Clinical significance of serum protease activated receptor1 levels in patients with lung cancer

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Abstract. – OBJECTIVE: Protease-activated receptors (PAR) are G protein coupled receptors and they regulate many biological processes, including coagulation and cell survival and they might be good markers in some types of malignant tumors, providing useful information in diagnosis and prognosis. The objective of this study was to determine the clinical significance of the serum levels of PAR1 in lung cancer patients.

PATIENTS AND METHODS: Eighty patients with lung cancer were enrolled into this study. Serum PAR1 levels were determined by the solid-phase sandwich ELISA method. Median age was 58.5-years old, range 36 to 80 years.

RESULTS: The majority of the patients had NSCLC (85%) and stage IV disease (56%). The baseline serum PAR1 concentrations of the lung cancer patients were significantly higher than control group (median values 26.45 ng/mL vs 0.07 ng/mL, $p < 0.001$). However, clinical variables including age, gender, histology, stage of disease, and response to chemotherapy were not found to be correlated with serum PAR1 levels ($p > 0.05$). Moreover, it failed to show any prognostic value on the survival of the lung cancer patients.

CONCLUSIONS: The serum levels of PAR1 might have a diagnostic value in lung cancer patients. However, its predictive and prognostic values were not determined.

Key Words: PAR1, Serum, Lung cancer, Diagnostic.

Introduction

Lung cancer is the leading cause of cancer deaths and is the second most common cancer worldwide. It is a malignancy characterized by its highly invasive and metastatic nature. These properties require that the cancer interact with receptors that promote angiogenesis, basal membrane breakdown and extracellular matrix degradation.

Protease-activated receptors (PAR) are G protein coupled receptors and they regulate many biological processes, including coagulation and cell survival. Four of these receptors have been identified, PAR1, PAR2, PAR3 and PAR4. PAR1 is overexpressed in cancers of the head and neck, colon, breast, ovaries, prostate, pancreatic carcinomas, and melanoma and PAR-1 plays an important role in invasion and metastasis in breast and ovarian carcinoma xenograft models.

These receptors are activated by proteases, such as serine and cysteine proteases and matrix metalloproteinases (MMPs), that are highly upregulated in metastatic cancers, that are highly upregulated in metastatic cancers. The proteases, including thrombin, plasmin, factor Xa and activated protein C, cleave the amino-terminal exodomain of the receptor exposing a new amino-end that serves as a tethered ligand, thus activating the receptors.

PAR1 is found on platelets and is activated when it is cleaved especially by thrombin. MMP1 also cleaves and activates PAR1 at a different site. PAR1 has significant effects on endothelial barrier function, vasoreactivity, intimal hyperplasia, inflammation and hemostasis, and PAR1 is also involved in the regulation of survival, apoptosis and tumor growth.

However, the significance of PAR1 in lung cancer is yet to be understood. We evaluated the serum level of PAR1 in lung cancer patients with all clinical stages and questioned its correlation with the clinical variables in hope that it might be used as a new biomarker in the management of lung cancer.

Patients and Methods

Patients

Eighty patients admitted to Istanbul University, Institute of Oncology with histologically or cytologically confirmed non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) treated and followed up in our clinic were
Measurement of Serum PAR1 Levels

Serum samples were obtained on first admission before any adjuvant and metastatic treatment were given or follow-up patients. Blood samples were obtained from patients with lung cancer and healthy controls (n=30) by venipuncture and clotted at room temperature. The sera were collected following centrifugation and frozen immediately at -20°C until analysis.

Serum PAR1 (Eastbiopharm, Hangzhou, China) levels were determined by the solid-phase sandwich ELISA method. The PAR1 ELISA uses a double-antibody sandwich enzyme-linked immunosorbent assay to determine the level of PAR1 in samples. Serum samples and standards were added to the wells which were pre-coated with human PAR1 monoclonal antibody. Following incubation, PAR1 antibodies labeled with biotin and combined with streptavidin-HRP were added to form immune complex and allowed to incubate. Unbound material was washed away and then chromogen solution was added for the conversion of the colorless solution to a blue solution, the intensity of which was proportional to the amount of PAR1 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 (Chromate, 4300 Microplate Reader, Palm City, FL, USA). The results were expressed as ng/mL.

