Enzyme Immunoassay of Immunoreactive Progastrin-Releasing Peptide(31–98) as Tumor Marker for Small-Cell Lung Carcinoma: Development and Evaluation

Katsumi Aoyagi,1 Yoshio Miyake,2,3 Kenichi Urakami,1,4 Tomiko Kashiwakuma,1 Akira Hasegawa,1 Tetsuro Kodama,2,5 and Ken Yamaguchi2,6

Previously, using recombinant human progastrin-releasing peptide (ProGRP)(31–98), we developed a RIA for ProGRP(31–98) and demonstrated that the determination of serum ProGRP(31–98) was a reliable marker for small-cell lung carcinoma (SCLC) (Miyake et al., Cancer Res 1994;54:2136–40). Aiming for a more convenient assay system, we have now developed and evaluated a highly sensitive and specific ELISA for ProGRP(31–98). Only 50 μL of nonextracted serum is needed, and results are obtained in only 2 h. Intraassay and between-day CVs were 1.7–4.6% and 4.2–6.8%, respectively. The log-log calibration curve was linear to 1000 ng/L, and analytical recovery was 91.5–108.7%. The detection limit of this assay, 1.9 ng/L, means that basal concentrations of ProGRP(31–98) were detectable in all healthy subjects. The cutoff value, based on the mean + 3 SD of concentrations in 247 healthy subjects, was set to 45.1 ng/L. Serum concentrations exceeded this value in 18 of 25 SCLC patients, similar to the frequency of increased values found by RIA previously. In contrast, the frequency of increased serum ProGRP(31–98) in patients with nonmalignant pulmonary diseases or non-SCLC was quite low: 0% and 5.0%, respectively. Such results may justify a clinical trial for evaluating this ELISA for the diagnosis and monitoring of SCLC patients.

Indexing Terms: cancer/gastrin-releasing peptide/radioimmunoassay compared

Small-cell lung carcinoma (SCLC), an aggressive and rapidly growing neoplasm that tends to be disseminated by the time of diagnosis, has the worst prognosis of several subtypes of lung cancer. This cancer, however, is highly sensitive to systemic chemotherapy; thus, a reliable tumor marker for SCLC patients may play an important role in their treatment. The assay of neuron-specific enolase (NSE) has been approved for this purpose in several countries. However, clinical experience has revealed several disadvantages of NSE, including the relatively high false-positive rate in patients with non-SCLC (1, 2), a low true-positive rate in SCLC patients with limited diseases (3), and increased measured concentrations in the presence of hemolysis (2).

Gastrin-releasing peptide (GRP), a gut hormone originally isolated from porcine stomach (4), is widely distributed throughout the mammalian nervous system, gastrointestinal tract, and pulmonary tract (5–7). We previously reported that GRP is frequently produced by SCLC cells (8, 9), such that the determination of plasma GRP concentrations could serve as a useful tumor marker for SCLC patients (10); however, the instability of GRP in blood made it impossible to develop a clinically applicable system for this purpose.

The GRP gene encodes putative progastrin-releasing peptide (ProGRP) molecules, which consist of a signal peptide, native GRP(1–27), an amidation and tryptophan-like cleavage site(28–30), common extension peptides(31–98), and variable carboxyl-terminal regions, which are the result of alternative RNA splicing (11–13). Holst et al. (14) demonstrated that plasma ProGRP(42–53) detected by RIA was increased in extracts of plasma obtained from SCLC patients. Although the combination of RIA and plasma extraction was not convenient for clinical use, their findings stimulated us to develop a sensitive and reliable method for determining GRP gene products in blood.

Our idea was to establish an immunoassay to determine serum concentrations of the common extension peptide(31–98) of ProGRP, because the amino acid sequences of human ProGRP(31–98) are not homologous with other proteins. After purifying recombinant human ProGRP(31–98) that had been expressed in Escherichia coli, we used this ProGRP(31–98) to develop a RIA of ProGRP(31–98) with no extraction steps and showed that the determination of serum ProGRP(31–98) concentrations could serve as a reliable tumor marker in SCLC patients (13).

