

RESEARCH ARTICLE

Clinical Significance of Circulating Serum Cellular Heat Shock Protein 90 (HSP90) Level in Patients with Cutaneous Malignant Melanoma

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Abstract

Background: Cellular heat shock proteins (HSPs) play significant roles in sustaining normal cellular conditions. The stimulated expressions of HSPs result in cellular stabilization at times of stress, such as cancer. The objective of this study was to determine the clinical significance of the serum levels of HSP90 in melanoma patients. **Material and methods:** A total number of 98 melanoma patients were enrolled into this study. Serum HSP90 concentrations were determined by the solid-phase sandwich ELISA method. Age and sex matched 43 healthy controls were included in the analysis. **Results:** The median age of patients was 51 years, ranging from 16 to 85 years. The majority of patients were male (61%), had lesions in axial localizations (54%) and had metastatic disease (61%). Moreover, most of the patients with metastatic disease had M1c diseases (73%). The baseline serum HSP90 levels of melanoma patients were significantly higher than those of the control subjects (median values 49.76 v 27.07ng/ml, respectively, $p<0.001$). However, clinical variables, such as age, gender, site of lesion, histology, lymph node involvement, stage, serum LDH levels and response to chemotherapy, were found not correlated with serum HSP90 concentrations ($p>0.05$). Moreover, serum HSP90 level was found not prognostic on survival ($p=0.683$). **Conclusions:** Serum levels of HSP90 may have a diagnostic value in melanoma. However, its predictive and prognostic values were not determined.

Keywords: HSP90- melanoma- prognosis- serum- marker

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Introduction

Cellular heat shock proteins (HSPs) play significant roles in sustaining normal cellular conditions (Becker, 2004; Stickler, 2014; McCarthy, 2008). Not only do HSPs stabilize cellular stress in conditions like cancer they also act as chaperones (transport proteins) both across plasma membranes within the cell and to the proteasome for degradations.

HSPs are classified by their molecular weights. HSP90 is a 90-kDa protein and protects proteins from deterioration caused by environmental stress, thus promoting chemotherapy resistance and cell survival (Becker, 2004; Stickler, 2014; McCarthy, 2008). Inhibition of HSP90 impairs regulation of signal transduction, cell cycle control and apoptosis, thus devastating results on cell viability ensue (McCarthy, 2008).

HSP90 expression has been investigated in miscellaneous human tumors but its clinical significance in melanoma has been addressed in only a few studies so far (Becker, 2004; Stickler, 2014; McCarthy, 2008). To the best of our knowledge no clinical study investigating serum or plasma HSP90 isoform in melanoma has been published so far, instead all available data have been acquired from preclinical studies which used tissue

samples.

Therefore, we evaluated the soluble serum levels of HSP90 in cutaneous melanoma patients, and assessed associations with the prognosis and various known clinical parameters.

Materials and methods

Patients

This study included 98 consecutive melanoma patients from the Institute of Oncology, Istanbul University. None of the patients had received any type of therapy (chemotherapy or radiation) within last 6 months. The staging was done according to the AJCC staging system. Detailed patient history, physical examination and blood tests including CBC and biochemistry analyses were done for each patient. Patients with ECOG performance status equal or less than 2, and suitable blood tests received chemotherapy included different single or combinations of various chemotherapeutic agents, such as interferon alpha, cisplatin, dacarbazine or temozolomide compounds, novel agents including ipilimumab and vemurafenib with/without radiotherapy depending on the stage of disease. Chemotherapy responsiveness was determined by the revised RECIST criteria version 1.1.

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A total of 43 healthy control subjects were included in the analysis. Informed consents were provided from all the patients. Our local ethical committee reviewed and approved the study.

Measurement of serum HSP90 levels

Blood serum samples of patients were provided on first admission by venipuncture before chemotherapy or follow-up and clotted at room temperature. The sera were collected after centrifugation and frozen at -20°C until analysis. HSP90 ELISA (SUNRED Biotechnology Company, Shanghai, People Republic of China) uses a double-antibody sandwich enzyme-linked immunosorbent assay to determine the level of human HSP90 in samples.

