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A Case for Gastrointestinal Stromal Tumor (GIST) with Reference to Its Ultrastructure and 'Gain-of-Function' Mutation

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A case for primary gastrointestinal stromal tumor (GIST) is described with reference to its ultrastructural characteristics and mutation within the exon 11 of c-*kit* gene. A forty-seven years old woman complaining of dysphasia was examined by endoscopy, which depicted a submucosal tumor (70 mm in diameter) with ulcerations at the fundus of the stomach. Histopathologically, the tumor cells had large nuclei and eosinophilic cytoplasm and were frequently during mitosis phase. The tumor cells were immunopositive for KIT, CD 34 and vimentin, suggesting their fibroblast-like characteristics. In contrast, desmin and S-100, a smooth muscle and an enteroglial marker, were not immunopositive within the cells. At least 30 % of the tumor cells possessed MIB-1 and 20 % of them possessed p53, which are compatible with fast development of the tumor. By electron microscopy, the tumor cells possessed large oval nuclei, abundant mitochondria, caveolae and smooth endoplasmic reticulums, while no gap junctions were seen on the cells: The tumor cells thus possessed interstitial cells-like characteristics at least in part. DNA mutation search for the tumor cells however realized no gain-of-function mutation within the exon 11 of the *c-kit* gene, suggesting existence of other mechanism for neoplasmic growth of the tumor cells classified as gastrointestinal stromal tumors.

Key words : gastrointestinal stromal tumor (GIST) c-*kit* interstitial cells of Cajal (ICC) ultrastructure 'gain-of-function' mutation

INTRODUCTION

The gastrointestinal stromal tumor (GIST) is a myogenic neoplasm, which originates from a kind of mesenchymal cell within the *tunica muscularis* of the alimentary tract. GIST usually possess the receptor tyrosine kinase KIT, which has an essential role for development and maintenance of the phenotype of the interstitial cells of Cajal (ICC) [1]. The expression of KIT is therefore a good marker for the ICC, and GIST is believed to have some relationships with the interstitial cells [2]. The ICC are specialized myofibroblast-like cells, which generate electrical slow wave activity and intercalate neuromuscular transmission and thus act as gastroenteric pacemaker. The morphological criterion to identify ICC still depends on electron microscopy, although ultrastructural examinations of GIST are few and whether GISTs resemble to the interstitial cells seems still not to be elucidated [3].

Another topic for the current concept for the GIST is 'gain-of-function' mutation in the c-*kit* gene, which encodes KIT [4]. A class of mutations in the c-*kit* gene in the GIST was recently reported with reference to a possible growth mechanism of the GIST. That is, KIT with the 'gain-of-function' mutation automatically increases receptor tyrosine kinase activity without its ligand, suggesting a possible mechanism of the neoplasmic growth

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of the tumor cells [4]. In this study, we report a case of the gastrointestinal stromal tumor, which showed rapid macroscopic changes, with reference to both ultrastructural characteristics of the tumor cells and mutation within c-*kit* gene. Our investigation realized that the tumor cell remained the morphological features of the interstitial cells by electron microscopy, although no 'gain-of-function' mutation was identified within the tumor region.

CASE PRESENTATION

The present examination including DNA mutation search within human tumor cells was permitted by the ethical committee and performed under a guideline for medical researches at Tokai University School of Medicine, Isehara, Japan.

A forty-seven years old female began to feel minor obstructive symptoms at around the stomach. Then she felt definite nocturnal epigastralgia and came to out patient clinic of our department. Screening gastrointestinal endoscopy identified a large protruding tumor with ulcerations at the gastric fundus (Fig. 1A): She subsequently admitted in our Hospital. Her past history and family history had no specific problems. Tables 1 and 2 shows the results of the physical examinations and laboratory findings from blood sample on the day of admission, which were hard to identify specialized problems.