In the present study, for further clinical use, we developed a sensitive and convenient ELISA for serum ProGRP(31–98), using 96-well microplates. Here we report the performance of this ELISA.
Materials and Methods

Materials

Hemoglobin, bilirubin, lipemic interferences, and rheumatoid factor were purchased from International Reagents Corp. (Kobe, Japan); GRP, bombesin, neurokinin A, galanin, carotidin, carotidin gene-related peptide, and corticotropin were from Peninsula Lab. (Belmont, CA); NSE was from Eiken Chemicals (Tokyo, Japan); the mouse monoAb-ID kit was from Zymed (San Francisco, CA); pepsin was from Boehringer Mannheim (Mannheim, Germany); and the BCA protein assay kit was from Pierce Chemical Co. (Rockford, IL).

Preparation Procedures

Recombinant human ProGRP(31-98). The human ProGRP(31-98) gene synthesized chemically with a model 8700A DNA synthesizer (Milligen/Biosearch, San Rafael, CA) was inserted into the EcoRI and SalI site of the expression vector (pATTrp) described previously (15) (Fig. 1, A and B). ProGRP(31-98) was expressed as a fusion protein with an amino-terminal 17-amino-acid sequence of TrpE protein in E. coli. The bacterial cells were grown in M9CA medium and were disrupted enzymatically with lysozyme. The insoluble fraction was solubilized in 6 mol/L guanidine-HCl, then dialyzed against 6 mol/L urea. After the fusion protein was purified by ion-exchange chromatography on a Q-Sepharose column (Pharmacia, Uppsala, Sweden), it was cleaved with cyanogen bromide in formic acid. Recombinant ProGRP(31-98) was purified by gel-filtration chromatography on a Superdex 75 prep-grade (Pharmacia) column. The purity was >95%, as determined by densitometric analysis after Coomassie Blue staining and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (16). The amino acid composition analysis of recombinant ProGRP(31-98) corresponded to that of human ProGRP(31-98). Peptide sequence analysis revealed that at least 35 of the amino-terminal amino acid residues were identical to those of human ProGRP(31-98). The concentration of recombinant ProGRP(31-98) was measured by the BCA protein assay kit, which is calibrated with bovine serum albumin (BSA) (17). This grade of recombinant ProGRP(31-98) was used as an antigen for both monoclonal antibody (mAb) and antisera preparations and as a calibrator for a sandwich ELISA.

Monoclonal antibodies. BALB/c mice were immunized subcutaneously five times with 50 μg/L recombinant ProGRP(31-98) in an equal volume of complete Freund's adjuvant (CFA). Splenocytes from the immunized mice were fused with myeloma cells (P3X63Ag8) by a modification of the method described by Kohler and Milstein (18). The cell mixture was cultured in RPMI-1640 supplemented with 100 mL/L fetal calf serum and selected in hypoxanthine–aminopterin–thymidine medium. The culture supernate of each hybridoma was used to determine anti-ProGRP(31-98) antibodies by ELISA. Four hybridomas secreting antibodies to ProGRP(31-98) were established and all mAbs were prepared from mouse ascites fluid. Three IgG-type mAbs were purified by chromatography on a Protein A column (Pharmacia), and one IgM type was purified by (NH₄)₂SO₄ precipitation and gel-filtration.

Fig. 1. (A) DNA sequence and amino acid sequence of synthetic human ProGRP(31-98); (B) ProGRP(31-98) expression plasmid; (C) SDS-PAGE analysis of recombinant ProGRP(31-98).

(A, B) The chemically synthesized ProGRP(31-98) gene was inserted into the EcoRI and SalI site of the expression vector (pATTrp); this gene was expressed in E. coli under the tryptophan operon promoter. (C) Recombinant ProGRP(31-98) purified by ion-exchange chromatography and gel filtration was separated on a gel and stained with Coomassie Brilliant Blue.

