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Original Article

HPSE expression correlates with invasive behaviors in lung cancer

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Abstract: Objective: Heparanase (HPSE), the only enzyme which can degrade the extracellular matrix and heparin sulfate in basement membrane, plays an important role in invasion and metastasis of tumor cells. The aim of this study is to investigate the expression of HPSE protein in lung cancer and its association with clinicopathological parameters and prognosis. Materials and methods: Expression of HPSE protein was detected immunohistochemically in paraffin-embedded specimens of 65 cases of lung cancer. Relationship between HPSE protein expression and clinicopathological parameters, prognosis was analyzed. Results: HPSE protein expressed positively in lung cancer (33/65 cases, 50.8%) compared with the adjacent normal tissue samples (6/65 cases, 9.2%) and normal lung tissue (3/65 cases, 4.6%) (P<0.05). There was no difference between adjacent lung tissue and normal lung tissue (P>0.05). HPSE protein expression did not correlate with the pathological type and grade of the tumor (P>0.05), but it correlated with the clinical stage and survival time of the patients (P<0.05). Survival in patients with high expression was different from those with low expression (P<0.05). Conclusion: HPSE is a reliable prognostic factor for this malignancy and an attractive target for anticancer drug development.

Keywords: Heparanase, lung cancer, invasive behaviors, prognosis

Introduction

The incidence of lung cancer is high, and the fatality rate is even higher due to the general ineffectiveness of conventional surgery and chemotherapy. Approximately 80% of lung cancer patients are inoperable at diagnosis, and normal chemotherapy is unable to effectively prevent the growth of tumor [1]. Multidisciplinary treatment of lung cancer has reached a new level, but long-term treatment is still unsatisfactory. Tumor invasion and metastasis, as the most essential biological characteristics, are related directly to poor prognosis and mortality of patients. HSPE can specifically degrade the extracellular matrix and heparin sulfate proteoglycans of basement membrane, and promote invasion and metastasis directly and indirectly [2]. Early treatment targeting lung cancer might be important for improving patient survival. The precise mechanisms in lung cancer should be further clarified.

HPSE is an endo-β-D-glucuronidase [3] involved in the degradation of cell surface (extracellular matrix and Heparan sulfate proteoglycans) of a wide range of normal and neoplastic tissues [4] and a molecular determinant of metastatic events. Many literatures have shown that there was high expression of HPSE in patients with ovarian cancer [5], nasopharyngeal carcinoma [6], oral carcinoma [7], hepatocellular carcinoma [8], breast cance [9] and pancreatic cancer [10]. The most important was that the HPSE expression and patients survival time was negatively correlated.

We demonstrated previously that low expression of HPSE protein and mRNA in vitro after successful transfection of HPSE antisense oligoxydeonucleotide (ASODN), and invasive ability of A549 cells was significantly reduced by inhibiting HPSE expression using liposome-mediated ASODN gene delivery strategy. The aim of the present study is to explore the role of HPSE protein in lung cancer development and prognosis. For this purpose, HPSE protein expression was detected in lung cancer and normal lung tissues by immunohistochemistry. Additionally, the correlation of HPSE protein

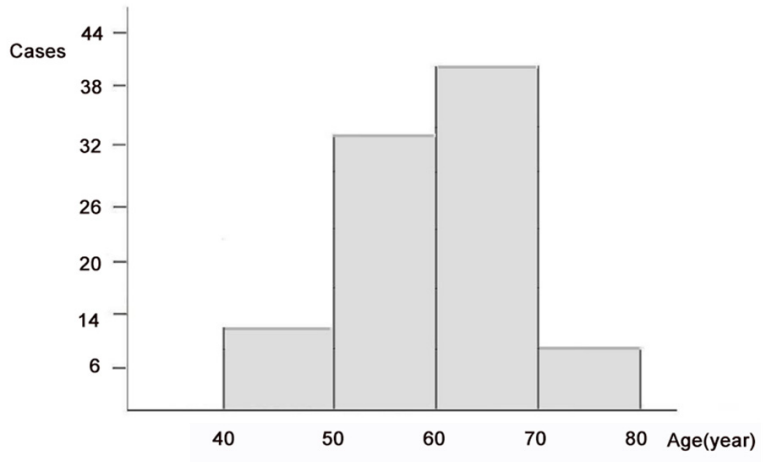

Figure 1. Age distribution in all patients.

expression with the clinicopathological data and prognostic variables was analyzed.

