ProGRP is a possible tumor marker for patients with Ewing sarcoma

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ABSTRACT

We analyzed serum ProGRP levels in patients with Ewing sarcoma, and found that 5 out of 9 patients had elevated levels; the values range equally with those of patients with limited disease of small-cell lung carcinoma. Serum ProGRP levels in patients with bone and soft tissue malignancies other than Ewing sarcoma are not elevated. Immunohistochemical studies demonstrated that ProGRP-like immunoreactivities were detected in Ewing sarcoma tissues obtained from 2 patients with elevated serum ProGRP levels, suggesting that ProGRP is a product of tumor cells of Ewing sarcoma. These results indicate that serum ProGRP could serve as a specific tumor marker for Ewing sarcoma. Since ProGRP is a major hormonal product of tumor cells of small-cell lung carcinoma, a typical neuroendocrine carcinoma, it is reasonable to postulate that the present study provides an evidence for Ewing sarcoma to possess neuroendocrine differentiation.

Ewing sarcoma is a clinical entity of aggressive bone and soft tissue malignancies that occur predominantly in children, adolescents and young adults (3, 5). This morbidity is also characterized by a genetic translocation that fuses *EWSR1* (22q12) to *FLII* (11q24) in approximately 85% of cases or in the remaining cases, to a member of the *ETS* transcription factor family other than *FLII* (11). During the process of searching for specific genes activated by *EWS/ETS* chimeric transcription factors, Lawlor *et al.* (6) found that Ewing sarcoma frequently expressed gastrin-releasing peptide (GRP) gene. Although they concluded that GRP gene expression was not directly activated by the fused gene, and that GRP gene expression could reflect the cellular

differentiation of these tumors, they also postulated that GRP could be a useful marker for patients with Ewing sarcoma by quoting our study on small-cell lung carcinoma (7).

In 1982, we firstly reported that GRP-like immunoreactivity was frequently produced by small-cell lung carcinoma cells (12, 13), and that the determination of plasma GRP levels could serve as a useful tumor marker for small-cell lung carcinoma patients (7); however, instability of GRP in blood made it difficult to develop a clinically applicable system for the measurement of plasma GRP. In 1994, we developed an improved immunoassay to measure a precursor form of GRP, pro-gastrin-releasing peptide (ProGRP), and demonstrated that ProGRP could serve as a reliable tumor marker for small-cell lung carcinoma (8). Soon after, enzyme-linked immunosorbent assay for ProGRP was approved by the Japanese Government for clinical use, which is now available worldwide (2, 14). Until now, however, the study has not yet been done to know whether

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Table 1 Clinical, pathological and tumor marker data of the patients examined

Patient No.	Age	Sex	Pathological diagnosis	Immunostaining for CD99	Origin and site of tumor	Tumor status	Serum levels of	
							ProGRP (pg/mL)	NSE (ng/mL)
1	10	Male	EWS	+	Bone (pelvis)	Primary	18.6	13.6
2	13	Male	EWS	+	Bone (chest wall)	Primary	22.7	17.4
3	51	Female	EWS	NE	Bone (face)	Relapse	155	15
4	58	Female	EWS	++	Bone (pelvis)	Primary	153	11.8
5	15	Male	EWS	+	Soft tissue (nasal cavity)	Primary	17.8	14.8
6	19	Male	EWS	+	Soft tissue (thorax)	Relapse	125	9.9
7	23	Male	EWS	++	Soft tissue (mediastinum)	Primary	96	12
8	45	Female	EWS	+	Soft tissue (chest wall)	Primary	683	29.2
9	63	Female	EWS	++	Soft tissue (shoulder)	Primary	12.9	36.2
							≤46.0*	≤16.3**

EWS, Ewing sarcoma; NE, not evaluated; *normal range of ProGRP; **normal range of NSE.

ProGRP could serve as a tumor marker for the patients with the Ewing sarcoma, except for a few case reports indicating serum ProGRP elevation (10, 15). In the present study, we evaluate serum ProGRP levels in an appreciable number of patients with Ewing sarcoma. The research plan was designed according to the Ethical Guideline for Clinical Research in Japan and was approved by the Institutional Review Board of the Shizuoka Cancer Center. All data and samples were obtained in routine clinics or from stored samples obtained with informed consent.

Serum ProGRP levels were evaluated by the method reported previously (2, 14), with fully automated chemiluminescent enzyme immunoassay system (LUMIPULSE Presto II, Fujirebio). We evaluated serum ProGRP levels in 9 patients with Ewing sarcoma (Table 1). The ages of all patients are greater than 10, and all were pathologically diagnosed as Ewing sarcoma. All tissue specimens examined showed positive immunostaining for CD99 (Cluster of differentiation 99), a heavily O-glycosylated transmembrane protein, which is frequently expressed in tumor tissues of Ewing sarcoma; this finding indicates that the pathological diagnosis of Ewing sarcoma could be appropriate. In 4 cases, the tumors were bone origin, and the remaining 5 were soft tissue origin. In 7 out of 9 patients, serum levels of ProGRP and NSE were analyzed at the time of initial diagnosis, and in the remaining 2, they were evaluated at the time of relapse. Serum ProGRP levels were high in 5 out of 9 patients, and serum NSE levels were high in 3 patients.