Statistical Analysis

Parameters were classified as median values as cut-off point. Comparisons between clinical/laboratory parameters and serum HSP90 assay levels were done using Mann-Whitney U test. Survival estimations of patients were determined by Kaplan-Meier method and differences of survivals were done by the log-rank statistics. A p value ≤ 0.05 was considered as significant. The SPSS 21.0 software (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses.

Results

Ninety-eight melanoma patients were enrolled into this study. The median age of patients was 51 years, ranging from 16 to 85 years. The majority of patients were male (61%), had lesions in axial localizations (54%) and had metastatic disease (61%). Moreover, most of the patients with metastatic disease had M1c diseases (73%).

The baseline serum HSP90 levels of melanoma patients were significantly higher than those of the control subjects (median values 49.76 v 27.07ng/ml, respectively, p<0.001) (Table 1). However, clinical variables, such as age, gender, site of lesion, histology, lymph node involvement, stage, serum LDH levels and response to chemotherapy, were found not correlated with serum HSP90 concentrations (p>0.05) (Table 2).

The median survival for all patients was 20.8 months (%95CI=11.1-30.4). The 1- and 2-year overall survival rates were 66.7% and 43.1%, respectively. Patients with metastasis (M1) (p<0.001), advanced metastatic disease (M1c) (p<0.001), anemia (p<0.001), elevated ESR (p=0.002), unresponsiveness to chemotherapy (p=0.003), axial localization (p=0.027), and multiple lymph node involvement in nonmetastatic disease (p=0.047) had worse survival (Table 2). However, serum HSP90 concentrations was not prognostic on survival in melanoma patients (p=0.683) (Table 2 and Figure 1).

Table 2. Distribution and Survival Comparisons of Serum HSP90 Levels on Various Patient/Clinical Parameters in Patients with Melanoma

Parameter	HSP90	
	Distribution p	Survival p
Age, years		
<50/≥50years	0.99	0.72
Sex		
male/female	0.33	0.67
Site of lesion		
axial/extremity	0.34	0.027
Histology*		
nodular/nonnodular	0.8	0.41
Breslow thickness*		
≤4mm)/>4mm	0.51	0.74
Clark invasion level*		
I-III/IV-V	0.58	0.88
Ulceration*		
yes/no	0.54	0.33
Mitotic rate*		
0-2/≥3	0.44	0.11
Regression*		
yes/no	0.76	0.62
Tumor infiltrating lymphocyte*		
yes/no	0.54	0.19
Nodal involvement*		
yes/no	0.59	0.08
Type of node involvement*		
single/multiple	0.38	0.047
Metastasis		
yes/no	0.34	<0.001
M1 status		
ab/c	0.43	<0.001
Serum LDH level		
high/normal	0.89	0.91
Anemia		
yes/no	0.56	<0.001
Erythrocyte sedimentation rate (ESR)		
high/normal	0.69	0.002
Response to chemotherapy		
yes/no	0.83	0.003
Serum HSP90 level		
low<median>high	-	0.683

*, only in patients with nonmetastatic melanoma

Table 1. The Values of Serum Assay HSP90 Levels in Melanoma Patients and Healthy Controls

Assay	Controls (n=43)		Patients (n=98)		p
	Median	Range	Median	Range	
HSP90 (ng/ml)	25.07	0.00-136.20	49.76	19.47-135.44	< 0.001

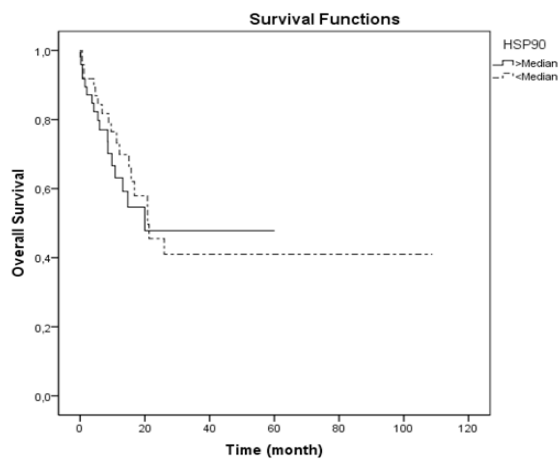


Figure 1. Overall Survival Curves in Melanoma Patients According to Serum HSP90 Levels ($p=0.683$)