Materials and methods

Patients and tissue samples

65 cases who were surgically resected with lymph node dissection for lung carcinoma in the Second Hospital of Shandong University between 2003 and 2008 were included in the study. Prior to surgery, none of the patients underwent comprehensive therapy, such as radiotherapy, chemotherapy, and biological therapy. All cases were confirmed by pathological diagnosis after surgery. Clinical data were complete for all patients, and all subjects signed an informed consent form. Samples were provided as formalin-fixed and paraffin-embedded tissue specimens, and tumor nearby normal lung tissues and normal lung tissues were also included. The patients included 51 males and 14 females with a mean age of 61 years (range 41-76 years). **Figure 1** show the age distribution. The pathological types were 31 squamous cell carcinomas, 25 adenocarcinomas, 3 large cell carcinoma and 6 small cell carcinoma (well differentiated in 17, moderately in 23, poorly in 16 and undifferentiated in 9). The pathological stages were evaluated as stage I: 12, II: 31, III: 18 and IV: 4. With no hospital death, 65 patients were followed up to December 2012. 4 cases were lost, and the follow-up rate was 93.8%.

Immunohistochemistry

The expression of HPSE was detected by streptavidin-peroxidase-biotin (SP) immunohistochemical method according to the manufacturer's instructions. In brief, paraffin-embedded specimens were cut into 4 μm sections and baked at 60°C for 60 min. The sections were deparaffinized with xylenes and rehydrated. Then sections were submerged into EDTA antigenic retrieval buffer in a pressure cooker for 10 minutes and then cooled at room temperature for 20 minutes. The sections were treated with 3% hydrogen peroxide in methanol to quench the endogenous peroxidase activity, followed by incubation with normal serum to block nonspecific binding. The sections were incubated with HPSE monoclonal antibody (1:200) overnight at 4°C. After washing, the tissue sections were incubated with biotinylated secondary antibody for 1 h at room temperature, followed by incubation with streptavidin-horseradish peroxidase for 20 minutes. After washing with PBS, diaminobenzidine (DAB) was added for visualization. The sections were counterstained with haematoxylin.

Evaluation of immunohistochemical staining

The criteria for interpreting the results of immune staining were based on the literature [11]: HPSE expression was scored positive based on the presence of brownish-yellow precipitates in the cytoplasm; two experienced pathologists interpreted the results using a double-blind method; each section was examined at high magnification (400×) to count cells in 15 fields of view; the ratio of positive cells was calculated from the mean number of HPSE-positive cells in 100 cells; the mean value from 15 fields of view was taken as the final result of each pathological section; four section specimens were selected from each patient, and the mean value of the four sections was taken as the final result of the patiet. Specimens were regarded as HPSE negative if <5% of the cells were stained, 5%-25% as '+'. 25%-50% as '++', and > 50% as '+++' according to many previous reports [12, 13]. The stained slides were reviewed and scored independently by two observers blinded to the patients' information.