Figures 2A-C shows enhanced roentgenog-

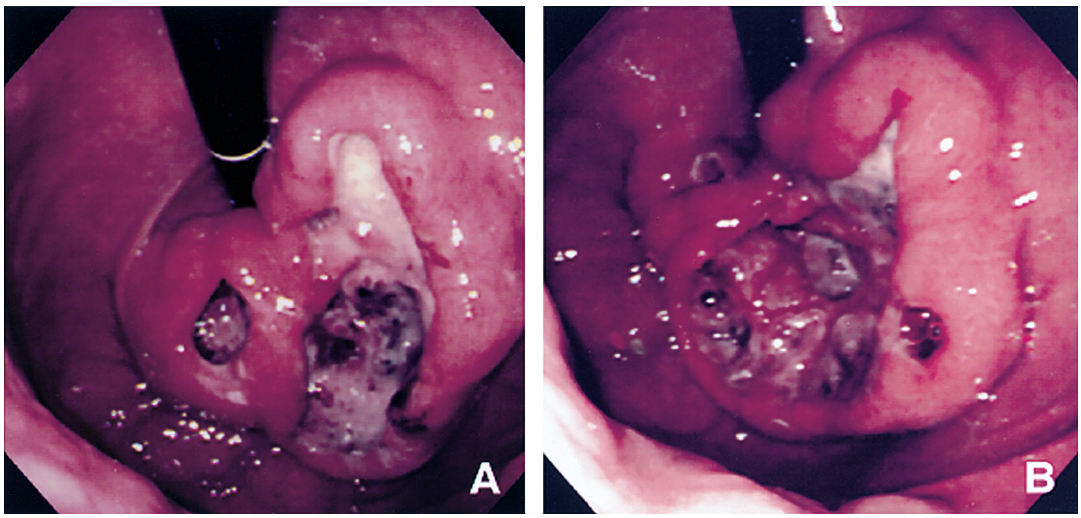

Fig. 1 **A-B:** Endoscopic views of the gastrointestinal stromal tumor (GIST) of the present case. **A:** A finding of the tumor by screening endoscopic examination performed on June 9th, 2001. Note large ulcerative regions on the tumor. **B:** An endoscopic finding of the same region by follow up examination performed on June 29th, 2001, about eight weeks after the first gastroscopy. Note both of the ulcerations on the tumor that were identified by the first examination become larger to make a huge ulcer together with.

Table 1 The results of physical examination on admission day.

Height	153 cm	Weight	47.0 kg
Body Temperature	36.4 °C	Blood Pressure	128/60 mmHg
Anemia	(−)	Jaundice	(−)
Lymph Nodes	Not Swollen		
Chest	No Rales	Heart	No Murmur
Abdomen	Soft & Flat	Bowel Sound	Active
Edema	(−)		

raphy of the stomach, which was performed just after her admission. The tumor was a large protrusion, which was about 70 mm in diameter and had two ulcerations within it. Gastrointestinal endoscopy subsequently re-identified the tumor (Fig. 1B), which was larger than the tumor identified three weeks before and also had larger ulceration suggesting the necrotic feature of the tumor. A surgical resection was done (Fig. 3), which

Table 2 Laboratory Data from Blood Sample on Admission Day.

WBC	11200 / μl	RBC	409 10^4/ μl
Hb	12.9 g/dl	Ht	36.9 %
Plt	23.2 10^4/ μl		
TP	7.0 g/dl		
GOT	19 IU/l	GPT	10 IU/l
LDH	262 IU/l	T. Bil	0.1 mg/dl
BUN	11 mg/dl	Crtn	0.6 mg/dl
Na	142 mEq/l	K	3.7 mEq/l
Cl	108 mEq/l	BS	121 mg/dl
CRP	< 0.3 mg/dl		
CEA	4.1 ng/dl	CA 19-9	< 1.0 U/ml

