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ORIGINAL ARTICLE

Pro-gastrin-releasing peptide as a marker for the Ewing sarcoma family of tumors

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Abstract

Background Pro-gastrin-releasing peptide (ProGRP) is an established tumor marker of small cell lung cancer. The purpose of this study was to determine if ProGRP could serve as a tumor marker for the Ewing sarcoma family of tumors (ESFTs).

Methods Sixteen patients with ESFTs (mean age 32 years) were included in this study. As a control group, 42 patients with other tumor types that clinically or pathologically mimic ESFTs were also analyzed. Pre-treatment serum ProGRP and neuron-specific enolase (NSE) levels, the relationships between these levels, and tumor volume were investigated. In addition, serial changes in the serum or plasma ProGRP (6 patients) and NSE levels (5 patients) were measured over the course of treatment.

Results Pre-treatment serum ProGRP levels were higher than the normal range in 8 of 16 patients; for these eight patients, ProGRP levels positively correlated with tumor volume ($R = 0.99$). In the control group, ProGRP levels were within the normal range, except for the two patients. Changes in ProGRP levels during treatment were consistent with tumor volume. Serum NSE levels were elevated in 14 of 16 patients with ESFTs and 8 of 42 patients with other tumor types. The range of NSE elevation was much smaller compared to that of ProGRP. Our data indicate that ProGRP is superior to NSE in terms of specificity.

Conclusions Serum ProGRP levels were elevated in half of the patients with ESFTs and reflected therapeutic response. ProGRP is a reliable tumor marker for the diagnosis of ESFTs and evaluation of treatment response.

Keywords Pro-gastrin-releasing peptide · Ewing sarcoma family of tumors · Neuron-specific enolase

Introduction

Ewing sarcoma (ES) is a highly malignant tumor composed of small round cells, as first described by Lücke in 1866 [1]. Over the last 40 years, the occurrence of histologically similar tumors has been reported. In 1975, Angervall and Enzinger reported the existence of extraskeletal-originating ES [2]. In 1976, Nesbitt and Vidone described neuroepithelioma as a primitive neuroectodermal tumor (PNET), and the term has since been used [3]. In 1979, Askin et al. [4] described a small round cell tumor that originated in the thoraco-pulmonary region. Currently, the above-mentioned small round cell tumors are classified as a single disease entity, the ES family of tumors (ESFTs), since they exhibit similar types of chromosomal translocation (e.g., t(11; 12)(q24; q12)) [5].

Until recently, the origin of ESFTs was unclear. In 1984, Jaffe et al. [6] reported a bone PNET composed of small

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round cells that exhibited a rosette formation and were positive for the neural marker neuron-specific enolase (NSE). The current practice of electron microscopy and immunohistochemical analyses suggests that they are of neurogenic origin.

ESFTs are often associated with symptoms such as inflammation. Elevated white blood cell counts, blood sedimentation rates, serum levels of lactate dehydrogenase (LDH), alkaline phosphatase, and C-reactive protein, are often observed in ESFTs. Elevated LDH levels are associated with poor prognoses [7]. However, none of these symptoms are specific to ESFTs, which have no known tumor marker.

During the search for specific genes activated by EWS/ETS chimeric transcription factors, Lawlor et al. found that ES was frequently associated with the gene expression for gastrin-releasing peptide (GRP) [8], and they also postulated that GRP could be a useful marker for ES patients.

In 1982, GRP-like immunoreactivity was first reported to be frequently observed in small cell lung cancer (SCLC) cells [9]. Subsequently, the determination of plasma GRP levels as useful tumor markers for SCLC was suggested [8]; however, the instability of GRP in the blood leads to difficulties in the development of a clinically useful measurement of plasma GRP. In 1994, Miyake et al. reported an improved immunoassay to measure a precursor form of GRP, progastrin-releasing peptide (ProGRP), and demonstrated that ProGRP could serve as a reliable tumor marker for SCLC [10]. ProGRP is now used as a tumor marker for SCLC [11].

However, no study has determined whether ProGRP could serve as a tumor marker for ESFTs, except for a few case reports in which serum ProGRP elevation was indicated [12, 13]. In 2015, we published a preliminary report suggesting the usefulness of serum ProGRP measurement in the diagnosis of ESFT [14].

The aim of this study was to verify the rate of ProGRP elevation in a larger number of patients and to determine whether serum ProGRP levels can act as tumor markers among ESFTs by examining ProGRP changes pre- and post-treatment.

Materials and methods

Between 2002 and 2016, we treated 43 ESFT patients. Of them, 16 patients who had radiological-measurable lesions and whose serum ProGRP levels were measurable before treatment were enrolled. Children below 10 years of age were excluded because a previous report suggested that serum ProGRP levels are high in children younger than 5 years [15]. In 8 of the 16 patients, SCLC or metastatic bone tumor was initially suspected; therefore, serum ProGRP levels were previously measured in the clinics. In the remaining eight patients, pre-treatment serum ProGRP levels were measured from stored samples obtained at the initial visit to our institution.

The characteristics of the study population are listed in Table 1. More than two senior pathologists agreed with the diagnosis of ESFT. Furthermore, all the examined tissue specimens exhibited positivity in the immunostaining for CD99 (cluster of differentiation 99), a product of the MIC2 gene, which is expressed in the cytoplasmic membrane of ES cells in over 90% of ESFTs [16]. This finding indicates that the pathological diagnosis of ESFT is appropriate. In addition, in all but three patients, molecular diagnostic techniques were performed, and EWSR1 translocation—one of the major chromosomal translocations in ESFT—was found either with reverse transcription polymerase chain reaction (RT-PCT) or fluorescent in situ hybridization (FISH) in 12 patients (Table 1).

