[Article ID] 1000 - 4718(2006)11 - 2223 - 04

# hTERT antisense oligodeoxynucleotide enhances the sensitivity of acute lymphoblastic leukemia cells from relapse patients to cisplatin

LI Wen - yu[1], ZHANG Yuan[2]

([1] *Guangdong Provincial People's Hospital, Guangzhou* 510080, *China*;
[2] *Institute of Hematology, Medical College of Jinan University, Guangzhou* 510632, *China*)

[**ABSTRACT**] **AIM**: To explore whether human telomerase reverse transcriptase (hTERT) antisense oligodeoxynucleotide (ASODN) could enhance the sensitivity of acute lymphoblastic leukemia cells from relapse patients to cisplatin. **METHODS**: The expression levels of hTERT protein were detected by immunofluorescence using fluorescence isothiocyanate (FITC), the number of viable cells was determined using the trypan blue dye exclusion assay, and apoptosis was detected by morphological observation and flow cytometric cell cycle analysis. **RESULTS**: The expression of hTERT protein was inhibited after treatment with hTERT ASODN. Treatment with cisplatin combined with hTERT ASODN had significantly reduced the number of viable acute lymphoblastic leukemic (ALL) cells ($P<0.05$). In morphological observation of apoptotic cells using Hoechst33258 and PI double staining techniques, cells displayed classic apoptotic changes in the presence of cisplatin or cisplatin combined with hTERT ASODN or ASODN at 48 h. Apoptotic rates of cells treated with cisplatin and ASODN were higher than that of cells treated with cisplatin alone ($P<0.05$). **CONCLUSION**: hTERT ASODN could increase sensitivity of cultured primary acute lymphoblastic leukemia cells from relapse patients to cisplatin.

[**KEY WORDS**] Genes, hTERT; Oligonucleotides, antisense; Telomerase; Leukemia; Apoptosis; Cisplatin
[**CLC number**] R363 [**Document code**] A

Telomerase is a unique reverse transcriptase consisting of two major components, the RNA moiety (hTER) and the catalytic subunit (hTERT) with reverse transcriptase activity, which is responsible for telomere protection and maintenance. Telomerase is specifically activated in most malignant tumors and usually inactive in normal somatic cells, suggesting that telomerase plays an important role in cellular immortalization and tumorigenesis[1,2]. It has been indicated that hTERT expression and telomerase activity can serve as a molecular marker of the clinical progression and prognosis of most leukemia[3]. The recent study suggests that the activation of telomerase in leukemia cells is connected with amplification of hTERT and hTERC genes[4]. These observations suggest telomerase as an important target for the development of new anti - cancer drugs and strategies based on the reversal of tumor growth by telomerase inhibition[5,6]. In our lab, one antisense sequence has been identified in the translation initiation region of hTERT mRNA that effectively down - regulates expression of telomerase activity and can increase the sensitivity of jurkat and CEM cell lines to cisplatin[7,8].

Relapsed acute lymphoblastic leukemic (ALL) patients always resist to many chemotherapic drugs. So it is important to enhance the sensitivity of ALL cells at relapse to chemotherapic drugs.

The aim of the present study was to further compare the effect of the antisense oligodeoxynucleotide (ASODN) on apoptosis in cultured primary ALL cells from relapse patients. It may be useful for searching for a new anti - telomerase drug.

## MATERIALS AND METHODS

### 1 Tissue samples

Bone marrow specimens from 6 acute lymphoblastic leukemia patients at relapse were analyzed. The diagnosis of these ALL patients was based on the morphologic and cytochemical criteria of the French - American - British (FAB) Cooperative Working Group. Ranging of the age was 14 - 27 years, three patients were male and three female. All of the ALL specimens were obtained

[**Received date**] 2006 - 03 - 08 [**Accepted**] 2006 - 05 - 10

from the first affiliated Hospital of Jinan University Medical College, People's Hospital of Guangdong Province. The mononuclear cells were isolated by centrifugation.

**2 Cell culture and reagents**

The mononuclear cells isolated were cultured in 5% $CO_2$ at 37 ℃ in RPMI – 1640. The medium was supplemented with 10% newborn calf serum and 1 × $10^5$ units/L of penicillin plus 100 mg/L streptomycin. The sequence of the ASODN targeting hTERT mRNA was 5' – GGAGCGCGCGGCATCGCGGG – 3'. For control, sense oligodeoxynucleotide (SODN) was used in the experiment. The sequence was 5' – CCCGCGATGCCGCGCGCTCC – 3'. Phosphorothioate ODN was synthesized and purified by Shanghai Sangon Biology Engineer Corporation. RPMI – 1640 and newborn calf serum were purchase from Gibco – BRL.

