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Prognostic significance of serum sMICA levels in non-small cell lung cancer

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Abstract. – OBJECTIVE: The soluble form of major histocompatibility complex class I-related chain A (MICA) is released from the surface of tumor cells of epithelial origin. Serum levels of soluble MHC class I-related chain A (sMICA) is related with the prognosis of various types of cancer. However, there are studies on the prognostic value of sMICA in non-small cell carcinoma (NSCLC). In this study, we retrospectively investigated the relationship between sMICA levels and clinical features of NSCLC, and we assessed the prognostic value of sMICA in NSCLC.

PATIENTS AND METHODS: sMICA levels were detected in 207 NSCLC patients and 207 normal control individuals with using enzyme-linked immunosorbent assay (ELISA), and its associations with clinicopathological parameters were evaluated. Survival curves were compared using the Kaplan-Meier method and log-rank tests. Univariate Cox regression was used on each clinical covariate to examine its influence on patient survival. Multivariate models were based on step-wise addition.

RESULTS: Serum sMICA levels were significantly higher in NSCLC patients than in healthy controls (mean ± SD [pg/ml], 143.52 ± 27.6 vs. 32.4 ± 7.53 $p < 0.01$) and were significantly correlated with TNM stage, poorer differentiation, lymph node metastases and distant metastases. Survival analysis showed that a low sMICA level had longer survival time than those with high serum sMICA. Multivariate analyses indicated that high sMICA proved to be an independent predictor of survival time.

CONCLUSIONS: Serum sMICA level in NSCLC patients is associated with metastasis. It is an indicator of a poorer survival probability. Serum sMICA levels may be an independent prognostic factor for NSCLC.

Key Words:
Non-small cell carcinoma, Soluble MHC class I-related chain A, Prognosis.

Introduction

Lung cancer is the most frequently diagnosed cancer and the leading cause of cancer-related mortality in the world. Approximately 80% of deaths are non-small cell carcinoma (NSCLC)[1]. At the time of diagnosis, most of the NSCLC patients are at advanced stage, so the prognosis of patients with NSCLC are very poor[2]. Therefore, to identify the potential of established biomarkers that can facilitate a better assessment the outcome and the response to specific therapies is crucial to improve the prognosis of patients with NSCLC. Serum levels of marker proteins, including CEA, NSE, CYFRA21-1, CA19-9, CA15-3, CA242 and CA50 are commonly used to determine the prognosis of NSCLC[3]. Pathologic TNM stage, age, sex, and cell type are important prognostic factors for the patients with NSCLC[4]. However, relying on a single marker as a prognostic indicator for NSCLC diagnosis would be considerable inaccuracy. Therefore, it is necessary to find more accurate serum markers for a reliable prognostic assessment, which would provide highly effective treatment.

The nonclassical major histocompatibility class I molecule A (MICA) is a natural ligand, which could activate receptor natural killer group 2, member D (NKG2D) expressed on the surface of natural killer (NK) cells[5] and many cancer cells such as in lung[6], breast[7], ovary[8], colon[9] and prostate cancer[10]. The binding of MICA and NKG2D could activate NK cells and subsequently cause NK cells to identify and lyse target cells[5]. Thus, the expression level of MICA on the tumor cell surface may determine the antitumor efficacy.

In colorectal cancer, the patients with low levels of MICA have a poor prognosis and may be good candidates for aggressive chemotherapy. In contrast, patients with high expression of MI-

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CA may be candidates for the antibody therapies[11]. In breast cancer, the expression of MICA may be an indicator of poor prognosis and it is indicative of a tumour environment that has undergone stresses such as apoptosis, necrosis, or hypoxia[12]. In lung cancer, the high expression of MICA is one of the indicators of a poor prognosis for advanced non-small cell lung cancer patients and the high expression of MICA might be one of the predictive factors for successful CIK therapy[13].

Recent studies[14] found MICA is also secreted into the serum to generate a soluble form (sMICA). This process leads to a decrease of membrane-bound MICA and to an increase of sMICA, and inhibits the anti-tumor effect of natural killer cells and CD8+ T cells by blocking their action[15,16]. It has found that sMICA level is positive correlated with breast cancer TNM stage[17]. However, sMICA and its prognostic value in NSCLC have not been studied. In the present work, we determined the serum levels of sMICA in patients with NSCLC, and we evaluated its correlation with prognosis; we estimated the value of using serum sMICA level as a prognostic indicator for NSCLC patients, too.

Patients and Methods

Ethics Statements

The study was approved by the Ethics Committee of the People's Hospital of Weifang, Shandong, China and followed the Declaration of Helsinki protocol. All patients were approached based on approved ethical guidelines, agreed to participate in this study, and could refuse entry and discontinue participation at any time. All participants proved written consent.