The control group comprised 42 cases of other types of bone or soft-tissue tumors that include ESFT in the differential diagnosis, either clinically or pathologically. There were 8 patients with osteosarcoma, seven with rhabdomyosarcoma, seven with malignant lymphoma, six with synovial sarcoma, five with malignant peripheral nerve sheath tumors (MPNST), three with spindle cell sarcoma, two with Langerhans cell histiocytosis (LCH), and one each

Table 1 Demographics of the ESFT patients

Parameter	Mean
Age at diagnosis (years)	32 (10–63)
	Number of patients
Sex	
Male	10
Female	6
Origin	
Bone	5
Soft tissue	11
Location	
Chest	9
Non-chest	7
Stage	
Primary	14
Recurrence	1
Metastasis	1
Molecular confirmation	
EWSR1 Break apart (FISH)	10
EWSR1: FLI1 (RT-PCR)	2
No EWSR1 rearrangement	1

FISH fluorescence in situ hybridization, *RT-PCR* reverse transcriptase-polymerase chain reaction, *ESFT* ewing sarcoma family of tumors

with mesenchymal chondrosarcoma, myxofibro-sarcoma, neuroblastoma, and undifferentiated pleomorphic sarcoma (Table 2).

Osteosarcoma has similar clinical features, such as age distribution and radiological findings, as ESFT. Rhabdomyosarcoma, malignant lymphoma, and mesenchymal chondrosarcoma contain small and/or round cell tumors that are similar to those in ESFT, and they need to be pathologically differentiated. Neuroblastoma affects children and needs to be histologically distinguished from ESFT. Similar to extraskeletal Ewing's sarcoma, synovial sarcoma and MPNST are frequently observed in children and young adults. ESFTs in soft tissue show no specific radiological findings such as other soft tissue sarcomas; thus, common soft tissue sarcomas such as undifferentiated pleomorphic sarcoma were included in the control group.

Although LCH is not a malignant tumor, whenever a young patient with possible bone sarcomas is examined, LCH should be differentiated from osteosarcoma or ESFT because LCH can exhibit imaging features mimicking malignant bone tumors such as ESFT, with bone destruction and sometimes periosteal reaction. Furthermore, LCH commonly affects the vertebral column and pelvis in children similarly to ESFT. Therefore, we have included LCH in the control group [17].

We compared the serum ProGRP and NSE levels before the start of treatment in both the ESFT and control groups. The relationships between tumor volume and serum ProGRP levels, as well as tumor volume and NSE levels were investigated. In addition, we measured serum or plasma ProGRP levels over the course of treatment in six patients and serial changes to serum NSE levels in five patients. Volumetric tumor measurement was performed using an area measurement function installed in our picture archiving and communication system (Synapse software, Fujifilm Medical Systems U.S.A Inc., Stamford, CT) on a magnetic resonance imaging or computed tomography (CT) scan. By manually tracing the lesion boundary, the lesion cross-section was measured. Lesion volume was calculated through the addition of the 2D volumes of the entire lesion (multiplying 2D area by reconstruction interval) [18]. Pearson's correlation coefficients were used to examine the association between tumor volume and serum ProGRP and NSE levels.

Serum ProGRP levels were evaluated using a previously reported method [10, 19], with a fully automated chemiluminescent enzyme immunoassay system (LUMIPULSE Presto II, Fujirebio Inc., Tokyo, Japan). The ProGRP measurement method was changed from serum to plasma in 2011. The normal range changed from < 41.3 pg/mL to < 81.6 pg/mL. Of the six patients with serial ProGRP measurements over the course of treatment, four visited our institution before 2011 and ProGRP levels were measured using serum samples; in the remaining two cases treated after 2011, ProGRP levels were measured using plasma. The serum NSE measurement method was also changed from radioimmunoassay (RIA) to electro-chemiluminescent immunoassay (ECLIA) in 2011. Consequently, the normal range changed from < 10 ng/mL to < 16.3 ng/mL. As for the initial serum NES level, in nine cases they were measured by ECLIA and in the remaining seven cases by RIA (Table 3). Furthermore, we conducted immunohistochemical studies to identify the cellular location of ProGRP immunoreactivity using the 16 ESFT tissues and 25 tumor tissues from the control group.

The research plan was designed according to the Ethical Guideline for Clinical Research in Japan and was approved by the Institutional Review Board of our institute. All data and samples were collected from routine clinics or stored samples obtained with informed consent.

Table 2 Demographics of patients in the control group

Parameter	Mean
Age at diagnosis (years)	40 (9–82)
	Number of patients
Sex	
Male	30
Female	12
Origin	
Bone	18
Soft tissue	24
Pathological diagnosis	
Osteosarcoma	8
Rhabdomyosarcoma	7
Malignant lymphoma	7
Synovial sarcoma	6
MPNST	5
Spindle cell sarcoma	3
LCH	2
Other	4

MPNST malignant peripheral nerve sheath tumor, *LCH* langerhans cell histiocytosis

Results

Serum ProGRP level

The mean initial serum ProGRP levels in ESFT patients was 214 pg/mL (3.2–1720.0) and was elevated in 8 of the 16 patients; in these patients, ProGRP levels were more than twice as high as the upper limit of the normal range. The mean ProGRP levels of the patients in the control group were 22.9 pg/mL (9.8–68.8); all were within the normal range, except for two patients who displayed only a slight elevation. These patients included an 11-year-old

