Clinical Significance of Telomerase Activity and p170 Coexpression in Acute Leukemia*

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Abstract **Objective** To explore the relationship between the coexpression of telomerase and p170 and clinical curative effect in acute leukemia(AL) cells. **Methods** The expression of p170 in bone marrow cells of 43 patients with AL were detected by flow cytometry and telomerase activity were detected by TRAP. **Results** There was positive correlation between telomerase activity and p170(r=0.745 P=0.02), The difference of complete remission rate in both positive and in both negative patients was significant (83.3% and 29.4%, P=0.04). **Conclusion** There were lower clinical complete remission and poorer prognosis in patients who expressed both telomerase activity and p170.

Key Words Acute leukemia; Lymphocytic; Myelocytic; Telomere; Prognosis

Multidrug resistance (MDR) is one of the important causes resulting in resistance and relapse acute leukemia (AL) and its mechanism is very complicated. Presently it is thought that MDR gene expression is the cross-drug resistance expression participated with multi-mechanisms [1], such as lung resistance-related protein (LRP), overexpression of MDR and bcl-2 mediated by DNA topoisomerase Ⅱ (TOPQⅡ), overexpression of p170 mediated by MDR-1 and high expression of telomerase. To investigate the relationship between telomerase activity, p170 protein expression and clinical drug resistance, we used telomerase repeat amplification protocol (TRAP), two-step fluorescence-labeled immunohistochemistry and flow cytometry to detect the telomerase activity and p170 protein expression in the leukemic cells of 43 patients with AL.

MATERIALS AND METHODS

Clinical data

From Aug. 1, 2001 to Dec. 30, 2002, we investigated 43 patients with AL hospitalized from Department of Hematology in General Military Regional Hospital of Guangzhou (23 cases) and Institute of Hematology in Jinan University (20 cases), including 27 males and 16 females. The age of patients ranged from 16 to 68 years old and the median was 39 years old. These patients included 26 cases of acute myeloid leukemia (AML) (3 cases of M1, 9 cases of M2, 3 cases of M3, 6 cases of M4, 4 cases of M5 and 1 cases of M6)and 17cases of acute lymphoblastic leukemia (ALL). There were 38 cases of initial treatment AL and 5 cases of relapse AL among them. The patients with ALL were administered with VDCP/VDLP scheme (V: vincristine; D: daunomycin; C: cyclophosphamide; L: L-asparaginase; P: prednisone), while these with AML were treated with HA/DA/MAE scheme (H: harringtonine; M: mitoxantrone; A: arabinosylcytosine; E: etoposide). Firstly the patients with M3 were induced to achieve remission with retinoic acid, secondly were maintained with HA/retinoic acid/DA schemes by turns for more than 3 years. The clinical curative effect was evaluated by the Criterion of National Leukemia Chemotherapy Conference in 1989 [2]. The 12 cases in normal control group were the healthy people doing physical examination in clinic during the same period, including 8 males and 4 females. The age ranged from 18 to 52 years old and the median was 41 years old. The expressions of p170 protein were simultaneously detected in all patients.

Preparation of samples

The peripheral blood of patients and healthy control group was taken in sterile condition, then was injected into the sterilized centrifuge tube (containing 2ml RPMI1640 culture medium and

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50U/ml heparin sodium). Four milliliters lymphocyte separation medium was put into the 10ml sterilized centrifuge tube. Then the peripheral blood samples were slightly transferred into the tube along the wall and were centrifuged at 1200r/min for 10 minutes. Mononuclear cells were sampled and washed with RPMI1640 culture medium, then were centrifuged at 1200r/min for 10 minutes. Consequently the upper layer liquid was discarded. The above process was repeated once. Finally the cells were suspended in the RPMI1640 culture medium containing 10% fetal bovine serum.

Quantitative detection of telomerase activity

TRAP-PCR-ELISA method and telomerase PCR-ELISA detection kit (Boehrioger mannheim company, Germany) were employed.

