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Relationship between telomerase activity and expression of p15INK4b gene in children with acute leukemia

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Abstract: Objective To study the relationship between telomerase activity and p15INK4b (p15) expression in the development of childhood acute leukemia (AL). **Methods** Telomerase activity and p15 expression in bone marrow (BM) of 27 cases of childhood AL were evaluated using a modified telomeric repeat amplification protocol assay and reverse-polymerase chain reaction respectively. For comparative analysis, BM from control donors ($n = 9$) were analyzed. **Results** At diagnosis, the telomerase activity detected in BM from AL children was significantly higher than that from control donors (34.5 ± 37.0 TPG vs 2.4 ± 2.2 TPG, $P < 0.001$). The p15 expression in BM of AL children was significantly lower than that of the controls (9.8 ± 16.2% vs 45.8 ± 16.9%, $P < 0.001$). No significant correlation was observed between p15 expression and telomerase activity ($r = -0.01304$, $P > 0.05$). **Conclusions** The telomerase up-regulation and lower expression of p15 gene may play an important role in the development of AL by different mechanisms.

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Key words: Leukemia; Telomerase; p15INK4B; Gene expression; Child

儿童急性白血病端粒酶活性与 p15 基因表达关系的研究

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[**摘 要**] **目的** 探讨端粒酶活性与 pINK4b(p15)基因表达在儿童急性白血病(AL)发病中的关系。**方法** 采用改良端粒重复序列 PCR 扩增和逆转录聚合酶链反应检测 27 例儿童 AL 骨髓单个核细胞端粒酶活性和 p15 基因表达情况,并与 9 例正常骨髓单个核细胞端粒酶活性及 p15 基因表达进行比较。**结果** 初诊时,AL 患儿骨髓细胞端粒酶活性(34.5 ± 37.0)TPG 较对照组(2.4 ± 2.2)TPG 明显增高($P < 0.001$); AL 患儿 p15 基因表达水平(9.8 ± 16.2)%, 明显低于对照组(45.8 ± 16.9)% ($P < 0.001$)。AL 患儿骨髓细胞端粒酶活性与 p15 基因表达水平之间无相关关系($r = -0.01304$, $P > 0.05$)。**结论** 端粒酶活化和 p15 基因表达水平降低与急性髓性白血病发展有关,但两者可能通过不同的机制作用于白血病。 [**中国当代儿科杂志,2005, 7(2):112-114**]

[**关 键 词**] 白血病;端粒酶;p15 INK4B;基因表达;儿童

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Telomerase (TS) is the only known ribonucleoprotein in human cells with reverse transcriptase activity. It contains an RNA component that provides a template for the synthesis of repeated telomeric sequences. These repeats are attached to the ends of existing telomeres to maintain telomere lengths. TS activation is an important step for the development of malignant tumor[1]. Telomerase activity is present in almost all types of malignances, including hematologic malignancies, but essentially is absent in the great majority of normal somatic tissues[2]. The p15INK4B (p15) gene is located in a region on chromosome 9p21. The protein encoded by the p15 gene inhibits kinase activity thereby suppressing cell proliferation. Inactivation of this gene has been found in many malignant tumors, suggesting that they are candidate tumor

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suppressor genes[3]. This study examined both the transcriptase activity and p15 gene expression in children with acute leukemia to explore their relationship in the development of acute leukemia.

Subjects and methods

Patients

Twenty-seven children with newly acute leukemia (AL, 16 males and 11 females aged from 3 to 14 years) were included in this study. The French-American-British classification was used to diagnose and classify AL. There were 13 cases of acute lymphatic leukemia (ALL) and 14 cases of acute myeloid leukemia (AML). Bone marrow specimens were obtained at initial diagnosis (before chemotherapy). Nine bone marrow specimens from children with immune thrombocytopenia were used as controls (5 males and 4 females aged from 3 to 10 years). Consent for specimen collection was obtained following institutional guidelines.

Assessment of telomerase activity in leukemic bone marrow cells

Mononuclear cells (MNCs) were isolated from heparinized bone marrow by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. After being washed with PBS for twice MNCs were incubated with 1 × CHAPS (10^6 cells/200 μL) and were then centrifuged at 12 000 g for 30 minutes at 4℃. Cell lysates from each patient's sample and positive control were assayed in 50 μL of reaction mixture. After 30 minutes of incubation at 30℃, the reaction mixture was subjected to 33 PCR cycles at 94℃ for 30 seconds, at 60℃ for 30 seconds, and then incubated at 72℃ for 10 minutes in a thermocycler. The PCR product was electrophoreses on a 10% acrylamide gel, and silver stain (Pharmacia Biotech) signals were scanned with Gel DOC200 analytic system of gel image (Bio-Rad). Negative and heat-inactivation controls were used for each amplification.