**3 Measurement of hTERT protein by flow cytometry**

The mononuclear cells isolated were harvested with 5 μmol/L cisplatin after 24 h of exposure to 10 μmol/L hTERT ASODN or SODN, collected, centrifuged, and washed with PBS. After fixation with methanol for 30 min and washing twice with PBS(containing permeabilization of 1 % Tween – 20), cells were incubated with polyclonal antibody against hTERT (Gene Co.) for 30 min at room temperature in the dark. After washing twice with PBS, cells were incubated with FITC – conjugated secondary antibody (Sigma Co.) for 30 min at room temperature in the dark. Then, the cells were measured by flow cytometry and mean fluorescence intensity for hTERT protein was determined.

**4 Analysis of cell apoptosis**

The mononuclear cells isolated were harvested after treatment with cisplatin after 24 h of exposure to ASODN, and fixed in methanol. Morphology was determined with Hoechst33258 and propidium iodide (PI) staining. The morphology of cells was examined under a fluorescence microscope. The flow cytometry evaluation of the cell – cycle status and apoptosis was performed. Briefly, untreated or treated cells were centrifuged, washed in phosphate – buffered saline (PBS), and fixed in 70% ethanol. The tubes containing the cell pellets were stored at 20 ℃ for at least 24 h. After this, the cells were centrifuged at 800 × $g$ for 15 min, and the supernatant was discarded to remove ethanol completely. The pellets were resuspended in 0.5 mL PBS and stained with PI solution (20 mg/L PI and 20 mg/L RNase A in PBS) for 30 min. Cells with DNA content below $G_1$ phase were regarded as apoptotic cells. The percentage of cells in the apoptotic sub – $G_1$ was analyzed by multicycle software (Phoenix Flow Systems, San Diego, CA).

**5 Statistical analyses**

Data are expressed as the mean ± standard deviations ($\bar{x} \pm s$). Comparisons were analyzed by ANOVA.

## RESULTS

**1 Effect of hTERT ASODN on hTERT protein in ALL cells from relapse patients**

Expression of hTERT protein significantly decreased in 6 case of cultured ALL cells treated with 10 μmol/L hTERT antisense ODN for 48 h as compared to the untreated cells and sense – ODN treated cells ($P < 0.05$). At 72 h after hTERT antisense ODN treatment, ALL cells showed more reduction in the levels of hTERT protein compared with cells treated with sense ODN ($P < 0.05$) (Tab 1).

**Tab 1 Effects of hTERT ASODN on hTERT protein level ($\bar{x} \pm s$. $n = 6$)**

| Group | Time | |
|---|---|---|
| | 48 h | 72 h |
| Control | 72.31 ± 3.45 | 67.20 ± 2.83 |
| SODN | 73.28 ± 4.17 | 65.83 ± 6.58 |
| ASODN | 47.07 ± 1.02* | 31.94 ± 4.53* |

*$P < 0.05$ *vs* control and SODN group at the same time.

**2 Effect of hTERT ASODN on viability of ALL cells**

10 μmol/L antisense hTERT inhibited the survival of ALL cells after incubation (Fig 1). However, there was no difference on ALL cells growth between ASODN and SODN ($P > 0.05$). Treatment with 5 μmol/L cisplatin after 24 h of exposure to 10 μmol/L ASODN had enhanced inhibition on growth of 6 ALL cells from 6 case of patients ($P < 0.05$), but there was no difference on ALL cells growth of 6 case between SODN plus cisplatin and cisplatin alone ($P > 0.05$).

**3 hTERT ASODN induced apoptosis of ALL cells**

Morphologically, treatment with 5 μmol/L cisplatin for 48 h after 24 h of exposure to hTERT ASODN, ALL cells presented characteristic of apoptosis (mem-

brane blebbing , nuclear condensation and fragmentation and formation of apoptotic bodies) (data not shown). ALL cells after treatment with hTERT ASODN (10 μmol/L) and cisplatin (5 μmol/L) for 48 or 72 h exhibited a sub – $G_1$ peak, and the apoptotic rate had significant difference, as compared with hTERT ASODN, cisplatin or hTERT SODN in combination with cisplatin treated cells, respectively ($P < 0.05$). No significant difference in apoptosis was identified between cells treated cells SODN plus cisplatin and cisplatin alone ($P > 0.05$) (Fig 2,3).

