Published in final edited form as:

Leuk Res. 2010 May ; 34(5): 605–609. doi:10.1016/j.leukres.2009.09.014.

Clinical Correlation of Circulating Heat Shock Protein 70 in Acute Leukemia

Chen-Hsiung Yeh¹, Richard Tseng¹, Alison Hannah³, Zeev Estrov², Elihu Estey², Hagop Kantarjian², and Maher Albitar¹

¹Oncology/Hematology, Quest Diagnostics Nichols Institute, San Juan Capistrano, California, USA

²Leukemia, M.D. Anderson Cancer Center, University of Texas, Houston, Texas, USA

³Clinical Trial, Kosan Biosciences, Hayward, California, USA

Abstract

The heat shock protein 70 (HSP70) is one of the molecular chaperone family involved in the protection of cells upon exposure to various types of stresses. Plasma circulating HSP70 (cHSP70) is believed to play a role in the anti-tumor immune responses and its levels may reflect the levels of severity or the disease condition. Using electrochemiluminescence protein detection immunoassay, we measured the cHSP70 levels in the plasma of patients with acute myeloid leukemia (AML) (n=96), myelodysplastic syndrome (MDS) (n=28), and acute lymphoblastic leukemia (ALL) (n=40) and compared with those in normal individuals (n=99). cHSP70 levels were significantly higher in AML (median: 10.71 ng/mL, range: 1.93-79.0 ng/mL) and ALL (median: 27.59 ng/mL, range: 5.09-129.6 ng/mL) as compared to those in MDS (median: 4.54 ng/mL, range: 1.35-58.3 ng/mL) or healthy controls (median: 4.13 ng/mL, range: 1.75-13.6 ng/mL). Levels of cHSP70 showed significant positive correlation with lactate dehydrogenase (LDH) and white blood cells (WBC) in AML and ALL patients, which may reflect overall tumor load. Furthermore, patients with higher levels of cHSP70 had significantly shorter survival in AML (P=0.04) and ALL (P=0.05), suggesting that in these two acute diseases, cHSP70 is an indicator for poor prognosis. Our data support the potential of using free cHSP70 as a biomarker in leukemias and potentially other types of cancers.

Keywords

circulating heat shock protein; acute leukemia; survival

© 2009 Elsevier Ltd. All rights reserved.

Corresponding author: Maher Albitar, Department of Oncology/Hematology, Quest Diagnostics Nichols Institute, 33608 Ortega Highway, San Juan Capistrano, CA 92690, USA, Tel: 949-728-4784, Fax: 949-728-7781, Maher.X.Albitar@questdiagnostics.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

There are no conflicts of interests.

1. Introduction

HSP70 is a vital molecular chaperone induced by cellular stresses due to misfolded and denatured proteins (1). Although expression of HSP70 is relatively low in normal cells, it is abundantly expressed in most cancer cells (1–3). As a result, in experimental models, HSP70 down-regulation strongly decreases tumorigenicity (4). HSP70 has a dual function depending on their intracellular or membrane location. While induction of cytosolic HSP70 confers cytoprotection to further stress, tumor-specific cell surface expression of HSP70 elicits anti-tumor immune responses (5). Cellular HSP70 supports the folding of newly synthesized polypeptides, transport of proteins across membranes, prevent protein aggregation, and enhances the ability of the cell to survive those otherwise lethal conditions by counteracting the apoptotic pathway at multiple levels (1, 2, 5). On the other hand, membrane-associated HSP70 detected specifically on tumor cells, but not on normal cell types, provides a recognition structure for the cytolytic attack mediated by natural killer (NK) cells (6, 7). Furthermore, the amount of membrane-bound HSP70 correlated positively with susceptibility to cell lysis by NK cells (8). A growing interest is thus targeting membrane HSP70 to improve patient outcome in different cancer immunotherapies. HSP70 membrane phenotype was also found to serve as a prognostic indicator of overall patient survival in leukemia, lower rectal and non small cell lung carcinomas (5, 9, 29).

