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RAPID COMMUNICATION

Expression of Ets-1 proto-oncoprotein in gastrointestinal stromal tumors, leiomyomas and schwannomas

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Abstract

AIM: Gastrointestinal stromal tumors (GISTs) are rare. GISTs differ from other mesenchymal tumors of the gastrointestinal tract (e.g. leiomyomas and schwannomas). The purpose of this study was to investigate the role of Ets-1 in the growth and differentiation of GISTs.

METHODS: Twenty-eight GISTs, nine leiomyomas and six schwannomas were examined by immunohistochemical staining method for Ets-1 in this study. Specimens were selected from surgical pathology archival tissues at Nagasaki University Hospital.

RESULTS: Ets-1 protein was expressed in the cytoplasm of cells in all of these tumors. Immunohistochemical staining revealed that 27 GISTs (96.4%), six leiomyomas (66.7%), and five schwannomas (83.3%) were positive for Ets-1. Ets-1 expression was statistically different between GISTs and leiomyomas ($P < 0.005$). However, there was no correlation between Ets-1 expression and clinical risk categories.

CONCLUSION: Ets-1 plays an important role in the growth and differentiation of GISTs, leiomyomas and schwannomas.

Key words: Ets-1; GISTs; Leiomyoma; Schwannoma; Immunohistochemistry; Risk category

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are rare mesenchymal tumors of the gastrointestinal tract that may occur from the oesophagus to the anus, including the omentum. These tumors have a wide clinical spectrum from benign, incidentally detected nodules to frankly malignant tumors[1]. Small GISTs are often detected incidentally during surgery for other conditions, during gastroscopy, or on routine X-ray[1,2]. GISTs may present with bleeding, perforation, pain, obstruction or a combination of these symptoms[3-6]. The mechanisms of tumorigenesis, progression and differentiation of GISTs are unknown. Traditionally, all primary mesnchymal spindle cell tumors of the gastrointestinal (GI) tract were uniformly classified as smooth muscle tumors (e.g., leiomyomas, cellular leiomyomas or leiomyosarcomas). Tumors with epithelioid cytologic features were designated leiomyoblastomas or epithelioid leiomyosarcomas[7]. Recently, Sircar *et al* postulated that GISTs origin-ate from Cajal cells in the gastrointestinal tract and differ from leiomyomas and schwannomas, which are of mesenchymal cell origin[8]. Cajal cells are thought to be gastrointestinal pacemaker cells that regulate intestinal motility[9]. GISTs are characterized by frequent expression of the bone marrow leukocytic progenitor cell antigen CD34[10] and the c-kit proto-oncogene[8,11,12]. Ets-1 was characterized originally as the v-ets retroviral gene, one of two oncogenes (v-myb and v-ets) of the avian leukemia retrovirus, E26[13]. The ets family of genes encodes transcription factors for mesodermal cell development during embryogenesis[14, 15].

Ets-1 plays a role in the regulation of physiological processes such as cell proliferation and differentiation[16]. Ets-1 also is expressed in astrocytes and vascular smooth muscle cells[17,18] but its expression has not been reported in Cajal cells. Increased Ets-1 expression was observed in several tumors in our previous studies[19-22]. We reported

Table 1 Ets-1 immunohistochemistry in intestinal stromal tumors. *n* (%)

	n	-	+	++
GISTs	28	1 (3.6)	4 (14.3)	23 (82.1)[a]
Leiomyomas	9	3 (33.3)	3 (33.3)	3 (33.3)
Schwannomas	6	1 (16.7)	1 (16.7)	4 (66.7)

[a]$P<0.005$ *vs* leiomyomas.

that Ets-1 is correlated with the progression of carcinoma cells of the stomach, pancreas, and thyroid and cells of astrocytic tumors[19-22]. These studies suggest that Ets-1 is involved in tumor growth and differentiation. However, there are no data concerning Ets-1 expression in GISTs, leiomyomas or schwannomas or the role of Ets-1 in the etiology of these tumors. The purpose of this study was to investigate the expression of Ets-1 in GISTs.

MATERIALS AND METHODS

Samples

A total of twenty-eight GISTs included 24 cases from the stomach and four from the small intestine. Nine leiomyomas included four from the oesophagus, two from the stomach and three from the large intestine, and five schwannomas included four from the stomach and one from the large intestine. Specimens were selected from surgical pathology archival tissues at Nagasaki University Hospital between 1999 and 2004. The GISTs were 0.8 - 12.0 cm in diameter, the leiomyomas were 0.1 - 4.5 cm, and the schwannomas were 0.6 - 5.0 cm. In this study, GISTs were defined as expressing both c-kit and CD34 surface antigens. GISTs were classified by risk categories, mitosis counts and tumor size[23]. The number of mitoses was determined by counting 50 high-power fields (HPF, x400) in Nikon (Tokyo, Japan) E400 microscope. Leiomyomas were defined as expressing α-smooth muscle actin (SMA) but not c-kit, CD34 and S100-protein. Schwannomas were defined as expressing S100-protein but not c-kit, CD34 and SMA. Tumor identification/classification was determined by two independent pathologists (T. Nakayama and I. Sekine).