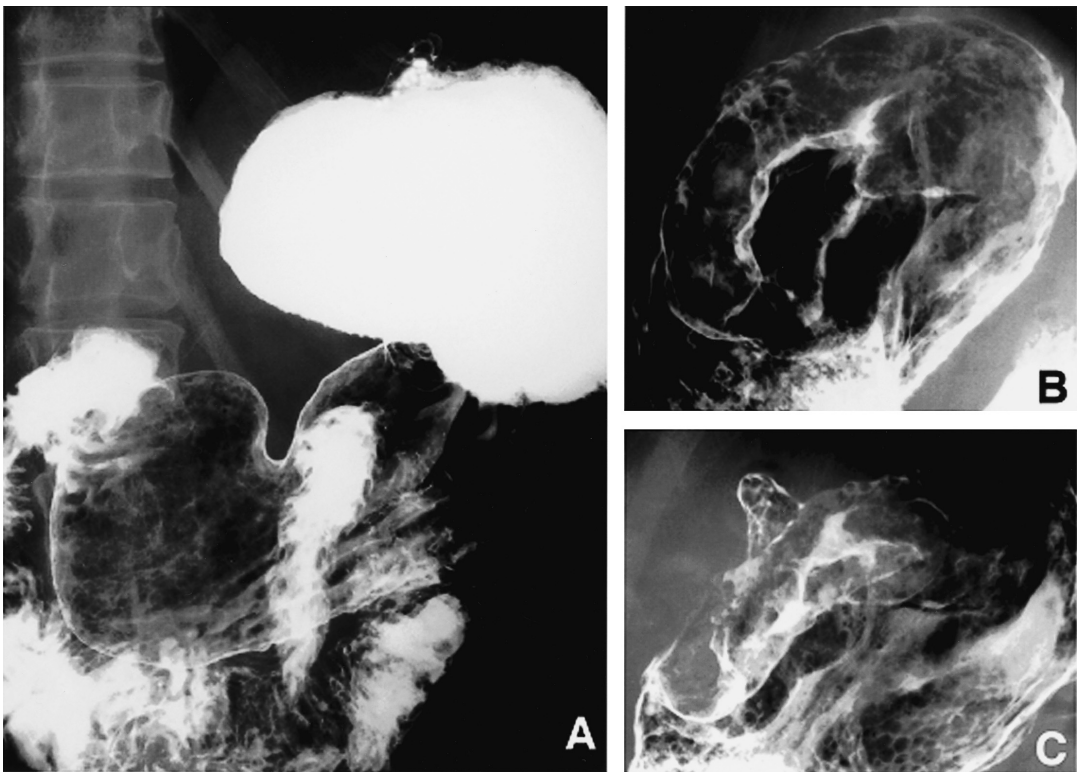

Fig. 2 A–C: The views of fluorescence roentgenography of the GIST of the present patient. The tumor exists at the fundus and is about seven centimeter in diameter. The borderline between the neoplasm and the gastric mucosa seems to be equivocal, suggesting the feature of submucosal tumor of identified region.

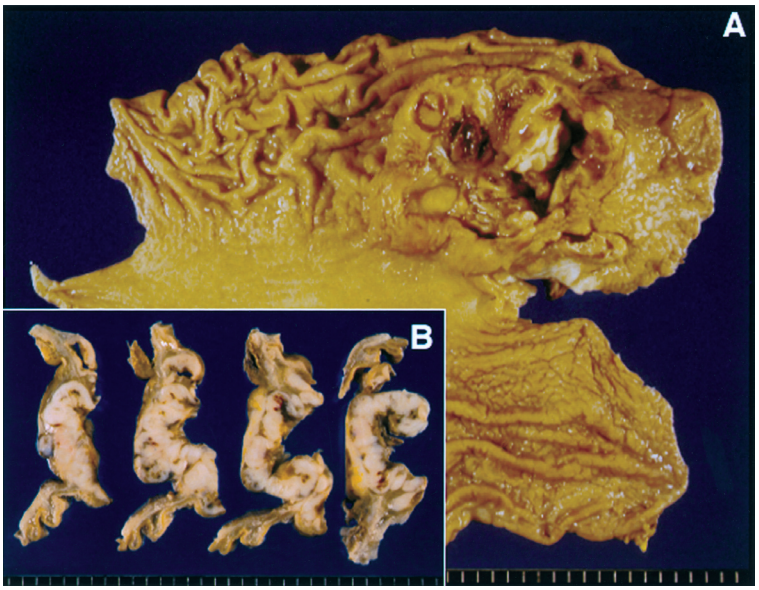

Fig. 3 Macroscopic findings of surgically resected tissue after immersion fixation in buffered formalin. *Inset;* macroscopic views of cut specimens depicting invasion of the tumor within the *tunica muscularis*.

