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Elevation of a Novel Pituitary Protein (7B2) in the Plasma in Small Cell Carcinoma of the Lung

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Abstract—*7B2 is a new protein isolated from human pituitary glands and distributed widely, particularly with high concentrations in the neuroendocrine tissues in the rat. We measured plasma 7B2 concentrations in* 333 *normal subjects,* 20 *patients with benign pulmonary disease and* 111 *patients with primary lung cancer* (21 *small cell carcinoma,* 90 *non-small cell carcinoma). A normal range for plasma 7B2 concentrations was defined as less than the mean + 3 S.D. value* (110 pg/ml) *based on plasma 7B2 concentrations in* 333 *normal subjects. Elevation of the plasma 7B2 concentration over the normal range was observed in* 15 *of* 21 *patients* (71.4%) *with small cell carcinoma, eight of* 90 (8.9%) *with non-small cell carcinoma and four of* 20 (20%) *with benign pulmonary disease. Plasma 7B2 concentrations correlated with the clinical course on chemotherapy in some patients with small cell carcinoma. Immunocytochemical studies revealed numerous 7B2-positive cells in the small cell carcinoma specimen, while 7B2 staining was not observed in the non-small cell carcinoma and the normal lung specimens. These findings suggest that 7B2 is secreted by the small cell carcinoma of the lung, which caused elevation of plasma 7B2 in these patients. 7B2 might be a possible plasma tumor marker for the small cell carcinoma of the lung.*

INTRODUCTION

7B2 was initially isolated from the porcine and human pituitary gland in 1982 [1, 2]. 7B2 is distributed in various tissues, particularly with high concentrations in the neuroendocrine tissues in the rat [3]. Secretory characteristics of 7B2 were well documented using cultured cells of rat pituitary gland [3, 4], rat pheochromocytoma (PC 12) [5], bovine adrenal medulla [6], and human growth hormone-producing pituitary adenoma [7]. 7B2 has been shown to be localized in the secretory granules of A and B cells of the human and rat pancreatic islets [8] and gonadotrophs and thyrotrophs of the rat pituitary gland [9], immunocytochemically. These findings indicate that 7B2 is a secretory protein in these tissues. 7B2 is present in human plasma [10, 11] and elevation of plasma 7B2 levels was noted in some patients with various endocrine tumors [10].

In the present study, we investigated the role of plasma 7B2 as a tumor marker for small cell carcinoma of the lung (SCCL). Immunocytochemistry and gel permeation chromatography of the plasma were also performed.

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MATERIALS AND METHODS

Plasma samples were collected from 111 patients with primary lung cancer (87 men and 24 women, 37–87 years of age), 20 with benign pulmonary disease (14 men and six women, 21–82 years of age) and 333 with normal subjects (128 men and 205 women, 40–87 years of age). Diagnosis of primary lung cancer was histologically and/or cytologically proven, 51 adenocarcinoma, 25 squamous cell carcinoma, 21 small cell carcinoma and 14 large cell carcinoma. Clinical staging of these patients was determined according to the International Union Against Cancer (UICC) staging system [12]. Normal subjects were defined as no evidence of hepatic, renal and endocrine disorders. To assess the effect of response to chemotherapy or relapse of the disease on plasma 7B2 levels, plasma samples were collected from nine patients with SCCL. The treatment response was evaluated according to the criteria defined by the Japan Lung Cancer Society [13]; six patients had partial remission (PR) and three had

relapsed. All samples were stored at −20°C until assay.

7B2 was measured by radioimmunoassay (RIA), as described previously [3]. The antiserum used was raised against a synthetic fragment of 7B2 corresponding to residues 23–39 of authentic 7B2 (7B2 23–39) in rabbits. The specificity of the antiserum was described previously [10]. 7B2 23–39 and [^{125}I]7B2 23–39 were used as standard and tracer, respectively. The sensitivity of the RIA was 2 pg/tube, and intra- and interassay coefficients of variation were below 12%, respectively ($n = 5$).

Neuron specific enolase (NSE) was measured by RIA using a RIA kit (Pharmacia Japan, Tokyo, Japan) and carcinoembryonic antigen (CEA) was measured by enzyme immunoassay (EIA) using a EIA kit (Boehringer-Mannheim-Yamanouchi Co., Tokyo, Japan).

Lung cancer and normal lung tissues, obtained at surgery, were fixed in 10% formalin and embedded in paraffin. Immunoperoxidase staining was performed on 5 μm cryostat sections using a 7B2 antiserum, which was used in RIA, according to the ABC procedure described by Hsu *et al.* [14].