Apart from its respective subcellular compartments, in the cytosol and on membrane, accumulating evidence suggests that HSP70 is actively secreted and has important extracellular functions (10–12). In humans, HSP70 is released into serum in response to stress conditions including inflammation, bacterial, or viral infections. The active secretion of HSP70 from viable tumor cells has been documented and could be further enhanced by exogenous stress, e.g., proinflammatory cytokines (11). In vitro, HSP70 can be detected in the culture medium of antigen-presenting cells (APCs) (9). Since HSP70 is an intracellular protein and lacks any secretory signal sequence, one would hypothesize that it is not exported through the classical exocytic route (12). Several mechanisms may account for the cellular release of HSP70, including the turnover of the cells (13), exocytosis through membrane microdomains, termed lipid rafts (12), or exosome-dependent trafficking (14). A growing body of evidence suggests that extracellular HSP70, released either as a result of cellular necrosis or via an active stress-induced exporting pathway, may act as a potent danger signal to the immune system (5, 10–12). Actually, recombinant HSP70 protein and HSP70 peptides, which mimic the secreted HSP70, have been shown to activate NK cells directly in some systems (15). The clinical use of purified, tumor-derived HSP70 as a prophylactic and therapeutic cancer vaccine has also been reported (16).

In the present study, we determined the plasma levels of circulating HSP70 (cHSP70) from 263 subjects including 96 with acute myeloid leukemia (AML), 40 with acute lymphocytic leukemia (ALL), 28 with myelodysplastic syndromes (MDS) and 99 healthy donors by electrochemiluminescence immunoassay. We also evaluated the clinical relevance of cHSP70 as an independent clinicopathological and prognostic marker for acute leukemia. Our results revealed that higher levels of cHSP70 in plasma confer a poor prognosis in AML and ALL. Plasma cHSP70 therefore could be a valuable prognostic indicator in leukemias.

2. Materials and methods

2.1. Patients and samples

The general characteristics of the patients with acute leukemia are listed in Table 1. All patient samples, as well as samples from normal controls, were collected under an Internal Review Board (IRB)-approved protocol with written informed consent. Pathologic diagnoses, based on examining peripheral blood and bone marrow samples, were reviewed centrally and classified according to the criteria of French-American-British (FAB) as well as World Health Organization (WHO). However, for the purpose of this paper patients with refractory anemia and excess blasts in transformation (RAEB-T) were considered as MDS. Blood counts, flow cytometry, molecular study, FISH and cytogenetic analysis were performed, reviewed, and classified accordingly. Cytogenetic status of both AML and MDS was classified as favorable (t(15;17), t(8;21), or inv16), unfavorable (-5, -7, or complex (3) abnormalities), or intermediate (all others). Most patients with ALL were classified as L1 and L2 (87.5%) and only 5 patients had Burkitt disease. Three of the ALL patients were Philadelphia chromosome positive. AML patients were treated at MD Anderson Cancer Center with standard therapy based on idarubicine+ ara-C, except when the diagnosis was APL, in which they were treated with ATRA-based therapy. Patients who achieved complete remission, they were given reduced dose of the same therapy. Patients with precursor-ALL were treated with Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, adriamycin, and dexamethasone). Patients with Burkitt leukemia were treated with Hyper-CVAD/methotrexate/Ara-C. Plasma was separated from EDTA peripheral blood samples by centrifuging samples at 5000 rpm for 10 minutes. Plasma samples obtained from all study subjects were aliquoted and stored at -70°C until analysis.

2.2. Measurement of plasma cHSP70 by electrochemiluminescence immunoassay

cHSP70 in plasma was measured using MA2400 total HSP70 whole cell lysate kits (Meso Scale Discovery, Gaithersburg, MD) according to the manufacturer's instructions. Briefly, 25 µL of plasma, in triplicate, was applied onto MSD Single-Spot 96-well plates that had been pre-coated with anti-HSP70 monoclonal antibody and incubated for 1 hour at room temperature. Sulfo-Tagged detection antibody was then added after washing the plate four times with Wash buffer. After the final wash, 150 µL of Read buffer T was added to each well and the plates were analyzed with an MSD SECTOR Imager 2400 reader. Each run included a 7-point standard prepared by two-fold serial dilution of recombinant human HSP70 (Epoch Biolabs, Sugar Land, TX) to a dynamic range between 625 and 40,000 pg/mL. Three positive plasma (high, medium, low) and two negative controls (normal plasma and blank) were also included in parallel. Sensitivity of this assay as determined by LOD (limit of detection) and LOQ (limit of quantitation) are 76 and 625 pg/mL, respectively. Specificity of this assay is 97% based on plasma samples from 100 healthy subjects. The intra-assay and inter-assay variations (%CV) of this assay on plasma samples are below 15%.

