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Clinical significance of serum Protease-Activated Receptor-1 (PAR-1) levels in patients with cutaneous melanoma

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ABSTRACT

Background: Protease-Activated Receptor-1 (PAR-1) plays an important role in the pathogenesis of multiple malignancies and its expression strongly also affects the outcomes of cancer patients. The objective of this study was to determine the clinical significance of the serum levels of PAR-1in cutaneous melanoma patients.
Methods: A total of 60 patients with a pathologically confirmed diagnosis of cutaneous melanoma were enrolled into this study. Serum PAR-1concentrations were determined by the solid-phase sandwich ELISA method.
Results: No significant difference in serum PAR-1 levels between melanoma patients and healthy controls was found ($p = 0.07$). The known clinical variables including age of patient, gender, site of lesion, histology, stage of disease, serum LDH levels and chemotherapy responsiveness were not correlated with serum PAR-1 concentrations ($p > 0.05$). Likewise, serum PAR-1 concentration had also no prognostic role on survival ($p = 0.41$).
Conclusion: Serum levels of PAR-1 have no diagnostic, predictive and prognostic roles in cutaneous melanoma patients.
General significance: Measurement of PAR-1 in serum is not a clinical significance in cutaneous melanoma patients.

1. Introduction

Protease Activated Receptor-1 (PAR-1), the prototypic member of the PAR family, is activated by thrombin following cleavage of its extracellular amino terminus domain [1]. PAR-1 and its activating factors, which are expressed on tumor cells and the surrounding stroma, induce not only coagulation, but also play an important role in promoting cancer progression in several malignancies such as lung, breast, prostate and melanoma [1].

Melanoma displays multifactorial etiology and its genetic and immunological background has not yet been fully elucidated. In vitro trials showed that cultured melanoma cell lines produce excessive levels of cytokines and growth factors with pleiotropic biological activities. Among them, PAR-1 functions as an autocrine and paracrine factor that drives many cellular processes such as tumor growth, invasion, angiogenesis and metastasis [1]. Increased expression and secretion of PAR-1 isoform in melanoma cells has been documented by several trials when compared with normal melanocytes [1–4]. Cell activation of the PAR-1 pathway in melanoma cell lines has been well documented. Increased expression of PAR-1 was found as closely associated with melanoma progression and metastasis in many various studies [1–10].

Although almost all available findings were provided from preclinical trials, so far, no clinical study to investigate the clinical significance of PAR-1 isoform in plasma/serum in melanoma patients. Thus, the significance of the serological levels of PAR-1in melanoma patients is not known yet. Therefore, we evaluated the soluble serum levels of PAR-1 in melanoma patients, and assessed associations with the prognosis, various known clinical variables, and response to chemotherapy, in order to examine whether these are potential new biomarkers, for use in the treatment of melanoma in this study.

2. Material and methods

2.1. Patients

This study comprised 60 patients admitted to Istanbul University, Institute of Oncology with histologically confirmed cutaneous melanoma. Patients with bidimensionally measurable disease without history of chemo/radiotherapy in the last six months were included in the study. The staging was determined according to the American Joint Committee on Cancer (AJCC) staging system. The pretreatment evaluation included detailed clinical history and physical examination with a series of biochemistry tests including LDH and complete blood cell counts. Those with ECOG performance status ≤2 and appropriate blood chemistry tests received chemotherapy on outpatient basis comprising interferon alpha, cisplatin, dacarbazine or temozolomide compounds with/

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without radiotherapy depending on the stage of disease. Follow up programs consisted of clinical, laboratory, radiological assessments performed at 8 week intervals during chemotherapy or every 12 weeks for no anticancer treatment. Response to treatment was determined according to revised RECIST criteria version 1.1.

For comparison of serum levels of PAR-1, age and sex matched 30 healthy controls were included in the analysis. Written informed consent was obtained from the patients and this study was approved by our ethical committee.