Gel permeation chromatography of pooled plasma obtained from SCCL patients and that from normal subjects as a control was performed on a Sephadex G-100 column (95 × 1.4 cm). Two ml of pooled plasma was applied to the column and eluted with 1 M acetic acid at a flow rate of 7 ml/h at 4°C. Fractions (1.3 ml) were collected, dried using a centrifugal concentrator (Taiyo VC-36, Tokyo, Japan) and reconstituted with RIA buffer before assay.

Statistical analysis of the results was performed using Student's *t*-test. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

The mean (± S.D.) value of plasma 7B2 concentrations in normal subjects was 55 ± 18 pg/ml, and the normal range for plasma 7B2 concentrations was defined as less than the mean + 3 S.D. value (110 pg/ml). Plasma 7B2 concentrations in patients with primary lung cancer and benign pulmonary disease are shown in Fig. 1. The mean (± S.D.) concentrations (pg/ml) of plasma 7B2 were 86 ± 34 in adenocarcinoma, 74 ± 21 in squamous cell carcinoma, 164 ± 128 in small cell carcinoma, 72 ± 29 in large cell carcinoma and 82 ± 31 in benign pulmonary disease, respectively. The mean level of plasma 7B2 concentrations in the SCCL patients were significantly higher than those in patients with non-small cell carcinoma of the lung (NSCCL) ($P < 0.01$) or benign pulmonary disease ($P < 0.01$). Fifteen of 21 patients (71.4%) with SCCL had plasma 7B2 concentrations exceeding 110 pg/ml, while six of 51 patients (11.8%) with adenocarcinoma, one of 25 (4.0%) with squamous cell carcinoma, one of 14 (7.1%) with large cell carcinoma and four of 20 (20%) with benign pulmonary disease had plasma 7B2 concentrations over 110 pg/ml, respectively (Fig. 1).

In the SCCL patients, plasma 7B2 concentrations tended to be higher in those in the advanced clinical stage (stage III, IV), as compared to those in an earlier stage (stage I, II) (Fig. 2, left). Similarly, plasma 7B2 concentrations tended to be higher in those with a large tumor size (> 3 cm) and a more distant lymph node metastasis (N_2), as compared to those in the smaller tumor size (< 3 cm) and the limited lymph node metastasis (N_0, N_1) (Fig. 2, right).

As shown in Fig. 3, there were no significant correlations between plasma levels of 7B2 and those of NSE or CEA.

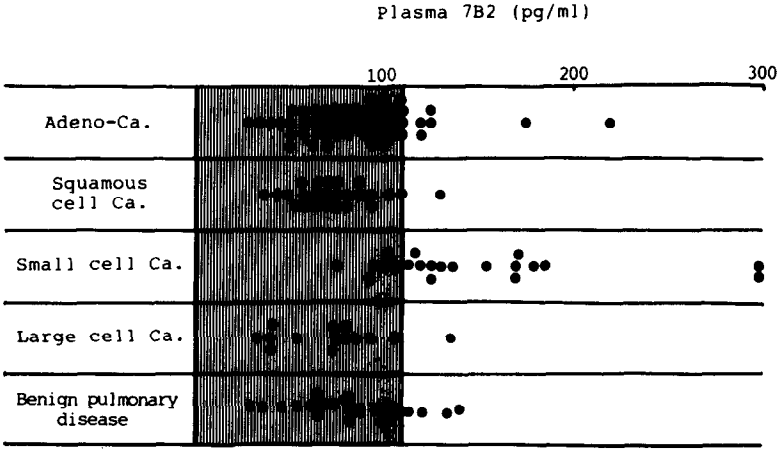

Fig. 1. Plasma 7B2 concentrations in 111 patients with primary lung cancer and 20 with benign pulmonary disease. Shaded: normal range.