2.3. Statistical analysis

Descriptive statistics were analyzed and univariate analyses were performed. Relationships between continuous variables were assessed with Spearman rank correlations. The chi-

square or Kruskal-Wallis test was used to assess relationships between categorical variables. Kaplan-Meier plots of survival were performed separately for each diagnostic group. Univariate Cox proportional hazards models were fit to evaluate the predictive effect of each factor alone. Factors found to be statistically significant at the 5% level in the univariate models were included in multivariate models. Multivariate models were reduced one factor at a time such that all factors remaining in the model were statistically significant at the 5% level. Cut-off points used for Kaplan-Meier were median or upper quartile for each disease group. Survival times were compared by means of the log-rank test.

3. Results

3.1. High levels of cHSP70 in AML and ALL but not in MDS

We measured plasma cHSP70 levels in a prospectively identified cohort of 164 patients with acute leukemia. The characteristics of the studied patients are shown in Table 1. AML and ALL patients had significantly higher cHSP70 levels than did healthy volunteers ($P < 0.0001$), whereas MDS patients did not ($P = 0.36$) (Fig. 1). When we compared AML with RAEB-T, there was significant difference between the two groups. The patients with RAEB-T had significantly lower levels ($P = 0.005$), confirming that RAEB-T disease is closer to MDS than to AML. In addition when we group the RAEB-T with the AML cases as recommended by the WHO classification, there was significant correlation between blast count in bone marrow and cHSP70 levels ($P = 0.04$), and correlated cHSP70 levels were also significantly higher in ALL than AML patients ($P < 0.001$). The median (range) plasma cHSP70 levels in the 4 groups were as follows: AML, 10.71 (1.93-79.0) ng/mL; ALL, 27.59 (5.09-129.6) ng/mL; MDS, 4.54 (1.35-58.3) ng/mL; normal controls, 4.13 (1.75-13.6) ng/mL. Therefore, ALL patients appear to express the highest levels of cHSP70.

3.2. Correlation with clinical and laboratory data

We next evaluated the association of cHSP70 with a series of clinical and hematological variables. Plasma cHSP70 levels correlated significantly and positively with $\beta 2$ microglobulin ($\beta 2M$), white blood cell (WBC) count, and lactate dehydrogenase (LDH) in AML and ALL patients (Table 2). In ALL patients, plasma cHSP70 levels correlated negatively with CD34 expression on blast cells. The association with WBC and blast count in peripheral blood in AML patients may explain why cHSP70 levels were not significantly high in patients with MDS. cHSP70 levels did not correlate with age, gender, cytogenetic grouping, performance status (according to Zubrod system), platelet count, hemoglobin levels, or CD markers (except CD34 and CD64) in AML or ALL. There was no correlation between pre- and post-menopause in women. The number of MDS patients was relatively small for statistical analysis, but several trends were noted (Table 2). There was a positive correlation between cHSP70 and $\beta 2M$ ($r = 0.28$), % monocytes ($r = 0.44$), absolute number of lymphocytes ($r = 0.50$), and platelet count ($r = 0.34$).

3.3. Association of cHSP70 levels with clinical behavior in AML and ALL

Although cytogenetic grouping did not correlate with cHSP70 in AML patients, those with cHSP70 levels in the upper quartile had significantly shorter survival ($P = 0.04$) (Fig. 2). Median survival was 20 weeks for patients with cHSP70 levels in the upper quartile (> 20