S38 CLINICAL CHEMISTRY, Vol. 41, No. 4, 1995
chromatography on Superdex 200 (Pharmacia). We determined the subclasses of the purified mAbs with the mouse monoAb-ID kit (Zymed). The protein concentration was determined by absorbance at 280 nm by use of the absorbivity of the protein.

Preparation of biotinylated mAbs. Hydroxysuccinimidyl-6-(biotinamido)hexanoato (NHS-biotin) (Pierce), 10 g/L dissolved in dimethyl sulfoxide, was added to the solution of four mAbs (5 g/L each) prepared in 0.1 mol/L carbonate buffer (0.1 mol/L NaHCO₃, 0.5 mol/L NaCl, pH 8.5) in a 50:1 molar ratio of NHS-biotin:mAb. The mixture was incubated for 2 h at room temperature. To the reaction solution we added 20 μL of 1 mol/L NH₄Cl and incubated for 30 min at room temperature. Biotinylated mAbs were purified by chromatography on a Sephadex G-25 column (Pharmacia).

Horseradish peroxidase (HRP)-conjugated polyclonal antibodies (pAbs). New Zealand White rabbits were immunized subcutaneously six times with 100 μg/L recombinant ProGRP(31–98) in an equal volume of FCA. The IgG fraction in the pAb was purified by chromatography on a protein A column. The IgG was digested with peptic in 0.1 mol/L acetic buffer (pH 4.5), and the Fab fragment was isolated by gel-filtration chromatography on Superdex 200. The Fab fragment was prepared by reducing the Fab fragment and conjugating it to HRP (Toyobo, Osaka, Japan) by the maleimide hinge method (19). The conjugated material was purified by gel-filtration chromatography on Superdex 200.

Assays

Competitive-binding ELISA. Microtiter plates (96-well) were coated with recombinant ProGRP(31–98) at a concentration of 1 mg/L in coating buffer (0.15 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.3) overnight at 4°C, incubated with blocking buffer (10 g/L BSA and 0.15 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.3) for 2 h. Then, 50 μL of 10 mg/L mAbs in assay buffer (10 g/L BSA, 0.5 mL/L Tween 20, and 0.15 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.3) was added to each well. After the plates were incubated for 40 min at 37°C, 50 μL of 100 μg/L biotinylated mAbs in assay buffer was added, and the samples were incubated for another 40 min at 37°C. After the wells were washed four times with washing buffer (0.15 mol/L NaCl and 0.5 mL/L Tween 20 in 0.1 mol/L phosphate buffer, pH 7.3), to each well was added 100 μL of HRP-conjugated avidin D (Vector Labs., Burlingame, CA) in assay buffer, and the plates were incubated for 30 min at room temperature. The plates were then washed five times with washing buffer, and the enzyme color reaction was developed by adding substrate solution (2 g/L o-phenylenediamine and 0.4 mL/L 30% hydrogen peroxide), letting the reaction to proceed for 30 min at room temperature, and stopping the reaction by adding 100 μL of 1 mol/L sulfuric acid. The absorbance of each well was measured at 492 nm (vs 630 nm as the reference wavelength) with a plate reader.