Immunohistochemical staining

The subcellular localization of Ets-1 was determined in GISTs using a monoclonal antibody directed against the unique middle sequence of Ets-1 and this antibody was devoid of any cross-reaction with other proteins in the Ets family. Formalin-fixed and paraffin-embedded specimens were cut into 4 μm thick sections, deparaffinized and preincubated with normal bovine serum to prevent non-specific binding. The sections were incubated overnight at 4°C with the primary monoclonal antibody to human Ets-1 (1 g/L; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and then with a horseraddish peroxidase conjugated goat anti-mouse IgG antibody (0.4 g/L; Santa Cruz Biotechnology, Santa Cruz, CA). The reaction products were resolved using diaminobenzidine (DAB; DAKO Ltd., Glostrap, Denmark). Negative controls involved replacing the primary antibody with non-immunized mouse serum and human gastric cancer tissue served as the positive control[19]. Ets-1 expression was classified into three categories depending upon the percentage of cells stained and/or the intensity of staining: -, 0% to 10% tumor cells positive; +, 10% to 50% tumor cells positive; and ++, >50% tumor cells positive.

Table 2 Ets-1 immunohistochemistry and risk categories in GIST. *n* (%)

	n	-	+	++
Total	28	1 (3.6)	4 (14.3)	23 (82.1)
Risk categories				
High	4	0 (0.0)	0 (0.0)	4 (100.0)
Intermediate	5	0 (0.0)	0 (0.0)	5 (100.0)
Low	14	1 (7.1)	3 (21.4)	10 (71.4)
Very low	5	0 (0.0)	1 (20.0)	4 (80.0)
Mitosis counts (per 50 HPF)				
<2	14	1 (7.1)	3 (21.4)	10 (71.4)
2-5	6	0 (0.0)	1 (1.7)	5 (83.3)
6-10	3	0 (0.0)	0 (0.0)	3 (100.0)
10<	5	0 (0.0)	0 (0.0)	5 (100.0)
Tumour size (cm)				
<2	5	0 (0.0)	1 (20.0)	4 (80.0)
2-<5	18	1 (5.6)	3 (16.7)	14 (77.8)
5-<10	4	0 (0.0)	0 (0.0)	4 (100.0)
10<	1	0 (0.0)	0 (0.0)	1 (100.0)

Statistical analysis

The Stat View II program (Abacus Concepts, Inc., Berkeley, CA) was used for statistical analyses. Analyses comparing the degree of Ets-1 expression in GISTs, leiomyomas and schwannomas were performed using the Mann-Whitney's test.

RESULTS

The results of immunohistochemical staining for Ets-1 are summarized in the Table 1. Ets-1 expression was heterogeneous in GISTs and localized to the cytoplasm (Figure 1). Twenty-three of the GISTs (82.1%) were strongly positive, four (14.3%) were positive and one (3.6%) was negative for Ets-1. Similarly, four of the schwannomas (66.7%) were strongly positive, one (16.7%) was positive and one (16.7%) was negative. However, only three of the leiomyomas (33.3%) were strongly positive, three (33.3%) were positive and three (33.3%) were negative. There is a statistical difference in Ets-1 expression between the GISTs and the leiomyomas ($P<0.005$). Positively stained cells, i.e. those classified as ++ or +, were found in 96.4% of the GISTs, 66.7% of the leiomyomas and 83.3% of the schwannomas. Ets-1 was expressed in the cytoplasm of cells in all three tumors. However, normal stromal cells and smooth muscle cells showed faint or focal positivity of expression. GISTs were classified by risk categories, mitosis counts and tumor size in Table 2. In risk categories, all nine cases of high and intermediate groups were strongly expressed Ets-1 protein. In mitosis counts, all eight cases with over 6 mitoses per 50 HPF strongly expressed Ets-1. In tumor size, all five cases with over 5 cm strongly expressed Ets-1. However, there was no correlation between