ng/mL), compared with 51 weeks for patients with lower levels. However, these effects were dependent on age. cHSP70 was not predictor of survival in patients younger than 60 years (# 40). In contrast, in patients older than 60 years, cHSP70 was strongly predictor of survival ($p=0.02$). There was no significant difference in cHSP70 levels between the two age groups ($P=0.43$). In the AML group, we usually exclude the favorable cytogenetic group from the analysis due to their specific course and outcome, hence the low percentage of patients with favorable cytogenetics (table 1). There were 2 patients with APL and 3 patients with inversion 16 and t(8,21). Since these patients have specific clinical course and were treated differently from the rest of the patients, we excluded these patients. However, there was no change in the value of cHSP70 in predicting survival when these patients were excluded. In addition, since the RAEB-T patients are grouped with the AML patients in the current WHO classification, we analyzed survival using the same cut-off point (20 ng/ml) and found that the conclusion is the same. Patients with levels higher than 20 ng/ml had significantly ($P=0.04$) shorter survival in AML patients classified according to the WHO classification despite that RAEB-T patients had significantly lower levels of cHSP70. There was no correlation with survival when the median was used for dichotomizing patients. cHSP70 levels were not correlated with response to therapy. As expected, cytogenetic, age, performance status (according to Zubrod system) were all strong predictor of survival in this group of patients ($P<0.001$). However, LDH and WBC were not predictor of survival. Multivariate Cox proportional hazard model incorporating all significant factors along with the cHSP70 showed that the cHSP70 is not independent of cytogenetics, performance status or age. However, AML patients who had high cHSP70 and had shorter survival also had significantly higher LDH ($P=0.0013$), WBC ($P=0.0001$), and platelets ($p=0.03$). There was no significant difference between the two groups in age, hemoglobin levels, BUN, or creatinine.

Similarly, high cHSP70 levels were also associated with shorter survival in ALL patients ($P=0.05$). Median survival was 123 weeks for ALL patients in the upper cHSP70 quartile (>55 ng/mL), but well beyond 350 weeks in those with lower levels (Fig 3). However, when we considered only patients younger than 60 years-old, cHSP70 levels were stronger predictor of survival with P-value of 0.006. Although the number is small, but in multivariate models incorporating WBC, platelets, hemoglobin, performance status and LDH, cHSP70 was independent of performance status, platelet count, hemoglobin, but not WBC or LDH. There was no significant difference in cHSP70 levels between the two age groups ($P=0.72$). There was no correlation with survival when the median was used for dichotomizing the ALL patients. There were only 5 patients with L3 diagnosis (Burkitt leukemia) and the rest of the patients had L1 or L2 morphology. Excluding Burkitt's cases did not change the findings. Patients with high levels of cHSP70 (>55 ng/mL) had significantly higher WBC ($P=0.009$) and LDH (0.008). There was no significant difference between the two groups in age, HGB, platelets, creatinine, or BUN.

4. Discussion

HSP70 is currently receiving considerable attention as a potential anti-cancer immunotherapeutic target. Elevated expression of HSP70 has been reported in several high-grade malignant tumors such as breast, endometrial, gastric cancers, osteosarcoma, and renal

cell tumors as well as Bcr-Abl-positive leukemia (17–19). High levels of HSP70 in breast, endometrial, or gastric cancer are also associated with metastasis, poor prognosis, and resistance to chemotherapy or radiation therapy (20, 21). On one hand, high intracellular HSP70 levels contribute to cell protection mechanisms; on the other hand, tumor-specific membrane-associated HSP70 expression has been shown to function as a danger signal that can increase the immunogenicity of tumors and induce NK and CTL responses (5, 15). In this context, either cytosolic or membrane-bound HSP70 released into the blood stream by tumor cells could serve as a prognostic factor for cancer progression. More importantly, secreted HSP70 is the consequence of immune responses, and HSP70 is among the dominant antigens recognized by the immune system. HSP70 released from tumor cells may allow the transfer of antigenic peptides to APCs or may enhance the ability of APCs ability to process and present tumor antigens to specific T cells, thereby augmenting the immune responses (22, 23). Thus, cHSP in plasma could also represent an indicator of both innate and adaptive immunity, as well as an indicator of tumor load.

Plasma HSP70 has been reported to be a reliable tumor marker in patients with early-stage prostate cancer when combined with PSA (24); cHSP70 has also been shown to serve as a danger marker for septic shock (25) and postoperative organ dysfunction (26), and its levels are directly linked to the inflammatory status (27). However, the clinical association between free cHSP70 levels and acute leukemia is largely unknown. Despite a variety of induction and consolidation regimens employed, most patients with acute leukemia ultimately relapse and die with drug-resistant disease (28).

