

HSP10 selective preference for myeloid and megakaryocytic precursors in normal human bone marrow

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Heat shock proteins (HSPs) constitute a heterogeneous family of proteins involved in cell homeostasis. During cell life they are involved in harmful insults, as well as in immune and inflammatory reactions. It is known that they regulate gene expression, and cell proliferation, differentiation and death. HSP60 is a mitochondrial chaperonin, highly preserved during evolution, responsible of protein folding. Its function is strictly dependent on HSP10 in both prokaryotic and eukaryotic elements. We investigated the presence and the expression of HSP60 and HSP10 in a series of 20 normal human bone marrow specimens (NHBM) by the means of immunohistochemistry. NHBM showed no expression of HSP60, probably due to its being below the detectable threshold, as already demonstrated in other normal human tissues. By contrast, HSP10 showed a selective positivity for myeloid and megakaryocytic lineages. The positivity was restricted to precursor cells, while mature elements were constantly negative. We postulate that HSP10 plays a role in bone marrow cell differentiation other than being a mitochondrial co-chaperonin. The present data emphasize the role of HSP10 during cellular homeostasis and encourage further investigations in this field.

Key words: HSP10, HSP60, normal human bone marrow, cell differentiation

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The *Heat Shock Proteins* (HSPs) are a family of molecules that play many critical roles during cell life (Ryan et al., 1997). HSP60 and HSP10 are mitochondrial chaperons involved in protein folding (Dubaque et al., 1998; Nielsen et al., 1999; Richardson et al., 2001). In particular, HSP60, the eukaryotic homologue of prokaryotic GroEL, is a 60 kDa protein with a cylindrical structure enclosing a ring-like central cavity (Weissman et al., 1995). HSP60 function critically depends on physical interaction with HSP10, the 10 kDa eukaryotic protein homologue of prokaryotic GroES (Hartman et al., 1992). An overexpression of HSP60 and HSP10 has been described, both *in vitro* and *in vivo*, in a number of cell-stress conditions, i.e. myocardial ischemia-reoxygenation (Lau et al., 1997; Lin et al., 2001) and tumours (Hettinga et al., 1996; Kobayashi et al., 1998; Cornford et al., 2000; Feng et al., 2001). Moreover, Samali et al. (1999) and Lin et al. (2001) have shown that extramitochondrial HSP60 and HSP10 overexpression could protect against apoptosis, postulating also other unknown functions of these proteins during cellular homeostasis.

Our previous studies performed on two different carcinogenetic models (the *dysplasia-carcinoma* sequence of uterine exocervix and the *adenoma-carcinoma* sequence of large bowel) showed the expression of HSP10 in the germinal cells of the colonic and uterine epithelium (Cappello et al., 2003). By contrast, mature elements lost this positivity. HSP10 thus was supposed to be involved in regeneration of normal tissues.

Since the bone marrow is a highly regulated and constantly regenerating tissue, responsible for hematopoiesis in adults; the aim of this study was to evaluate the presence and expression of HSP60 and HSP10 in a series of normal human bone marrow specimens (NHBM), to better understand the putative role of these proteins during homeostasis of normal tissues.

Materials and Methods

For the present study, twenty bone marrow biopsies diagnosed as normal were selected from the archival data of the Istituto di Anatomia e Istologia Patologica of the University of Palermo. In addition, five normal fresh samples of bone marrow were also collected. All the biopsies were taken from the posterior iliac crest with a Jamshidi needle, fixed in Schaffer solution and, after decalcification in EDTA, embedded in paraffin. The age of the subjects ranged between 19 and 67 years (median age 53). The bone marrow biopsies were morphologically evaluated on routine histologic sections stained with hematoxylin-eosin, PAS, Giemsa and Gomori. The immunohistochemical analyses were performed with the streptavidin-biotin complex method (LSAB2 kit peroxidase, DAKO Corporation, Carpinteria CA, Cat. No K0677), on 6 μ thick sections of tissues using primary antibodies against HSP60 (monoclonal mouse, SIGMA, Cat. No. H4149) at the dilution of 1:500, and HSP10 (Rabbit Anti-Cpn10 polyclonal antibody, StressGen, Biotechnologies Corp., Victoria BC, Canada, Cat. No. SPA-110), at the dilution of 1:400. We chose these dilutions on the basis of our previous experiments (Cappello et al., 2002; 2003a; 2003b; 2003c). We stained both paraffin-embedded and fresh tissues to evaluate if deparaffining procedures could change HSPs expression. After a 10 min of incubation with a serum-free protein block (DAKO Corporation, Carpinteria CA, Cat. No X0909), the primary antibody was added to the sections. A non-immune mouse serum was substituted for negative controls. Finally, AEC was used as developing chromogen (DAKO Corporation, Carpinteria CA, Cat. No K0677), and hematoxylin aqueous formula (DAKO Corporation, Carpinteria CA, Cat. No S2020) was used as nuclear counterstaining. Three observers (F.C., C.T. and V.F.) independently evaluated the type and the percentage of positive cells on 10 HPF. Inter-observer discrepancies were minimal and, where present, the mean value was considered as the result.