1) TRAP reaction: Two microliters peripheral mononuclear cells separation medium in the above process, 25μl reactive mixture and 23μl sterilized DEPC water were added into the PCR amplification tube. Then the primer was extended at 25℃ for 10 minutes, then the telomerase was inactivated at 94℃ for 5 minutes. Consequently PCR amplification was performed. The 50μl reactive system was amplified for 30 cycles in the condition of 94℃ for 30 seconds, 50℃ for 30 seconds and 72℃ for 90 seconds and finally was equilibrated at 72℃ for 10 minutes, which contained dNTP, Taq enzyme, primer Ⅰ and primer Ⅱ labeled by biotin.

2) Detection of amplification products by ELISA Five microliters PCR amplification products mixed with 20μl degeneration agent were incubated at room temperature (20℃) for 10 minutes, then were sufficiently mixed with 225μl hybridization mixture liquid (containing probe labeled by digoxin). One hundred microliters liquid was transferred from the above liquid into micropore plate coated with antibiotic protein and hybridized in the swing bed at 37℃ for 2 hours, then was washed with 250μl washing buffer 3 times after the hybridization liquid being discarding. Consequently it was mixed with 100μl anti-digoxin antibody coupled with peroxidase (anti-DIG-POD) and incubated at room temperature for 30 minutes. Being vibrated and washed, it was mixed with 100μl POD´s substrate TMB to show color for 10 minutes and finally 100μl termination liquid was added into. Wave length 690nm serving as control, the degree of light absorbed (A) of sample was detected at the wave length 450nm by ELISA meter during 30 minutes. Telomerase activity was calculated according to the formula A = A450−A690.

Detection of p170 expression

Two-step fluorescence-labeled immunohistochemistry and flow cytometry were employed. About 2ml patient´s bone marrow was aspired and coagulated with heparin, then was diluted with equal Hanks liquid and centrifuged with Ficoll liquid at 1500r/min for 15minutes. Consequently all mononuclear cells were isolated, sampled and washed with 100μl PBS twice and were suspended to fix in the 1% polyformaldehyde for 20 minutes. Then they were (1) washed with 100μl PBS containing 0.1% Tween-20 three times; (2) blocked with PBS containing 20% bovine calf serum for 30 minutes; (3) sufficiently mixed with anti-p170 glycoprotein monoclonal antibody; (4) incubated at 37℃ for 1 hour; (5)washed with 100μl PBS twice(experimental group and control group were set up); (6) suspended in 25μl secondary antibody working liquid; (7) incubated in the wet box at 37℃ for 1 hour; (8) washed with 100μl PBS twice and suspended in PBS again. The percentage of cells expressing p170 protein in the whole was detected by flow cytometry.

Statistical analysis

The data were analyzed with X^2 test and linear correlation analysis by software SPSS 8.0, while $\alpha = 0.05$ was designated as significant difference level.

RESULTS

Relationship between telomerase activity and complete remission (CR) rate of AL

Among 43 cases of AL, the CR rate is 65.12% (28/43) and the positive rate of telomerase activity is 53.49% (23/43). The CR rate of patients with positive telomerase is 47.83% (11/23), while that of patients with negative telomerase is 85.00% (17/20). The difference between them is significant ($X^2 = 6.508$, $P=0.011$). Among 5 cases of relapse AL, there are 4 cases of positive telomerase (including 1 case of CR) and 1 case of negative telomerase (1 case of CR).

Expression of telomerase and p170 in different prognosis group

There are 8 cases of positive telomerase among the 24 cases of non-relapse and resistance AL

(33.33%), while 15 cases of positive telomerase among the 19 cases of relapse and resistance AL (78.95%). The percentage in the latter is higher than that in the former (X^2=7.130, P=0.008). The expression of p170 is accordance with that of telomerase (X^2=4.408, P=0.036, see Table.1).

Correlation of telomerase activity with p170 expression

There are 24 cases of positive expression p170 protein among the 43 cases of AL (55.81%) and 15 cases of positive expression p170 in the positive telomerase group (65.22%). The difference of p170 protein expression rate between the positive telomerase group and the negative telomerase group is significant (X^2=4.369, P=0.018). The expression rate of telomerase activity is positively related with activity of $p170$ lineally (r=0.745, α=0.05, see Tab. 1).