Patients and Serum Samples

From February 2006 to October 2014, a continuous series of 207 patients with NSCLC and 207 normal control individuals in our hospital had been recruited in this study. Clinical and biological data were prospectively collected. The patients were confirmed pathologically with NSCLC and had no previous history of other cancers. The clinical characteristic of 207 patients with NSCLC was summarized in Table I. Fasting blood was taken for all participants and serum was collected and stored at -80°C. Blood data were collected from the patients at the time of diagnosis, before any kind of treatment.

Table I. Clinicopathologic correlation and serum sMICA level in NSCLC patients.

		Serum sMICA level		
	Cases	High	Low	*p*-value
Factors				
Gender				0.263
Male	149	118	31	
Female	58	38	20	
Age (years)				0.172
≤60	75	53	22	
>60	132	103	29	
Differentiation				0.042
Low	114	97	17	
Moderate/high	93	59	34	
Tumor size				0.084
T1+T2	125	96	29	
T3+T4	82	60	22	
Lymph node metastasis				0.028
Yes	65	31	34	
No	142	125	17	
Distant metastasis				0.01
Yes	34	28	6	
No	173	128	45	
TNM stage				0.036
I+II	125	82	43	
III+IV	82	74	8	
Smoking history				0.145
Yes	134	101	33	
No	73	55	18	

Detection of Serum sMICA by ELISA

Venous blood (3.5 ml) was collected, then clotted, centrifuged and stored at -80°C as a routine. sMICA levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA) as the manufacturer's instructions. The optical density (OD) was measured at 450 nm on the microplate reader (Roche, Nutley, NY, USA), and the sMICA concentration determined. Results are reported as the concentration of sMICA (pg/ml) in the serum sample.

Statistical Analysis

Statistical analyses were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The data were recorded as the means ± SD and analyzed using independent t tests. The sMICA levels results and their associations with clinical characteristics were analyzed using the chi-square test. Survival curves were compared using the Kaplan-Meier method and log-rank tests. Univariate Cox regression was used on each clinical covariate to examine its influence on patient survival. Multivariate models were based on step-wise addition. p values < 0.05 were considered to be significant.

Results

Serum Level of sMICA is Enhanced in Patients with NSCLC

Serum sMICA level was found to be significantly higher in patients with NSCLC than that of controls. Mean serum sMICA level was 143.52 ± 27.6 pg/ml in the NSCLC group and 32.4 ± 7.53 pg/ml in the control group ($p < 0.01$, shown in Figure 1).

Serum sMICA Level and its Relationship with ClinicopathologiCal Variables in NSCLC Patients

In this study, serum sMICA levels were 32.4 ± 7.53 pg/ml in the control group. 39.93 pg/ml was used as the cutoff value. NSCLC patients with serum sMICA levels values ≤ 39.93 pg/ml were assigned to the low level group (n = 51), whereas those with values >39.93 pg/ml were assigned to the high level group (n = 156). As shown in Table I, high serum sMICA level was correlated with differentiation, lymph node metastases, TNM stage, and distant metastases. However, high serum sMICA level was not associated with age, sex, size and smoking history (all $p > 0.05$).

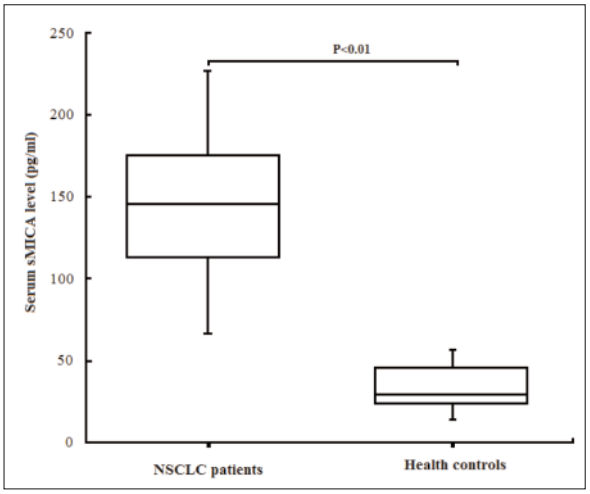

Figure 1. Serum sMICA levels in NSCLC patients and healthy controls. Mean serum sMICA level was 143.52 ± 27.6 pg/ml in the NSCLC group and 32.4 ± 7.53 pg/ml in the control group ($p < 0.01$).