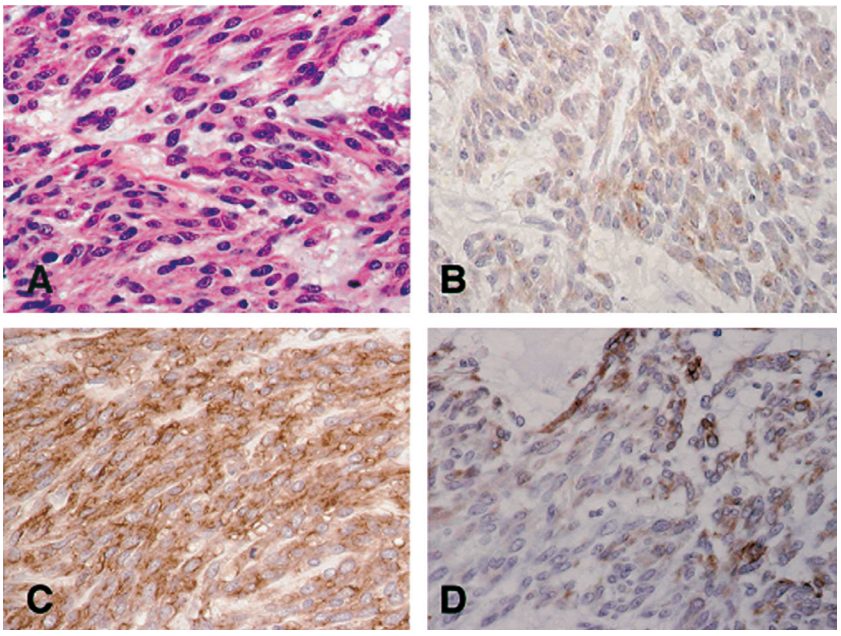

Fig. 4 **A–D:** Light micrographs of the present gastrointestinal stromal tumor (GIST). **A:** A preparation stained by hematoxylin-eosin represents that the tumor cells possess large nucleus and eosinophilic cytoplasm. **B:** Immunohistochemistry for KIT receptor tyrosine kinase. All neoplasmic cells show definite immunoreactivity for KIT. **C:** Immunohistochemistry for vimentin. All neoplasmic cells possess positive immunoreactions for vimentin, which indicate cell biological characteristics of the tumor cells as fibroblast-like ones. **D:** Immunohistochemistry for CD 34. All neoplasmic cells are positive for CD 34. *Original Magnifications*: × 400.

identified no lymph node metastasis and a pleural invasion of the tumor.

Histopathological examination was performed. The tumor cells possessed large clear nucleus with small dense nucleolus and eosinophilic cytoplasm (Fig. 4A). The cells during mitosis were frequently seen. Immunohistochemically, almost tumor cells possessed KIT-like immunoreactions (Fig. 4B) and also realized positive reactions for both CD 34 and vimentin (Figs. 4C-D), which suggested the features of the tumor cells as pacemaker interstitial cell-like. In contrast, desmin-like immunoreactivity was not identified within the tumor cells (Fig. 5A), as well as S-100b protein (data not shown). The smooth muscle actin (SMA), a bulk smooth muscle cell marker, was positive just in part of the tumor cells (Fig. 5B). MIB-1-like immunoreactivity was seen in 30 % of the tumor cells, and p53 could be identified about 20 % of the cells, which suggested fast development of the tumor (Figs. 5C-D).

By electron microscopy, the tumor cells possessed oval or spindle contours and formed whorls (Figs. 6a-b). They had large light nuclei with irregular contours and distinct nucleolus. Many tumor cells seemed to be during mitotic condition. The Golgi's apparatus, endoplasmic reticulum (ER), large mitochondria and small vesicles were observed in their cytoplasm (Fig. 6c). The neighbouring cells frequently possessed interdigitations of cellular membranes (Fig. 6d). Desmosomes were also seen between neighbouring cells however the gap junctions were hardly to be realized (Figs. 6a & e). Some rough-surfaced ER seemed to contain electron dense elements (Fig. 6f). As shown in a thick section (Fig. 6b), the extracellular