Statistical Analysis

Continuous variables were categorized using median values as cut-off point. Assessment of relationships, comparisons between various clinical/laboratory parameters 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 distribution and differences in survival 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 (SPSS Inc., Chicago, IL, USA).

Results

Eighty consecutive patients with pathologically confirmed diagnosis of lung cancer were enrolled into this study. Baseline histopathological characteristics and demographic features of patients are listed in Table I. The median age at diagnosis was 58.5-years old, range 36 to 80 years, where males constituted the majority of the group (n=72, 90%). The majority of the patients had NSCLC (n=68, 85%) and stage IV disease (n=45, 56%).
The levels of serum PAR1 in patients with lung cancer and healthy controls are shown in Table II. The baseline serum PAR1 concentrations of the lung cancer patients were significantly higher than those in the control group (median values 26.45 ng/mL vs 0.07 ng/mL, \( p < 0.001 \)) (Figure 1).

Table III shows the correlation between serum PAR1 levels and clinicopathological variables. Known clinical variables including age of patient, gender, histology, stage of disease, and response to chemotherapy were not found to be correlated with serum PAR1 concentrations (\( p > 0.05 \)).

The median follow-up time was 58 weeks, range 3.7 to 149.3 weeks. The median survival for all patients was 94.4 weeks (%95CI=73.1-115.7). The 1- and 2-year overall survival rates were 68.5% and 40.6%, respectively.

As expected, histology (\( p = 0.004 \)), metastasis (\( p = 0.005 \)), and response to chemotherapy (\( p = 0.009 \)) had prognostic factors on survival (Table III). However, serum PAR1 level was no associated with outcome (\( p = 0.21 \)) (Table III, Figure 2).

**Table I.** Patient and disease characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>80</td>
</tr>
<tr>
<td>Age of patients</td>
<td></td>
</tr>
<tr>
<td>( \geq 60 )</td>
<td>37</td>
</tr>
<tr>
<td>(&lt; 60 )</td>
<td>43</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>72</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>68</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>33</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>27</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>8</td>
</tr>
<tr>
<td>SCLC</td>
<td>12</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>30</td>
</tr>
<tr>
<td>IV</td>
<td>34</td>
</tr>
<tr>
<td>Limited</td>
<td>1</td>
</tr>
<tr>
<td>Extended</td>
<td>11</td>
</tr>
<tr>
<td>Response to chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>41</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
</tr>
</tbody>
</table>

**Figure 1.** The values of serum PAR1 assay in lung cancer patients and healthy controls (\( p < 0.001 \)).

**Table II.** The values of serum PAR1 levels in lung cancer patients and healthy controls.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patients (( n = 80 ))</th>
<th>Controls (( n = 30 ))</th>
<th>( \rho )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PAR-1 level (ng/mL)</td>
<td>26.45 5.88-396</td>
<td>0.07 0.02-23.62</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Lung cancer with a high metastatic rate requires the identification of potential biomarkers to diagnose patients and then to screen them after their primary treatment. In the present study we determined that serum PAR1 level had a diagnostic value as the baseline serum PAR1 levels were significantly higher in lung cancer patients compared to the control group ($p < 0.001$). However, there was no significant correlation between serum PAR1 concentration and age, gender, response to chemotherapy, histology of the disease and stage in NSCLC. Furthermore, serum PAR1 level failed to show any prognostic value on the survival of the lung cancer patients.

Available data about PAR1 in different types of cancers were obtained only from tissue cultures as paraffin-embedded materials and cell line trials. To the best of our knowledge, our study is the first in that matter that used serum samples instead of tissue. Thus, we discuss our results with findings that have been provided from tissue, not sera.

