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Prognostic relevance of soluble human leukocyte antigen–G and total human leukocyte antigen class I molecules in lung cancer patients

Philipp Schütt [a,*], Birgit Schütt [b,c], Magdalena Switala [b], Sebastian Bauer [a], Georgios Stamatis [d], Bertram Opalka [e], Wilfried Eberhardt [a], Martin Schuler [a], Peter A. Horn [b], Vera Rebmann [b]

[a] Department of Medicine (Cancer Research), West German Cancer Center, University of Duisburg-Essen, Essen, Germany
[b] Institute for Transfusion Medicine, University of Duisburg-Essen, Essen, Germany
[c] Department of Haematology, Kliniken Essen-Süd, Essen, Germany
[d] Department of Thoracic Surgery, Ruhrlandklinik, University of Duisburg-Essen, Essen, Germany
[e] Department of Haematology, West German Cancer Center, University of Duisburg-Essen, Essen, Germany

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ABSTRACT

The aim of this study was to determine the prognostic significance of soluble human leukocyte antigen (HLA) class I (sHLA-I) and HLA-G molecules in lung cancer patients. A total of 23 small-cell lung cancer (SCLC) and 114 non–small-cell lung cancer (NSCLC) patients, including 55 adenocarcinoma, 46 squamous cell carcinoma (SCC), and 13 patients with undifferentiated carcinoma, were prospectively enrolled. Levels of sHLA-G and sHLA-I were analyzed by specific enzyme-linked immunosorbent assay. Median levels of sHLA-G and sHLA-I were significantly increased in patients compared with controls (34 ng/ml [3.6–160] vs 14 ng/ml [0–98], $p < 0.0001$; 2580 ng/ml [749–5770] vs 1370 ng/ml [274–2670], $p < 0.0001$, respectively). Regarding the different subgroups, patients with NSCLC or SCLC showed increased sHLA-I levels, whereas sHLA-G was exclusively elevated in NSCLC, especially in patients with SCC. Patients with sHLA-I<2800 ng/ml ($p = 0.008$) or sHLA-G<40 ng/ml ($p = 0.073$) showed prolonged overall survival (OS). Using these cut-offs in patients with SCC, a pronounced prognostic significance for sHLA-G ($p = 0.003$) and sHLA-I ($p = 0.004$) was observed for the prediction of OS. Here, multivariate analysis confirmed sHLA-G and sHLA-I in addition to disease stage as independent prognostic factors. The prognostic power was further enhanced by combining the two factors and comparing the OS of patients with low sHLA-I and low sHLA-G against the remaining ones. In conclusion, plasma levels of sHLA-G and sHLA-I are potent predictors for OS in lung cancer patients.

1. Introduction

Lung cancer is the leading cause of cancer mortality, resulting in more than 160,000 deaths annually in the United States. Surgery, radiotherapy, and medical treatment using classical chemotherapeutic drugs or target therapies are standard treatments for lung cancer patients. In addition, certain immune therapeutic strategies have been shown in clinical trials to elicit or strengthen a cellular immune response against the tumor [1,2]. The limited effectiveness of such approaches in lung cancer may indicate that mechanisms are operative *in vivo,* enabling tumor cells to escape from immunosurveillance.

Among various molecules implicated in immune escape mechanisms of tumor cells, the nonclassical human leukocyte antigen–G (HLA-G) seems to be one of the most powerful molecules for the suppression of the innate and/or adaptive immune response by multiple pathways of the immune system [3]. In contrast to classical HLA class I molecules, HLA-G displays a limited polymorphism but can exists in seven different isoforms known to be expressed as membrane-anchored molecules (HLA-G1, G2, G3, and G4 isoforms) and as secreted soluble ones (HLA-G5, G6, and G7 isoforms) [4]. With regard to function, HLA-G molecules suppress the effector functions of T cells and natural killer (NK) cells by the engagement with immunoglobulin-like transcript (ILT) receptors ILT2 (LILRB1/CD85j), ILT4 (LILRB2/CD85d), CD8 α-chain, killer immunoglobulin-like receptor 2DL4 (KIR2DL4/ CD158d), and CD160 [5–7]. The interaction of ILT2 and ILT4 with HLA-G on dendritic cells (DC) results in the downregulation of co-stimulatory molecules as well as in the downregulation of HLA class II antigens. Such modified DC enable the differentiation of anergic and immunosuppressive CD4- and CD8-positive T cells [8]. Interestingly, the modulation of DC differentiation by HLA-G and ILT4 requires the IL-6 STAT3 signaling pathway [9]. As soluble and membrane-anchored HLA-G exhibit the same receptor specificity, both types of molecules are potent regulators for the innate and acquired cellular immune response. In view of the functional properties, it is not surprising that, under physiologic conditions, HLA-G expression is confined to immune privileged sites, such as fetal tissue, cornea, pancreatic islets, and

