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Original Article

Significance of plasma hepatocyte growth factor in diagnosis of benign and malignant solitary pulmonary nodules

Haixin Yu[1], Yan Wang[1], Qian Yu[2], Haiyan Zhang[2], Wenduan Ma[1], Shengtao Shang[1], Donglin Wang[3]

[1]Department of Thoracic Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, China; [2]Department of Pharmacy, China-Japan Union Hospital of Jilin University, Changchun 130033, China; [3]Green Garden District Changchun City Hospital, No. 1015, Chuncheng Avenue, Changchun 130062, China

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Abstract: Purpose: We aimed to figure out the difference of serum hepatocyte growth factor (S-HGF) level between benign and malignant solitary pulmonary nodules (SPNs) patients. Methods: The study comprised 42 serum samples from SPNs patients and 10 serum samples of healthy donors. The HGF level was measured by the commercially enzyme-linked immunosorbent assay (ELISA) kit. Results: By statistical analysis, the S-HGF levels of the malignant SPNs patients were significantly higher than that of control group ($P < 0.05$). Moreover, the levels of S-HGF in malignant group were also significantly higher than that in benign group ($P < 0.05$), while there was no significant difference between the benign and control group ($P > 0.05$). The levels of S-HGF were also shown no statistically significant difference ($P > 0.05$) in different pathologic types of lung cancer patients. In addition, the incidence of malignant SPNs increased when the S-HGF level ≥ 250 pg/ml. Conclusion: The detection of S-HGF level may be a new detection method used for the rapid diagnosis of benign and malignant SPNs.

Keywords: Diagnosis, hepatocyte growth factor, solitary pulmonary nodules

Introduction

The solitary pulmonary nodules (SPNs) are spheroid parenchymal lung lesions less than or equal to 3 cm in diameter that are surrounded by lung parenchyma [1]. In the general population, it has been reported that approximately 5% of SPNs patients will deteriorate into lung cancer [2], which is considered one of the most common forms of cancer with a high death incidence ratio in the world [3]. So effective diagnosis of SPNs contributes to the early therapy of lung cancer [4]. However, diagnosis of benign and malignant SPNs is still a challenge for clinicians in recent decades [5, 6].

With the development of modern medical science and technology, several detection and monitoring methods have been used in screening the SPNs and lung cancer [7-9]. For example, Momen et al. [10] applied three main detection methods (positron emission tomography imaging, dynamic computed tomography (CT) and CT-guided needle biopsy) to identify SPNs, and found these methods could be used for the diagnosis of SPNs. However, all these assay methods need the help of instruments which lead to high cost and time consuming. Therefore, it is still necessary to find a more convenient detection method for SPNs.

Serum-hepatocyte growth factor (S-HGF, Serum-HGF) is an important fibroblast-secreted protein that mediates development and progression of cancers [11]. Early in 1984, hepatocyte growth factor (HGF) from the serum of hepatectomized rats has been partially purified and described by Nakamura [12]. HGF receptor encoded by the *c-met* proto-oncogene is a member of the cell surface receptors. As a kind of cytokine, the HGF possess widely biological activities, including regeneration, antifibrosis, cytoprotection, and differentiation [13]. Moreover, HGF is a predominant fibroblast-derived factor that stimulates the invasion and metastasis of human carcinoma cells [14]. Telega et al. have reported that HGF may be useful in the clinical diagnosis of bronchopul-

Table 1. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in healthy control group, benign and malignant solitary pulmonary nodules (SPNs) groups

Groups	Number of cases	ALT (IU/L)	AST (IU/L)
Healthy control group	10	21.6 (11.1~37.8)	21.2 (17.3~33.6)
Benign SPNs group	12	28.8 (16.8~36.5)	26.6 (18.3~31.3)
Malignant SPNs group	30	24.2 (16.1~38.1)	25.6 (13.6~38.6)

Table 2. Serum hepatocyte growth factor levels (pg/ml) of healthy control group, benign and malignant solitary pulmonary nodules groups

Groups	Number of cases	S-HGF (x ± s)	Median
Healthy control group	10	185.00 ± 75.02	180 (100~300)
Benign SPNs group	12	197.50 ± 101.19	165 (100~400)
Malignant SPNs group	30	467.67 ± 424.25	395 (100~1550)

monary carcinoids [15]. Resent researches showed that HGF level was closely associated with lung cancer [16-18]. Since malignant SPNs are likely to represent the early form of lung cancer, we give the hypothesis that S-HGF level may be a potential target in diagnosis of benign and malignant SPNs. In present study, we detected and analyzed the S-HGF levels of different serum samples from healthy controls, benign and malignant SPNs groups to investigate a new diagnostic method for SPNs.