High Serum sMICA Level is Associated with Poor Prognosis for NSCLC

As shown in Figure 2, patients with high serum sMICA level had a significantly lower 5-year OS rate (37.4.0% vs. 61.2%; $p = 0.012$) than those with low serum sMICA level. Univariate analysis showed tumor size, lymph node metastasis, TNM stage and serum sMICA levels were associated with prognosis (Table II). Moreover,

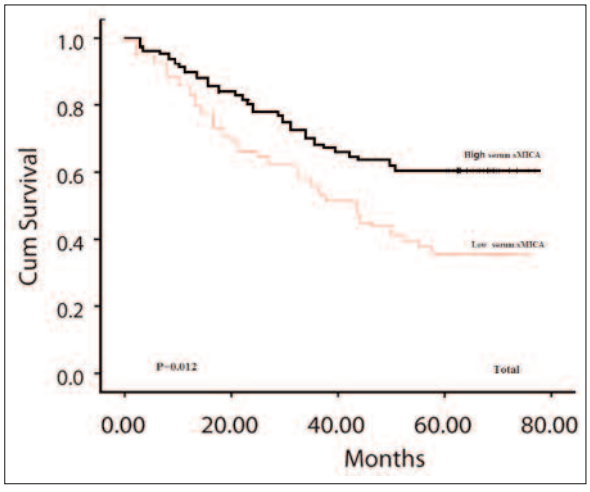

Figure 2. Kaplan-Meier survival curves in relation to serum sMICA level in patients with NSCLC. Survival curves were analyzed by Kaplan-Meier method and log-rank test. Patients with high sMICA levels had a significantly poorer survival than those with low sMICA levels ($p = 0.012$).

Table II. CClinicopathologic correlation and serum sMICA level in NSCLC patients.

	Univariate analysis			Multivariate analysis		
Variables	HR	95% CI	*p*-value	HR	95% CI	*p*-value
sMICA level						
low vs. high	1.893	1.1061-3.724	0.038	2.386	1.304-4.982	0.002
Age						
60 vs. <60	1.174	0.653-2.072	0.512	1.174	0.642-2.174	0.528
Sex						
male vs. female	0.731	0.40-1.332	0.547	0.782	0.402-1.214	0.430
Differentiation						
Low vs. Moderate/high	1.765	1.164-2.843	0.02	1.920	1.203-2.962	0.018
Tumor size						
T1+T2 vs. T3+T4	2.137	1.402-3.421	0.015	1.984	1.120-2.673	0.043
Lymph node metastasis	3.106	1.953-4.756	0.002	2.282	1.543-5.02	0.01
Yes vs. No						
Distant metastasis						
Yes vs. No	1.307	0.625-2.382	0.52	1.314	0.637-2.386	0.481
TNM stage						
I-II vs. III-IV	2.148	1.453-3.269	0.003	1.02	0.560-1.838	0.763
Smoking history						
Yes vs. No	1.296	0.745-2.384	0.336	0.903	0.430-2.651	0.542

multivariate Cox regression analysis revealed that tumor size, lymph node metastasis and serum sMICA level were independent predictors of NSCLC prognosis (Table II).

Discussion

Up to data, the best prognostic system for monitoring NSCLC is still the TNM staging system. However, relying on the single marker as a prognostic indicator for NSCLC would be very inaccuracy. Identification of novel molecular markers which can improve diagnosis and prognostic stratification will be of great importance in the near future.

Circulating biomarkers are soluble molecules released into the blood stream by different cell types[18]. The plasma samples are easily obtainable; therefore, measurement of these molecules is an economic and non-invasive diagnostic method which will be able to give information about the disease. A number of novel markers for lung cancer have been identified in recent years, such as CEA, CA19-9 and CA15-3[3]; however, because of their relatively low sensitivity and specificity, they are not satisfactory for diagnosis of the disease.

It has found that high serum sMICA levels in HCC patients were significantly related with poor prognosis and sMICA was an independent prognostic factor. In addition, sMICA level was negatively correlated with the level of $NKG2D^+$ NK cells[19]. In oral squamous cell carcinoma (OSCC), patients with stage IV disease and/or regional lymph node metastasis exhibited significantly higher serum levels of sMICA than control individuals. Overall survival rates were significantly higher for OSCC patients with low sMICA levels. Therefore, serum levels of sMICA may be useful in the diagnosis of advanced stage OSCC and as an indicator of regional lymph node metastasis[20]. sMICA may be also as a potent prognostic marker in multiple myeloma (MM)[21] and cervical adenocarcinoma[22], which may be useful to identify risk patients.

In the present study, we found that serum sMICA level was found to be significantly higher in patients with NSCLC, and high serum sMICA level was correlated with differentiation, lymph node metastases, TNM stage, and distant metastases. Kaplan-Meier method found that patients with high serum sMICA level had a significantly lower 5-year OS rate. Univariate analysis showed tumor size, lymph node metastasis, TNM stage and serum sMICA levels were associated with prognosis. Multivariate Cox regression analysis revealed that tumor size, lymph node metastasis and serum sMICA level were independent predictors of NSCLC prognosis.

Conclusions

Serum sMICA level in NSCLC is significantly higher, and high sMICA level was associated with poor prognosis in patients with NSCLC. The data suggests that serum sMICA level may be an useful molecular markers in patients with NSCLC.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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