Sandwich ELISA for ProGRP(31–98). We applied 100 μL of an equimolar mixture of anti-ProGRP(31–98) mAbs 2B10 and 3G2 at a total concentration of 10 mg/L to the microtiter plate wells. Reaction buffer (10 g/L BSA, 20 mmol/L EDTA, 0.5 mol/L NaCl, and 0.5 mL/L Tween 20 in 0.1 mol/L phosphate buffer, pH 7.0), 100 μL, was added to each well. We then added 50 μL of human sera or recombinant ProGRP(31–98) in sample dilution buffer (10 g/L BSA, 20 mmol/L EDTA, and 0.15 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.3) to each well. The plates were incubated for 60 min at 37°C and then washed five times with washing buffer. HRP-conjugated pAb in dilution buffer (10 g/L BSA, 10 mmol/L EDTA, 0.15 mol/L NaCl, and 0.5 mL/L Tween 20 in 0.1 mol/L phosphate buffer, pH 7.3) was added to each well, and the plates were incubated 30 min at room temperature. After washing the plates five times with washing buffer, we measured the enzyme activities as described above. A calibration curve was generated by plotting the absorbance vs the log concentration of recombinant ProGRP(31–98). Concentrations of ProGRP(31–98) in samples were obtained by interpolation of their absorbance from the calibration curve.

Gel-filtration chromatography. The extracts (50 μL) from frozen tissue of SCLC or the serum (200 μL) from SCLC patients were chromatographed on a Sephadex G-75 fine column (Pharmacia; 1.0 × 56.0 cm) that had been equilibrated with 1 mol/L acetic acid and eluted with the same solution. The molecular mass of each fraction was calibrated with cytochrome c (Mr, 12 384), human ProGRP(31–98) (Mr, 7 726), and human GRP (1–27) (Mr, 2 859). The total amount of ProGRP(31–98) in each fraction was measured by ELISA as described above.

Subjects

Serum samples were obtained from 247 healthy control subjects, 20 patients with nonmalignant pulmonary diseases (pneumonia, pulmonary suppuration, interstitial pneumonia, chronic bronchitis, bronchiectasis, and pulmonary sequestration), 20 patients with non–SCLC (8 with adenocarcinoma, 7 with squamous cell carcinoma, and 5 with large cell carcinoma), and 25 patients with untreated SCLC. In SCLC patients, the stage of disease (limited vs extensive) was determined according to the Veterans Administration Lung Study Group criteria (20). Frozen SCLC tissue at autopsy was extracted by the boiling water method (8), reconstituted in 1 mL of 1 mol/L acetic acid, and stored at −20°C. The venous blood samples were centrifuged at 1500g for 10 min and stored at −20°C. The ProGRP(31–98) immunoreactivity in these specimens stored at −20°C was stable for 1 year (data not shown).

Results

Analytical Variables

Antigens. Recombinant ProGRP(31–98) was expressed in large quantities as a TrpE fusion protein in E. coli (Fig. 1B) and was purified from the cells carry-
ing this expression plasmid by ion-exchange chromatography and gel filtration. The purified recombinant ProGRP(31–98) was analyzed by SDS-PAGE; the Coomassie Brilliant Blue-stained gel showed a single band with a molecular mass of ~7.7 kDa (Fig. 1C). The purity of the recombinant ProGRP(31–98) was >95% by densitometric analysis.

Antibodies. Four mAbs for ProGRP(31–98) were generated. Three of the mAbs had the γ1 heavy chain and were called 2B10, 3G2, and 1E2; the other, which had the μ heavy chain, was termed 3H1.

To assess whether the four mAbs bound the same or different epitopes on ProGRP(31–98), we used a competitive-binding ELISA to determine the ability of each biotinylated mAb to bind recombinant ProGRP(31–98) in the presence of 100-fold excess of unlabeled mAbs (data not shown). The binding of all biotinylated anti-ProGRP(31–98) mAbs was inhibited by the unlabeled mAbs. The binding of biotinylated 2B10 and 1E2 was inhibited strongly by each other, and not by 3G2. Biotinylated 3G2 and 3H1 bound to ProGRP(31–98) very competitively. These results suggested that 2B10 and 1E2 recognized the same epitope or a closely related binding site, and that the epitope recognized by 3G2 was near the site of that recognized by 3H1. To select the best mAb for an immobilized antibody on solid phase in a sandwich ELISA, we examined various combinations of the four mAbs and a rabbit pAb. Separately, 2B10 and 3G2 bound to distinct epitopes on ProGRP(31–98), and a mixed coating of 2B10 and 3G2 in equal quantities on the plate produced in the lowest detection limit with HRP-conjugated rabbit pAb (data not shown). Therefore, we selected 2B10 and 3G2 for immobilized antibodies.

