Myelodysplastic Syndromes

Expression and prognostic significance of heat-shock proteins in myelodysplastic syndromes

Using flow cytometry, we investigated the clinical and hematologic relevance of expression of heat-shock proteins (HSP) HSP27, HSP60, HSP70, HSP90 and HSP110 in bone marrow of 142 patients with newly diagnosed myelodysplastic syndromes, together with that of the membrane differentiation antigen CD34 and the drug-resistance related protein, P170 (Pgp).

Haematologica 2006; 91:713-714
(*http://www.haematologica.org/journal/2006/05/713.html*)

Excessive apoptosis is believed to contribute to the ineffective hematopieisis that characterizes myelodysplastic syndromes (MDS) but mechanisms underlying this phenomenon remain elusive.[1] Heat-shock proteins (HSP) are constitutive or induced intracytoplasmic proteins found in both cancer and normal cells.[2] Abnormal expression of HSP may be implicated in the regulation of apoptosis.[3] Moreover, many tumor cells appear to constitutively express high HSP levels, which may result in chemo-resistant tumors.[4,5] Thus, we sought to determine whether HSP expression in MDS has any clinical or hematologic relevance.

We studied HSP expression in bone marrow mononuclear cells from 142 patients with newly diagnosed MDS before treatment, except transfusion. The characteristics of these patients are listed in Table 1. All samples were collected after obtaining informed consent.

HSP70 displayed the highest and HSP110 the lowest level of expression. A single sample was negative (<20% stained cells) for all HSP, whereas 35.2% of the samples expressed at least two HSP, 25.3% expressed three HSP, 14.8% expressed four HSP and 9.8% expressed five HSP.

In 42 samples, multicolor staining for CD45, CD34 and one of the HSP allowed comparison of HSP expression between the whole $CD45^+$ population and $CD34^+$ cells. Expression of all HSP was higher in the $CD34^+$ population (p<0.04). Interestingly, the difference was less marked for HSP70 than for the other proteins.

We also examined the correlation between HSP expression and some patients' characteristics. Expression of all HSP, except HSP70, strongly correlated with FAB subtype (p<0.0001) and was higher in more blastic MDS. Logically, HSP expression correlated significantly with the marrow blast percentage (p<0.0001) and CD34 (p<0.0001) expression. It was also positively associated with protein glycoprotein (Pgp) (p<0.0001), Bcl-2 and Bcl-xL expression (p<0.01). Expression of all HSP, except HSP70, negatively correlated with that of the pro-apoptotic proteins Bad and Bak (p<0.01). Mcl-1 was positively and Bcl-xS negatively associated only with HSP60, HSP90 and HSP110 (p<0.01). There was no correlation for Bax. Results regarding expression of Bcl-2 family proteins have been published separately.[6] Significant correlations were observed between expression of all HSP, except HSP70 (p<0.05). No correlation between HSP expression and any specific chromosomal abnormality was observed. When grouped according to prognostic groups, HSP27 (p=0.005), HSP60 (p=0.002), HSP90 (p=0.001) and HSP110 (p<0.0001) expression was significantly higher in poor and intermediate-risk groups than in the good-risk group. When considering clinical outcome, the median overall survival of the entire cohort was 514 days. As expected, bone marrow blast percentage (p=0.0005), karyotype (p=0.012), FAB subgroup and International Prognostic Scoring System (IPSS) score (p<0.00001 in each case) were prognostic factors for overall survival. Low CD34 and Pgp expression (<20% and <5% respectively) correlated with better overall survival (p<0.00001). HSP expression, except HSP70, increased with higher IPSS score (p<0.05). Overall survival was significantly better with lower expression of all HSP, except HSP70. Survival curves are shown in Figure 1. All these parameters had the same relation with time to leukemic transformation as with overall survival (*data not shown*).

Finally, we tested a prognostic multivariate model entering percentage of blasts, cytogenetics, CD34, Pgp and significant HSP expression. CD34 expression was a significant prognostic variable for both survival and leukemic transformation (p=0.02 and 0.01 respectively) but not Pgp expression. However, when added to potential prognostic factors, blast percentage remained the only prognostic parameter (p=0.001 for survival and p<0.00001 for trans-

Table 1. Initial characteristics and outcome of 142 patients with MDS.