Quantitation of telomerase activity

The TRAPEZE™ telomerase detection kit (Oncor Corporation, USA) was used to detect the telomerase activity. Each reaction mixture included TS primer, cell extracts from samples, 36 bp TSK1 template (internal standard) and TSR8 control template for quantitation. Each unit of TPG (total product generated) corresponded to the number of TS primers (in 1×10^{-3} amole or 600 molecules) extended with at least 4 telomeric repeats by telomerase in the extract in a 10 minute incubation at 30℃. The assay had a linear range of 1 to 300 TPG, which is equivalent to telomerase activity from approximately 30 to 10 000 control cells.

RT-PCR analysis of p15 gene expression

Total cellular RNA was extracted from pelleted bone marrow MNCs (5×10^6 cells) by RNAsol methods[2]. Complementary DNA (cDNA) was made using the Universal RiboClone cDNA synthesis system (Promega, Madison, WI). Each reaction contained 2.5 μL 10 × reaction buffer, 0.75 μL 10mM dNTPs, 5 μL of each cDNA, 2 μL and 1.25 μL of p15 and β-actin primers respectively, 2 μL 25 μM $MgCl_2$, 0.25μL Taq polymerase (Fisher Biotech, Pittsburgh, PA), 3.1 μL 40% glycerol and nuclease-free water to a final volume of 25 μL. Temperature conditions for PCR were as follows: 32 cycles of 94 ℃ for 50 seconds, 62 ℃ for 45 seconds, 72 ℃ for 45 seconds. RNA of HL-60 cell was used as a positive control. Primer sequences: for p15, 5′-CCAGAAGCAATCCAGGCGCG and 5′-CGATGAAGGCTGCCAACG, and for β-actin, 5′-GGGTCGGAAGGATTCCTATG and 5′-TCTCAAACATGATCTGGGTC. For relative quantitation, p15 expression level was based on ratio of density of ethidium staining of p15 and β-actin.

Statistical analysis

SPSS 8.0 software was used and the data were presented as $\bar{x} \pm s$. One-way ANOVA was applied for analysis of differences among groups. Correlation was evaluated with Spearman analysis.

Results

Telomerase activity and p15 gene expression

The telomerase activity was from 1 to 135 TPG in the BM cells from 27 AL children, with an average of 34.5 ± 37.0 TPG. That of the 9 control donors was 1 to 7 TPG, with the average of 2.4 ± 2.2 TPG. There were significant differences in the telomerase activity between the AL children and control donors ($P < 0.001$). The expression level of p15 in AL children was from 0 to 57.2%, with the average level of 10.1 ± 17.3%, while the expression level of the controls was 45.8 ± 16.9%. A significant difference was found between the two groups for the p15 expression ($P < 0.001$). There were no significant differences between the AML and ALL children for both the telomerase activity and p15 expression ($P > 0.05$).

Relationship between telomerase activity and p15 expression

The telomerase activity and p15 expression level

in 27 cases of childhood leukemia are shown in Figure 1. The correlation coefficient was -0.0130 by Spearman analysis ($P > 0.05$), suggesting that there was no correlation between the telomerase activity and p15 expression level.

Table1 Telomerase activity and p15 gene expression in MNCs ($\bar{x} \pm s$)

Group	n	p15 expression(%)	TS (TPG)
Control	9	45.8 ±16.9	2.4 ±2.2
AL	27	9.8 ±16.2[a]	34.5 ±37.0[a]
ALL	13	10.6 ±17.3[a]	35.2 ±39.4[a]
AML	14	9.0 ±15.8[a]	33.8 ±36.0[a]

a Compared with the Control group $P < 0.001$

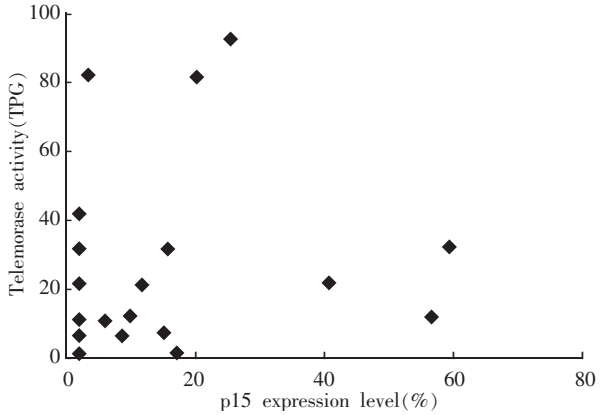

Figure 1 Relationship between telomerase activity and p15 expression in AL children