To know whether serum ProGRP elevation is specific for Ewing sarcoma or not, we examined 16 patients with bone and soft tissue malignancies other

than Ewing sarcoma, including osteosarcoma, rhabdomyosarcoma and diffuse large B-cell lymphoma. Furthermore, 31 patients with small-cell lung carcinoma, 12 with the stage of limited disease and 19 with that of extensive disease, were also analyzed for the comparison of serum ProGRP levels. All of these patients were treated at the Shizuoka Cancer Center Hospital, and serum NSE levels were also evaluated in all samples. In the case of Ewing sarcoma and bone and soft tissue malignancies other than Ewing sarcoma, serum samples stored in the Biobank of the Shizuoka Cancer Center Hospital were analyzed, and in the case of small-cell lung carcinoma, serum levels of ProGRP and NSE were examined in the routine clinics for the diagnosis of lung cancer.

Serum ProGRP and NSE levels in patients with Ewing sarcoma, other bone and soft tissue malignancies and small-cell lung carcinoma are demonstrated in Fig. 1. In patients with Ewing sarcoma, 5 out of 9 had elevated serum ProGRP levels, and the levels were not so much different when compared with those of limited disease of small-cell lung carcinoma patients. The levels of ProGRP in patients with bone and soft tissue malignancies other than Ewing sarcoma were in the normal range. These results indicate that serum ProGRP could serve as a reliable tumor marker for a part of patients with Ewing sarcoma. Meanwhile, serum NSE levels were not likely to become a reliable tumor marker.

Immunohistochemical studies were performed using tumor tissues obtained from Ewing sarcoma and rhabdomyosarcoma for the cellular location of ProGRP-like immunoreactivity. Briefly, tissue sections of 3-μm thick processed for immunohistochemistry were incubated with mouse anti-ProGRP(31–98)

Fig. 1 Serum ProGRP (A) and NSE (B) levels and frequencies of their elevation in patients with tumors belonging to Ewing sarcoma, other bone and soft tissue malignancies and small-cell lung carcinoma.

monoclonal antibody PGCY9 (1 : 400 in dilution), which was kindly provided by Advanced Life Science Institute, Inc. (Saitama, Japan). Following the peroxidase reaction, the existence of specific immunostaining was evaluated.

As shown in Fig. 2A and 2B, the anti-ProGRP monoclonal antibody demonstrated positive results in small-cell lung carcinoma and pulmonary tumorlet. When antigen-preabsorbed antiserum was used, positively stained cells disappeared. In 2 cases of Ewing sarcoma with elevated serum ProGRP levels (Patient 6 and 8 in Table 1), positive immunostaining was observed (Fig. 2C and 2D), and it disappeared by antigen-preabsorption. The distribution of immunostaining was irregular, and the immunoreactivity appeared most often as a perinuclear dot-like pattern. Meanwhile, when tumor tissues from 2 patients of Ewing sarcoma with normal serum ProGRP levels (patients 1 and 9 in Table 1) were examined, negative results of ProGRP immunostaining were obtained. Furthermore, in two patients with rhabdomyosarcoma, the results were also negative. These results indicate that ProGRP immunoreactivity is a product of tumor cells of Ewing sarcoma, and suggest that elevated serum ProGRP levels are derived from ProGRP immunoreactivity produced by tumor cells. This finding also provides information to the histogenesis of Ewing sarcoma. Since the first de-

scription of this clinical entity by Ewing J in 1921 (4), there have been numerous discussions on the origin of Ewing sarcoma. The term “sarcoma” means that the tumor is mesenchymal origin; however, recent studies using immunohistochemistry, molecular genetics and tissue culture indicate that the tumor shows various degrees of neuroectodermal differentiation (3). The present study supports neuroendocrine nature of Ewing sarcoma by demonstrating the production of ProGRP, a major hormonal product of tumor cells of small-cell lung carcinoma.

The present study demonstrated that Ewing sarcoma frequently produces ProGRP and appreciable number of patients had elevated serum ProGRP levels. Since serum ProGRP levels are not elevated in other types of bone and soft tissue malignancies, its determination is useful for differential diagnosis of Ewing sarcoma. However, further studies are required to know the difference of clinical and pathological features between Ewing sarcoma with elevated ProGRP levels and without.

For the clinical use of ProGRP, clinicians should keep in mind following facts. First, in the case of bone and soft tissue malignancies, serum ProGRP levels should be carefully evaluated in pediatric patients with the ages lower than 5. This is because we found that serum ProGRP levels were high in infants and children younger than 5-years-old (1). Second,

Fig. 2 Immunohistochemical studies for ProGRP with anti-ProGRP monoclonal antibody PGY9. A, small-cell lung carcinoma; B, pulmonary tumorlet; C, Ewing sarcoma (Patient 6 in Table 1); D, Ewing sarcoma (Patient 8 in Table 1).

in lung cancer clinics, we insisted that the elevation of serum ProGRP was specific for small-cell lung carcinoma and pulmonary neuroendocrine tumors (14). However, the present study indicates that Ewing sarcoma should be added to the list of diseases showing serum ProGRP elevation.

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DISCLOSURE STATEMENT

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 Advanced Life Science Institute, Inc. (Saitama, Japan), a company distributing reagents to several in vitro diagnostics companies, for constructing the assay system.

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