Discussion

HSP90, a significant member of the HSPs family, has been identified as having stabilizing effects on cellular functions in malignant tumors and thus considered to be one of the main integral components for tumor progression and metastatic dissemination. Although HSP90 expression has been investigated in miscellaneous human malignancies, its overall functions still remain indeterminate in human melanoma. To date, there are only a few studies addressing the clinical significance of HSP90 expression in melanoma (Becker, 2004; Stickler, 2014; McCarthy, 2008).

In a small trial, HSP90 mRNA expressions were detected in 22% (2/9) of nevi, in 71% (10/14) of melanomas, in 100% (6/6) of melanoma metastases, and in all melanoma cell lines (100%, 9/9) (Becker, 2004). There were significant differences between nevi and melanoma data in terms of HSP90 mRNA expressions ($p=0.036$, $p=0.007$, and $p=0.002$, respectively). It was suggested that HSP90 mRNA expression was induced more strongly in malignant melanocytic lesions and melanoma cell lines than in benign nevi. Similar results were also found with the confirmative IHC assay, i.e. positive stainings for HSP90 were 22% (2/9), 77% (7/9), and 92% (13/14) in melanocytic nevi, melanoma and melanoma metastases, respectively. This trial was the first to demonstrate in vivo that HSP90 was upregulated more in malignant melanoma cells than in benign melanocytic lesions. In another similar small study, paraffin-embedded biopsy samples of 10 nevi and 10 malignant melanomas were compared (Stickler, 2014). The HSP90 proteins were expressed at higher levels in melanomas than in nevi. Even though HSP90 staining was present in benign nevi, staining scores in melanomas were significantly higher ($p<0.001$).

In a large-scale study, in which HSP90 expression patterns in melanocytic lesions were investigated, tissue microarrays comprising of 414 nevi, 198 primary melanomas, and 270 metastatic melanomas were assessed by automated quantitative analysis (McCarthy, 2008). HSP90 protein expression was higher in melanomas and metastatic melanomas than in nevi and primary melanomas, respectively ($p<0.0001$ for both). In primary

melanomas, HSP90 expression was associated with higher Clark level (IV-V) ($p=0.0167$) and increased Breslow thickness ($>2\text{mm}$) ($p<0.0001$). However, no significant correlation was found between HSP90 expression and other parameters, such as age, sex and ulceration. Similarly, there was no association between HSP90 expression and survival in primary and metastatic melanomas. Thus, it was suggested in this study that HSP90 might be used as a diagnostic indicator.

To the best of our knowledge no clinical study investigating serum or plasma HSP90 isoform in melanoma has been published so far, instead all available data have been acquired from preclinical studies which used tissue samples. So, the significance of serologic levels of HSP90 in melanoma patients has yet to be elucidated. The data of 98 patients with different stages of melanoma were analyzed in this study. We found a statistically significant difference in serum HSP90 level between melanoma patients and healthy controls, so we suggest that serum HSP90 might be considered as a diagnostic marker for melanoma. However, serum HSP90 concentrations were found not correlated with either known clinical variables, such as stage, or outcome, which indicates that serum HSP90 is not prognostic in melanoma.

In conclusion, even though serum level of HSP90 might be a diagnostic indicator for melanoma its predictive and prognostic values have yet to be determined. However, the small sample size and short follow-up time of our study are significant limitations. Yet, we believe that our study will be a contribution to the literature since to the best of our knowledge it is the first publication that was conducted on a patients group that comprised of all disease stages. Further studies with larger cohorts in longer time intervals are required to determine the potential clinical significance of this biomarker in melanoma.

Conflict of Interest Statement

None

Role of the funding source

None

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