Here we demonstrate high levels of cHSP70 in the plasma of patients with AML and ALL, but not MDS, in which the immune response plays a dominant role. Furthermore, patients with RAEB-T had significantly lower levels than patients with AML, which support the concept that despite they are currently considered AML according to the WHO classification, they are biologically closer to MDS than to AML. The association with WBC and blast count in AML patients may also explain why cHSP70 levels were not significantly elevated in patients with MDS. More importantly, cHSP70 correlated positively with risk factors such as WBC count, LDH, and β 2M, all of which have prognostic values and are associated with adverse outcomes in AML and ALL. Higher levels of cHSP70 could also reflect higher levels of cellular and/or membrane-bound HSP70, which are frequently associated with unfavorable and intermediate cytogenetics in AML (29). We also demonstrate that the clinical correlation between cHSP70 and survival was mainly in patients younger than 60 year of age in ALL and in patients older than 60 year of age in the AML group. Levels of cHSP70 did not play any role in predicting response nor relapse in either AML or ALL patients. Identification of plasma cHSP70 as a prognostic factor for survival in AML and ALL may support the concept that cHSP70 reflects the severity of the disease as well as the condition of the patient. We believe the bulk of cHSP70 in AML and ALL patients to be derived from turnover of leukemic cells. However, it could also be released or secreted actively or passively, by lymphocytes or other organs and tissues, in response to certain stresses and pathological conditions. It remains unclear whether cHSP70 is released as free soluble protein, in detergent-soluble membrane vesicles, or in or cHSP70-peptide complexes. Additional research is needed to determine the nature and form of the circulating HSP70. Nevertheless, the clinical significance of plasma cHSP70 as an adverse

independent prognostic indicator for survival in AML and ALL patients in this study is remarkable. Further studies are needed to better understand the role of cHSP70 in the prognosis of leukemia and the prediction of therapeutic response.

Acknowledgments

Chen-Hsiung Yeh performed testing and helped write paper.

Richard Tseng performed testing.

Alison Hannah provided clinical data.

Zeev Estrov provided clinical data and analyzed data.

Hagop Kantarjian provided clinical data and analyzed data.

Maier Albitar wrote paper, provided clinical data, analyzed data and designed study.

References

1. Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science*. 2002; 295:1852–1858. [PubMed: 11884745]
2. Jolly C, Morimoto RI. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *J Natl Cancer Inst*. 2000; 92:1564–1572. [PubMed: 11018092]
3. Creagh EM, Sheehan D, Cotter TG. Heat shock proteins: modulators of apoptosis in tumour cells. *Leukemia*. 2000; 14:1161–1173. [PubMed: 10914538]
4. Gurbuxani S, Bruey JM, Fromentin A, Larmonier N, Parcellier A, Jaattela M, et al. Selective depletion of inducible HSP70 enhances immunogenicity of rat colon cancer cells. *Oncogene*. 2001; 20:7478–7485. [PubMed: 11709719]
5. Schmitt E, Gehrman M, Brunet M, Multhoff G, Garrido C. Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. *J Leukocyte Biol*. 2007; 81:15–27. [PubMed: 16931602]
6. Multhoff G, Botzler C, Wiesnet M, Müller E, Meier T, Wilmanns W, Issels RD. A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. *Int J Cancer*. 1995; 61:272–279. [PubMed: 7705958]
7. Gehrman M, Schmetzer H, Eissner G, Haferlach T, Hiddemann W, Multhoff G. Membrane-bound heat shock protein 70 (Hsp70) in acute myeloid leukemia: a tumor specific recognition structure for the cytolytic activity of autologous NK cells. *Haematologica*. 2003; 88:474–476. [PubMed: 12681978]
8. Gehrman M, Brunner M, Pfister K, Reichle A, Kremmer E, Multhoff G. Differential up-regulation of cytosolic and membrane-bound heat shock protein 70 in tumor cells by anti-inflammatory drugs. *Clinical Cancer Research*. 2004; 10:3354–3364. [PubMed: 15161689]
9. Gehrman M, Liebisch G, Schmitz G, Anderson R, Steinem C, De Maio A, et al. Tumor-specific Hsp70 plasma membrane localization is enabled by the glycosphingolipid Gb3. *PLoS ONE*. 2008; 3:e1925. [PubMed: 18382692]
10. Calderwood SK, Mambula SS, Gray PJ Jr, Theriault JR. Extracellular heat shock proteins in cell signaling. *FEBS Lett*. 2007; 581:3689–3694. [PubMed: 17499247]
11. Barreto A, Gonzalez JM, Kabingu E, Asea A, Fiorentino S. Stress-induced release of HSC70 from human tumors. *Cell Immunol*. 2003; 222:97–104. [PubMed: 12826079]
12. Broquet AH, Thomas G, Masliah J, Trugnan G, Bachelet M. Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. *J Biol Chem*. 2003; 278:21601–21606. [PubMed: 12682040]
13. Saito K, Dai Y, Ohtsuka K. Enhanced expression of heat shock proteins in gradually dying cells and their release from necrotically dead cells. *Exp Cell Res*. 2005; 310:229–236. [PubMed: 16129430]