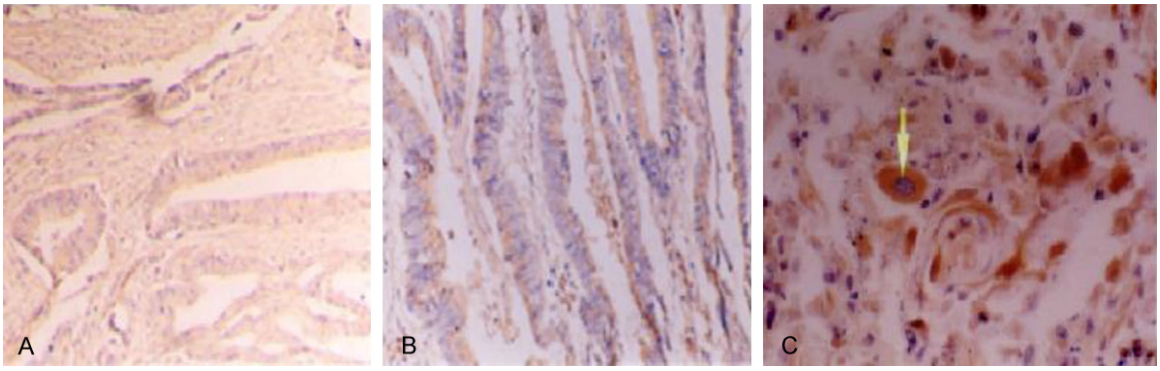

Figure 2. HPSE protein expression in lung cancer cell. A: HPSE-negative in lung adenocarcinoma (-, ×10); B: HPSE-positive in lung adenocarcinoma (++, ×10); C: HPSE-positive in lung squamous cell carcinoma (+++, ×400).

Table 1. Data analysis of HPSE protein expression in different tissues

	N	HPSE expression			Positive rate (%)	*P*
		+	++	+++		
Lung cancer	65	11	12	10	50.8	
Adjacent normal tissue	65	3	2	1	9.2	<0.05[(a)]
Normal lung tissue	65	2	1	0	4.6	<0.05[(b)]

a: lung cancer compared with adjacent normal tissue; b: lung cancer compared with normal tissue.

Statistical analysis

Analysis of variance and t-test were used for measurement data and multiple linear regression analysis for relationship between multiple measurement data. Correlations between the HPSE expression and clinical characteristics were assessed by means of chi-square test or Fisher's exact probability test. The survival time of patients with different HPSE expression was estimated by Kaplan-Meier method with log-rank test for statistical significance.The statistical package SPSS Version 12.0 was applied to complete data processing, values of $P<0.05$ were considered statistically.

Results

HPSE protein expression in tissues

Positive expression of HPSE reflected by the presence of brownish-yellow granules, mainly distributed in the cytoplasm (**Figure 2**). By comparison, the positive expression of HPSE was significantly increased in tumor tissue. Statistical analysis showed that the positive expression ratios of HPSE were 50.8% in lung cancer compared with 9.2% in the adjacent normal tissue samples and 4.6% in normal lung tissue ($P<0.05$), while there was no difference between adjacent lung tissue and normal lung tissue ($P>0.05$) (**Table 1**).

Correlation between HPSE expression and clinical features of lung cancer

It was shown that HPSE protein expression did not correlate with the pathological type and grade of the tumor ($P>0.05$), but it correlated with the clinical stage and survival time of the patients ($P<0.05$). HPSE protein expression were significantly lower in stage I and II than that in stage III and IV. Expression rate was lower in patients with more than 3 years survival time than those with less than 3 years. Survival in patients with high expression of HPSE protein was different from those with low expression ($P<0.05$) (**Table 2**).

Correlation between HPSE expression and survival

Using Kaplan-Meier analysis we found that postoperative survival in low HPSE protein expression was significantly longer than that of high expression (**Figure 3**).

Discussion

Since Ogren and Lindahl first reported HPSE in mouse mast cells and demonstrated its digestive function on macromolecular heparin at specific sites [14], HPSE has been reported to be widely expressed in cells of normal tissues and malignant tumors. The gene sequences of the human HPSE gene were first determined in 1999, and the molecular structure, synthesis and action mechanism of HPSE were further studied [15, 16]. HPSE is the only known human

Table 2. Correlation between HPSE expression and clinicopathological grading of lung cancer