Variable	*No of patients*	*% of patients*	*Mean survival (days)*	*p value*
Sex				
Male	82	57.7	692	0.71
Female	60	42.3	729.6	
Age (years)				
≤60	45	31.7	886	0.41
>60	97	68.3	624.6	
FAB				
RA	35	24.6	not reached	$<10^{-5}$
RARS	25	17.6	not reached	
RAEB	63	44.4	626.4	
CMML	19	13.4	487.2	
Hemoglobin (g/L)				
<100	102	71.8	688	0.019
≥100	40	28.2	758.4	
Absolute neutrophil count (10^9/L)				
<1.5	8	5.67	555.1	0.3
≥1.5	134	94.47	717.2	
Platelets (10^9/L)				
<100	65	45.8	617.5	0.03
≥100	77	54.2	785.4	
% bone marrow blasts				
<5	67	47.2	not reached	
5-10	48	33.8	611.3	$5x10^{-4}$
11-20	27	19	571	
Cytogenetics*				
not evaluable	17	12		
good	70	49.3	809.4	0.012
intermediate	27	19	701.7	
poor	28	19.7	583	
IPSS score‡				
low	33	27.7	966	$<10^{-5}$
Intermediate-1	41	34.4	764.8	
Intermediate-2	30	25.3	503	
high	15	12.6	621.9	

RA: refractory anemia; RARS: refractory anemia with ringed sideroblasts; RAEB: refractory anemia with excess blasts; CMML: chronic myelomonocytic leukemia; + Cytogenetic subgroups: good, normal, $5q^-$, $20q^-$, -Y; poor, chromosome 7 abnormalities, complexe (≥3 abnormalities); intermediate, +8, other abnormalities. ‡IPSS score was calculated for 119 patients, excluding six patients with proliferative-type chronic myelomonocytic leukemia (ie WBC >12×10^9/L) and 17 cases without cytogenetic study.

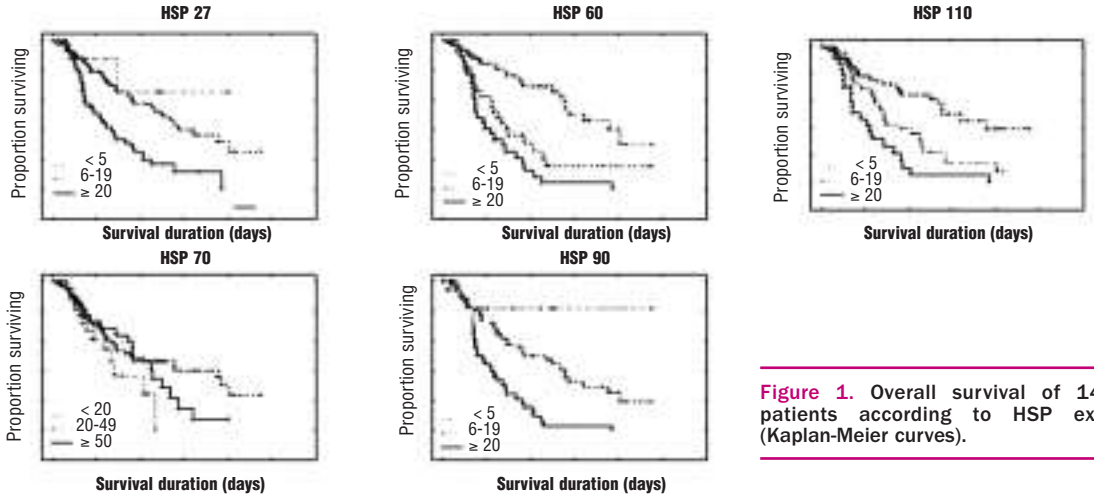

Figure 1. Overall survival of 142 MDS patients according to HSP expression (Kaplan-Meier curves).

formation). Expression of each HSP alone did not have any independent prognostic significance because of their correlation with blast percentage and FAB subtype. However, positive expression of at least two HSP, excluding HSP70, was significant for overall survival and leukemic transformation. The more significant combinations were HSP60 + HSP90 (p<0.001) and HSP27 + HSP60 (p=0.0013).

