Antisense human telomerase reverse transcriptase inhibits leukemia cell proliferation in vitro
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Abstract: Objective To study the inhibitory effect of antisense human telomerase reverse transcriptase (hTERT) on leukemia cell proliferation in vitro. Methods Sense and antisense hTERT eukaryotic expression vectors previously constructed were transfected into leukemia cell line HL60 using SuperFect transfection reagent (Qiagen) to obtain HL60-s and HL60-as, and the G418-resistant colonies were identified for the presence of hTERT insert by PCR with T7 and pcDNA3.1/BGH reverse primers. Endogenous hTERT mRNA expression and telomerase activity were then detected by quantitative real-time RT-PCR and telomerase-associated protein -silver staining in each cell line. MTT cellular proliferation assay, soft agar colony formation assay and flow cytometry were also employed to analyze the changes in proliferation capacity of leukemia cell in vitro and apoptosis of the tumor cells induced by antisense hTERT. Results Antisense hTERT remarkably reduced endogenous hTERT mRNA expression (P<0.01) and down-regulated telomerase activity in HL60 as compared with the blank control and sense hTERT. After 25 passages of the 3 cell lines, a 7-day cell growth curve and the numbers (size) of soft agar colony formation showed that the proliferation rates and the anchorage-independent growth ability of HL60-as cells were significantly decreased in comparison with HL60 and HL60-s cells, but a significant increase in apoptosis of HL60-as cells occurred as determined by flow cytometry. Conclusions Antisense hTERT can obviously inhibit leukemia cell growth and proliferation in vitro, and this telomerase-targeted molecular biotherapy may be achieved by apoptosis pathway through down-regulation of hTERT mRNA and telomerase activity.

Key words: antisense human telomerase reverse transcriptase; leukemia; quantitative real-time RT-PCR; cell proliferation; molecular biotherapy

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1. 材料与方法
1.1 基于正向克隆的基因真核表达载体的构建与鉴定
首先将上述含有正确克隆的正向克隆载体多克隆位点两侧的序列经限制性内切酶剪切后，用DNA连接酶连接到质粒载体多克隆位点两侧的相应位点上，形成基因真核表达载体，以继续维持筛选作用。所有转染及筛选所用的质粒载体均为高效真核表达质粒，具体逆转录及PCR反应混合物准备如下：

1.2 材料

反转录试剂盒、质粒试剂盒及质粒试剂盒为美国公司产品，T2/F2胎牛血清、双抗等试剂、试剂盒立即提取细胞总RNA，回收试剂盒为上海公司产品，公司产品凝胶回收试剂盒，反转录试剂盒为美国公司产品，试剂盒大量提取并定量为美国公司产品，试剂盒为上海公司产品

1.3 方法

反转录试剂盒为美国公司产品，试剂盒大量提取并定量为美国公司产品，试剂盒为上海公司产品

1.4 试剂

EndoFree、SuperFect Transfection Reagent、Qiagen

1.5 反转录

RT-PCR

PCR

LightCycler

LightCycler TeloTAGGG hTERT Quantification Kit，PCR

2. 统计学方法

3. 结果和讨论
Table 1: Quantitative real-time RT-PCR results of hTERT mRNA expression in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean NhTERT Hlmo (M±SD)</th>
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<tbody>
<tr>
<td>Control</td>
<td>38.3±2.32</td>
</tr>
<tr>
<td>Sense hTERT</td>
<td>36.4±2.78*</td>
</tr>
<tr>
<td>Antisense hTERT</td>
<td>6.33±0.96**</td>
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*P<0.05, **P<0.01 vs control

Fig. 1: PCR amplification of the exogenous gene fragment in G418-resistant colonies
Lane 1: 100 bp ladder marker; Lane 2: HLmo-as; Lane 3: HLmo-s; Lane 4: HLmo

Fig. 2: TRAP-silver staining results of HLmo telomerase activity
M: PBR322 DNA/Hoe III marker; Lane 1: HLmo-s group; Lane 2: Negative control; Lane 3: Blank control; Lane 4: HLmo-as group

Fig. 3: Growth curves of the 25th generation of the 3 cell lines in a 7-day incubation

Fig. 4: Apoptotic rate of the 25th generation of the 3 cell lines
在抗肿瘤靶向分子生物学治疗中有着高度特异性。尽管传统的手术及放化疗方法不断对患者的治愈率仍不理想，但对患者的治愈率仍不理想。因此尽管转入了反义的构建与鉴定有意义。

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