Table 3 ProGRP and NSE levels in the study patients

Pt. No	Patient's element					Blood level		Immunostaining		Tumor volume (cm^3)	EWSR1 rearrangement
	Age (years)	Sex	Origin	Site	Current disease status	ProGRP (pg/mL)	NSE[a] (ng/mL)	CD99	ProGRP		
1	10	M	Bone	Pelvis	DOD (39 M)	18.6	***17.2***[a]	+	–	168	Break apart
2	13	M	Bone	Chest wall	CDF (61 M)	22.7	***19.6***[a]	+	–	11	No valid data
3	15	M	Soft	Nasal cavity	CDF (38 M)	17.8	***17.6***[a]	+	–	88	Break apart
4	63	F	Soft	Shoulder	DOD (10 M)	12.9	***43.1***[a]	+	–	289	Break apart
5	58	F	Bone	Pelvis	NED (41 M)	***153***	12.2*	+	+		Not confirmed
6	29	M	Soft	Mediastinum	DOD (12 M)	***240***	***18.5***[a]	+	–	199	EWS-FLI1
7	19	M	Soft	Chest wall	DOD (17 M)	***215***	25	+	+	98	No valid data
8	23	M	Soft	Mediastinum	CDF (111 M)	***96***	***12***	+	–	219	EWS-FLI1
9	51	F	Bone	Face bone	DOD (35 M)	***155***	***15***	+	+	73	No valid data
10	45	F	Soft	Chest wall	DOD (49 M)	***683***	***32***[a]	+	+	406	Break apart
11	29	M	Bone	Scapula	DOD (17 M)	***1720***	***120***	+	+	1128	Break apart
12	55	M	Soft	Retroperitoneum	DOD (3 M)	27.3	***230***	+	–	767	Break apart
13	17	F	Soft	Chest wall	CDF' (124 M)	22.2	***12***	+	–	17	Break apart
14	42	F	Soft	Chest wall	CDF (116 M)	3.2	***23***	+	–	330	Break apart
15	14	M	Bone	Femoral bone	CDF (21 M)	17.3	***48.6****	+	–	96	Break apart
16	21	M	Soft	Chest wall	CDF (13 M)	***110***	**44.9**[a]	+	+	171	Break apart

[a]Serum NSE measured by an ECLIA; in remaining seven patients measured by a RIA.

Numerals that are italic and in bold indicate values over the upper limit; Serum ProGRP >46 pg/ml or NSE (ECLIA) >16.3 ng/ml, NSE (RIA) >10 ng/ml

NSE neuron-specific enolase, *ProGRP* pro-gastrin-releasing peptide, *ECLIA* electro-chemiluminescent immunoassay, *RIA* radioimmunoassay

male with LCH of the scapula and a 53-year-old male with MPNST in the forearm; ProGRP levels of these patients were 50.4 and 68.8 pg/mL, respectively (Fig. 1 and Table 3). This difference was statistically significant (Fisher's exact test, $p < 0.05$). The sensitivity of the ProGRP elevation in the ESFT patients was 50%, and the specificity was as high as 95%.

Since ProGRP is a tumor marker for SCLC, we compared the ProGRP levels of the patients with thoraco-pulmonary ESFT and that of those with ESFT of other locations. Three out of eight patients with thoraco-pulmonary ESFTs and 5 with ESFTs at other locations showed elevated ProGRP levels (Fig. 2). In terms of origin, the ProGRP levels were elevated in 3 of 6 patients with ESFTs of bone origin, and in 5 of 10 patients with ESFTs of soft tissue origin (Fig. 3). These results suggest that ESFTs of bone and non-thoracic origin produce ProGRP.

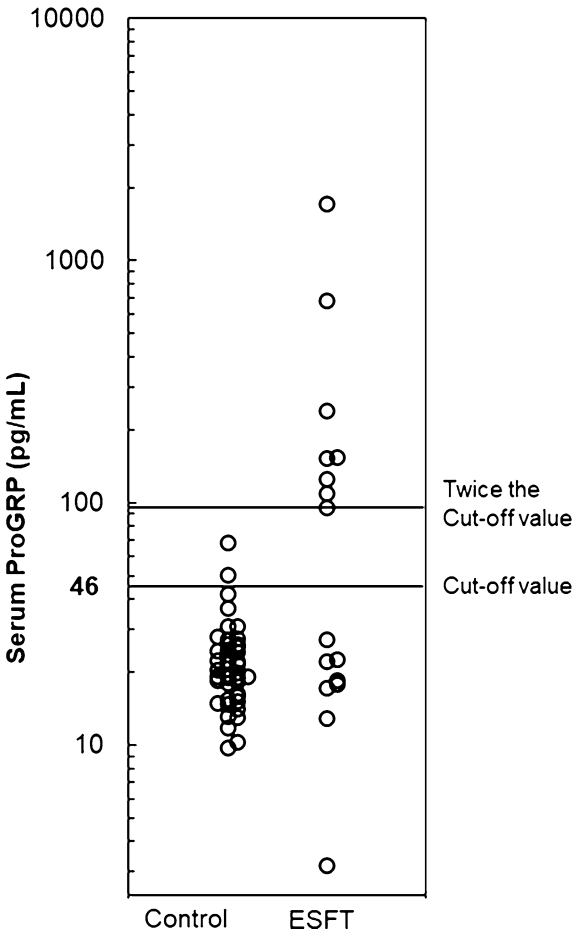

Fig. 1 Serum ProGRP levels of both ESFT patients and control patients. *ProGRP* pro-gastrin-releasing peptide, *ESFT* Ewing sarcoma family of tumors