We observed over-expression of HSP27, HSP60, HSP70, HSP90 and HSP110 in patients with poor prognosis MDS. Moreover, all HSP were more expressed in CD34$^+$ cells. Indeed, expression of all HSP, except HSP70, correlated with FAB subtype, IPSS score, Bcl-2 related proteins, CD34 and Pgp expression and all these features correlated with bone marrow blast percentage. Parker *et al*. have previously reported expression of Bcl-2 related proteins on CD34$^+$ blasts.[7] This would indicate that HSP over-expression in refractory anemia with excess blasts (RAEB) is due to the increased blast percentage.

In conclusion, this work shows that types of MDS with a good prognosis (refractory anemia/refractory anemia with ringed sideroblasts) have lower HSP expression while RAEB has higher HSP expression, associated with the increased blast percentage. As HSP have a role in apoptosis,[3,8] studying their expression in blasts could provide information for understanding disease progression. Moreover, HSP may also be implicated in chemo-resistance as blocking HSP90 activity restores drug sensitivity.[9] Thus, it could be of interest to study whether HSP antagonists can prevent or at least delay blastic evolution of MDS.

Amélie Duval, Daniela Olaru,* Lydia Campos,*° Pascale Flandrin,*° Nathalie Nadal,° Denis Guyotat*#*

**Equipe 'Adhérence et Survie cellulaires dans les cancers et les greffes', Faculté de Médecine, Saint-Etienne; °Laboratoire d'Hématologie, Hôpital Nord, CHU de Saint-Etienne, France; #Département d'Hématologie, Institut de Cancérologie de la Loire, Saint-Etienne, France*

Acknowledgments: we thank Mohammed Rehailia for his help with statistical analysis, and Sylvianne Chautard, Salima Goumeziane-Dris and Simone Piselli for excellent technical support.

Funding: this study was supported by a grant from Comité Départemental de La Ligue contre le Cancer de la Loire.

Key words: MDS, HSP, apoptosis, prognosis.

Correspondence: Amélie Duval, EA3063 Adhérence et survie cellulaires dans les cancers et les greffes, Faculté de Médecine Jacques Lisfranc, 15 rue Ambroise Paré, 42023 Saint Etienne, France. Phone: international +33.477421494. Fax: international +33.477421485. E-mail: amelie.duval@univ-st-etienne.fr

References

1. Parker JE, Mufti GJ. Ineffective haemopoiesis and apoptosis in myelodysplastic syndromes. Br J Haematol 1998;101:220-30.
2. Lindquist S, Craig EA. The heat-shock proteins. Annu Rev Genet 1988;22:631-77.
3. Creagh EM, Sheehan D, Cotter TG. Heat shock proteins-modulators of apoptosis in tumour cells. Leukemia 2000; 14:1161-73.
4. Kim SH, Yeo GS, Lim YS, Kang CD, Kim CM, Chung BS. Suppression of multidrug resistance via inhibition of heat shock factor by quercetin in MDR cells. Exp Mol Med 1998; 30:87-92.
5. Mosser DD, Caron AW, Bourget L, Denis-Larose C, Massie B. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. Mol Cell Biol 1997;17:5317-27.
6. Boudard D, Vasselon C, Bertheas MF, Jaubert J, Mounier C, Reynaud J, et al. Expression and prognostic significance of Bcl-2 family proteins in myelodysplastic syndromes. Am J Hematol 2002;70:115-25.
7. Parker JE, Mufti GJ, Rasool F, Mijovic A, Devereux S, Pagliuca A. The role of apoptosis, proliferation, and the Bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. Blood 2000;96:3932-8.
8. Mehlen P, Schulze-Osthoff K, Arrigo AP. Small stress proteins as novel regulators of apoptosis. Heat shock protein 27 blocks Fas/APO-1- and staurosporine-induced cell death. J Biol Chem 1996;271:16510-4.
9. Thomas X, Campos L, Le QH, Guyotat D. Heat shock proteins and acute leukemias. Hematology 2005;10:225-35.