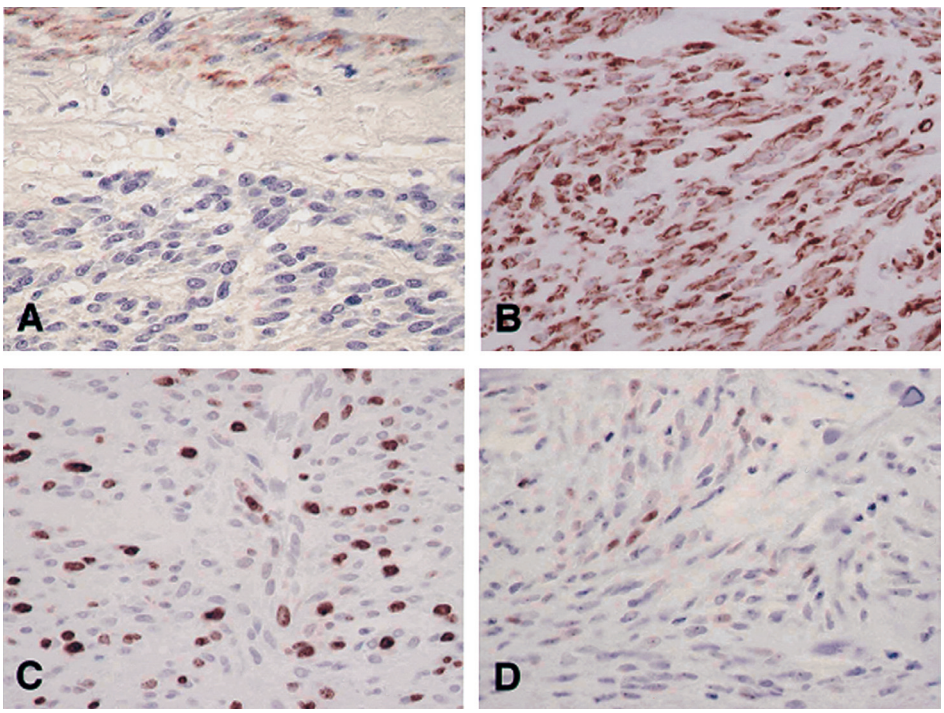

Fig. 5 **A-D:** Light micrographs of the present gastrointestinal stromal tumor (GIST). **A:** Immunohistochemistry for desmin. Desmin-like immunoreactivity was faint within the tumor cells suggesting that cell biological features of the tumor cells are not smooth muscle cell-like ones. **B:** Immunohistochemistry for smooth muscle actin. Some cells show positive immunoreactions for the protein. **C:** Expression of MIB-1 within the tumor cells. About thirty percent of the cells represent MIB-1 suggesting rapid growth of the tumor cells. **D:** Expression of p53 within the tumor cells. Twenty percent of the cells represent p53 suggesting malignant potential of the tumor cells is high. *Original Magnifications*: × 400.