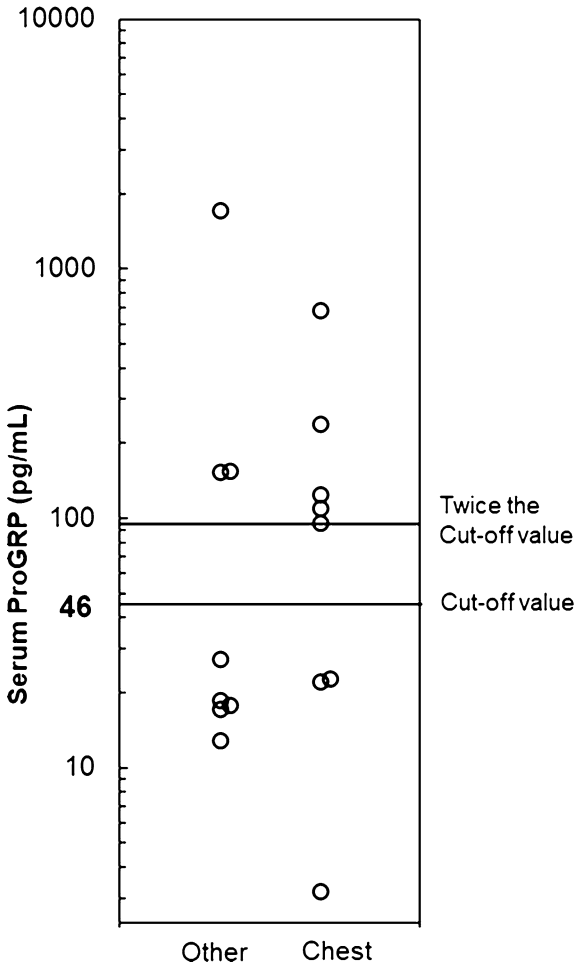

Fig. 2 Serum ProGRP levels of ESFT patients, as classified according to tumor origin. *ProGRP* pro-gastrin-releasing peptide, *ESFT* Ewing sarcoma family of tumors

Serum NSE levels

Serum NSE levels were elevated in 14 of the 16 ESFT patients and 8 of the 42 patients in the control group. This difference was also significant (Fisher's exact test, $p < 0.05$). The mean serum NSE values in the ESFT group were 60.3 pg/mL and 28.1 pg/mL by RIA and ECLIA, respectively; NSE levels were 12.7 pg/mL (ECLIA) in the control group. Although the sensitivity of NSE elevation in the ESFT group was higher (88%) than that of ProGRP elevation (50%), the extent of NSE elevation in the ESFT patients was much lower than ProGRP elevation. Only six of 14 patients with elevated NSE levels, had values that were more than twice as high as the cutoff value (Fig. 4).

Tumor volume and serum ProGRP and NSE levels

In one patient (case 5), the tumor volume was not measured since the patient had undergone decompression

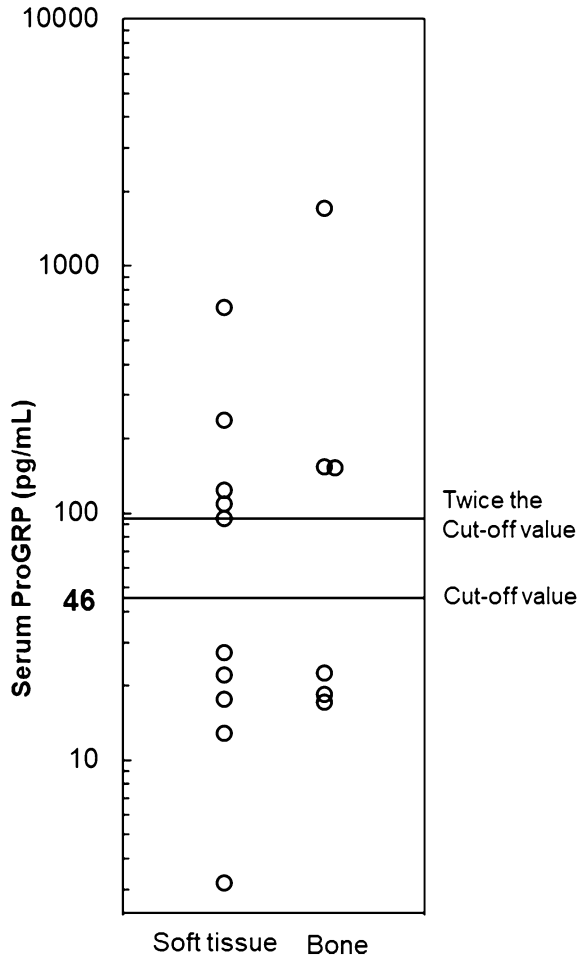

Fig. 3 Serum ProGRP levels of ESFT patients, as classified according to location. *ProGRP* pro-gastrin-releasing peptide, *ESFT* Ewing sarcoma family of tumors

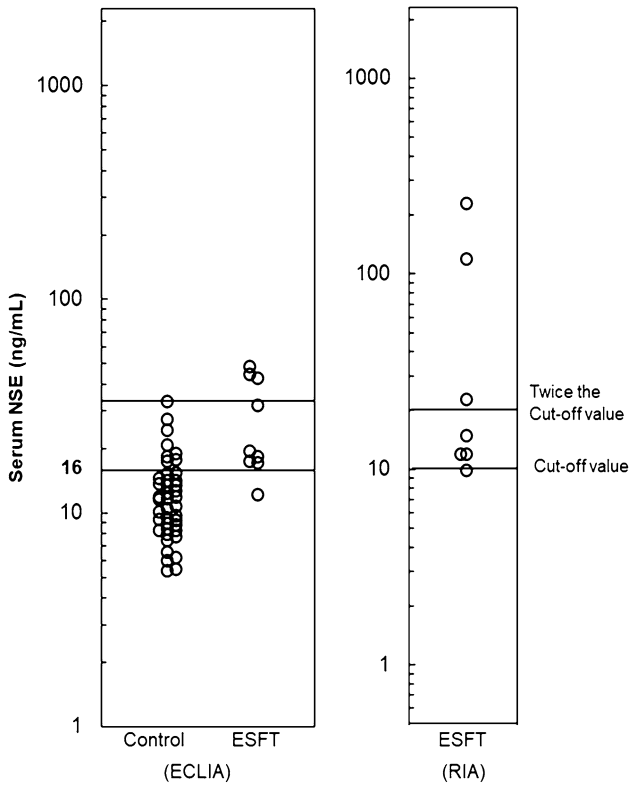

Fig. 4 Serum NSE levels of both ESFT patients and control patients, as measured by ECLIA, with serum NSE levels of the ESFT patients being measured by RIA. *NSE* neuron-specific enolase, *ESFT* Ewing sarcoma family of tumors, *ECLIA* electro-chemiluminescent immunoassay, *RIA* radioimmunoassay