Pathological type	N	HPSE protein expression		P
		Positive cases	Negative cases	
Squamous cell carcinoma	31	15	16	>0.05
Adenocarcinoma	25	13	12	
Large cell carcinoma	3	2	1	
Small cell carcinoma	6	3	3	
Differentiation				
Well-differentiated	17	8	9	>0.05
Moderately differentiated	23	11	12	
Poorly differentiated	16	9	7	
Undifferentiated	9	5	4	
Clinical stage				<0.05
I	12	4	8	
II	31	8	23	
III	18	17	1	
IV	4	4	0	
Survival time after <0.05				
Operation (year)				<0.05
<3	25	22	3	
>3	36	10	26	

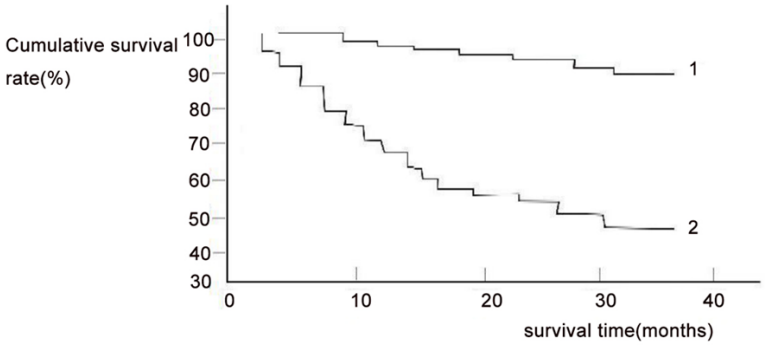

Figure 3. Kaplan-Meier survival curves of Heparanase expression. Log-rank timing test χ^2=8.42 *P*=0.0044. 1: low expression, 2: high expression.

endoglycosidase that plays an irreplaceable role in physiological and pathological processes. It has been a subject of intense research in molecular biology [17]. Heparan sulfate proteoglycans are essential and ubiquitous macromolecules associated with the cell surface and extracellular matrix of a wide range of cells and tissues. HPSE is an extracellular matrix degradative enzyme, which degrades the heparan sulfate (HS) chains of Heparan sulfate proteoglycans at specific intrachain sites. The enzymatic activity of HPSE is characterized by specific intrachain heparan sulfate cleavage of glycosidic bonds with a hydrolase (but not eliminase) type of action, therefore facilitating the release of several protein modulators of cell function, including migration, adhesion, inflammation, angiogenesis, embryogenesis, and metastatic invasion [18].

In the present study, we examined the HPSE expression in paraffin-embedded tumor samples using the immunohistochemical method with the monoclonal antibody. Our results showed that HPSE is in the cytoplasm of cells. Positive HPSE expression in the lung cancer tissues was detected in 33 cases of the patients. Expression level of HPSE was significantly increased in the lung cancer compared with the normal lung cell. These results suggest HPSE might be a biological indicator of malignant potential of lung cancer. Furthermore, HPSE expression level is correlated with clinical stage and survival time, which suggests that HPSE may have some correlation with worse biological behavior and clinical aggressiveness of lung cancer.

In our previous study, we transfected HPSE ASODN into A549 cell lines and examined its invasive ability and expression of HPSE. We found that HPSE ASODN could downregulate the expression of HPSE protein and mRNA in the A549 cell line and could obviously inhibit its invasive ability in a dose-dependent manner in vitro. In this study, we found the same results, they both indicated that HPSE involved in lung cancer cell invasion and migration and played an important role. These studies led us to hypothesize that inhibition of HPSE expression could inhibit tumor cell invasion and prolong patients' survival time.

In summary, High levels of HPSE protein are expressed in most malignant tumors including

lung cancer and are closely associated with tumor metastasis, angiogenesis and other diverse pathological and physiological processes [9, 19]. The specific mechanism of participation of HPSE in invasion and prognosis of lung cancer is worthy of further investigation. This is likely to contribute to an in-depth understanding of mechanism, as well as create conditions for exploring more effective clinical treatment methods.

Disclosure of conflict of interest

None.

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