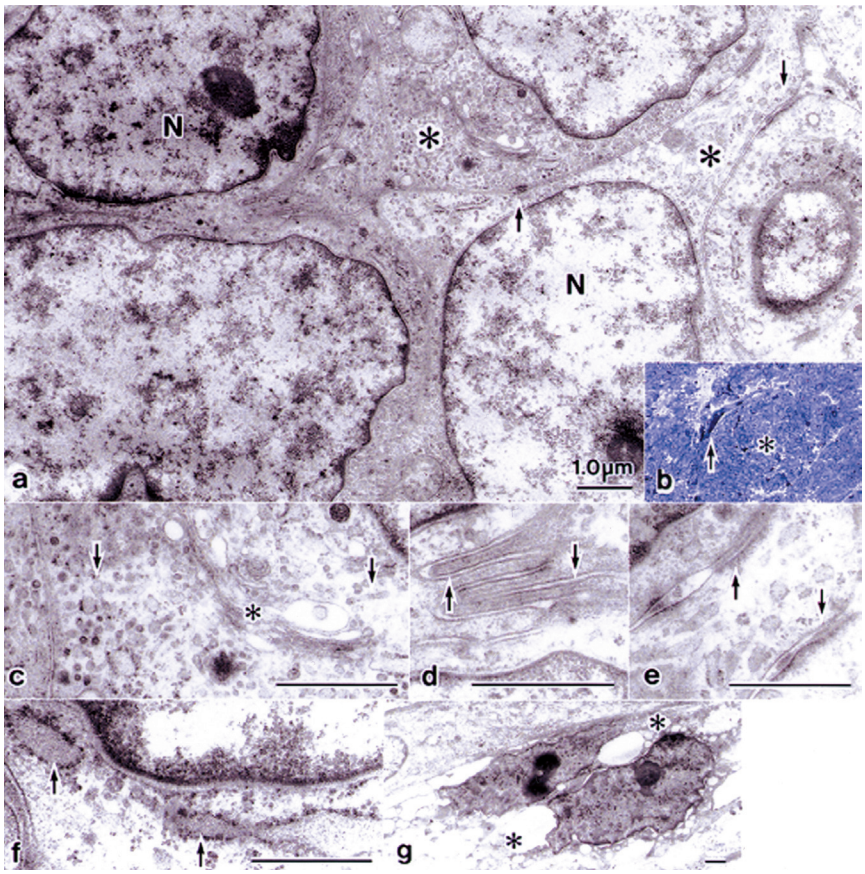

Fig. 6 Electron micrographs of the present case of the GIST. **a** An overview of the tumor cells. The tumor cells have large light nucleus (N), numerous organelles (asterisks), and desmosomes (arrows). **b** A light microscopic view of a thick section of the tumor tissue stained by toluidine blue. The blood capillaries are abundant (an arrow) around the tumor cells that possess whorl formations (an asterisk). *Magnification:* × 100. **c** Higher magnified view of cytoplasmic regions of the tumor cells. The Golgi's apparatus (an asterisk) and many membranous organelles (arrows) are seen. **d** Higher magnified view, which demonstrates interdigitations of the neighbouring cellular membranes (arrows). **e** Higher magnified view, which demonstrates desmosomes between neighbouring cellular membranes (arrows). **f** Higher magnified view of rough-surfaced endoplasmic reticulum, which contains atypical electron-dense materials (arrows). **g** An over view of endothelial cells that have various sizes of cytoplasmic pores (asterisks). *Bars:* 1 μm

Table 3 Primer sets for DNA mutation search of exon 11 in *c-kit* gene.

Set one	Forward	AGA GTG CTC TAA TGA CTG AGA CAA
	Reverse	AAG CCC CTG TTT CAT ACT GAC
Set two	Forward	GTG CAT TAT TGT GAT GAT TCT GA
	Reverse	GGA AAC TCC CAT TTG TGA TCA T

matrices were not abundant around tumor cells, while minor septa were intercalated between the nests of the tumor cells.

Part of the tumor tissue was quickly frozen in liquid nitrogen just after surgical resection to proceed with mutation search in exon 11 of c-*kit* gene by using polymerase chain reaction (PCR) direct sequencing method with two sets of primers (Table 3), although we could not detect any mutation in this region in the tumor tissue obtained from the present case.

DISCUSSION

Recent concept for the gastrointestinal stromal tumor (GIST) is a mesenchymal tumor, which possess both KIT and CD 34. Tumor KIT often demonstrates 'gain-of-function' mutation [4-6]. KIT has a pivotal role for the development and maintenance of a phenotype of the interstitial cells of Cajal (ICC), the gastroenteric pacemaker cells, and the GIST is believed to have a close relationship to the interstitial cells. A standard criterion to recognize ICC is established by electron microscopy [7, 8], although most of previous studies did not observe the ultrastructural features of the tumor cells in GISTs. In addition, many papers confirmed just expression of KIT receptor or c-*kit* RNA in the tumor cells and ignore whether any mutations exist in the c-*kit* gene. The present report is one of the first papers of the GIST, which examined both ultrastructure and mutation of the c-*kit* gene at the same time.

We have examined the ultrastructure of the GIST by a conventional electron microscopy [8]. As shown in *the Result* section, the ultrastructural characteristics of the tumor cells resembled to the features of the interstitial cells in part (Table 4). In general, ICC are characterized by numerous mitochondria, abundant intermediate filaments and large gap junctions, which connect the cells with each other and with smooth muscle cells [3, 7, 8]. Due to their location in the gut and the specific species, the ICC are markedly heterogeneous in appearance, ranging from cells closely resembling smooth muscle cells to those similar to fibroblasts.