surgery for sacral ESFT at another hospital, which resulted in an unclearly delineated tumor border on the image for postoperative effusion or edema. The correlation between tumor volume and serum ProGRP levels is shown in Fig. 5. In 7 patients with elevated initial serum ProGRP levels, the pre-treatment tumor volume was positively correlated with ProGRP levels (coefficient of correlation, $R = 0.99$). The remaining 8 patients showed no ProGRP elevation, irrespective of tumor volume.

As for the relationship between pre-treatment tumor volume and NSE levels, the coefficient of correlation, as measured by RIA and ECLIA, was 0.79 and 0.26, respectively. The pre-treatment tumor volume was positively correlated with NSE levels as measured by RIA, but not ECLIA (Fig. 6).

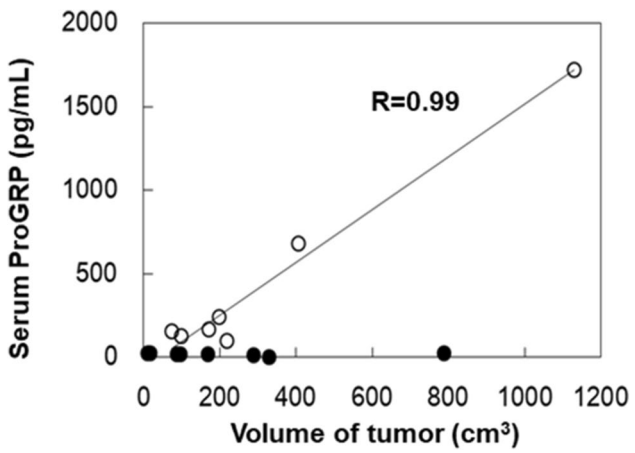

Fig. 5 Correlation between tumor volume and serum ProGRP levels before treatment, (empty circle) ProGRP values of patients with ProGRP levels above the cutoff value, (filled circle) ProGRP values of patients with no ProGRP elevation. *ProGRP* pro-gastrin-releasing peptide

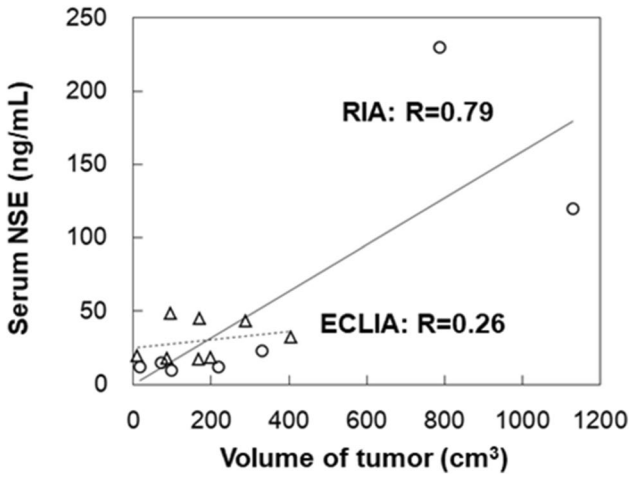

Fig. 6 Correlation between tumor volume and serum NSE levels, as measured by RIA (circle) or ECLIA (triangle), before treatment. *NSE* neuron-specific enolase, *ECLIA* electro-chemiluminescent immunoassay, *RIA* radioimmunoassay

ProGRP level during the course of treatment

We measured ProGRP levels more than once over the course of treatment and/or during the follow-up in 6 of 8 patients with initially elevated ProGRP levels. Currently, the disease status of these six patients was complete remission (CR) in one patient, no evidence of disease (NED) in two, and death due to progressive disease (DOD) in three. In the three patients with CR and NED, elevated pre-treatment ProGRP levels returned to normal post-treatment. In patients with DOD status, the ProGRP levels fluctuated with tumor size, as indicated on CT.

Case presentation

Case 1

A 21-year-old man presented with a mass in the anterior chest wall. A CT image revealed a subcutaneous tumor in his chest wall (Fig. 7). Needle biopsy was performed, and the tumor was diagnosed as ESFT, pathologically. His initial plasma ProGRP level was elevated, at 169 pg/mL. After diagnosis, six courses of preoperative chemotherapy, composed of vincristine, doxorubicin, and cyclophosphamide alternating with ifosfamide and etoposide (VDC/IE) were administered. After two courses of chemotherapy, the tumor shrunk and the plasma ProGRP level decreased to a normal value. After six courses of chemotherapy, surgery was performed, and 2 weeks later, the administration of postoperative chemotherapy was resumed. The patient is disease-free and the plasma ProGRP level continued to be within the normal range.