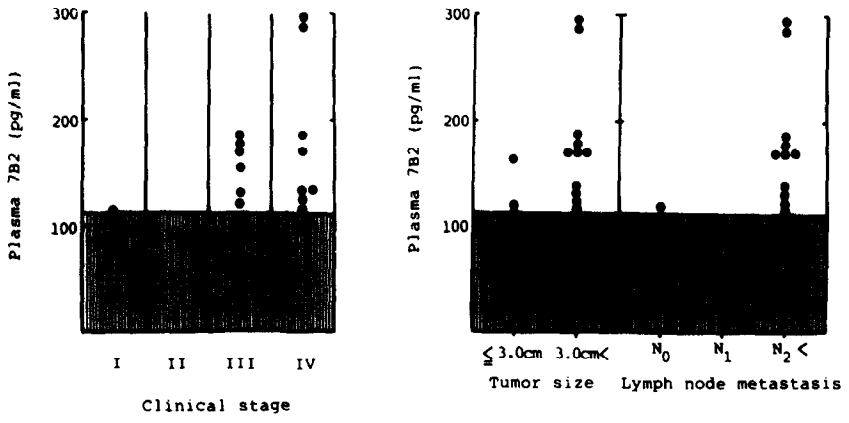

Fig. 2. Relationship between 7B2 concentrations and clinical stages (left) or tumor size and extent of lymph node metastasis (right) in 21 SCCL patients. Shaded: normal range.

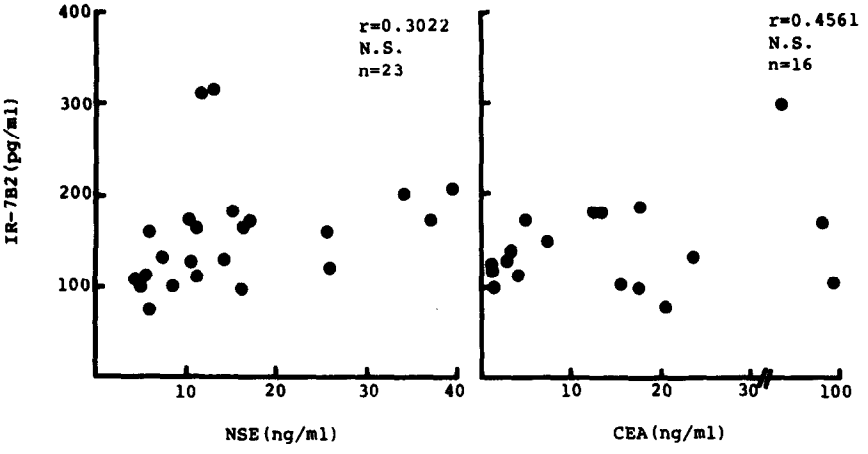

Fig. 3. Correlations of plasma 7B2 levels with those of NSE (left) and CEA (right) in SCCL patients.

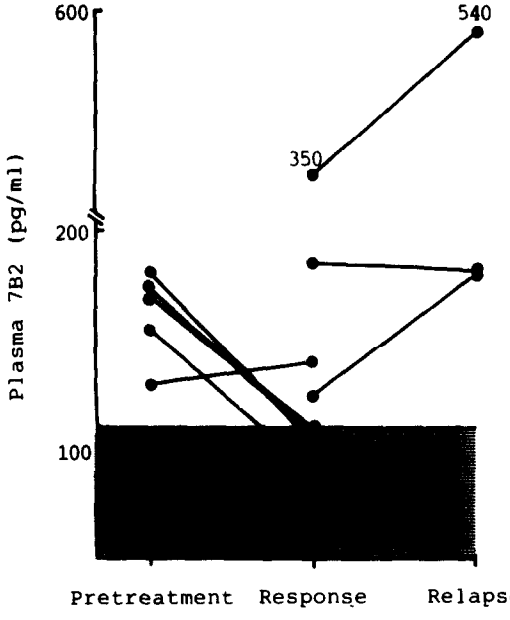

Fig. 4. Changes of plasma 7B2 levels at the time of response to chemotherapy or relapse of the disease in nine SCCL patients. Shaded: normal range.

Among six SCCL patients with PR, the plasma 7B2 levels dropped to the normal range in four out of six, but did not alter in one patient. In the remaining patient, the plasma 7B2 level decreased after treatment but its level before treatment was within the normal range. In three patients with relapse, the plasma 7B2 levels rose in two but did not change in the remaining patient (Fig. 4).

Immunocytochemical studies, as shown in Fig. 5, revealed numerous 7B2-positive cells in the SCCL specimen (a), whereas 7B2 staining was not found in the normal lung (b) or NSCCL specimens (data not shown).

Figure 6 depicts an elution profile of the pooled plasma obtained from SCCL patients on Sephadex G-100. A major peak of 7B2 immunoreactivity was eluted at an apparent molecular weight of 20,000. In the analysis of the pooled plasma from normal subjects, a similar elution profile was obtained (data not shown).

