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High expression of heat shock protein 90 alpha and its significance in human acute leukemia cells

Wen-Liang Tian [a,1], Fei He [b,1], Xue Fu [a], Jun-Tang Lin [c], Ping Tang [a], Yu-Min Huang [a], Rong Guo [a,*], Ling Sun [a,*]

[a] Department of Hematology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province 450052, China
[b] Department of Cardiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province 450052, China
[c] Key Laboratory for Medical Tissue Regeneration of Henan Province, Xinxiang Medical University, Xinxiang, Henan Province 453003, China

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ABSTRACT

This study investigated the expression of heat shock protein 90 alpha (Hsp90α) in acute leukemia cells. The expression of Hsp90α was investigated in leukemia cell lines and human bone marrow mononuclear cells derived from acute leukemia patients and from healthy individuals using polymerase chain reaction, Western blot, and enzyme-linked immunosorbent assay. Compared with cells from healthy individuals, the expression of Hsp90α in the untreated patients was higher. Similarly high levels were observed in remission patients. Significantly higher expression levels were observed in all the tested cell lines, and in cells from refractory and relapsed patients. No obvious relationship was observed between the occurrence of graft versus host disease and the expression of Hsp90α. The untreated patients showing higher expression levels of Hsp90α had lower complete remission rates. During remission of untreated patients, the expression of Hsp90α decreased and reached the lowest level after transplantation, but the expression increased again before relapse. Hsp90α was highly expressed in leukemia cells. The expression level of Hsp90α was associated with leukemia prognosis. However, no obvious relationship was observed between the occurrence of graft versus host disease and the expression of Hsp90α.

1. Introduction

Heat shock protein 90 (Hsp90) is a type of molecular chaperone that is found in various cell lines. Hsp90 was initially observed in cells exposed to elevated temperatures (Hartl and Hayer-Hartl, 2002; Young et al., 2004). Recent studies have shown that Hsp90 has an important function in the conformational maturation and stabilization of signaling proteins involved in cell growth and survival (Thomas et al., 2005; Whitesell and Lindquist, 2005). By regulating the function of various cancer proteins, Hsp90 participates in regulating tumor cell proliferation, survival, invasion, metastasis, angiogenesis, and other important processes (Didelot et al., 2007; Pearl et al., 2008). Inhibiting the expression of Hsp90 can simultaneously regulate a wide variety of tumor signal pathways, and thus, it may have a critical function in tumor activity (Kamal et al., 2003, 2004; Lin et al., 2008; Solit et al., 2007).

Depending on whether or not the protein contains rich regions of glutamine, human Hsp90 is divided into two categories: Hsp90 alpha (Hsp90α) and Hsp90 beta (Hsp90β). Hsp90α is important for the proliferation of tumor cells, whereas Hsp90β is associated with cell differentiation and structure building. Yano et al. (1996) found that Hsp90α mRNA expression is significantly higher in breast cancer tissue than in non-cancerous tissue, and that its expression is closely related to the expression of the nuclear antigen index of the proliferating cell, suggesting that high expression levels of Hsp90α are important for cell proliferation. Hsp90α gene shows high expression levels in pancreatic cancer and laryngeal cancer tissues (Gress et al., 1994). Significantly higher gene expression of Hsp90α was observed in the peripheral blood of patients with untreated acute leukemia (AL) compared with samples from healthy controls (Sedlackova et al., 2011; Xiao et al., 1996; Yufu et al., 1992). In previous studies, the expression of Hsp90α in patients with relapses, refractory disease, remission, or transplantation was not examined. Only the gene expression of Hsp90α was examined, and information on the protein expression of Hsp90α inside and outside the cells is lacking. We observed the differences in the expression of Hsp90α in different AL states to determine whether or not a correlation exists

Abbreviations: Hsp90α, heat shock protein 90 alpha; Hsp90α, Hsp90 alpha; Hsp90β, Hsp90 beta; AL, acute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ELISA, enzyme-linked immunosorbent assay; BMMCs, bone marrow mononuclear cells; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; CR, complete remission; NR, non-remission; GVHD, graft versus host disease.

* Corresponding authors at: Department of Hematology, The First Affiliated Hospital of Zhengzhou University, No. 1 Jianshe East Road, Zhengzhou, Henan Province 450052, China.
E-mail addresses: gh7311@aliyun.com (R. Guo), lingsuncn@126.com (L. Sun).

[1] Wen-Liang Tian and Fei He contributed equally as co-first authors.

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between Hsp90α protein and gene expression, and to examine the association between Hsp90α and AL patient clinical outcomes. No similar reports have been published.

2. Patients and methods

2.1. Leukemia cell lines

The cell lines used were K562 cells, a human chronic myeloid leukemia cell line (Center of Stem Cells, Zhengzhou University, China), Jurkat and molt-4 cells, a human acute lymphoblastic leukemia (ALL) cell line (Teaching And Research Section of Immunity, XinXiang Medical University, China), HL-60 and NB4 cells, and human acute myeloid leukemia (AML) cell line (Center of Stem Cells, Zhengzhou University, Zhengzhou, China). The five types of cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum and penicillin/streptomycin in a humidified incubator at 37 °C with 5% CO_2.