2.2. Measurement of serum PAR-1 levels

Serum samples were obtained on first admission before any adjuvant and metastatic treatment was given or follow-up patients. Blood samples were obtained from patients with malignant melanoma and healthy controls by venipuncture and clotted at room temperature. The sera were collected following centrifugation and frozen immediately at −20 °C until analysis.

The Human Protease Activated Receptor 1 (PAR-1) ELISA (Wuhan EIAab Science, China) uses a double-antibody sandwich enzyme-linked immunosorbent assay to determine the level of Human PAR-1 in samples. Serum samples and standards were added to the wells which were pre-coated with Human PAR-1 monoclonal antibody and allowed to incubate for 2 h. Unbound material was washed away and PAR-1 combined with Streptavidin-HRP were added to form immune complex and then allowed to incubate for 1 h. Unbound material was washed away. Chromogen solution was added and incubated for 15–25 min (protect from light) for the conversion of the colorless solution to a blue solution, the intensity of which was proportional to the amount of PAR-1 in the sample. As the effect of the acidic stop solution, the color has become yellow. The colored reaction product was measured using an automated ELISA reader (Rayto, RT-1904C Chemistry Analyzer, Atlanta, GA, USA) at 450 nm. The results were expressed as ng/mL.

2.3. Statistical analysis

Continuous variables were categorized using median values as cut-off point. Assessment of relationships, comparisons between various clinical/laboratory parameters and serum levels of PAR-1 assay were accomplished using Mann–Whitney U test. Survival was calculated from the date of first admission to hospital to death resulting from any cause or to last contact with the patient or any family member. Kaplan–Meier method was used for estimation of survival of patient and differences in survivals were assessed by the log-rank statistics. A p value ≤0.05 was considered significant. Statistical analysis was carried out using SPSS 16.0 software.

3. Results

A total of 60 cutaneous melanoma patients enrolled into this study. The baseline histopathological and the demographic characteristics of the patients are listed in Table 1. The median age at diagnosis was 53.5 years, range 16–88 years.

There was no significant difference in serum PAR-1 levels between melanoma patients and healthy controls ($p = 0.07$) (Table 2). The known clinical variables including age of patient, gender, site of lesion, histology, stage of disease, serum LDH levels and chemotherapy responsiveness were not correlated with serum PAR-1 concentrations ($p > 0.05$) (Table 3).

The median follow-up time was 11.1 months (range 6–39 months). The median survival for all patients was 26.0 months (%95 CI = 21–30). The 1-, 2-, and 3-year overall survival rates were 76.3% (%95 CI = 64–88), 55.6% (%95 CI = 39–72), and 51.0% (%95 CI = 33–69), respectively. As expected, the presence of metastasis ($p < 0.001$), advanced metastatic disease (M1c) ($p = 0.007$), elevated erythrocyte sedimentation rate ($p < 0.001$), higher serum LDH levels ($p < 0.001$), and unresponsiveness to chemotherapy ($p = 0.01$) had statistically significant worse survival (Table 3). However, serum PAR-1 concentration was not associated with outcome ($p = 0.41$) (Table 3 and Fig. 1).

Table 1
Patient and disease characteristics.

Variables	n
No. of patients	60
Age, years	
<50/≥50	26/34
Gender	
Male/female	33/27
Site of lesion	
Axial/extremity/unknown	38/16/6
Histology	
Nodular/nonnodular/unknown	9/31/20
Stage of disease	
I–II/III/IV/unknown	13/14/31/2
Tumor status[a]	
Thin (T1–2)/thick (T3–4)/unknown	10/16/1
Node status[a]	
Negative/positive	12/14
M1 status	
M1a + b/M1c	13/18
Serum hemoglobin level (12 g/dL)	
Low/normal/unknown	19/40/1
Serum White blood cell (WBC) count (10000)	
Normal/elevated/unknown	50/9/1
Serum LDH level (450 U/L)	
Normal/elevated	48/12
Erythrocyte sedimentation rate (ESR) (40/h)	
Normal/elevated/unknown	28/20/12
Response to chemotherapy[b]	
Yes/no/unknown	10/11/10
Last status	
Alive/dead	41/19

[a] In nonmetastatic patients.
[b] Metastatic patients.

