J Korean Med Sci 1999; 14: 401-4
ISSN 1011-8934

Serum Insulin-like Growth Factor (IGF)-I and IGF-Binding Proteins in Lung Cancer Patients

Many studies have shown that insulin-like growth factors (IGF-I & IGF-II) are implicated in the autocrine and paracrine growth of various tumors. Alterations in serum IGFs and IGF-binding proteins (IGFBPs) profiles have been reported in lung cancer. In this study, we measured serum levels of IGF-I and IGFBPs in 41 patients with lung cancer (small cell lung cancer, SCLC, 9; non-small cell lung cancer, NSCLC, 32) by radioimmunoassay and Western ligand blot (WLB). The serum IGF-I level in patients with lung cancer was significantly lower than in controls (207.9±62.6 vs 281.3±53.9 ng/mL, $p<0.01$). Patients with NSCLC showed significantly lower serum levels of IGF-I compared with SCLC patients (194.0±62.9 vs 258.4±27.8 ng/mL, $p<0.01$). Patients with squamous cell carcinoma tended to show lower serum levels of IGF-I than in those with adenocarcinoma (187.9±63.6 vs 215.9±59.5 ng/mL, $p>0.05$). The concentration of IGFBP-3 in lung cancer was 48% of that found in controls by WLB. The serum level of IGFBP-2 was markedly elevated in patients with lung cancer compared with controls (1303.7±618.0 vs 696.2±300.5, $p<0.01$). However, there was no significant difference between SCLC and NSCLC groups. This result showed that serum level of IGF-I/IGFBPs may be useful markers for diagnosing and identifying tumor types in lung cancer and further studies are needed.

Key Words: *Serum; Insulin-like growth factor-I; Insulin-like growth factor binding proteins; Lung neoplasms*

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Received: 24 December 1998
Accepted: 24 February 1999

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* This work was supported by a grant from Chonbuk National University Hospital (1997).

INTRODUCTION

The insulin-like growth factors, IGF-I and -II, are important mitogens of normal and malignant cells. They regulate cellular proliferation in an autocrine/paracrine way (1, 2). In the serum of normal adults, most IGFs are bound in a 150 kDa complex of IGF-I or -II, acid labile subunit (ALS) and IGF-binding protein (IGFBP)-3 (3).

A wide variety of human tumors have been characterized as expressing unusually large amount of IGF mRNA or protein and IGFBPs (4, 5). Several reports have been shown that human lung tumor cell lines secrete IGFs and IGFBPs as well as the alteration of serum IGFs and IGFBPs levels in human lung cancer (6-8).

In this study, we measured serum IGF-I, IGFBP-2 and -3 to determine if these indices are useful in the diagnosis and identification of tumor types in lung cancer.

MATERIALS AND METHODS

Human subjects

A total of 41 patients were enrolled in this study. There were 31 males and ten females, ranging from 42-74 years old. Nine patients had histologically confirmed small cell lung cancer (SCLC), and 32 had non-small cell lung cancer (NSCLC). The NSCLC group comprised 25 squamous cell carcinoma and seven adenocarcinoma. There was no significant demographic differences between SCLC and NSCLC groups. The control groups comprised age and sex-matched 20 healthy individuals.

This study was approved by the Ethics Committee of the Chonbuk National University Hospital.

Samples

Blood samples were obtained from 41 newly diagnosed untreated patients with lung cancer and 20 normal controls. Aliquots of sera were stored at -70℃ until used.

Peptide and anti-sera

Recombinant human IGF-I was purchased from Bachem (Torance, CA, USA). IGF-I was iodinated by modifying chloramine T method to a specific activity of

150-300 μCi/μg. Polyclonal anti-IGF-I antiserum, a generous gift of Drs. L E Underwood and J J Van Wyk (University of North Carolina at Chapel Hill) was distributed through the Hormone Distribution Program of the NIDDK to the National Hormone and Pituitary Program.

