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# Clinicopathological findings of non-small-cell lung cancer with high serum progastrin-releasing peptide concentrations

Keita Kudo[a], Fumiyoshi Ohyanagi[a], Atushi Horiike[a], Eisaku Miyauchi[a], Noriko Yanagitani[a], Rira Hoshi[c], Yukitoshi Satoh[d], Noriko Motoi[b], Wakako Hamanaka[b], Yuichi Ishikawa[b], Mingyon Mun[a], Yukinori Sakao[a], Sakae Okumura[a], Ken Nakagawa[a], Takeshi Horai[a], Makoto Nishio[a,*]

[a] Thoracic Oncology Center, Cancer Institute Hospital, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Kouto-ku, Tokyo 135-8550, Japan
[b] Department of Pathology, Cancer Institute, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Kouto-ku, Tokyo, Japan
[c] Department of Cytology, Cancer Institute, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Kouto-ku, Tokyo, Japan
[d] Department of Thoracic Surgery, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamiharashi, Kanagawa, Japan

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ABSTRACT

Although progastrin-releasing peptide (proGRP) is used as a serum tumor marker for small cell lung cancer (SCLC), high serum pro-GRP concentrations are observed in some non-small-cell lung cancers (NSCLCs). The characteristics of these NSCLCs are not well known. To determine the clinicopathological features of NSCLC in patients with elevated serum proGRP concentrations, serum proGRP values were assessed in 654 advanced lung cancer patients, and positive (>46 pg/mL) NSCLC specimens were subjected to cytological and histopathological reevaluation. Serum proGRP concentrations were positive in 34 of 421 NSCLC patients (8.1%) and 186 of 233 SCLC patients (80%). Histological subtypes of the 34 NSCLC patients at diagnosis were 20 adenocarcinomas, 5 squamous cell carcinomas, 4 large cell carcinomas, and 5 large cell neuroendocrine carcinomas. Six of 27 cytology specimens contained characteristic neuroendocrine morphology. Immunohistochemical analysis showed that 11 of 17 tumors were positive for neuroendocrine markers (64.7%). Twenty of 34 serum proGRP-positive NSCLC patients received platinum-based chemotherapy, and the response rate was 55.0%. These results suggest that serum proGRP-positive NSCLCs may have neuroendocrine differentiation. In addition, serum proGRP-positive NSCLCs may have clinical characteristics that are different from other NSCLCs.



## 1. Introduction

Lung cancer is the leading cause of cancer death worldwide. In 2005, the number of deaths due to lung cancer in Japan exceeded 60,000 [1]. Conventionally, lung cancer is classified into small cell lung cancer (SCLC) and non-small-cell carcinoma (NSCLC). Because SCLC has neuroendocrine features, it has a poorer prognosis and shows greater sensitivity to chemotherapy than NSCLC. Although NSCLC is subclassified into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, some NSCLCs have neuroendocrine differentiation. In 1999, the World Health Organization categorized large cell neuroendocrine carcinoma (LCNEC) as a variant of large cell carcinoma [2]. LCNEC has been reported to have a poor prognosis, even for early-stage disease [3,4]. Different types of NSCLCs differ in their clinical behavior according to the presence or absence of neuroendocrine differentiation. Neuroendocrine differentiation in a tumor is generally determined by immunohistochemistry and/or electron microscopy, which reveal the characteristic neuroendocrine morphology [2,5]. However, it is difficult to obtain sufficient tissue by biopsy, and limited tumor tissue sampling may make it difficult to diagnose neuroendocrine differentiation in NSCLC. Therefore, the development of a sensitive serum marker for the detection of neuroendocrine differentiation is greatly desired to facilitate the diagnosis of NSCLCs and neuroendocrine tumors.

Progastrin-releasing peptide (proGRP) is a signal peptide that is produced by small cell lung cancer cells (SCLC). Serum proGRP is considered to be a sensitive tumor marker for SCLC. The sensitivity and specificity of serum proGRP as a tumor marker for SCLC is 60–70% and 96%, respectively [6]. Elevated serum pro-GRP concentrations have been observed in some NSCLC patients, especially LCNEC patients [6,7], suggesting that serum proGRP is a potentially good marker not only for SCLC but also for NSCLC with neuroendocrine features. However, the clinical and pathological characteristics of NSCLCs with elevated serum proGRP concentrations have not been well studied. In the present study, serum proGRP levels were measured in 654 lung cancer patients and the clinical characteristics of serum proGRP-positive NSCLC patients

* Corresponding author. Tel.: +81 03 3520 0111; fax: +81 03 3570 0343.
*E-mail address:* mnishio@jfcr.or.jp (M. Nishio).

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were analyzed; the histopathology of the surgical or biopsy specimens of the positive patients were also evaluated.

## 2. Patients and methods

Serum proGRP concentrations were measured in 654 patients who were diagnosed with lung cancer by histology or cytology at the Cancer Institute Hospital of the Japanese Foundation for Cancer Research between April 1998 and April 2006.

An enzyme-linked immunosorbent assay (ELISA) kit (serumlabo ProGRP; Fujirebio Diagnostics Inc., Tokyo, Japan) was used to determine serum proGRP concentrations, and samples were considered positive when their values exceeded 46 pg/mL [8].

The clinical characteristics of serum proGRP-positive NSCLC patients were retrospectively analyzed, including age at diagnosis, gender, smoking history, and TNM stage. Response to platinum-based chemotherapy in serum proGRP-positive NSCLC patients was determined according to RECIST criteria (without confirmation).