14. Lancaster GI, Febbraio MA. Exosome-dependent trafficking of HSP70. A novel secretory pathway for cellular stress proteins. *J Biol Chem.* 2005; 280:23349–23355. [PubMed: 15826944]
15. Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroeder JA, Multhoff G. Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Res.* 2005; 65:5238–5247. [PubMed: 15958569]
16. Calderwood SK. Chaperones and slow death: a recipe for tumor immunotherapy. *Trends Biotechnol.* 2005; 23:57–59. [PubMed: 15661338]
17. Santarosa M, Favaro D, Quaia M, Galligioni E. Expression of heat shock protein 72 in renal cell carcinoma: possible role and prognostic implications in cancer patients. *Eur J Cancer.* 1997; 33:873–877. [PubMed: 9291808]
18. Nanbu K, Konishi I, Mandai M, Kuroda H, Hamid AA, Komatsu T, Mori T. Prognostic significance of heat shock proteins HSP70 and HSP90 in endometrial carcinomas. *Cancer Detect Prev.* 1998; 22:549–555. [PubMed: 9824379]
19. Ray S, Lu Y, Kaufmann SH, Gustafson WC, Karp JE, Boldogh I, et al. Genomic mechanisms of p210BCR-ABL signaling: induction of heat shock protein 70 through the GATA response element confers resistance to paclitaxel-induced apoptosis. *J Biol Chem.* 2004; 279:35604–35615. [PubMed: 15155749]
20. Vargas-Roig LM, Gago FE, Tello O, Aznar JC, Ciocca DR. Heat shock protein expression and drug resistance in breast cancer patients treated with induction chemotherapy. *Int J Cancer.* 1998; 79:468–475. [PubMed: 9761114]
21. Brondani Da Rocha A, Regner A, Grivicich I, Pretto Schunemann D, Diel C, Kovaleski G, et al. Radioresistance is associated to increased Hsp70 content in human glioblastoma cell lines. *Int J Oncol.* 2004; 25:777–785. [PubMed: 15289883]
22. Wells AD, Malkovsky M. Heat shock proteins, tumor immunogenicity and antigen presentation: an integrated view. *Immunol Today.* 2000; 21:129–132. [PubMed: 10689300]
23. Massa C, Guiducci C, Arioli I, Parenza M, Colombo MP, Melani C. Enhanced efficacy of tumor cell vaccines transfected with secreted HSP70. *Cancer Research.* 2004; 64:1502–1508. [PubMed: 14973071]
24. Abe M, Manola JB, Oh WK, Parslow DL, George DJ, Austin CL, Kantoff PW. Plasma levels of heat shock protein 70 in patients with prostate cancer: a potential biomarker for prostate cancer. *Clin Prostate Cancer.* 2004; 3:49–53. [PubMed: 15279691]
25. Wheeler DS, Fisher LE Jr, Catravas JD, Jacobs BR, Carcillo JA, Wong HR. Extracellular HSP70 levels in children with septic shock. *Pediatr Crit Care Med.* 2005; 6:308–311. [PubMed: 15857530]
26. Kimura F, Itoh H, Ambiru S, Shimizu H, Togawa A, Yoshidome H, et al. Circulating heat-shock protein 70 is associated with postoperative infection and organ dysfunction after liver resection. *Am J Surg.* 2004; 187:777–784. [PubMed: 15191875]
27. Njemini R, Demanet C, Mets T. Inflammatory status as an important determinant of heat shock protein 70 serum concentrations during aging. *Biogerontology.* 2004; 5:31–38. [PubMed: 15138379]
28. Tallman MS. Therapy of acute myeloid leukemia. *Cancer Control.* 2001; 8:62–78. [PubMed: 11176038]
29. Steiner K, Graf M, Hecht K, Reif S, Rossbacher L, Pfister K, Kolb H-J, Schmetzer HM, Multhoff G. High HSP70-membrane expression on leukemic cells from patients with acute myeloid leukemia is associated with a worse prognosis. *Leukemia.* 2006; 20:2076–2079. [PubMed: 16990768]

Figure 1.