4. Discussion

Tissue microarray trials showed that PAR-1 was highly expressed in melanoma as compared to melanocytic nevi and normal skin [1]. Moreover, a significantly elevated PAR-1 expression level in clinical samples of atypical nevi and melanoma compared to melanocytic nevi [2]. In addition to these trials, PAR-1 expression correlated with their metastatic potential in melanoma cell lines [3,4]. PAR-1 regulates melanoma cell growth and metastasis by affecting both invasive and angiogenic factors because of displaying decreased blood vessel density [3]. These factors can act in both an autocrine and paracrine fashion, influencing both melanoma tumor cells, as well as cells in the tumor microenvironment [1,3].

Tumor and stromal interactions are backbone to melanoma growth and metastasis [1]. PAR-1 is not only expressed on melanoma cells but is also expressed on several cell types in the melanoma microenvironment, such as endothelial cells, platelets, fibroblasts and macrophages. Activation of PAR-1 in melanoma cells results in secretion of cytokines, expression of adhesion molecules and increased vascular permeability in multiple steps during melanoma carcinogenesis, including proliferation, angiogenesis, invasion, and survival [1]. In the past years, significant research efforts have focused on determining the role of PAR-1 in

Table 2
The values of serum PAR-1 levels in melanoma patients and healthy controls.

Assay	Patients (n = 60)		Controls (n = 30)		p
	Median	Range	Median	Range	
PAR-1(ng/mL)	0.052	0.00–6.110	0.079	0.030–0.230	0.07

Table 3
Distribution and survival comparisons of serum PAR-1 levels on various clinical parameters in melanoma patients.

Parameter	Serum PAR-1 level p value	
	Distribution	Survival
Age, years <50/≥50	0.91	0.20
Gender Male/female	0.82	0.35
Site of lesion Axial/extremity	0.68	0.35
Histology Nodular/others	0.32	0.50
Tumor status (in M0 disease) T1–2/T3–4	0.24	0.61
Node status (in M0 disease) Negative/positive	0.16	0.48
Metastasis status Yes/no	0.23	<0.001
M1 status M1a-b/M1c	0.65	0.007
Erythrocyte sedimentation rate Normal/elevated	0.67	<0.001
Serum LDH level Normal/elevated	0.90	<0.001
Response to chemotherapy No yes	0.81	0.01
Serum PAR-1 level <median/≥median	–	0.41

melanoma progression by examining its interactions with other signaling molecules [1]. A link between PAR-1 and platelet activating factor receptor (PAFR) was established [5]. Moreover, two genes that are regulated by PAR-1, connexin 43 and maspin, both involved in modulating the interactions between melanoma cells and the stroma [6,7]. Thus, PAR-1 is both essential in promoting cross-talk between metastatic melanoma cells and the microenvironment and important in transcriptionally regulating various genes involved in the metastatic process in melanoma [1]. PAR-1 also plays a significant role in promoting melanoma cell migration, motility, antiapoptotic behavior, and survival [1,8]. Moreover, overexpression of PAR-1 also promotes metastatic melanoma as it can function as a growth factor [1]. Activation of PAR-1 by MMP-1 in melanoma cells has been shown to induce the expression of growth factors, as well as promote the invasiveness of melanoma cells [1,9].

Targeting PAR-1 utilizing siRNA incorporated DOPC liposomes inhibited melanoma tumor growth, metastases and angiogenesis in nude mice [3]. Thus, decreased melanoma growth and metastasis can be achieved directly by inhibiting PAR-1 on melanoma cells, indirectly by inhibiting PAR-1 activity on platelets [1,3,10]. Taken together, these data suggest that PAR-1 could be a potential therapeutic target for metastatic melanoma.