Subjects and methods

Patients

This study was approved by the institutional research ethics committee of China-Japan Union Hospital, and the protocol number was 20141103. All the 42 patients participated in this study were consecutively treated in the Thoracic Surgery Department of China-Japan Union Hospital. All of them signed the written informed consent after introducing our study. The diagnosis of SPNs was based on CT scan of the chest. The diagnosis was further confirmed by a serious of routine inspection, including tracheoscopy, electrocardiography, blood biochemistry and blood routine examination. In addition, 10 healthy adults were chosen as control and signed the written informed consent as well. The inclusion criteria of SPNs patients are as follows: (1) Spheroid lung lesions ≤ 3 cm in diameter in the lung parenchyma; (2) Patients without hilar lymphadenopathy, atelectasis, pneumonia or chest wall lesions; (3) Patients are irrespective of age and gender. Considering some influencing factors, exclusion criteria are as follows: (1) SPNs patients undergo inflammation or infection within a month. (2) SPNs patients undergo surgery or trauma within 6 months. (3) SPNs patients with various liver diseases. (4) SPNs patients with chronic renal failure, arteriosclerosis, rheumatoid arthritis, osteoarthritis and diabetes mellitus. Furthermore, the liver enzyme levels of patients and healthy donors are in the normal range, as shown in **Table 1**.

Specimen collection

All the patients did not receive neoadjuvant therapies before sample collection. The fasting venous blood samples of 42 patients were obtained in the morning before operation. The venous blood samples of healthy adults were also collected after informed consent was obtained. Then we used sterile polypropylene tubes containing ethylenediaminetetraacetic acid (EDTA) to collect the blood samples, and the samples were centrifuged at 400 rpm for 10 min. At last, the plasma was stored at -70°C until the assays were performed.

Assay for S-HGF

We used sandwich enzyme-linked immunosorbent assay (ELISA) to measure S-HGF. The HGF monoclonal antibody and standard substance for the assays were purchased from American R&D systems. Goat-anti-human HGF polyclonal antibody was used as the primary antibody, while donkey-anti-goat IgG polyclonal antibody labeled with horseradish peroxidase was served as the secondary antibody. Both of the antibodies were purchased from Abcam (Cambridge, MA, USA).

Statistical analysis

Because the measured data were manifested as skewed distribution, geometrical mean G (logG ± s) was calculated in each group after logarithmic transformation. Then t test (two-sides) was performed using SPSS 19.0 statisti-

Table 3. Comparison of serum hepatocyte growth factor levels of each group after logarithmic transformation had been carried out on each datum

Groups	Number of cases	S-HGF (pg/ml)	
		G (logG ± s)	95% CI
Healthy control group	10	185.00 (2.24 ± 0.17)	131.33~238.67
Benign SPNs group	12	197.50 (2.25 ± 0.20)[a]	133.21~261.79
Malignant SPNs group	30	467.67 (2.48 ± 0.43)[b,c]	309.25~626.09

[a]Benign SPNs group vs healthy control group, $P > 0.05$. [b]Malignant SPNs group vs healthy control group, $P < 0.05$. [c]Malignant SPNs group vs benign SPNs group, $P < 0.05$.

Table 4. Comparison of serum hepatocyte growth factor levels of adenocarcinoma and squamous carcinoma

Pathological types	Number of cases	S-HGF [G (logG ± s), pg/ml]
Adenocarcinoma	17	415.29 (2.43 ± 0.43)
Squamous carcinoma	13	536.15 (2.54 ± 0.45)[a]

[a]Squamous cell carcinoma vs adenocarcinoma, $P > 0.05$.

cal software. $P < 0.05$ was considered statistically significant.

Results

All the 42 patients with SPNs obtained pathological diagnosis after operation. The results showed that 12 cases were benign nodules and 30 cases were malignant nodules (17 were adenocarcinoma, 13 were squamous carcinoma).

The S-HGF levels of the healthy control, benign and malignant SPNs groups were shown in **Table 2**. The median level of S-HGF was 180 (from 100 to 300) pg/ml in the healthy control group, 165 (from 100 to 400) pg/ml in benign SPNs group while 395 (from 100 to 1550) pg/ml in malignant SPNs group. The data with geometrical mean G (logG ± s) was shown in **Table 3**. The results showed that there was no significant difference between benign SPNs group and healthy control group ($P > 0.05$). The S-HGF levels of malignant SPNs group were significantly higher than that of healthy control group ($P < 0.05$) and benign SPNs group ($P < 0.05$). In addition, the S-HGF levels of patients with squamous carcinoma and adenocarcinoma were shown in **Table 4**. We easily found that the S-HGF levels of squamous carcinoma were higher than that of adenocarcinoma, but no significant difference was observed ($P > 0.05$).