Scatter plot of plasma cHSP70 levels in patients with ALL, AML, and MDS and in normal healthy subjects (n). Total patient number enrolled in each group is indicated in parentheses. cHSP70 levels are significantly higher in ALL and AML patients than in control subjects ($P < 0.0001$), but did not differ significantly between MDS patients control subjects ($P = 0.36$).

Figure 2.

Association of plasma cHSP70 levels with overall survival in 96 AML patients. The cumulative proportion of surviving patients (as a percentage of the total patients) with baseline cHSP70 values below (top or blue line) and above the upper quartile (bottom or red line) is shown. The two curves are significantly different ($P = 0.04$). (N refers to number of patients and E refers to patients with an event, which is defined here as death)

Figure 3.

Association of plasma cHSP70 levels with overall survival in 40 ALL patients. The cumulative proportion of surviving patients (as a percentage of the total patients) with baseline cHSP70 values below (top or blue line) and above the upper quartile (bottom or red line) is shown. The two curves are significantly different ($P = 0.05$). (N refers to number of patients and E refers to patients with an event, which is defined here as death)

Table 1

Characteristics of patients with acute leukemia.

Characteristic	AML	MDS	ALL
Number	96	28	40
Median (range) age, yr	63 (18-84)	64 (24-75)	38 (18-78)
Performance Status, No.			
0-1	69 (72%)	28 (100%)	30 (75%)
2-4	30 (28%)	0 (0%)	10 (25%)
Cytogenetics, No.			
Favorable	5 (5%)	0	0 (0%)*
Unfavorable	46 (34.5%)	12 (43%)	3 (8%)*
Intermediate	55 (60.5%)	16 (57%)	37 (93%)*
Median (range) white blood cell count, $\times 10^9/L$	3.8 (0.4-161.0)	2.8 (0.8-24.0)	7.75 (0.9-74.9)
Median (range) hemoglobin, g/dL	7.8 (3.4-13.1)	7.5 (2.2-11.0)	7.95 (4.0-13.0)
Median (range) platelets, $\times 10^9/L$	55 (8-293)	40.5 (11-270)	67 (11-393)
FAB classification			
M0	13 (14%)		
M1	20 (21%)		
M2	31 (32%)		
M3	2 (2%)		
M4	18 (19%)		
M5	3 (3%)		
M6	6 (6%)		
M7	3 (3%)		
Pre-B ALL			29 (72.5%)
Pre-T ALL			6 (15%)
Burkitt			5 (12.5%)
RA/RARS		3 (11%)	
RAEB		5 (18%)	
RAEB-T		20 (71%)	

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphocytic leukemia;

* For patients with ALL, we considered the t(9,22) translocation as unfavorable, hyperdiploid as favorable, and all others as intermediate.

Table 2

Spearman correlations of cHSP70 with clinical and hematological parameters*

	AML		ALL		MDS	
	Spearman	p-level	Spearman	p-level	Spearman	p-level
Beta-2 Microglobulin	0.32	0.002	0.33	0.043	0.28	0.161
White Blood Count	0.47	0.000	0.40	0.010	0.19	0.332
PB Blasts (%)	0.39	0.000	0.22	0.175	0.08	0.696
PB Monocytes (%)	0.06	0.578	0.20	0.219	0.38	0.049
Absolute count, lymphocytes	0.27	0.057	0.31	0.188	0.50	0.012
Platelets	0.20	0.055	-0.10	0.533	0.34	0.028
BM Monocytes (%)	0.01	0.959	0.09	0.589	0.44	0.019
Lactate Dehydrogenase	0.50	0.000	0.49	0.001	0.18	0.364
CD34	-0.04	0.727	-0.51	0.002	-0.21	0.358
CD64	0.12	0.279	0.51	0.002	0.28	0.212

* Significant correlations, either positive or negative, are marked in bold type.