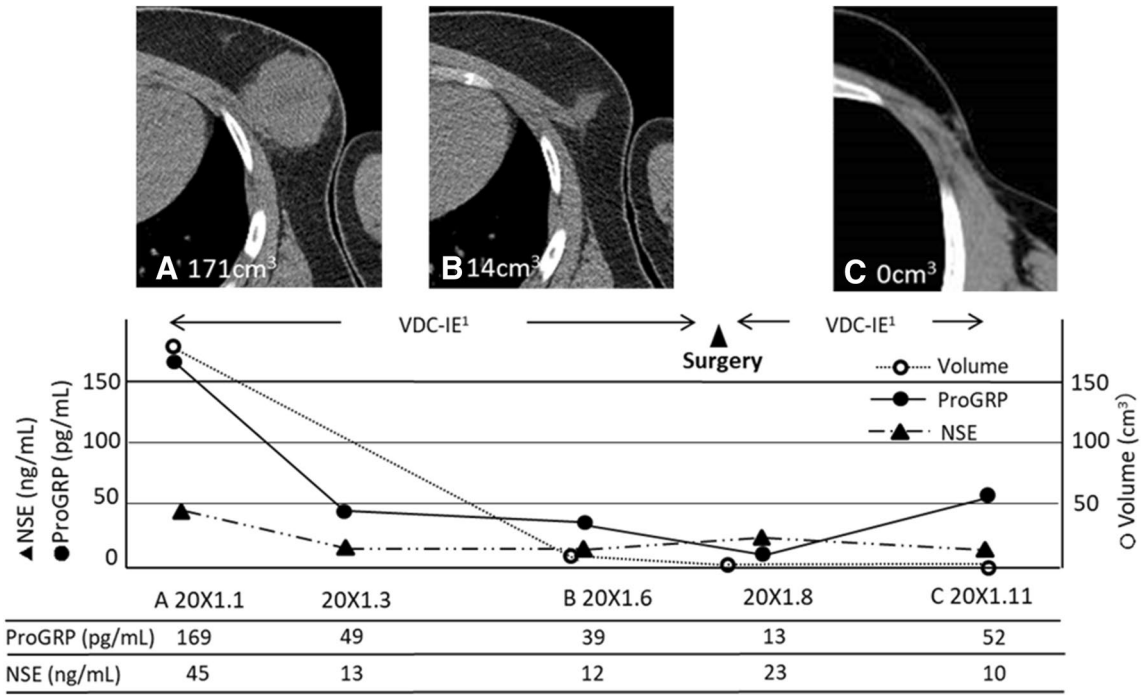

Fig. 7 Changes in tumor volume on enhanced CT imaging, ProGRP levels, and NES levels, over the course of treatment for a patient with ESFT of the thoracic cavity (Patient No. 16; Table 3).[1]VDC-IE: vincristine (1.5 mg/m^2), CPA (1200.0 mg/m^2), adriamycin (75.0 mg/m^2), etoposide (100.0 mg/m^2), ifosfamide (1.8 g/m^2); with four courses administered preoperatively and three postoperatively. *ProGRP* progastrin-releasing peptide, *NSE* neuron-specific enolase, *CT* computed tomography, *ESFT* Ewing sarcoma family of tumor

Case 2

A 45-year-old woman was referred to our hospital because of the presence of an abnormal shadow on her chest X-ray image. A CT image revealed the presence of pleural effusion and an intrathoracic tumor, which was diagnosed as ESFT according to needle biopsy (Fig. 8). The plasma ProGRP levels before treatment were elevated (1700.0 pg/mL). First, 5 courses of chemotherapy, comprising vincristine, actinomycin D, ifosfamide, and adriamycin (VAIA) were administered, followed by seven courses of chemotherapy consisting of ifosfamide, carboplatin, and etoposide (ICE). Simultaneously, she underwent proton beam radiotherapy (dose 60 Gy). The tumor shrunk and serum ProGRP levels decreased to 136 pg/mL. As abnormal uptake on fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) almost completely resolved, we considered this as metabolic CR, and chemotherapy was interrupted. Eight months later, a CT image revealed the presence of multiple lung and lymph node metastases. Although chemotherapy was performed, the serum ProGRP level gradually increased and the tumor increased in size. Finally, the serum ProGRP levels reached 1570.0 pg/mL and the tumor volume exceeded that at the initial diagnosis.

Immunohistochemical staining of ProGRP

Positive immunostaining for ProGRP was observed in 6 of the 16 ESFT cases, with all these cases showing elevated serum ProGRP levels (Table 3). Positive immunostaining disappeared by antigen-preabsorption. The immunostaining distribution was predominantly focal and irregular, and immunoreactivity appeared most often as a perinuclear dot-like pattern (Fig. 9). In the control group, ProGRP immunostaining was negative in all but one case that showed weak-positive staining. These results indicate that the ProGRP immunoreactivity in ESFT tissues is a product of the tumor cells, suggesting that elevated serum ProGRP levels are derived from the ProGRP immunoreactivity produced by these tumor cells.

Discussion

Serum ProGRP elevations are specific to SCLCs, pulmonary carcinoid tumors and several types of neuroendocrine tumors [10]. In Japan, ProGRP assay was approved by the government in 1995 and is widely used by clinicians. Over the past 20 years, extremely high ProGRP levels have been observed in some patients with large ESFTs of the chest or pelvis [12, 13]. Accordingly, we performed a preliminary study to evaluate if ProGRP could be a tumor marker for