Discussion

Early in 1992, Adamson[5] reported that the length of the telomere sequence of the blast phase cells was shortened when compared with that of the cells examined during remission in children ALL. The telomerase activity detected in AL children was significantly higher than that in the control donors, and the result was consistent with that reported at home and abroad[6,7]. The data suggested that telomerase was generally activated in the early stage of AL. The p15 gene is considered as an important tumor suppressor gene and is located in a region on chromosome 9p21. The p15 protein prevents hyperphosphoralation of Rb gene and disassociation of transcription factor E_2F, which then leads to inhibition of G1/S transition. This gene is a negative regulator of the cell cycle[8]. This study showed that p15 expression level in AL children was significantly lower than that in the controls, suggesting that telomerase activity up-regulation and lower expression of p15 gene play an important role in the development of AL.

Telomeres, the ends of linear chromosomes, shorten with each round of DNA replication. Loss of telomeric DNA can lead to senescence, a state in which cells no longer divide, and crisis, which triggers cell death. To prevent these phenomena, cancer and stem cells must maintain their telomeres, for example, by expressing telomerase, an enzyme that can extend telomeres. Activity of telomerase is mainly associated with velocity of cell division, cell cycle regulation and cell differentiation. Up-regulation of telomerase activity is considered to be responsible for immortalization and carcinogenesis and it may be a molecular marker of vigorous cell proliferation. The p15 gene is a candidate tumor suppressor gene with direct effects on cell cycle. Loss of function of this cyclin-dependent inhibitor leads to unrestrained proliferation of cells as cells with damaged DNA cannot be stopped at G1 phase and the changed genomic DNA cannot be repaired or cleared. This study showed that there was no correlation between telomerase activity and p15 expression. It is suggested that telomerase activity up-regulation and lower expression of p15 gene may be involved in the pathogenesis of AL by different mechanisms.

[**References**]

[1] Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, et al. Telomerase maintains telomere structure in normal human cells [J]. Cell, 2003, 114(2): 241-253.

[2] Djojosubroto MW, Choi YS, Lee HW, Rudolph KL. Telomeres and telomerase in aging, regeneration and cancer [J]. Mol Cells, 2003,15(2): 164-175.

[3] Teofili L, Martini M, Luongo M, Diverio D, Capelli G, Breccia M, et al. Hypermethylation of GpG islands in the promoter region of p15 (INK4b) in acute promyelocytic leukemia represses p15 (INK4b) expression and correlates with poor prognosis [J]. Leukemia, 2003, 17(5): 919-924.

[4] Gao XZ, Bi S, Copra H, Devemy E, Venugopal P, Li B, et al. Cytokine gene activity in AML cells in vivo in patients [J]. Leuk Res, 1998, 22(5): 429-438.

[5] Adamson DJ, King DJ, Haites NE. Significant telomere shortening in childhood leukemia [J]. Cancer Genet Cytogenet, 1992, 61(2): 204-206.

[6] Engelhardt M, Ozkaynak MF, Drullinsky P, Sandoval C, Tugal O, Jayabose S, et al. Telomerase activity and telomere length in pediatric patients with malignancies undergoing chemotherapy [J]. Leukemia. 1998, 12(1): 13-24.

[7] Wen F-Q, Cao L-Z, Chen Y-X, Li B, Zhou K-Y, Li Y-H, et al. Detection of telomerase activity in acute myeloid leukemia of children and its clinical significance(in Chinese) [J]. Chin J Pract Pediatr, 2004, 19(9): 549-551

[8] Hitomi T, Matsuzaki Y, Yokota T, Takaoka Y, Sakai T. p15 (INK4b) in HDAC inhibitor-induced growth arrest [J]. FEBS Lett. 2003, 554(3): 347-350.

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