2.2. Subjects

The protocol was approved by the Ethical Committee of the First Affiliated Hospital of Zhengzhou University, and informed consents were obtained from all the participants prior to testing. The untreated patients were sampled at the onset of the disease and prior to chemotherapy. The refractory and relapsed patients were sampled after diagnosis of refractory status or relapse but did not continue treatment. The remission patients were sampled after diagnosis of complete remission. Several cohorts of leukemia patients were included in the study. Patient data are shown in Table 1.

All the cases were diagnosed at the Department of Hematology, The First Affiliated Hospital of Zhengzhou University. The healthy controls in the study included 14 healthy individuals (10 females and 4 males; age range, 14 years old to 52 years old; median age, 29 years old). The cohort was collected based on a suspicion of a hematological diagnosis that was ultimately determined as non-malignant or free of disease. Fresh bone marrow samples (4 ml) were collected from each subject. We centrifuged the samples, removed the supernatant, and stored the plasma samples in single-use tubes at −80 °C for further enzyme-linked immunosorbent assay (ELISA) tests. We avoided multiple freeze–thaw cycles. The bone marrow mononuclear cells (BMMCs) were collected. A portion of the total BMMCs was used for isolation of total RNA, and another portion was used for isolation of total protein.

2.3. Real-time quantitative PCR (qPCR) analysis

Total RNA was extracted from cells using the RNeasy kit (Sangon Biotech, Shanghai, China). The cDNA was prepared by reverse transcription of the total RNA using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Beijing, China). The cDNA samples were stored at −80 °C until use.

The experiment was performed using the Maxima SYBR qPCR Master Mix kit (Fermentas, Harrington, Canada) and the StepOne™ Real-Time PCR System (Applied Biosystem, Foster City, CA, USA). The system was applied according to the manufacturer's instructions. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as reference gene. The following primers were used: (1) Hsp90α forward: 5 -ACCCAGACCC AAGACCAACCG-3 , reverse: 5 -ATTTGAAATGAGCTCTCTCAG-3 ; (2) GAPDH forward: 5 -TGCCCTCAACGACCACTTTG-3 , reverse: 5 -TCTC TCTTCCTCTTGTGCTCTTGC-3 . The primers were designed by the software primer3 (http://bioinfo.ut.ee/primer3-0.4.0/). For the real-time quantitative RT-PCR assay, the Hsp90α gene and the endogenous control GAPDH were amplified in different wells of a 96-well plate. Each well contained a reaction volume of 25 μl comprising 1 × Maxima SYBR qPCR Master Mix, 0.2 μM of the forward primer, 0.2 μM of the reverse primer, and 25 ng of cDNA. Each sample was analyzed in triplicate. The thermal profile used for the one-step real-time RT-PCR included denaturation at 95 °C for 10 min, followed by 40 cycles of PCR with denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. To eliminate the need for standard curves, the comparative Ct method (Kenneth and Thomas, 2001) was used to interpret the data. The difference (ΔCt) between the Ct values of Hsp90α and the endogenous control was calculated for each sample. The RNA isolated from the bone marrow of a randomly selected healthy control was used as the reference for each comparison. The comparative ΔΔCt calculation involved the determination of the difference between the ΔCt of each sample and the ΔCt of the reference. The ΔΔCt values were transformed to absolute values using the formula 2-ΔΔCt. Multiple negative water blanks (no template controls) were included in all the analyses. The samples were tested using a complete master mix without reverse transcriptase or primers to ensure that they were negative for DNA. No amplification was observed for these controls, indicating the specificity of the assays for the respective mRNAs.

2.4. Western blot

Protein from the BMMCs was isolated using a protein isolation kit (Sangon Biotech, Shanghai, China). Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with two gels simultaneously. Fifty micrograms of protein from the total cell lysate was concentrated on 5% SDS gels for 30 min at 100 V and separated on 10% SDS gels for 70 min at 120 V. The gels were transferred to polyvinylidenefluoride membranes (BBI, Canada) and blotted in parallel at 200 mA for 2 h. After blotting, the gels (Coomassie) and the membranes (Ponceau S) were stained to document successful blotting. The membranes were dipped in blocking buffer for 1 h and incubated with a polyclonal anti-hsp90α antibody (BBI, Canada) at 4 °C overnight. After washing three times with PBS-T, the membranes were incubated with a goat anti-rabbit secondary antibody (BBI) at 25 °C for 2 h. After washing three times with PBS-T, the bands were visualized on an X-ray film using the electrochemiluminescence method according to the manufacturer's instructions. The bands of the Hsp90α protein appeared at approximately 90 kDa, and the bands of the β-actin protein appeared at approximately 40 kDa. After scanning the X-ray film, image analysis was performed on a computer using the image processing software Image J.

2.5. ELISA

To perform the ELISA detection of plasma from the bone marrow, the plasma was centrifuged at 2400 ×*g* for 10 min. Precautions were taken

Table 1
Patients data in this study.

Cohorts	Diagnosis	N	Females	Males	ALL	AML	Age range (years)	Median age (years)
First	De novo AL	48	19	29	20	28	19–72	49
Second	Remittent AL	38	20	18	8	30	16–67	41
Third	Refractory AL	16	8	8	2	14	17–74	47
Fourth	After HSCT	41	24	17	25	16	10–28	22
Fifth	Relapsed AL after chemotherapy	5	2	3	3	2	27–49	37
Sixth	Relapsed AL after HSCT	3	3	0	2	1	22–26	24