ELISA for ProGRP(31–98). A sandwich ELISA for ProGRP(31–98) was established by using the optimized reaction components, concentrations, and assay conditions. The calibration curve was linear up to 1000 ng/L on a log-log scale (Fig. 2). The analytical detection limit of this assay is 1.9 ng/L, defined as the concentration corresponding to the mean + 2 SD absorbance of the zero calibrator (n = 6). To analyze the reactivity of native ProGRP(31–98) in serum in this ELISA, we diluted and measured the native sera from three SCLC patients who had high concentrations of ProGRP(31–98) as determined by RIA (13). The dilution curve of the three sera was parallel to that of recombinant ProGRP(31–98) as calibrator. The recovery of three concentrations of recombinant ProGRP(31–98) (130.0, 390.0, and 650.0 ng/L) added to three specimens having concentrations of 34.1, 55.6, and 121.3 ng/L was 91.5–108.7% (data not shown).

To analyze the immunoreactivity measured by this ELISA in biological fluids, we examined the gel-filtration study of extracts of SCLC tissue and serum from a SCLC patient (Fig. 3). The total amount of ProGRP(31–98) was measured in each fraction. This ELISA system recognized one major peak in the tissue sample as well as in the serum sample, each peak eluting at a position slightly in front of cytochrome c. The molecular size of this peak seemed to be greater than that of ProGRP(31–98).

We examined the cross-reactivity with other hormones from brains and intestines in this ELISA system. This system did not react with 1 mg/L concentrations of GRP, bombesin, neurokinin A, galanin, carcitonin, carcitonin gene-related peptide, corticotropin, or NSE (data not shown).

Assay imprecision. Intraassay imprecision was assessed from eight measurements of four specimens. For
mean ProGRP(31–98) values of 38.3, 134.4, 359.0, and 821.1 ng/L, the CVs ranged from 1.7% to 4.6%. The between-day reproducibility was assessed from three specimens, which had mean ProGRP(31–98) values of 55.7, 219.8, and 429.5 ng/L during 4 days of measurements; the CVs ranged from 4.2% to 6.8%.

Interference. We assessed the influence of anticoagulants and blood elements in serum on ELISA results. The anticoagulants EDTA (<20 g/L), heparin (<0.2 g/L), NaF (<2.0 g/L), sodium oxalate (<0.6 g/L), and sodium citrate (<100 g/L) showed no interference with the assay. Assessment of the influence of hemoglobin (<49 g/L), bilirubin (<0.19 g/L), lipemia (turbidity, with lipids up to 16.5 g/L), and rheumatoid factor (<400 kIU/L) also showed no interference in this assay (data not shown).

Serum ProGRP(31–98) Distribution in Healthy Subjects and Patients

The distribution of the serum ProGRP(31–98) concentrations in healthy subjects is shown in Fig. 4. The concentrations in these subjects ranged from 1.9 to 74.6 ng/L. The distribution pattern was gaussian after logarithmic transformation. Estimated after this transformation, the mean + 1.96 SD was 27.0 ng/L; the mean + 3 SD, which we tentatively set as the cutoff value of this assay, was 45.1 ng/L.

Serum ProGRP(31–98) concentrations and the frequency of their increase in healthy subjects, patients with nonmalignant pulmonary diseases, and those with lung carcinomas were assessed by this ELISA; the results are summarized in Table 1. For the control subjects and the patients with nonmalignant pulmonary diseases or with non-SCLC, 99.6% (246 of 247), 100% (20 of 20), and 95% (19 of 20), respectively, had ProGRP(31–98) <45.1 ng/L. Concentrations >45.1 ng/L were found in 72.0% (18 of 25) of the SCLC patients. Eight of 12 (66.7%) SCLC patients with limited disease (defined as tumor confined to the hemithorax or ipsilateral mediastinum) had concentrations exceeding the cutoff value; their mean concentration was 861.9 ng/L, 68-fold higher than that of the healthy subjects. Results for 10 of 13 (76.9%) extensive-disease patients also exceeded the cutoff, having a mean concentration of 1645.0 ng/L, 130-fold that of the healthy subjects.