Further analysis of 20 patients with high levels of S-HGF (≥ 250 pg/ml) indicated that there were 3 patients (15%) with benign SPNs and 17 patients (85%) with malignant SPNs (**Table 5**). Only 1 patient (6.25%) was diagnosed with benign SPNs when the levels of S-HGF ≥ 400 pg/ml (**Table 5**).

Discussion

In order to find a more convenient method for the diagnosis of benign and malignant SPNs, the S-HGF levels were used to diagnose this disease for the first time. In our study, the results showed that S-HGF levels of malignant SPNs group were significantly higher than that of benign SPNs group ($P < 0.05$) and healthy control ($P < 0.05$). Nevertheless, the difference between benign SPNs group and healthy control was not significant ($P > 0.05$). Further analysis indicated that more than 85% of pathological types were malignant SPNs when the S-HGF level ≥ 250 pg/ml.

Since the S-HGF levels were the crucial parameters in this study and a number of factors affected the S-HGF levels, inclusion and exclusion criteria of patients was necessary. The SPNs patients participated in this study should exclude the following situations. The S-HGF levels in patients with acute hepatitis, chronic hepatitis and cirrhosis were found to be slightly higher than those in normal subjects [19]. So the patients with various liver and gall diseases were first excluded. Some studies showed that the S-HGF levels were significantly increased in patients underwent inflammation, infection, surgery and traumas. Therefore, the patients suffered inflammation or infection within a month and the patients underwent surgery or traumas within 6 months were also excluded. Johanna et al. [20] had concluded that patients with chronic renal failure (CRF) have a systemic HGF profile reflecting a chronic inflammatory condition with high concentration of HGF. Furthermore, the S-HGF levels in patients with arteriosclerosis [21], rheumatoid arthritis [22], osteoarthritis [23], and diabetes mellitus [24] were reported to be significantly higher than that in healthy population. Thus, the patients with these diseases must be excluded as well.

Table 5. Analysis of patients with high levels of S-HGF

Pathological types	Number of cases	
	S-HGF ≥ 250 pg/ml	S-HGF ≥ 400 pg/ml
Benign SPNs	3 (15%)	1 (6.25%)
Malignant SPNs	17 (85%)	15 (93.75%)
In total	20	16

SPNs are extremely common in clinical practice and difficult to manage. In addition, malignant SPNs represent a potentially curable form of lung cancer [25, 26]. Therefore, a convenient detection method of SPNs is crucial. Tsao et al. [27] showed that the HGF messenger RNA (mRNA) and protein were predominantly expressed in the cells of non-small cell lung cancer (NSCLC). Furthermore, Nagio et al. [28] gave the evidence that the S-HGF levels of patients with small cell lung cancer (SCLC) were significantly higher than those of patients with benign SPNs and healthy subjects. A recent study indicated that HGF-positive serum was predictive of a negative response to gefitinib therapy in patients with advanced NSCLC [29]. Our study showed that the healthy control group contained low concentration of S-HGF, and the S-HGF levels of the patients with benign SPNs were almost the same as control group ($P > 0.05$). However, the S-HGF levels of the patients with malignant SPNs were significantly higher than that of healthy control group ($P < 0.05$) and benign SPNs group ($P < 0.05$). These results demonstrated that high levels of S-HGF might be closely associated with malignant SPNs and lung cancer. Ujiie et al [17] also proved that the levels of HGF in serum could be used as prognostic indicators of stage III NSCLC. However, the difference between squamous carcinoma and adenocarcinoma was not significant ($P > 0.05$). It means that S-HGF may be useless for identifying different pathologic types of lung cancer.

Further analysis of patients with high S-HGF levels revealed that 85% patients of SPNs were malignant SPNs. The incidence of malignant SPNs was over 90% when the S-HGF level ≥ 400 pg/ml. These results also indicated that high S-HGF levels might be closely related to malignant SPNs.

In conclusion, the detection of serum HGF level was meaningful for the diagnosis of benign and malignant SPNs. The results in present study showed that the high S-HGFs levels might be a useful indicator for the diagnosis and prognosis of malignant SPNs. The operative treatment could be recommended when the S-HGFs levels were over 250 pg/ml. However, there are some limitations in this study. First, the samples used in this study was relatively less, a larger sample size was needed for further validation. Second, the serum HGF levels were only detected by ELISA, further studies were needed to demonstrate the function of serum HGF in malignant SPNs.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yan Wang, Department of Thoracic Surgery, China-Japan Union Hospital of Jilin University, No. 126, Xiantai Avenue, Changchun 130033, China. Tel: +86-13944161699; E-mail: ougan888@hotmail.com

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