* Corresponding author.
E-mail address: philipp.schuett@uni-due.de (P. Schütt).

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adult thymic medulla, where it plays a crucial role in mediating immunotolerance [3]. Nevertheless, traces of HLA-G can be found in blood cells [10] and as soluble molecules in the blood of healthy individuals [11]. In recent years, a growing number of studies have documented an aberrant expression of membrane-anchored or soluble HLA-G molecules in cancer [3,12]. In addition, clinical studies have often found an association of high levels of membrane-anchored or soluble HLA-G molecules with a shortened overall and/or progression-free survival of patients [12–18]. In peripheral blood, the most abundant soluble HLA molecules are the classical class I molecules. With regard to functions, both types of soluble molecules, the classical HLA-I and the nonclassical HLA-G molecules, exhibit strong immunosuppressive properties; soluble HLA-G (sHLA-G) and soluble classical HLA-I molecules (sHLA-I) are able to induce apoptosis in activated $CD8^+$ T lymphocytes or NK cells through ligation with the CD8 coreceptor [19–21]. Although the classical HLA class I molecule displays a lower affinity to ILT-2 and ILT-4 than does HLA-G, both molecules are able to induce the inhibition of T and NK cell effector functions by interaction with these inhibitory receptors [22,23]. The clinical relevance of sHLA-I was studied for patients with pancreatic cancer, malignant lymphoma, acute myeloid leukemia, and multiple myeloma. Increased serum sHLA-I levels of the patients were associated with a poor prognosis [24–30]. A recent report revealed that, compared with controls, lung cancer patients with metastatic disease had significantly increased levels, whereas patients with localized disease exhibited diminished levels [31]. By contrast, downregulation or loss of HLA class I surface expression is frequently found in malignancy and has been associated with an undifferentiated cell phenotype and aneuploid cancer cells in lung cancer [32]. Kikuchi et al. demonstrated that downregulation of HLA-I on lung cancer cells was an independent poor prognostic factor for overall survival [33]. It is thought that loss of HLA-I expression on tumor cells is a mechanism for tumor cells to escape immunosurveillance. In view of this, we studied the clinical relevance of sHLA-G in comparison with the total amount of soluble HLA class I in various subgroups of lung cancer patients. To this end, plasma samples of lung cancer patients were prospectively collected and tested for their content of sHLA-I and sHLA-G by specific ELISA formats. The results obtained were correlated with clinical parameters, and their relevance for the prognosis of the patients was investigated.

2. Subjects and methods

2.1. Patients and procedure

From September 2001 to December 2007, plasma samples were collected from 137 lung cancer patients (95 males and 42 female), including 23 patients with small-cell lung cancer (SCLC) and 114 patients with non–small-cell lung cancer (NSCLC). The patients' characteristics are summarized in Table 1. Only patients with histologically or cytologically confirmed diagnoses of lung cancer were included in this study. Patients who underwent chemotherapy within 3 months before blood sampling or patients on corticosteroid treatment were excluded from the study. A total of 131 patients were chemotherapy-naive and six patients had already received at least one cycle of chemotherapy. For patients after chemotherapy, blood samples were collected in median 9.1 months after the last application of chemotherapeutic drugs (range 4.5–32 months). At the time of plasma collection, the stage of disease according to the International Union Against Cancer (UICC) classification was I/II, III, and IV in 37, 52, and 48 patients, respectively. The median age of the patients was 63 years (range 24–78 years). Because of loss to follow-up, Kaplan–Meier Curve analysis and multivariate Cox regression analysis were performed for 136 patients. The median follow-up of the patients was 9 months (range 0.6–77 months). Plasma samples from 84 age-matched healthy individuals served as control panel. Plasma samples of patients and controls were prepared from the peripheral blood by centrifugation at 1.500 *g* for 10 minutes. The supernatants were stored at −80°C until use. Written informed consent was obtained from each individual, and the study was approved by the local ethics committee.