Results

Since we did not record any change in HSPs expression comparing paraffin-embedded and fresh tissues, we performed a statistical evaluation of

HSP10 positivity on all 25 specimens. The bone marrow parenchyma showed a cellularity ranging from 40 to 60%; maturation was preserved in all three lineages. Immunohistochemistry for HSP60 resulted negative in all cellular lineages of all examined NHBM (Figure 1A). A HSP10 positivity in 20-35% (mean 30%) of cells was found in all examined specimens (Figure 1B). An abundant granular cytoplasmic positivity was present in 72.5% (range 60-85%) of myeloid precursors (Figures 1C, D, E), while erythroid precursors were commonly negative to HSP10. Although the vast majority (75%, range 70-90%) of mature megakaryocytes were negative to HSP10 (Figure 1F), we found a strong cytoplasmic positivity in 81.5% (range 60-90%) of their precursors (Figures 1B, C, G). Finally, mature granulocytes, monocytes, lymphocytes and erythrocytes were commonly negative to HSP10. Percentages of HSP10 positive precursors and mature cells are detailed in Table 1.

Discussion

Hematopoiesis takes place in the bone marrow where pluripotential stem cells proliferate and differentiate into erythroid, myeloid and megakaryocytic lineages. Mature elements derived from different stage precursors circulate in the blood stream. The morphological evaluation of bone marrow parenchyma is the first approach to assess the functional status of bone marrow and to verify the presence of diseases. Histochemical and immunohistochemical studies can add important information about the functional status of the bone marrow.

HSP60 and *HSP10* gene sequences have recently been localized on chromosome 2 (Hansen, et al., 2003). Although it is clear that the expression of many HSPs could be critical for many adult cell functions, their molecular mechanisms are not yet well understood. In particular, a role in neural and glial differentiation was suggested, *in vitro*, for HSP70 (Zhang et al., 2001; Calabrese et al., 2002), while HSP27 might play a role, *in vivo*, during the postnatal development of rat submandibular gland (Amano et al., 2001). Immunoregulatory function through monocytic and dendritic cells activation and maturation has also been postulated (Bethke, et al., 2002; Flohe, et al., 2003). An increase of the levels of HSP60 has been demon-