IGF-I radioimmunoassay (RIA)

To separate IGF peptides from their binding proteins, 500 μL of serum was eluted with 0.1% formic acid on 1.0×100 cm long Sephadex G-50 fine gel filtration. The fractions eluting between 50 and 70 mL, which contain 90% of the IGF peptide activity, were collected in glass tubes containing 1.0 mL of 1% BSA, lyophilized and reconstituted in 1 mL RIA buffer. Serum IGF-I concentration was determined by RIA using ^{125}I-IGF-I and a polyclonal antisomatomedin C antiserum. The inter- and intra-assay coefficients of variation were 7 and 5%, respectively, for the IGF-I RIA.

Western ligand blot (WLB)

Three μL of serum was electrophoresed on 10% SDS-PAGE under nonreducing conditions. The resolved proteins were electroblotted onto nitrocellulose, incubated with 2.0×10^6 cpm of ^{125}I-IGF-I for 48 hr and exposed to x-ray films for seven days (9).

IGFBP-2 RIA

Serum IGFBP-2 concentration was determined by RIA, using IGFBP-2 RIA kit (Diagnostic System Laboratories, Webster, Texas, U.S.A.). Samples were diluted 1:100 with RIA buffer. The specific and non-specific binding of radiolabeled IGFBP-3 were 32.9 and 2.8%, respectively.

Statistics

Values are expressed as mean±SD. Student's t-test was used for statistical analyses of serum IGF-I, IGFBP-2 and -3 values.

RESULTS

The serum concentration of IGF-I in lung cancer, as determined by RIA, was significantly lower (207.9±62.6 ng/mL, $p<0.01$) than that in normal controls (281.3±53.9 ng/mL, Table 1). Patients with NSCLC showed significantly lower level of serum IGF-I compared with SCLC patients (194.0±62.9 vs 258.4±27.8 ng/mL, $p<0.01$). Furthermore, patients with squamous cell carcinoma tended to show lower serum levels of IGF-I than these in patients with adenocarcinoma (187.9±63.6 vs 215.9±59.5 ng/mL, $p>0.05$).

The serum profiles of IGFBPs in lung cancer were significantly different from 20 normal controls. A western ligand blot (WLB) of sera from 41 patients with lung cancer and normal controls were performed. The typical finding of 13 patient samples was provided in Fig. 1. Pooled control serum (lane 1) has two major bands,

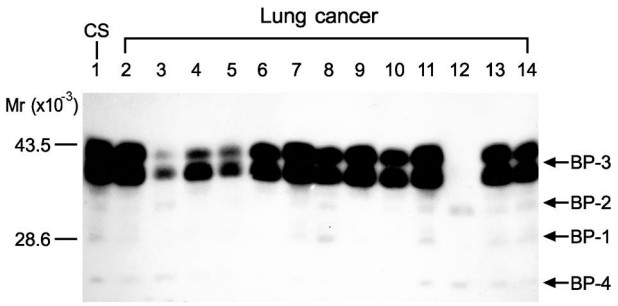

Fig. 1. Serum IGFBP pattern in 13 patients with lung cancer by western ligand blot. Lane 1, pooled control serum; lanes 2-14, sera from individual lung cancer patient. Arrows indicate IGFBP-1, -2, -3 and 4. A representative gel from one of 3 experiments is shown.

Table 1. Serum IGF-I, IGFBP-2 and -3 concentrations in lung cancer patients

	Normal controls (n=20)	Lung cancer			
		Total (n=41)	SCLC (n=9)	NSCLC	
				Squamous (n=25)	Adeno (n=7)
IGF-I (ng/mL)	281.3±53.9	207.9±62.9*	258.4±27.8	187.9±63.6†	215.9±59.5
IGFBP-2 (ng/mL)	696.2±200.5	1303.7±518.0*	1125.7±358.6	1275.4±453.5	1638.8±825.1
IGFBP-3 (ADU)	15.43±2.15	7.43±5.42†	7.43±5.42	7.65±5.09	7.21±5.97

ADU, arbitrary densitometric unit; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; Squamous, squamous cell carcinoma; Adeno, Adenocarcinoma

Values are expressed by mean ± standard deviation.