Table 1
Plasma levels of sHLA-G and sHLA-I in lung cancer patients

	No. of patients (%)	sHLA-G (ng/ml)	sHLA-I (ng/ml)
Age (y)			
<60	54 (40)	35 (3.6–128)	2850 (1090–5420)
≥60	83 (60)	33 (3.7–161)	2310 (749–5770)
Gender			
Male	95 (69)	34 (3.6–161)	2620 (749–5770)
Female	42 (31)	36 (3.7–88)	2510 (1090–5420)
Histology			
SCLC	23 (17)	23 (3.6–66)[b]	2790 (1020–5590)[a]
NSCLC	114 (83)	36 (6.0–161)[a,c]	2510 (749–5770)[a]
Adenocarcinoma	55 (41)	33 (9.5–117)[a,b,c]	2490 (1090–5770)[a]
SCC	46 (33)	45 (6.0–161)[a]	2280 (749–5340)[a]
Undifferentiated carcinoma	13 (9)	37 (18–85)[a,b,c]	2660 (1530–4980)[a]
Disease stage			
I/II	37 (27)	31 (3.7–117)	2320 (749–5770)
III	52 (38)	34 (6.0–161)	2550 (890–5630)
IV	48 (35)	34 (3.6–93)	2800 (1090–5590)
Previous chemotherapy			
No	131 (96)	34 (3.6–161)	2520 (749–5770)
Yes	6 (4.0)	45 (11–75)	3240 (1430–4350)

Abbreviations: SCLC, small-cell lung cancer; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma.
Differences between groups were assessed using the Mann–Whitney *U* test.
[a]Significant differences ($p < 0.002$) compared with healthy controls.
[b]Significant differences ($p < 0.05$) compared with patients with SCC.
[c]Significant differences ($p < 0.002$) compared with patients with SCLC. Results are presented as median and range.

2.2. Determination of total sHLA-I and sHLA-G plasma levels

The determination of sHLA-I and sHLA-G was performed as described previously [14,34] with minor modifications. For sHLA-I and sHLA-G the specific capture reagents were the monoclonal antibodies W6/32 and G233, respectively [35]. Bound molecules were detected by a polyclonal antiserum rabbit anti-human β_2-microglobulin (Dako, Hamburg, Germany) followed by Envision goat antirabbit horseradish peroxidase (Dako, Germany). Plasma samples were diluted either 1:26 (sHLA-I) or 1:2 (sHLA-G). In both assays, purified sHLA-G5 protein served as standard reagent [14] and 3,3′,5,5′-tetramethybenzidine as substrate solution. After stopping the enzyme reaction with 1 M H_2SO_4, the optical density was measured at 450 nm (Biotek Instruments, Winooski, VT). Determination of sHLA-I and sHLA-G plasma levels was performed by four-parameter curve fitting. For the calculation of the ELISA detection limits, a standard curve starting from a concentration of 40 ng/ml for the HLA-I ELISA and from 8 ng/ml for the sHLA-G ELISA was performed in equimolar dilution steps of 5 and 1 ng/ml, respectively. The results obtained were subjected to the software DINTEST (Institut für Rechts-und Verkehrsmedizin, Universitätsklinikum Heidelberg, Germany). According to this procedure, the detection limits of sHLA-I and sHLA-G ELISA were 4.58 and 0.25 ng/ml, respectively.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL), BIAS version 8.6 (Epsilon Verlag, Frankfurt, Germany), or GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). According to nonparametric distribution of sHLA-I and sHLA-G plasma levels, levels of respective groups are presented as median and range and differences between groups were assessed using the