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Original article

Soluble CD40 in plasma and malignant pleural effusion with non-small cell lung cancer: A potential marker of prognosis

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Abstract

Objective: Soluble CD40 (sCD40) is a potential modulator for both antitumor responses and CD40-based immunotherapy; however the levels and significance of sCD40 in non-small cell lung cancer (NSCLC) patients with malignant pleural effusion are unknown.

Methods: Forty-eight patients with lung cancer were treated in our institutions from January 2008 to January 2010. Peripheral blood and pleural effusion samples were collected from each subject. sCD40 levels in plasma and malignant pleural effusions supernatant were measured. The CD40L expression on CD3t T-cells was confirmed by flow cytometric direct immunofluorescence analysis. All patients were followed up after the study ended on January 1, 2010.

Results: Patients with malignant pleural effusion of NSCLC had elevated circulating and pleural effusion levels of sCD40, and these elevated sCD40 levels were associated with advanced diseases and a poor prognosis.

Conclusions: These findings indicate that elevated sCD40 may have a role in modulating antitumor responses and may also be a useful prognostic marker.

Keywords: Soluble CD40; Malignant pleural effusion; Prognosis

Introduction

Malignant pleural effusions (MPE) worsen the clinical course of patients with lung cancer.[1,2] The majority of the MPEs develop when tumor cells directly infiltrate the pleura. Due to mediastinal lymph node or bronchial obstruction, pulmonary embolism or superior vena cava syndrome, pleural effusion may also occur in patients with indirect malignant diseases.[3] CD40 is best appreciated as a critical regulator

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of cellular and humoral immunity via its expression on B lymphocytes, dendritic cells, and monocytes.[4,5] CD40 is also expressed on the surface of many other normal cells. The global physiologic effect of the CD40 signaling pathway is profound.[4] CD40 ligand (CD40L), which is also known as CD154, is the chief ligand described for CD40 and is expressed primarily by T lymphocytes and platelets. Atherosclerosis, graft rejection, coagulation, infection control, and autoimmunity are all regulated by CD40-CD40L interactions.[5,6] In the present study, we provide data about sCD40 levels in NSCLC patients with malignant pleural effusion and analyze the relationship between the levels of sCD40 and the prognosis of patients.

Materials and methods

Patients and study design

Forty-eight patients with lung cancer were treated in our institutions from January 2008 to January 2010. All patients (28 males, 20 females with an average age of 64.4 years, ranging from 35 to 82 years old) were histologically diagnosed with lung cancer and pathologically staged according to the Tumor-Node-Metastasis (TNM) classification.[7] Among them, 13 patients were stage I and II, and the other 35 patients were stage III and IV. No patients had chemotherapy or immunotherapy within the six weeks preceding the study. Out of 48 patients, 29 of them with MPE (when malignant cells were detected in the pleural fluid or in the pleura with a cytopathological examination) were enrolled in this study, 17 patients had Performance Status (PS) = 0–1, 12 patients had PS ≥2. Two samples of peripheral blood were successfully obtained from 26 patients before and after two cycles of chemotherapy. Eighteen patients experienced progressive disease (PD) and eight patients experienced stable disease and partial remission (SD + PR). The same procedure was also performed in 15 peripheral blood samples from healthy volunteers.

Enzyme immunoassays and flow cytometric immunofluorescence analysis

Peripheral blood and pleural effusion samples were withdrawn from each subject after they signed an informed consent, and anticoagulated in Natrium Citrate. Samples were immediately centrifuged at 3000 r/min for 10 min. sCD40 levels in plasma and MPE supernatant were measured with an sCD40 enzyme-linked immunosorbent assay kit (eBioscience, USA) according to the manufacturers' instructions. Peripheral blood mononuclear cells were obtained by Hypaque-Ficoll gradient centrifugation and then washed. The CD40L expression on $CD3^+$ T-cells was confirmed by flow cytometric direct immunofluorescence analysis, using CD40L-polyethylene (PE) and CD3-fluorescein isothiocyanate (FITC) monoclonal antibodies (BioLegend, USA). All samples were assayed twice.

Follow-up

All patients were followed up after the study ended on January 1, 2010 for at least three months unless deceased occurred. Before the end of the study, the patients (or their families) were contacted by telephone to assess the survival status or the date of death.

Statistical analysis

All analyses were performed using GraphPad Prism 5.0 (GraphPad Software, USA); Pearson's correlation, the *t*-test, one-way Analysis of Variance (ANOVA), and Log-rank (Mantel–Cox) Test were used. Statistical significance was assumed if a *P*-value (two tailed) was less than 0.05. Data are expressed as mean ± standard deviation (SD).

Results

sCD40 levels in patients' plasma with different TNM stages and PS score

Levels of sCD40 in the plasma of patients with lung cancer were higher than in healthy volunteers (249.60 ± 40.37 pg/ml vs. 128.70 ± 16.49 pg/ml, $P = 0.002$). Different sCD40 levels were detected in the patients' plasma with different TNM stages. The sCD40 levels in plasma from patients who at stage III–IV, 310.00 ± 51.55 pg/ml ($n = 35$), were higher than the levels in patients with stage I–II disease, 87.08 ± 16.74 pg/ml ($n = 13$) (Fig. 1A). The sCD40 levels from patients with the most advanced lung cancer stage IV ($n = 29$) whose PS ≥2 was 521.7 ± 124.7 pg/ml ($n = 12$), higher than in patients with PS = 0–1, 216.00 ± 30.76 pg/ml ($n = 17$) (Fig. 1B).

Different sCD40 levels pre-chemotherapy and post-chemotherapy

The sCD40 level of patients after two cycles of chemotherapy was higher, 193.70 ± 13.17 pg/ml