In order for the cells to accomplish migration, first the extracellular matrix should break down with proteolytic enzymes, such as matrix metalloproteinases (MMPs), then the cells should survive the distance to the target, then they should be able to intravasate into the target tissue, again with the help of proteolytic enzymes, and finally they should interact with the new microenvironment. Of 24 MMP members, MMP1’s type I collagenase ac-
Activity has long been associated with tumor growth, invasion, and metastasis by both modifying the matrix and promoting vessel formation. MMP1 also proteolytically activates PAR-1²¹.

MMP1 and PAR1 expressions have been demonstrated to correlate with tumor progression both in vitro and in vivo. After the potential significance of MMP1 and PAR1 in the tumorigenesis has been revealed the idea of the blockage of the MMP1/PAR1 axis by a monoclonal antibody against PAR1 has been developed. Then, it has been shown that this antibody significantly reduced the migration ability of human mesenchymal stem cells²². This finding also suggested that MMP1 expression and MMP1/PAR1 axis played a significant role in migration capacity of tumor cells²³,²⁴.

In light of these encouraging data, the association of MMP1 and/or PAR1 protein overexpression with the clinicopathological characteristics of several cancers has been studied and it has been concluded that higher levels of these proteins are strongly linked with poor outcome of the diseases²⁵,²⁶,²⁸-³⁰.

Boire et al⁵ used a xenograft model of MMP1/PAR1 in breast cancer cells and discovered that MMP1 that was reproduced from stromal fibroblasts instead of cancer cells induced cell migration and invasion via PAR1. Hernandez et al²⁵ showed in breast cancer patients that PAR1 protein expression is associated with higher grade, cerb-B2 positivity and advanced stage thus higher metastasis rate and mortality. Diaz et al²⁶ showed that PAR1 was elevated in progesterone receptor positive breast cancer patients and this finding suggested that adhesion and migration of cancer cells was enhanced through PAR1 that was resulted from progesterone induction.

Other cancers have also been reported with similar findings on the MMP1 and/or PAR1 protein overexpression. Granovsky-Grisaru et al⁶ found not only the association of PAR1 protein overexpression and endometrial carcinoma but also the correlation of PAR1 protein and higher grade of the tumor. Ben Nasr et al²⁷ noticed the correlation of MMP1 expression and lymph node metastasis in nasopharyngeal carcinoma. Zhu et al¹⁹ suggested that the proliferation and invasion of nasopharyngeal carcinoma cells was mediated by activation of PAR1. Yang et al²⁸ demonstrated that MMP1 and PAR1 were both overexpressed in nasopharyngeal carcinoma and that this finding was consistent with the previous studies of Ben Nasr et al²³ and Zhu et al¹⁹. The expression levels of MMP1 in the nasopharyngeal carcinoma specimens were correlated with those of PAR1, thus it suggested that there was a regulatory relationship between MMP1 and PAR1. Yang et al²⁸ also concluded that MMP1/PAR1 axis activation played crucial role in tumorigenesis and progression of nasopharyngeal carcinoma. It was also stated that the upregulation of MMP1 and PAR1 protein overexpression are unfavorable prognostic markers for nasopharyngeal cancer thus they might be targeted as a potential therapeutic approach.

Peng et al²⁹ reported that overexpression of MMP1 and PAR1 in 85 esophageal squamous cell carcinoma patients predicted poor prognosis after curative resection and this axis might be targeted for a future therapeutic method. Liao et al³⁰ showed in 106 hepatocellular patients that overexpression of MMP1 and PAR1 was associated with poor prognosis. Karabulut et al³¹ found that serum PAR1 levels had diagnostic value for the epithelial ovarian carcinoma. Like ours, this work had its unique value in that it used serum samples instead of tissue and its finding was consistent with the previous studies performed on tissues. However, it was found that serum PAR1 levels had neither predictive nor prognostic significance in epithelial ovarian carcinoma patients.

**Conclusions**

Our data provide evidences for the first time that serum PAR1 level might provide a diagnostic prediction of lung cancer. The response rate to chemotherapy in lung cancer also might be foreseen in accordance with serum PAR1 protein level. However, it failed to show any prognostic value on the survival of the lung cancer patients. Studies of a larger scale are required to determine the clinical significance of serum PAR1 levels in lung cancer.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

**References**