Relationship between telomerase and p170 coexpression and prognosis of AL

Among the 43 cases of AL, there are 12 cases of both negative telomerase and p170 (27.91%) and there CR rate is 83.33%(10/12); while there are 17 cases of both positive telomerase and p170 (39.53%) and its CR rate is 27.91% (5/17). The difference of CR rate between them is significant (X^2=8.191, P=0.004).

DISCUSSION

MDR is the main cause of acute leukemia chemotherapy failure and its mechanism is complicated. Presently it is thought that the main classical pathway is that overexpression of p170 coded by MDR-1 mediates drug resistance [3]. p170 is a kind of membrane-spanned glycoprotein which eliminates the drug from inner of cell to outer space along the membrane-penetrated passage by the means of energy-consumed. Therefore it is one of the causes resulting in cells´ drug resistance. Telomerase is a special kind of ribonucleoprotein reverse transcriptase, and is highly expressed in most malignant tumor to maintain the stability of tumor cells´ telomere length and to immortalize the tumor cells[4]. The telomerase activities of malignant tumor in hematopoietic system are not identical. In AL and myelodysplastic syndrome (MDS), the telomerase activity is highly expressed [5]. But it is expressed only in a part of chronic myeloid leukemia (CML) and its expression increases during the acute transformation phase[6,7]. Ohyashiki et al.[8] found that the telomerase activity of monocyte in ALL patients decreased 6-folds during the remission phase and restored to the former level after relapse. In acute and chronic leukemia, the more severe the disease is, the higher the telomerase activity is. Moreover, it is more difficult to treat the leukemia with high-level telomerase activity[8-10]. Telomerase activity is closely correlated with drug resistance of AL, but its molecular mechanism is still unclear. It is found that the telomerase activity in the colon cancer and non-small cell lung cancer is correlated with the mutation of p53 and high expression of p16, bcl-2 respectively[11-12].

Our results demonstrate that and protein were both highly expressed in AL (both about 50%), and the CR rate of patients with positive telomerase or p170 expression is significantly lower than that of patients with negative telomerase or p170 expression respectively (P<0.01). We also found that the CR rate of patients with both positive telomerase activity and p170 protein is significantly lower than that of patients with both negative expressions (P<0.01); the both positive expression rate of telomerase and p170 in the relapse and resistance patients is significantly higher than that in the non-relapse and resistance patients (P<0.01); the telomerase activity is positively correlated with p170

Table 1 Expression of telomerase activity and p170 in 43 patients with acute leukemia

Index	Relapse & resistance (n=19)	Non-relapse & resistance (n=24)	X^2 value	P value
Telomerase positive	15 (78.95)	8 (33.33)	7.130	0.008
p170 positive	14 (73.68)	10 (41.67)	4.408	0.036
Both positive	12 (63.16)	5 (20.83)	7.947	0.005
Both negative	2 (10.53)	10 (41.67)	5.111	0.024

protein expression (r=0.745, P<0.01), which suggested that MDR is related with telomerase activity. The cells´ drug resistance is to export the drug through high expression of p170, and MDR is also related with the reinforcement of cellular DNA´s ability of repair. One of the telomerase´s functions is to take part in the chromosomal repair because high level of telomerase can repair more chromosome break induced by chemotherapy drug. Meanwhile high expression of p170 can reduce the accumulation of drug in the cells. On the double effects of telomerase and p170, drug resistance cells can more easily avoid toxic effect of chemotherapy drug and proliferate quickly.

Our results illustrate that high expression of telomerase or p170 in patients with AL can result in apparent reduction of their complete remission rate and serve as an independent prognosis factor. Moreover high coexpression of telomerase and p170 can lead to lower clinical remission and is more significant to predict prognosis. Therefore, simultaneous detection of telomerase activity and p170 is valuable in the diagnosis, judgment of prognosis and choice of chemotherapy scheme. And further research should be made in the molecular mechanism of telomerase and p170 in MDR and their intrinsic relevance.

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