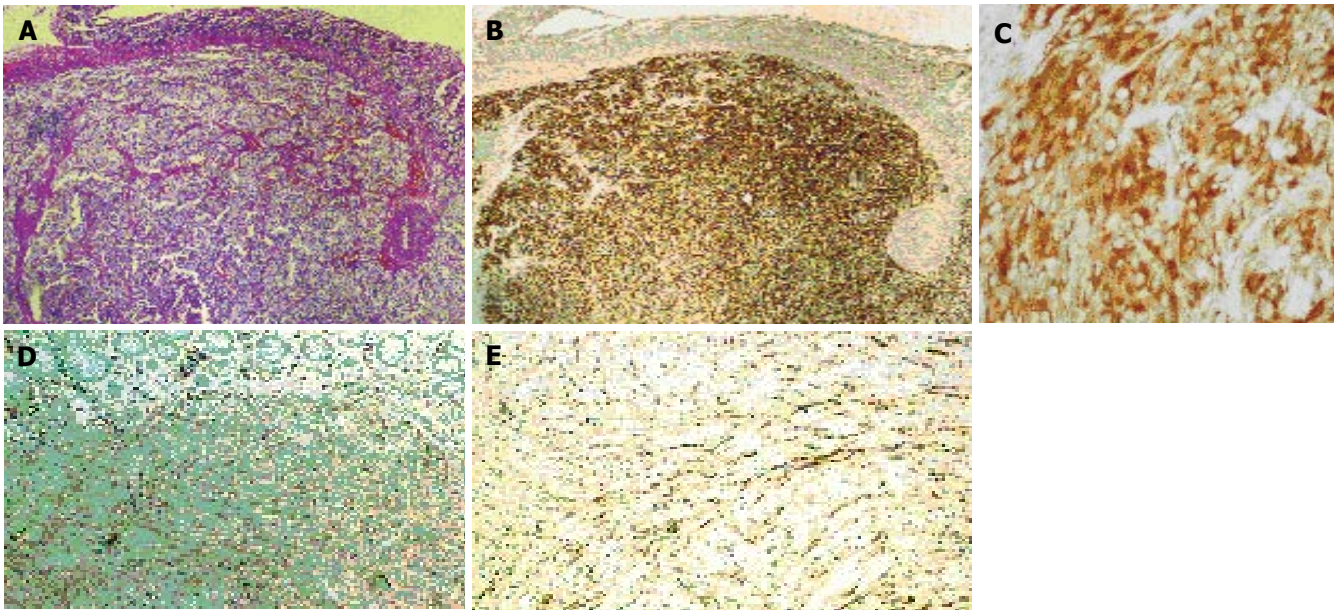

Figure 1 Ets-1 expression in GISTs (**A**-**C**), Leiomyomas (**D**) and Schwannomas (**E**). (magnification; **A**, **B**:x20, **C**: x 200, **D**, **E**: x100).

Ets-1 expression and each classification.

DISCUSSION

GISTs are known to originate from the Cajal cells of the neural crest[8] and schwannomas are thought to originate from the peripheral nerve sheath cell[24]. In this study, Ets-1 expression was higher in GISTs and schwannomas than in leiomyomas. Ets-1 expression has been reported in neural cells and astrocytes[22], but not yet in Cajal cells, cells that are all of neurons origin. Vascular smooth muscle cells also express Ets-1[18]. These findings suggest that Ets-1 may play a role in neural differentiation of intestinal stromal tumors. Previous studies have demonstrated Ets-1 expression in several tumors and normal stromal cells[19-22,25]. Furthermore, Ets-1 has been shown to play a role in the proliferation and/or differentiation of stromal cells[25]. We have shown already that Ets-1 may function as a growth factor in several tumors[19-22]. However, there have been no studies of Ets-1 expression in GISTs, leiomyomas and schwannomas, or of the potential role of Ets-1 in the growth of these tumors. Our results demonstrate substantial levels of Ets-1 expression in the cytoplasm of GIST, leiomyoma and schwannoma cells. These results suggest that Ets-1 may play a role in the growth and/or differentiation of intestinal tumors.

Ets-1 regulates the expression of many proteins, such as matrix metalloproteinases, urokinase type-plasminogen activator and parathyroid hormone-related peptide (PTHrP), which promote tumor growth and/or progression[26,27]. In our previous study, PTHrP and its receptor were found to be highly expressed in GISTs, leiomyomas and schwannomas[28]. Ets-1 may promote tumor growth and/or progression through regulating the expression of these proteins.

In recent studies, mutations affecting c-kit that cause constitutive tyrosine kinase activation have been shown to be important for the pathogenesis of GIST[29,30]. Joensuu *et al* reported a patient in whom STI-571 (imatinib, Gleevec), a tyrosine kinase inhibitor, was effective against a GIST[31]. And STI-571 has proven to be remarkably efficacious in heavily pretreated GISTs patients with advanced disease in phase II clinical trials[32]. The expression of the Ets family protein is upregulated by the activation of tyrosine kinase through the mitogen-activated protein kinase pathway[33]. Ets-1 expression may be upregulated by the c-kit/tyrosine kinase pathway.

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