In addition, the cytological and histological findings of the surgical or biopsy specimens of these patients were reevaluated. Immunohistochemical (IHC) staining was used to evaluate neuroendocrine differentiation in the tumors. Formalin-fixed paraffin-embedded sections were stained for a panel of epithelial markers, including thyroid transcription factor-1 (TTF-1; Dako EnVision+, Saitama, Japan) and carcinoembryonic antigen (CEA; Nichirei, Tokyo, Japan), and neuroendocrine markers, including chromogranin A (CGA) (Dako EnVision+, Saitama, Japan), synaptophysin (Dako EnVision+, Saitama, Japan), CD56 (neural cell adhesion molecule [NCAM]) (Clone 1B6; Novocastra, and proGRP (Advanced Life Science Institute Inc., Saitama, Japan). IHC staining was performed according to standard protocols with EnVision kits (Dako EnVision+, Saitama, Japan). IHC results were grouped into 3 categories – strongly positive, weakly positive, or negative – by well-trained pathologists (WH and NM).

Statistical calculations were performed using StatView version 5.0 for Windows XP (SAS Institute, Cary, NC). Associations between categorical variables and serum proGRP concentrations were evaluated using Student's *t* test. Survival was measured from the start of chemotherapy to the last follow-up evaluation or death, and survival rates were estimated using the Kaplan–Meier method.

## 3. Results

### 3.1. Patient characteristics

Of a total of 654 patients, 421 were diagnosed with NSCLC and 233 with SCLC. Serum proGRP samples were positive in 220 of 654 patients, of which 34 (8.1%) had NSCLC and 186 (80%) had SCLC.

The clinical characteristics of serum proGRP-positive and negative NSCLC patients are shown in Table 1. There were no significant differences in the clinical characteristics between the serum proGRP-positive and -negative NSCLC patients.

In serum ProGRP-positive NSCLC patients, the median age of these patients was 67 years (range, 49–77). There were 22 males and 12 females, and 65% of the patients were heavy smokers (smoking index > 400). Most of the patients (94%) had advanced NSCLC. Serum creatinine concentrations were less than 1.6 mg/dL in all 34 serum proGRP-positive NSCLC patients.

The histological subtypes of the 34 serum proGRP-positive NSCLCs at diagnosis were as follows: 20 adenocarcinomas, 5 squamous cell carcinomas, 4 large cell carcinomas, and 5 LCNECs. The rates of positive serum proGRP in each histological subtype were as follows: 7.7% in 260 adenocarcinomas, 5.9% in 85 squamous cell carcinomas, 9.3% in 43 large cell carcinomas, and 44.4% in 11 LCNECs.

**Table 1**
Clinical characteristics of NSCLC patients.

| Characteristics | ProGRP-positive NSCLC patients | ProGRP-negative NSCLC patients |
|---|---|---|
| Total no. of patients | 34 | 387 |
| Age, years | | |
| Median (range) | 67 (49–77) | 62 (29–87) |
| Sex | | |
| Male/female | 22/12 | 261/126 |
| Smoking index | | |
| Mean (range) | 807.5 (0–1400) | 661 (0–3000) |
| Never/≤400/>400 | 10/2/22 | 121/29/237 |
| Histological subtype at diagnosis | | |
| Adenocarcinoma | 20 | 240 |
| Squamous cell carcinoma | 5 | 80 |
| Large cell carcinoma | 4 | 48 |
| LCNEC | 5 | 6 |
| Adenosquamous | 0 | 2 |
| Other | 0 | 11 |
| Stage | | |
| I/II | 2 | 56 |
| IIIA | 7 | 63 |
| IIIB | 6 | 106 |
| IV | 16 | 144 |
| Recurrent | 3 | 18 |

LCNEC: large cell neuroendocrine carcinoma, ProGRP: progastrin-releasing peptide, NSCLC: non-small-cell lung cancer.

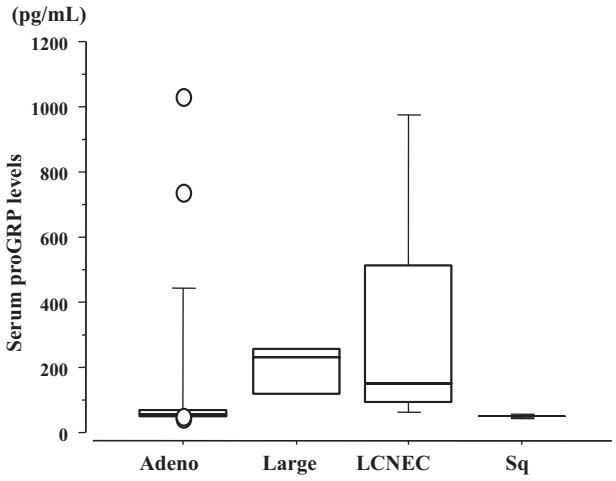


**Fig. 1.** Serum progastrin-releasing peptide (proGRP) concentrations of 34 non-small-cell lung cancer patients with elevated proGRP. Adeno: adenocarcinoma, Large: large cell carcinoma, LCNEC: large cell neuroendocrine carcinoma, Sq: squamous cell carcinoma.

The median serum proGRP concentration of the 34 NSCLC patients was 60.7 pg/mL and the range was 46.0–973.0 pg/mL. The serum proGRP concentrations in these 34 NSCLC patients were significantly lower than the serum concentrations in proGRP-positive SCLC patients (median, 469 pg/mL; range, 47.1–344,000 pg/mL) ($P < 0.05$).

Fig. 1 shows the serum proGRP concentrations for each histological subtype of serum proGRP-positive NSCLC. The mean serum proGRP concentration in LCNECs was 147 pg/mL and the range was 78.6–973 pg/mL. These concentrations are relatively high compared to other NSCLCs. On the other hand, serum proGRP concentrations were relatively low, even in serum proGRP-positive squamous cell carcinoma patients (median, 47.4 pg/mL; range, 46–56.7 pg/mL).