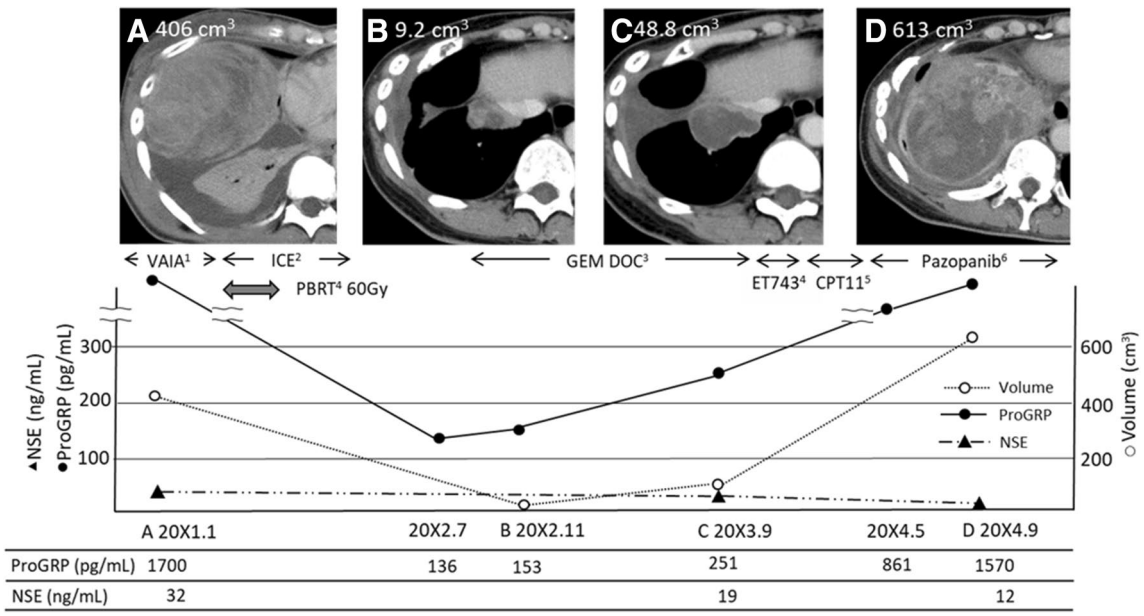

Fig. 8 Changes in tumor volume on enhanced CT imaging, ProGRP levels, and NES levels, over the course of treatment for a patient with ESFT of the thoracic cavity (patient no. 10; Table 3). [1]VAIA: vincristine (1.0 mg/m^2), actinomycin-D (0.36 mg/m^2), ifosfamide (2.0 g/m^2), doxorubicin (20.0 mg/m^2); 5 courses. [2]ICE: ifosfamide (1.5 g/m^2), carboplatin (390.0 mg/m^2), etoposide (75.0 mg/m^2); 7 courses. [3]GEM-DOC: gemcitabine (900.0 mg/m^2), docetaxel (70.0 mg/m^2); 14 courses. [4]ET473: trabectedine (1.2 mg/m^2); 2 courses. [5]CPT11: irinotecan hydrochloride (100.0 mg/m^2); 8 courses. [6]Pazopanib: (600.0 mg/day). [7]PBRT: Proton beam radiotherapy. *ProGRP* progastrin-releasing peptide, *NSE* neuron-specific enolase, *CT* computed tomography, *ESFT* Ewing sarcoma family of tumor

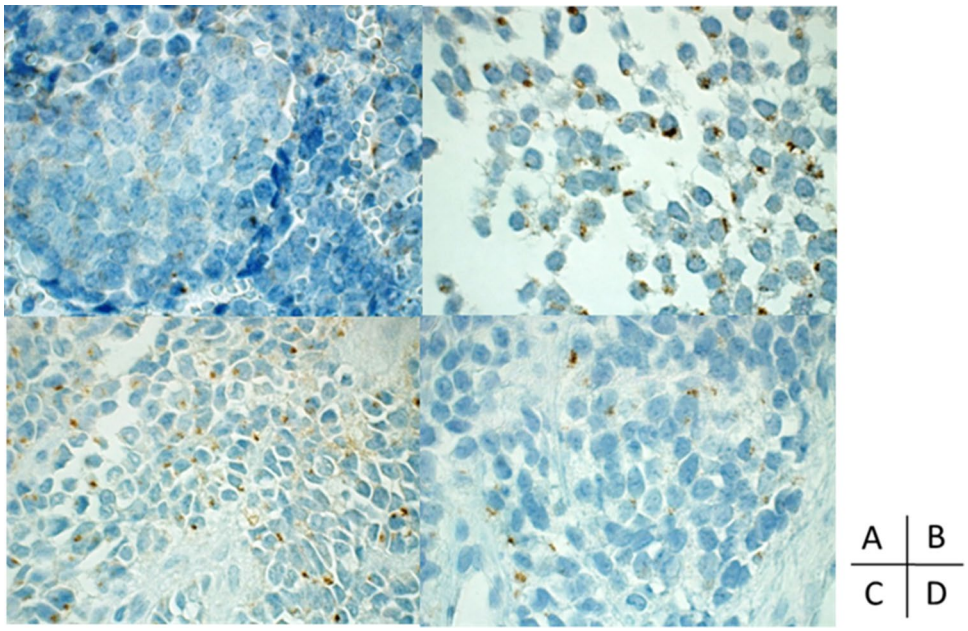

Fig. 9 Histological image of ESFT specimens positive for ProGRP according to immunostaining. Patient number and serum ProGRP levels: **a** (no. 5, 153.0 pg/mL), **b** (no. 10, 683.0 pg/mL), **c** (no. 11, 1720.0 pg/mL), **d** (no. 14, 110.0 pg/mL)

ESFT patients and found that serum ProGRP could serve as a tumor marker for ES [14]. In the present study, we analyzed a larger series of patients with ESFTs and other bone and soft tissue tumors that require a differential diagnosis. To confirm the reliability of ProGRP as a tumor marker, we monitored the ProGRP levels in ESFT patients during the course of treatment.

In this series, we found that 50% of the patients with ESFT had elevated serum ProGRP levels; this clearly indicates that ESFT should be added to the list of diseases associated with serum ProGRP elevation. ProGRP elevation has not been known to occur in ESFTs. Therefore, in lung cancer clinics, some ESFT patients with tumors arising around the thoracic region, such as Askin's tumors, may be misdiagnosed as SCLC based on their serum ProGRP elevation. Since serum ProGRP levels are not elevated in other types of bone and soft tissue malignancies that resemble ESFT, either histologically or radiologically, their determination may be useful for the differential diagnosis of ESFT.