DISCUSSION

SCCL has been shown to produce a variety of peptides or proteins [15]. Among them, serum levels of NSE [16], creatinine kinase-BB [17] or bombesin [18] were frequently elevated and correlated with the disease activity in the SCCL patients. Recently, elevation of serum chromogranin A concentrations was also noted in SCCL patients [19, 20] and expression of chromogranin A in SCCL was demonstrated using Northern blot and *in situ* hybridizing analysis [21]. The present study has shown elevation of a novel pituitary protein 7B2 in the plasma of SCCL patients as compared to plasma 7B2 levels in patients with NSCCL and benign pulmonary disease. Plasma 7B2 levels in the SCCL patients with advanced clinical stages (III, IV) tended to be higher than those in the SCCL patients with the earlier stage (I). Plasma 7B2 levels showed a good correlation with the disease activity during chemotherapy in SCCL patients. These findings suggest that plasma 7B2 levels correlate with SCCL tumor burden. 7B2 may be secreted by SCCL, which causes elevation of plasma 7B2 levels in SCCL patients. Immunocytochemical studies confirmed the presence of 7B2 in SCCL. This also supports the hypothesis of the 7B2 secretion by SCCL. 7B2 may be a useful plasma marker to monitor the disease activity in SCCL patients as is NSE. Suzuki *et al.* [10] reported elevation of plasma 7B2 levels in some patients with various endocrine tumors, however this elevation was noted in only one of six SCCL patients. We found that more than 70% of the SCCL patients had plasma 7B2 levels greater than the normal range. Such a difference may be due to the different normal range and/or different clinical stages of the SCCL patients studied. Significant correlations between plasma levels of 7B2 and those of NSE or CEA were not found in SCCL patients. This suggests that a combination of these markers may be more useful for monitoring.

Elevation of plasma 7B2 levels over the normal range was also noted in eight of 90 patients (8.9%) with NSCCL and four of 20 (20%) with benign pulmonary disease. Among the eight patients with NSCCL, seven were adenocarcinoma and the remaining was squamous cell carcinoma, and all patients revealed the advanced clinical stages (III or IV). Ariyoshi *et al.* [22] observed the elevation of serum NSE levels even in patients with NSCCL and raised the possibility that such NSCCL possessed the neuroendocrine properties like SCCL. Elevation of plasma 7B2 levels in NSCCL patients may be attributed to the similar mechanism. Among the four patients with benign pulmonary disease, on the other hand, three were pulmonary tuberculosis and the remaining was neurinoma in the mediastinum. Elevation of plasma 7B2 in the patient with neurinoma may be attributed to the secretion of 7B2 by the tumor since 7B2 was distributed, particularly in the neuroendocrine tissues [3, 10]. However, we cannot account for elevation of plasma 7B2 levels in the patients with pulmonary tuberculosis. Two patients did not show any incidence of cancer up to date, but the remaining patient died of gastric cancer a year after the blood sampling. Occult tumors might be present in addition to pulmonary tuberculosis. However, further studies will be definitely needed to lead a conclusion.

A biological function of 7B2 is still unclear. Martens [23] revealed a nucleotide sequence of the human pituitary 7B2-cDNA and suggested that 7B2 is a member of the GTP-binding proteins. Some of the GTP-binding proteins have been shown to be involved in the intracellular protein transport system [24–26]. 7B2 may have an important role

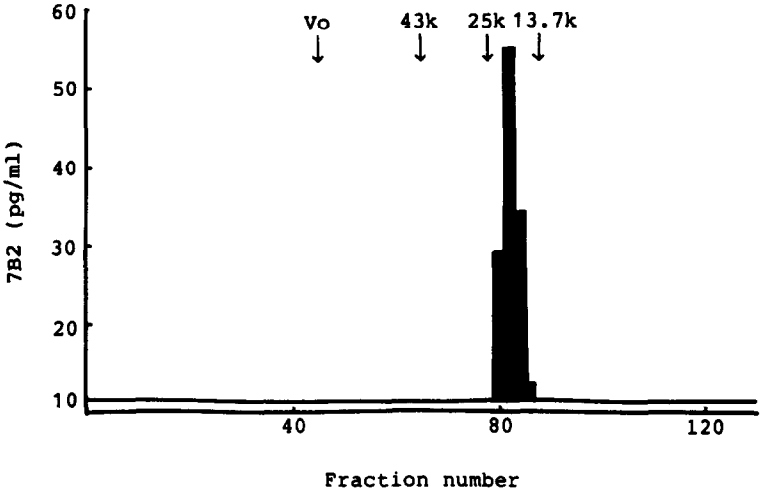

Fig. 6. Gel permeation chromatography of pooled plasma obtained from SCCL patients on a Sephadex G-100 column (95 × 1.4 cm). Molecular markers are V_0, catalase; 43K, ovalbumin; 25K, chymotrypsinogen A and 13.7K, ribonuclease.