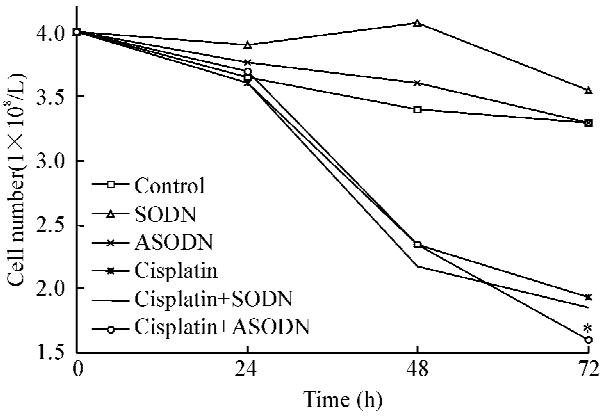


**Fig 1 The effect of hTERT ASODN and 5 μmol/L cisplatin used together on survival of primary cultured ALL cells of relapse patients. *$P < 0.05$ *vs* cisplatin + SODN or cisplatin. $n = 6$.**

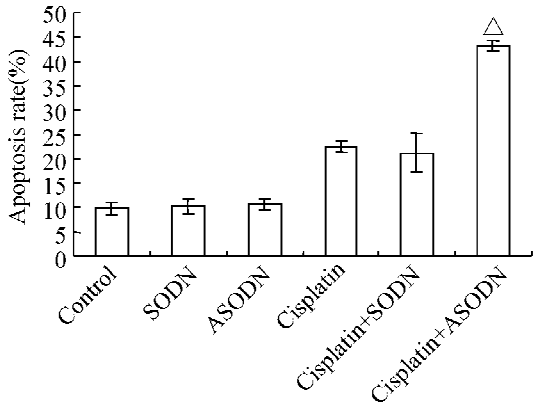


**Fig 2 The percentage of apoptotic cells of ALL cells treated with hTERT ASODN and 5 μmol/L cisplatin together in 48 h. $\bar{x} \pm s$. $n = 6$. △$P < 0.05$ *vs* cisplatin + SODN group and cisplatin group, respectively.**

## DISCUSSION

Human TERT is a limiting component for telomerase activity, the hTERT mRNA is often up – regulated in cells containing telomerase activity. Repression of telomerase activity was demonstrated to be associated with hTERT mRNA down – regulation[9,10]. The recent studies indicate that telomerase is endowed of additional functions in the control of growth and survival of tumor cells that do not depend only on the ability of this enzyme to maintain telomere length. This observation suggests that inhibiting telomerase or its synthesis may have additional anti – proliferation and apoptosis inducing effect, independently of the reduction of telomere length during cell divisions[11].

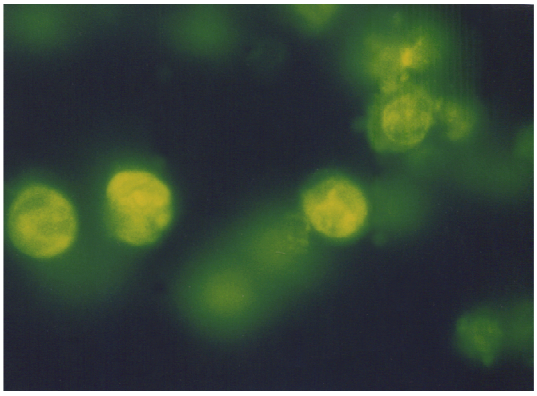

**Fig 3 Apoptotic cells of ALL cells treated with hTERT ASODN and 5 μmol/L cisplatin together in 48 h (Hoechst33258 and PI double staining).**

Our results indicate that expression of hTERT significantly decreased in cultured ALL cells treated with 10 μmol/L hTERT ASODN for 48 h and 72 h as compared with either hTERT SODN or untreated cells. The viability of cultured ALL cells exposed to 10 μmol/L hTERT ASODN, followed by cisplatin (5 μmol/L) was effectively reduced in a time – dependent fashion, compared to treatments with either hTERT SODN plus cisplatin or cisplatin alone. There was no difference in the viability of ALL cells between hTERT SODN in combination with cisplatin and cisplatin. Similarly, the apoptotic rate was also increased in combination – treated cells compared with either hTERT SODN and cisplatin – treated cells or cisplatin – treated cells. This observation is in agreement with other findings, in which suppression of telomerase can enhance chemotherapeutic agents[12]. We previously demonstrated that hTERT antisense oligonucleotide can increase the sensitivity of jurkat and CEM cell lines to cisplatin[7,8]. Our data indicated that the combination of hTERT ASODN and cisplatin produced a greater inhibitory effect on cell growth of ALL cells than the two treatments given separately, suggesting hTERT knock – down caused a marked syn-

ergistic enhancement of cisplatin – induced apoptosis. In our research, the ALL cells are cultured from relapsed patients which are not sensitive to drugs. So, this study may provide a new therapeutic approach to recurrent ALL patients.