Generally speaking, ESFT is a chemo-sensitive sarcoma. In 6 of 8 patients with elevated ProGRP levels, the levels were monitored during the course of treatment. All these patients showed a decrease in the ProGRP level, and this was well-correlated with tumor volume. In 2 out of 3 patients with tumor re-growth, the ProGRP levels initially decreased to the normal range after effective treatment, but the levels increased as the tumor grew. In a previous study of SCLC, it was reported that the ProGRP levels increased approximately 1 month before the detection of relapse [20]. These results suggest the ProGRP could be useful not only in evaluating treatment response, but also in early recurrence detection.

Until recently, immunohistochemical staining for ProGRP has not been performed in any case report describing serum ProGRP elevation of ESFT patients [12, 13]. In this study, ProGRP immunohistochemical staining was positive in 6 of 8 ESFT patients with elevated ProGRP levels, none of the ESFT patients without ProGRP elevation, and none of those with other bone and soft-tissue tumors. These results indicate that serum ProGRP is produced by neoplastic cells, and that immunostaining for ProGRP is useful for the diagnosis of ESFT in some cases.

Serum NSE is also known to be a tumor maker of SCLC. Serum NSE levels are elevated in about 60% of all SCLC patients, which reflects therapeutic effect. However, serum NSE levels are also elevated in some other diseases, including other neuroendocrine-derived tumors such as neuroblastomas and medullary carcinomas of the thyroid, as well as some types of sarcoma [20]. In current study, the sensitivity of serum NSE elevation was 88%, and the specificity was 81%. In the case of serum ProGRP, the sensitivity was 50% and specificity was 95%. While it appears that the sensitivity of NSE is better than that of ProGRP, the elevation range of serum NSE levels in 8 of 14 ESFT patients was slight and under twice the cutoff value. Meanwhile, all eight ESFT patients with elevated ProGRP had levels that were more than twice as high as the cutoff value, suggesting the clarity and superiority of ProGRP for diagnosis and treatment monitoring. This is in line with the findings of a previous study of SCLC, in which the degree of serum NSE elevation was reportedly smaller than that of ProGRP elevation; consequently, serum NSE levels are not useful in the early detection of

SCLC [20]. Our study's findings suggest that the results of the aforementioned study can be applied to ESFT as well.

We considered there would be no evident interaction between proGRP and NSE levels in ESFT. The reasons are first, that in those who had elevated proGRP level, the mean NSE level was 2.9 times as high as the upper limit, whereas in those without elevated proGRP level, the mean NSE level was 4.4 times as high as the upper limit. Second, among the 5 patients in whom proGRP and NSE level could be measured during the course of treatment, only one patient (case 16 in Table 3) showed a parallel change in proGRP and NSE levels. Third, in a patient who showed an extremely high NSE level (case 12 in Table 3), the ProGRP level was not elevated (27 pg/mL).

Several decades ago, gastrin-releasing peptide (GRP), an active form of ProGRP, was proposed to function as an autocrine growth factor for SCLC; several lines of evidence support this. First, a high frequency of human SCLCs produce GRP. Second, exogenous GRP stimulates cellular growth and DNA synthesis of SCLC cells in vitro. Third, SCLC cell lines sometimes express high-affinity receptors for GRP. Fourth, a GRP-specific monoclonal antibody inhibits the clonal growth of SCLC cells in vitro and the tumor growth of SCLC xenografts in vivo [21]. In the case of Ewing sarcoma, it was recently reported that cancer cells produce GRP; therefore, it is essential to determine whether GRP functions as an autocrine growth factor. GRP receptor is one of the classes of G protein-coupled receptors. Recent progress in cancer research revealed that G protein-coupled receptors, which contain seven transmembrane regions, play a role in oncogenesis [22]. Nonetheless, it is still possible that a GRP-related autocrine mechanism also plays a role in the growth of SCLC.

Based on these lines of evidence, there have been approaches to inhibit the autocrine effect of GRP on tumor growth in human and/or animal studies; these approaches include receptor antagonists, monoclonal antibodies, vaccination against GRP, antisense oligonucleotides, or bispecific molecules [23]. In a phase II clinical trial to treat 13 cases of SCLC using GRP monoclonal antibodies conducted at the US National Cancer Institute, there was one case of complete response and four cases of stable disease [24]. This suggests that similar to the attention given to treatment of SCLC using GRP monoclonal antibodies, inhibition of GRP will receive increased attention as a possible approach for the development of novel treatments in ESFTs.

The present study has several limitations. First, this was a retrospective study. Second, the sample size was small, owing to the rarity of ESFT occurrence and the single-center study design. Second, the correlation between ProGRP levels and clinical features such as tumor location, prognosis or chromosomal translocations was unclear. To draw definite conclusions with regard to the rate of plasma ProGRP elevation among ESFT patients, as well as to clarify the above-mentioned relationships, it is important to conduct a prospective, multi-institutional study with a larger study population. Third, this study excluded children younger than 10 years, although this demographic comprises 17% of all ESFT patients [25]. This is because serum ProGRP levels are reported to be high in children younger than 5 years [15]. Therefore, serum ProGRP levels cannot be reliable markers, especially in pediatric patients below 5 years of age.

In conclusion, in the current study, serum ProGRP elevations were observed in 50% of the ESFT cases with a remarkably high specificity. The ProGRP elevation range was much larger than the NSE elevation range. Pre-treatment tumor volume was significantly correlated to ProGRP levels, and serum ProGRP levels were clearly correlated to therapeutic responses. Therefore, ProGRP could serve as a useful tumor marker for ESFT in terms of diagnosis, response evaluation, and patient follow-up.

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Compliance with ethical standards

Conflict of interest Dr. Ken Yamaguchi is the inventor of the ProGRP assay system, and he holds patents on this method. He receives honoraria for patent royalties from the Advanced Life Science Institute, Inc. (Saitama, Japan), a company distributing reagents to several in vitro diagnostics companies, for constructing the assay system. None of other authors have any conflict of interest to declare.

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