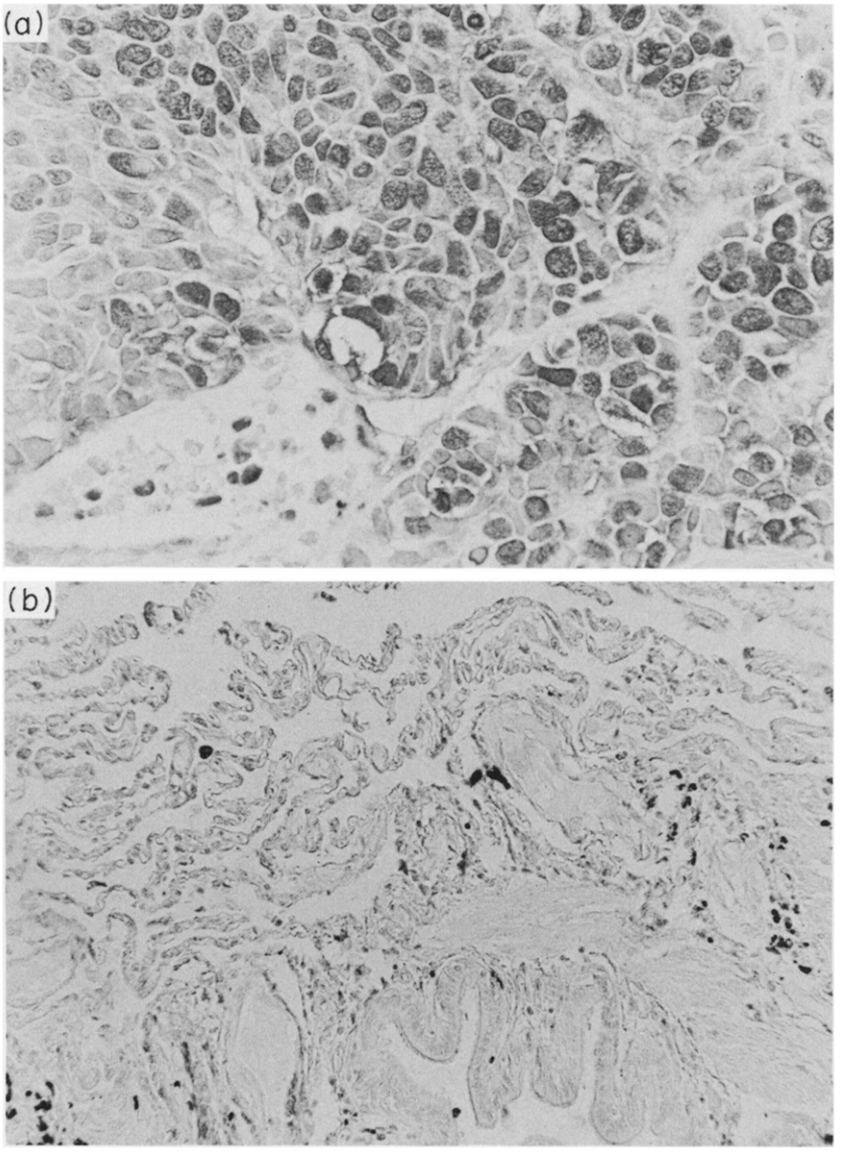

Fig. 5. Immunocytochemical staining of 7B2 in SCCL (a) and normal lung specimens (b).

in this system, particularly exocytosis of various substances packed in the secretory granules in the neuroendocrine tissues including SCCL.

Molecular heterogeneity of peptide or protein hormones is observed in plasma and/or tissues of cancer patients and this seems to be due to different posttranslational processing of the precursor molecule [27]. In this study, the elution profile of pooled plasma from SCCL patients was similar to that of pooled plasma from normal subjects, indicating no molecular heterogeneity of plasma 7B2 in SCCL.

In conclusion, 7B2 may be a new biomarker to assess the neuroendocrine properties of SCCL and measurement of plasma 7B2 levels may find future application in monitoring for SCCL. However, further studies in a larger number of patients will be required to ascertain the clinical utility of 7B2 as a tumor marker for SCCL.

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