Discussion

To set up this assay, we attempted to synthesize human ProGRP(31–98), a region common to three human ProGRP molecules (13). We designed and synthesized the human ProGRP(31–98) gene and produced recombinant ProGRP(31–98). Using this peptide, we previously generated a guinea pig antiserum and developed a RIA for ProGRP(31–98), with which we determined that ProGRP(31–98) concentrations could serve as potential reliable tumor markers in SCLC patients. We also demonstrated that SCLC cells produce equimolar amounts of GRP(1–27) and ProGRP(31–98), although the latter was more stable in the blood than the former (13).

We succeeded in generating four mouse mAbs and a rabbit polyclonal antiserum. Results from the competitive-binding ELISA suggested that our mAbs recognized mainly two epitopes. We assumed that the avidity between ProGRP(31–98) and the immobilized antibodies on solid phase became stronger when we immobilized two mAbs that recognized different epitopes on the ProGRP(31–98) molecule. On the basis of the combination of these two mAbs and HRP-conjugated rabbit pAb, we developed a sensitive and specific ELISA for ProGRP(31–98), using various optimized reaction variables. With this ELISA, recombinant ProGRP(31–98) could be used as a calibrator because both recombinant and native ProGRP(31–98) in serum had the same reactivity (Fig. 2), and because the analytical recovery of various concentrations of recombinant ProGRP(31–98) added to three sera was 91.5% to 108.7% (data not shown).

Gel-filtration studies revealed that this ELISA system recognized one major peak (eluting slightly ahead of cytochrome c) in tissue extracts and serum obtained from a SCLC patient (Fig. 3). The retention time of the peak by this ELISA was equal to that by RIA (13). This result suggests that each assay recognized the same immunoreactive molecules in tissue and in serum. Because three ProGRP molecules are produced from

| Table 1. Serum ProGRP(31–98) concentrations and frequency of increase in healthy subjects and patients with nonmalignant pulmonary diseases and lung cancers. |
|---------------------------------|-------|----------------|-----------------|----------------|
|                                | No.   | ELISA positive| RIA positive    | Conc by ELISA, ng/L (mean ± SD) |
| Healthy subjects               |       | rate, %        | rate, %         |                              |
| Nonmalignant pulmonary diseases|       |                |                 |                              |
| Lung cancer                    |       |                |                 |                              |
| Non-SCLC                       | 20    | 0              | 0               | 15.2 ± 6.7                  |
| SCLC                            |       |                |                 |                              |
| Limited disease                | 12    | 8              | 66.7            | 862 ± 1203                  |
| Extensive disease              | 13    | 10             | 76.9            | 1645 ± 1491                 |

* Frequency of serum ProGRP(31–98) exceeding the RIA cutoff value (13).
alternative RNA splicing (11, 12), we conclude that this peak, which is the native ProGRP recognized by the ELISA, represents a mixture of ProGRP(31-125), (31-118), and (31-115). Upon gel-filtration analysis (14), immunoreactive molecules in plasma of SCLC patients and extracts derived from SCLC cell lines by RIA for ProGRP(42-53) had masses of 8-10 kDa.

Western blotting analysis of the immunoreactivity of GRP gene-associated peptides by Cuttitta et al. (21) showed that the rabbit antiseraum against ProGRP(31-52) recognized the 14-kDa molecule in extracts derived from SCLC cell lines. This latter result almost corresponds with our results. However, the 27-28-kDa molecular form was not recognized by our ELISA system.

