Study of Heat Shock Protein HSP90α, HSP70, HSP27 mRNA Expression in Human Acute Leukemia Cells

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Summary: The expression of three heat shock proteins (HSPs)-HSP90α, HSP70, HSP27 in cells obtained from 22 patients with leukemia, K562 erythroleukemia cell line, and normal blood cells was observed by means of RNA dot blot analysis. The results showed that the expression of the HSP27 gene was enhanced in 4 cases of acute lymphoid leukemia (ALL), 7 cases of acute nonlymphoid leukemia (ANLL) and 2 cases of myelodysplastic syndrome (MDS) as compared with that of the normal blood cells, yet there was no significant difference in the HSP27 expression between the ALL and ANLL cells. The expression of HSP70 in all the 5 ALL and ANLL patients was much lower than that of the normal subjects, except 1 case of ALL and 1 case of MDS, in which the expression was obviously enhanced. All the cases including 11 ANLL, 5 ALL and 1 MDS had higher HSP90α expression than the normal subjects. The enhanced expression of HSP90α in leukemia cells may be associated with the active and indefinite proliferation of leukemia cells. Our results also suggest that the high expression of the HSP27 gene may not be confined to a specific type of acute leukemia.

Key words: acute leukemia; leukemia cell; heat shock protein

Heat Shock Protein (HSPs), which can protect the cells against heat-induced damage and other stressful conditions, have been known to have very wide spectrum of functions. Recent reports suggested that HSP90α (Molecular weight (MW): 84–90 ku), HSP70 (66–70 ku) and HSP27 (27–28 ku), may be involved in the proliferation, differentiation and heat tolerance of leukemia cells[1]. Therefore, in this study we investigated the expression of HSP gene in cells from patients with acute leukemia, normal hematopoietic cells and leukemia cell lines, with attempt to find any possible differences and its clinical implication.

1 MATERIAL AND METHODS

1.1 Patients and Cells

Mononuclear cells from the peripheral blood or bone marrow were obtained from 22 patients including 7 cases of acute lymphoid leukemia (ALL), 11 cases of acute nonlymphoid leukemia (ANLL), 2 cases of chronic granulocytic leukemia (CML, acute transformation), 2 cases of myelodysplastic syndrome (MDS), and 6 healthy donors by using centrifugation on a Ficoll-Hypaque gradient. The classification of leukemia was based on FAB criteria[2]. No patient had received any treatment with cytosine such as G-CSF etc. before the samples were taken. 4 cases were suffering from moderate fever during sampling. The liver functions of all the patients except one (GPT: 850.17 nmol -s⁻¹/L) were normal. More detailed clinical data of the patients are presented in table 1.

1.2 K-562 Myeloid/erythroid Leukemia Cell Line

K-562 was provided by the Department of Immunology, Tongji Medical University, Wuhan.

1.3 Detection of the Constitutive Expression of HSP27, HSP70, HSP90α in Leukemia Cells

1.3.1 Isolation of Total Cellular RNA

The total cellular RNA was isolated by a single-step procedure with acid guanidinium thiocyanate-phenol-chloroform mixture and quantitatively determined by ultraviolet
spectrophotometer (U-2000, Hitachi). Following denaturation, equal amount of total RNA (5 μg) were spotted on nitrocellulose membranes (LKB, Sweden) and then dried at 80°C for 2 h.

1.3.2 Preparation of HSP27, HSP70, HSP90α Probes

Plasmids PUC208, PUC709 and PUC801 containing cDNAs coding for human HSP27, HSP70, HSP90α respectively, were gifts from Dr. Hickey (University of Nevada, USA). After plasmid transformation, amplification and purification, the DNA fragments of HSP27, HSP70 and HSP90α were recovered and labeled by 32P-dCTP (Isotope Department, Atomic Energy Research Institute of China).

1.3.3 RNA Dot-blot Hybridization

The RNA membranes were pre-hybridized at 68°C for 4 h in pre-hybridization buffer (5×Denhardt, 5×SSPE, 1% SDS, 50% deionized formamide, 500 μg/ml heparin), and then added with denatured 32P-labeled HSP27, HSP70, HSP90α probes, hybridized at 68°C for 20 h. After having been washed for 3 times with different concentrations of SSC and SDS, the membranes were processed for autoradiography at -20°C.

1.3.4 Analysis of Hybridization Results

Quantitative measurements of hybridization dots were made by chromatosanner (CS-930, Shimadzu Co. Japan).

2 RESULTS

2.1 Expression of HSP27 in Leukemia Cells

As showed in fig. 1, 4 cases of ALL and 7 cases of ANLL showed a high level of expression of HSP27 mRNA, the other 3 cases of ALL, ANLL-M3 and CML (acute transformation) respectively, however, expressed less amounts than normal subjects. The expression of HSP27 mRNA in leukemia cells was significantly higher than that in normal blood cells (P<0.05, table 2). There was no obvious difference in the HSP27 expression between ALL and ANLL (P>0.05). We also found that 2 cases of MDS had high level expression of HSP27.

2.2 Expression of HSP70 in Leukemia Cells

In the 7 cases who were examined for expression of HSP70, 1 case of ALL expressed less amount of HSP70 mRNA than normal subjects, while another case of ALL expressed much higher amount. The HSP70 expression in all the 4 cases of ANLL decreased to the level of 6.38±4.31, which was significantly lower than that in normal blood cells (9±2.55; P<0.05). The amount of HSP70 mRNA expression in 1 case of MDS reached as high as 28.9, which was 3 times that in normal cells (fig. 2).

2.3 Expression of HSP90α in Leukemia Cells

All the 16 patients with acute leukemia (11 cases of ANLL, 5 cases of ALL) showed high levels of expression of HSP90α mRNA. The average value was 9.72±3.87, which was significantly higher than that of normal blood cells (3.6±3.0; P<0.05). 1 case of MDS also had high expression of HSP90α with the value being 9.6 (fig. 3).

Fig. 1 mRNA expression of HSP27 in 14 cases with leukemia and 2 cases with MDS
A: Control B: K-562 cell line
Fig. 2 mRNA expression of HSP70 in 7 cases
A: Control
Fig. 3 mRNA expression of HSP90α in 16 cases with leukemia and 1 cases with MDS
A: Control