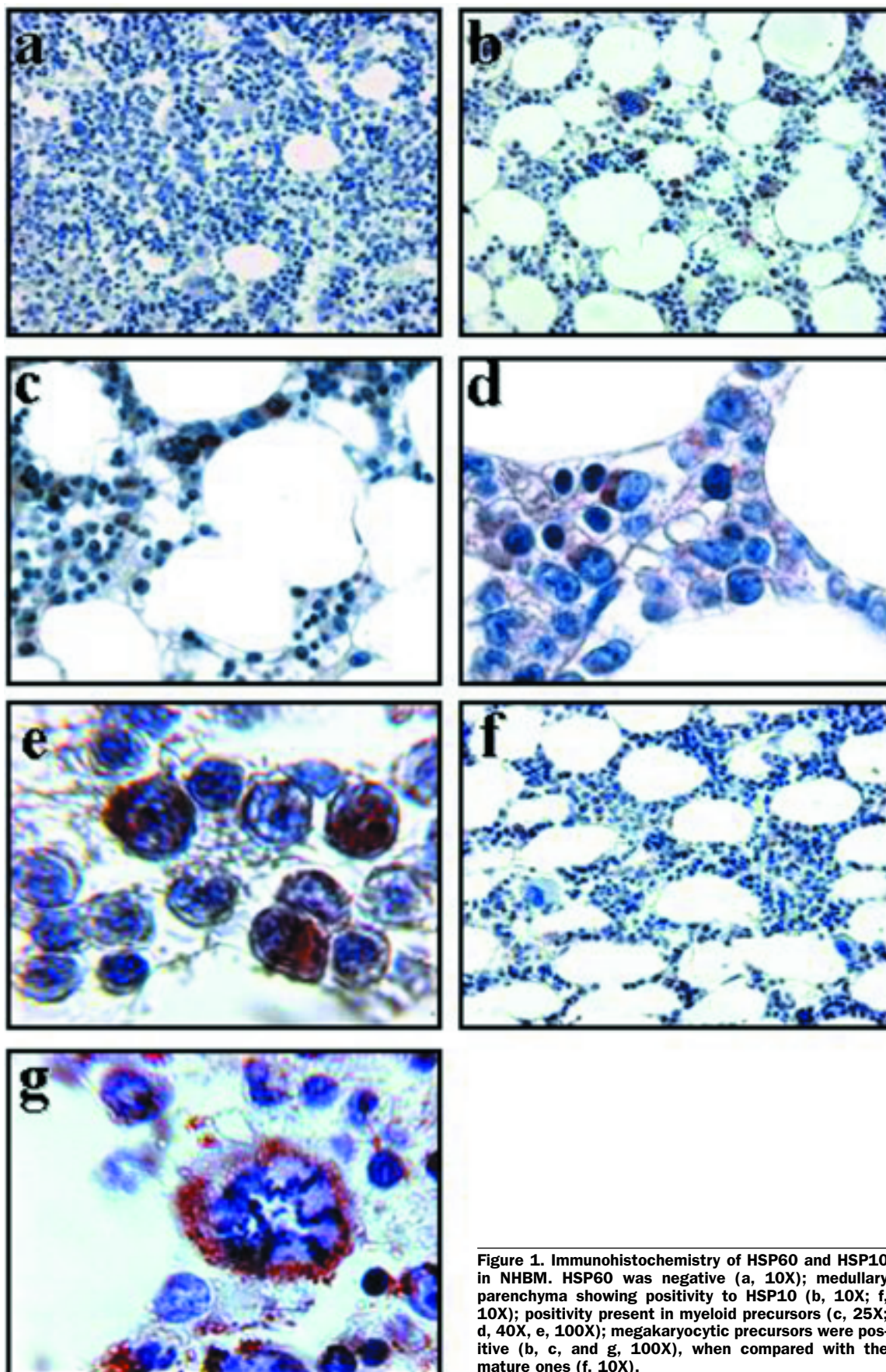


Figure 1. Immunohistochemistry of HSP60 and HSP10 in NHBM. HSP60 was negative (a, 10X); medullary parenchyma showing positivity to HSP10 (b, 10X; f, 10X); positivity present in myeloid precursors (c, 25X; d, 40X, e, 100X); megakaryocytic precursors were positive (b, c, and g, 100X), when compared with the mature ones (f, 10X).

Table 1. Percentages of HSP10 positive cells.

	<i>Precursors</i>	<i>Mature elements</i>
Myelocytes	60-85% (mean: 72.5%)	5-15% (10%)
Erythrocytes	0-10% (mean: 3%)	0%
Megakaryocytes	60-90% (mean: 81.5%)	10-30% (25%)

strated during the postnatal development of rat brain and kidney (D'Souza and Brown, 1998), as well as an increase in HSP27 expression during B cell differentiation in pediatric nonleukemic patients (Madsen et al., 1995).

In the present study, we compared the expression of HSP60 and HSP10 during cellular homeostasis of bone marrow. Although these two HSPs are known to cooperate in mitochondria, normal human bone marrow showed a positivity for HSP10, in particular for some precursors, while HSP60 was negative in all cellular elements. HSP60, as a mitochondrial chaperonin, is commonly below the threshold of detectability for immunohistochemistry in normal tissues (Cappello et al., 2002; 2003a; 2003c) and we expected its negativity in NHBM. By contrast, the cytoplasmic positivity of HSP10 during differentiation may indicate that this protein has other roles during proliferation and differentiation of these precursors besides that of co-chaperonin. Indeed, an increase in the synthesis of some HSPs happens during gene expression (Kiang and Tsokos, 1998) or harmful insults, such as a transient temperature rise (Ohtsuka and Hata, 2000). HSP over-expression is also critical in various cellular responses, as in immune and inflammatory processes (Moseley, 1998). Their synthesis can be modulated by cell signal transducers such as pH, cAMP, Ca²⁺, Na⁺, inositol triphosphate, etc. (Walsh et al., 1999). Molecular pathways between some HSPs, such as HSP70 and HSP90, and other proteins can regulate cellular proliferation and differentiation (Nollen and Morimoto, 2002), as well as prevent or enhance cell death (Garrido et al., 2001). Nevertheless, so far few studies have focused on the involvement of HSP10 during these cellular functions and responses.

In the present study, we found that HSP10 is selectively expressed in myeloid and megakaryocytic precursors and disappears during lineage maturation. Differentiation and maturation of hematopoietic lineages are under the influx of various molecules (Deutsch et al., 2000; Gazzero et

al., 2001). We postulate that HSP10 may have a role during differentiation and/or proliferation of these normal cellular lineages, but our results do not explain why HSP10 shows this selective expression. For instance, HSP10 could have a role in modulating the apoptotic phenomena of myeloid and megakaryocytic precursors, as already demonstrated *in vitro* in non-hematopoietic cell cultures (Samali et al., 1999; Lin et al., 2001), but this hypothesis needs to be confirmed.

In conclusion, our results may address further studies to understand better the role of HSP10 during normal human tissue homeostasis.

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