In the ProGRP(31-98) RIA reported previously, the minimum detectable amount in serum was 10 pmol/L (78 ng/L), which was insufficient to detect serum ProGRP(31-98) in healthy subjects. Moreover, this RIA system took 5 days to obtain the result and, of course, required radiolabeled materials (13). In contrast, the ELISA we developed had a minimum detection limit of 1.9 ng/L in serum, which is low enough to detect ProGRP(31-98) in healthy subjects; moreover, by easily yielding results within ~2 h, our ELISA is suitable for clinical use.

The performance of this ELISA system was examined by various tests. The CVs for both intraassay imprecision and between-day reproducibility for identifying ProGRP(31-98) in several sera ranged from 1.7% to 6.8%. These CVs are well acceptable in clinical practice. Blood elements in serum and added anticoagulants did not interfere with this ELISA, nor was there any cross-reactivity with various peptides, hormones, or enzymes from brains or intestines (data not shown).

The immunoreactivity of ProGRP(31-98) detected in serum of healthy subjects was specifically inhibited by two anti-ProGRP(31-98) mAbs. Therefore, we postulate that serum ProGRP(31-98) concentrations in normal subjects can be evaluated in terms of various criteria, e.g., subject's age, sex, and history of smoking.

In tumor marker studies, the establishment of a cutoff value is very important for evaluating a true-positive rate in cancer patients or a false-positive rate in normal subjects or patients with benign diseases. We tentatively set the cutoff value as the mean + 3 SD (45.1 ng/L) of the distribution pattern of serum ProGRP(31-98) in the healthy subjects tested. According to that criterion, the frequency of above-normal serum concentrations of ProGRP(31-98) in healthy subjects or patients with nonmalignant pulmonary diseases or in non-SCLC patients was almost equal to that determined with our RIA, i.e., 0% to 5% (Table 1); however, the number of cases examined in this study is not large. In contrast, ~72% of SCLC patients had above-normal serum ProGRP(31-98) concentrations, and positive rates between limited- and extensive-disease cases did not differ. Both sets of results indicated that this value was an appropriate cutoff value.

Moreover, the mean ProGRP(31-98) values in SCLC patients with limited disease and extensive disease were 68- and 130-fold higher, respectively, than that of healthy subjects. This ratio of increased serum concentrations is much greater than that for other tumor markers, e.g., NSE. Carney et al. (3) reported that the mean NSE value of healthy subjects was 5.2 μg/L, and those of SCLC patients with limited disease and extensive disease were 13.8 μg/L (2.7-fold greater) and 59.0 μg/L (11.3-fold greater), respectively. Thus the determination of serum ProGRP(31-98) concentrations by this ELISA could serve as a reliable tumor marker in SCLC patients.

In conclusion, the present ELISA is highly sensitive and specific for ProGRP(31-98), being able to measure serum concentrations in all healthy subjects and serve in routine clinical use. We also evaluated serum ProGRP(31-98) in patients with nonmalignant pulmonary diseases and various lung carcinomas; the results were about the same as those evaluated with RIA (Table 1) (13). This ELISA may therefore be a very useful tool for diagnosis and monitoring of SCLC patients.

We express great thanks to Hiroshi Yamamoto, Hiroshi Terai, and Shigehiro Yamamoto (Terumo Corp., Research & Development Center, Kanagawa, Japan) for useful discussion. This work was supported in part by a Research Grant from the Princess Takamatsu Cancer Research Fund, by a Grant-in-Aid from the Ministry of Health and Welfare for the 2nd-term Comprehensive 10-Year Strategy for Cancer Control, by Grants-in-Aid for Cancer Research (6·29) from the Ministry of Health and Welfare, and by the Special Coordination Funds from the Science and Technology Agency for Promoting Science and Technology.

References
11. Reeve JR Jr, Cuttitta F, Vigna SR, Heubner V, Lee TD,