[REFERENCES]

[1] Hiyama E, Hiyama K. Clinical utility of telomerase in cancer[J]. Oncogene, 2002, 21(21): 643 – 649.

[2] Hiyama E, Hiyama K. Telomerase as tumor marker[J]. Cancer Lett, 2003, 194(2): 221 – 233.

[3] Deville L, Hillion J, Lanotte M, et al. Diagnostics, prognostic and therapeutic exploitation of telomeres and telomerase in leukemias[J]. Curr Pharm Biotechnol, 2006, 7(3): 171 – 183.

[4] Nowak T, Januszkiewicz D, Zawada M, et al. Amplification of hTERT and hTERC genes in leukemic cells with high expression and activity of telomerase[J]. Oncol Rep, 2006, 16(2): 301 – 305.

[5] Kelland LR. Overcoming the immortality of tumour cells by telomere and telomerase based cancer therapeutics – current status and future prospects[J]. Eur J Cancer, 2005, 41(7): 971 – 979.

[6] Folini M, Zaffaroni N. Targeting telomerase by antisense – based approaches: perspectives for new anti – cancer therapies[J]. Curr Pharm Des, 2005, 11(9): 1105 – 1117.

[7] 李文瑜,张 洹,何冬梅. hTERT 基因反义核酸对 CEM 细胞端粒酶活性的影响[J]. 中国实验血液学杂志, 2003, 11(5): 450 – 453.

[8] 李文瑜,张 洹,何冬梅. hTERT 基因反义核酸对顺铂诱导 Jurkat 细胞凋亡的影响[J]. 中国病理生理杂志, 2003, 19(12): 1596 – 1600.

[9] Janknecht R. On the road to immortality: hTERT upregulation in cancer cells[J]. FEBS Lett, 2004, 564(1 – 2): 9 – 13.

[10] Chang JT, Chen YL, Yang HT, et al. Differential regulation of telomerase activity by six telomerase subunits[J]. Eur J Biochem, 2002, 269(14): 3442 – 3450.

[11] Pendino F, Tarkanyi I, Dudognon C, et al. Telomeres and telomerase: Pharmacological targets for new anticancer strategies? [J]. Curr Cancer Drug Targets, 2006, 6(2): 147 – 180.

[12] Nakamura M, Masutomi K, Kyo S, et al. Efficient inhibition of human telomerase reverse transcriptase expression by RNA interference sensitizes cancer cells to ionizing radiation and chemotherapy[J]. Hum Gene Ther, 2005, 16(7): 859 – 868.

# hTERT 基因反义核酸提高原代复发急性淋巴细胞白血病细胞对顺铂敏感性的研究

李文瑜[1], 张 洹[2]

([1] 广东省人民医院, 广东 广州 510080; [2] 暨南大学医学院血液病研究所, 广东 广州 510632)

[摘 要] **目的**:研究人类端粒酶逆转录酶(hTERT)基因的反义核酸(AS PS – ODN)能否增强复发急性淋巴细胞白血病原代细胞对顺铂的敏感性。**方法**:采用免疫荧光标记法检测 hTERT 蛋白表达水平,以台盼蓝拒染法计数细胞数,通过细胞形态学观察及流式细胞仪细胞周期的分析来检测凋亡。**结果**:hTERT 基因反义核酸能降低 hTERT 蛋白的表达。顺铂与 hTERT 基因反义核酸联合应用能明显抑制培养原代细胞的增殖,与单用顺铂组比较 $P<0.05$。凋亡检测,用药 48 h 后,单用顺铂、顺铂联用反义核酸或正义核酸组,经 Hoechst33258 和 PI 双染法观察细胞均表现典型的细胞凋亡的形态学改变;顺铂联用反义核酸组,细胞的凋亡率明显高于单用顺铂组($P<0.05$)。**结论**:hTERT 基因反义核酸能增强复发急性淋巴细胞白血病原代细胞对顺铂的敏感性。

[关键词] 基因,hTERT; 寡核苷酸类,反义; 端粒,末端转移酶; 白血病; 细胞凋亡; 顺铂

[中图分类号] R363 [文献标识码] A