Although all available findings were provided from preclinical trials using melanoma tissue sections, so far, no clinical study to investigate the clinical significance of PAR-1 isoform in serum or plasma in melanoma patients. Thus, the significance of the serological levels of PAR-1 in melanoma patients is not known yet. A total of 60 patients with different stages of melanoma were studied in this study. Serum PAR-1 concentrations were determined by the solid-phase sandwich ELISA method. The results demonstrated that the analysis of serum PAR-1 was not able to discriminate between the melanoma patients and healthy persons, indicating that PAR-1 was not a good serological diagnostic marker of melanoma patients. Likewise, there were no significant associations between serum PAR-1 levels and clinical characteristics including age of patient, gender, site of lesion, histology, stage of disease, and serum LDH level. Similarly, no link between serum PAR-1 concentrations and chemosensitivity has raised the possibility of using PAR-1 as predictors of response to chemotherapy in patients scheduled to undergo various chemotherapeutic regimens in our study. We showed that serum PAR-1 levels may not be a potential predictor of clinical response to chemotherapy in melanoma patients. Moreover, we also found that serum levels of PAR-1 were not associated with survival. Therefore, in this study, PAR-1 concentrations in serum could not be useful prognostic marker to predict tumor prognosis in melanoma patients. Overall, it means that these findings are inconsistent with the aforementioned data provided from preclinical studies. Furthermore, we have also previously reported similar observations in patients with epithelial ovarian carcinoma including small sample number ($n = 44$) using a different ELISA kit [11]. In this trial, we found that serum PAR-1 levels were significantly elevated in patients compared with healthy

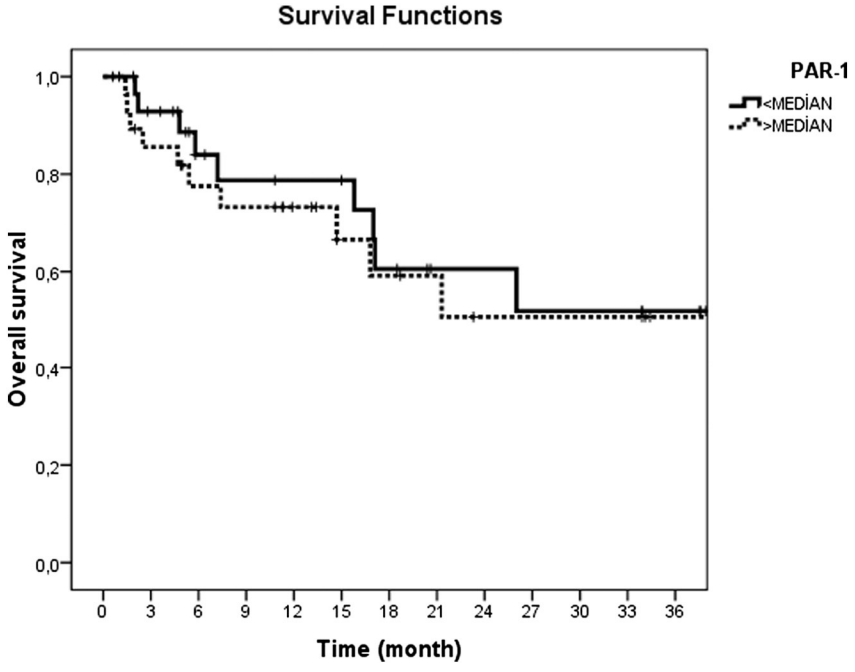

Fig. 1. Survival curves in melanoma patients according to serum PAR-1 levels ($p = 0.41$).

controls, whereas any clinical variables including chemotherapy responsiveness and survival did not associate with the serum PAR-1 assay. In our newly published study which consists of gastric cancer patients, identical results were also determined using same ELISA kit used in the current study [12].

In conclusion, we showed that serum levels of PAR-1 have no diagnostic, predictive and prognostic roles in melanoma patients. However, the small sample size and short follow-up time of our study could be considered as significant limitation and might have influenced these results. However, our study contributes to the literature, because we performed preliminarily in literature. Further studies in a larger patient population are necessary to determine the potential clinical significance of these assays in melanoma.

Conflict of interest statement

None.

Role of the funding source

None.

Transparency document

The Transparency document associated with this article can be found, in online version.

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