, hTERT, and an RNA component (hTR) containing the template for adding repeating units onto the ends of chromosomes.[9] The ability of cells to replicate indefinitely has been linked to telomerase expression.

A high percentage of tumor cells that take on immortalized characteristics show telomerase activity. Hara et al.[10] found that elevated levels of telomerase activity correlated with tumor stages as well as with the degree of nuclear RCC grades. The ubiquitous expression of telomerase in human tumors, including RCC, has supported the hypothesis that the enzyme is involved in cellular immortality and carcinogenesis. It has been demonstrated that activation of telomerase is one of the key steps in cell immortalization and carcinogenesis.[11]

In the present study, using a human renal carcinoma cell line, we evaluated the inhibitory effect of siRNA directed against the hTR region containing the telomere repeat template sequence on telomerase activity. Incubation of the human 786-0 RCC with appropriate siRNAs resulted in inhibition of hTR mRNA expression and telomerase activity. siRNA-mediated inhibition of telomerase activity subsequently led to significant inhibition of proliferation and apoptotic cell death.

**MATERIALS AND METHODS**

**siRNA preparation**

The siRNAs duplexes were synthesized, purified, and annealed by the Ambion Co. (USA). The siRNA targeted the hTR region containing the telomere repeat template sequence: sense sequence 5'-UUG UCU AAC CCU AAC UGA GTT-3' and antisense sequence 3'-TTA ACA GAU UGG GAU UGA CUC-5'. The selected sequence was submitted to BLAST search to assure that only the selected gene was targeted. A scrambled siRNA was purchased from Ambition (silencer™ Control siRNA#3) and used as a control.

**Cell culture and transfection**

Human renal carcinoma 786-0 cells were obtained from the Shanghai Cell Institute (Shanghai, China) and cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin and streptomycin. The cells were routinely passaged to maintain exponential growth. The day before transfection, the cells were trypsinized, diluted with fresh medium and transferred to 24-well plates. Transfection of siRNAs was carried out using siPORT™ lipid (Ambion). siPORT lipids and siRNAs were both diluted into OPTI-MEM I, respectively. Diluted siPORT lipids were mixed with diluted siRNAs and the mixture was incubated for 20 min at room temperature for complex formation. After addition of OPTI-MEM I to each well containing cells to a level of 200 μl, the entire mixture was added to the cells in one well resulting in a final concentrations of 10, 50 and 100 nM siRNAs. Cells were harvested and assayed at 24, 48 and 72 h after transfection. All experiments were repeated at least six times.

**RT-PCR**

Analysis of hTR RNA was performed by RT-PCR amplification. Total RNA was purified using a Total RNA isolation system and RT-PCR was performed using the Acess RT-PCR system (Promega). The upstream and downstream primers were 5'-CTG GGA GGG GTG TG G CCA TTT-3' and 5'-CGA ACG GGC CAG CAG CAC ATG ACAT-3', respectively. Reaction parameters were 94°C for 20 s, 50°C for 20 s, and 72°C for 30 s for 25 cycles. GAPDH was used as an internal control to assure the accuracy. Quantitation was performed with an image analyzer (LabWorks Software, UVP Upland, CA, USA).

**In situ hybridization.**

Chamber slides were permeabilized with proteinase K digestion (2.5 μg/ml) followed by acetylation. Hybridization was performed overnight at 37°C with a human hTR-biotinylated DNA probe that was a 30-mer oligonucleotide (5'-TTC TAC CGG AAG AGT TCG TGA GCA AGT TCG-3'), which was synthesized and biotinylated by the Boshide Biotechnology Co (Wuhan China). After post-hybridization washes, the signals were detected immunochemically by subsequent incubation with a streptavidin-horseradish peroxidase conjugate and developed by the DAB system.

**Telomerase activity**

Telomerase activity assay was performed according to a polymerase chain reaction-based telomeric repeat-amplification protocol (TRAP) as described by Mehle[12] using the Telomerase PCR ELISA Kit (Roche, Germany) following the provider's instructions.

**TUNEL assay**

The TUNEL technique was performed to detect and quantify apoptotic cell death using the In situ Cell Death Detection Kit (Roche Diagnostics, USA) following the instructions supplied by the manufacturer.