*$p<0.01$, †$p<0.05$ compared with normal controls, †$p<0.01$ compared with SCLC.

corresponding to the glycosylated form of IGFBP-3, with molecular weights (MW) ranging 37-43 kDa. Other bands were seen in control serum with MW of 31, 28 and 24 kDa, corresponding to IGFBP-2, -1 and -4, respectively. The IGFBP-3 band on autoradiograph of the blot was quantitated by scanning with a LKB Ultra-Scan XL densitometer. The results in arbitrary densitometry unit are shown in Table 1. In lung cancer sera, IGFBP-3 was 48% of that found in controls (7.4±5.4 vs 15.4± 2.2 ADU, $p<0.05$). The serum concentration of IGFBP-2, as determined by RIA, was significantly high (1303.7 ±518.0 ng/mL, $p<0.01$), when compared with normal controls (696.2±200.5 ng/mL as shown in Table 1). However, there was no significant differences in serum IGFBP-2 and -3 between SCLC and NSCLC groups.

DISCUSSION

It is well known that many pulmonary tumors secrete hormones and other substances ectopically. There have been many trials to make use of these substances to diagnose early, to identify tumor type and stage, and to monitor the response to treatment in lung cancer patients (10).

In the present study, we observed decreased levels of serum IGF-I, IGFBP-3 and increased serum IGFBP-2 levels in patients with lung cancer. The low serum IGF-I levels detected in patients with lung cancer could be due to decreased synthesis or increased catabolism of IGF-I. Serum IGF-I levels decrease due to protein-calorie malnutrition, fasting, severe insulin deficiency, liver disease and primary hypothyroidism (11). Even though our patients do not seem to have inadequate protein intake, the negative nitrogen balance may, in part, explain the low level of serum IGF-I. The elevated level of serum IGF-I in non-small cell lung cancer patients was observed by several investigators (6, 7, 12). Reeve et al. (8) reported that decreased serum IGF-I concentration is associated with abnormal glucose tolerance in small cell lung cancer. In our study, patients with NSCLC showed significantly lower serum IGF-I level compared with SCLC patients. This implied that serum concentration of IGF-I may be changed by the tumor cell type.

The serum concentration of IGFBP-2 has been found to be elevated in lung cancer patient compared to normal controls. The concentration of serum IGFBP-2 is not changed by acute metabolic events but is slightly increased after prolonged fasting (13). Plasma IGFBP-2 is increased in patients with chronic renal failure, hepatic failure, non-islet cell tumor hypoglycemia and leukemia (14). In general, there is an inverse relationship between the serum IGFBP-3 and IGFBP-2. Many studies have demonstrated that the increased concentration of serum IGFBP-2 in lung tumors probably results from tumor overproduction (6, 15). Cohen et al. (16) suggested that elevated levels of serum IGFBP-2 in prostate cancer patients was due to secretion by prostate tumor.

IGFBP-3, the predominant IGFBP in adult serum, was significantly decreased in lung cancer patient compared with normal controls. Plasma IGFBP-3 levels vary with the developmental age and growth hormone/IGF-I level. However, plasma IGFBP-3 levels show little diurnal variation and are not changed by acute metabolic events (17). Plasma IGF-I and IGFBP-3 often change coordinately, being decreased in growth hormone deficiency, and in catabolic states such as diabetes mellitus and dietary protein restriction. The reduction of intact IGFBP-3 may be due to proteolysis of IGFBP-3. Various proteases capable of cleaving IGFBP-3 have been reported (18, 19). While serum IGFBP-2 is increased and IGFBP-3 is decreased in patients with prostate cancer (20), we could not observe the significant difference of serum IGFBP-2 and -3 between SCLC and NSCLC groups.

In conclusion, patients with lung cancer had low serum levels of IGF-I and IGFBP-3, and increased IGFBP-2 levels, when compared with normal controls. The difference of serum IGF-I and IGFBPs levels between in lung cancer patients and in normal controls showed the possibility that IGFs and IGFBPs, especially IGF-I, may be a uesful tumor markers for lung cancer. Further study in the disturbance of IGF-IGFBP axis in lung cancer patients for early diagnosis, clinical staging, identification of tumor type and response to therapy in lung cancer will be needed.

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