The above-mentioned morphological features of ICC were shared with the tumor cells of the present case just in part. First, the tumor cells lost gap junctions, which suggested they had no connexins-mediated communication between the neighbouring cells and might be compatible with their neoplasmic changes. Secondarily, they possessed desmosomes and no typical *basal lamina* surrounding each tumor cell. These charac-

Table 4 Ultrastructural Features of the Tumor Cells of the Present Case.

	Shape	Nucleus	Mito	sER	Cav	Gap	Des	BL
Case	Spindle	Large & Light	+	+	+	−	+	−
ICC	Spindle	Large & Dark	+	+	+	+	−	+

Mito: mitochondria sER: smooth-surfaced endoplasmic reticulum Cav: caveolae
Gap: gap junction Des: desmin BL: *basal lamina*
ICC: interstitial cells of Cajal

Table 5 Proposed Classifications of the GIST with Reference to the Degree of Malignancy.

Signs for Malignancy	the Present Case	Features
Clinical Factor		
Diameter > 5 cm	9 × 6.5 cm	Compatible
Histological Factor		
Abundant Mitosis	Abundant	Compatible
Immunohistochemical Factor		
p53 Positive Cells > 5 %	20 %	Compatible
MIB-1 Positive Cells > 1 %	30 %	Compatible
Genomic Factor		
'Gain-of-Function' Mutation	No	Not Compatible

teristics advocate that the tumor cells have epithelial-like features possibly caused by rapid growth and increase of the number of the tumor cells. In contrast, the tumor cells were installed with large oval nuclei, abundant mitochondria, caveolae and smooth endoplasmic reticulums, which are compatible with the ultrastructural criterion for the ICC. Consequently, the ultrastructure of the present tumor is well matched to the interstitial cells, although it is just a little bit modified because of its neoplasmic characters.

In the present study we performed a mutation search for the change of c-*kit* gene. Kit, a c-*kit* product protein, has a key role for the differentiation and maintenance of the phenotype of the ICC. The mutation within c-*kit* in the gastrointestinal stromal tumor (GIST) has been reported by Hirota *et al*. as 'Gain-of-Function' mutation [4]. This type of mutations is frequently seen in exon 11 of the c-*kit* gene, however we could not identify any mutations in this exon known as a hot mutation spot in spite of existence lots of malignant features in the present case (Table 5). The 'gain-of-function' mutation has a possible role for neoplasmic growth of the tumor cells of the GIST. In contrast, some GISTs have been confirmed not to have mutations in c-*kit* region, suggesting existence of other pathways for proliferation and growth of the GIST as a malignant tumor [9]. Further molecular analyses are needed to reveal novel mechanisms of the cell biological features of the GIST.

REFERENCES

1) Fletcher CD, Berman JJ, Corless C, *et al*.: Diagnosis of gastrointestinal stromal tumors: a consensus approach. Int J Surg Pathol 10: 81-89, 2002.
2) Strickland L, Letson GD, Muro-Gacho CA.: Gastrointestinal stromal tumors. Cancer Control 8: 252-261, 2001.
3) Thuneberg L, Rumessen JJ, Mikkelesen SP, *et al*.: Structural aspects of interstitial cells of Cajal as intestinal pacemaker cells. In: Huizinga JD ed. Pacemaker activity and intercellular communication. CRC press, Boca Raton, 193-222, 1994.
4) Hirota S, Isozaki K, Moriyama Y, *et al*.: Gain-of-Function Mutations of c-kit in Human Gastrointestinal Stromal Tumors. Science 279: 577-580, 1998.
5) Nishida T, Hirota S, Taniguchi M *et al*.: Familial gastrointestinal stromal tumors with germ line mutation of the KIT gene. Nature Genet 19: 323-324, 1998.
6) Hirota S, Nishida T, Isozaki K, *et al*.: Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumors. J Pathol 193: 505-510, 2001.
7) Takayama I, Horiguchi K, Daigo Y, *et al*.: The interstitial cells of Cajal and a gastroenteric pacemaker system. Arch Histol Cytol 65: 1-26, 2002.
8) Komuro T, Seki K, Horiguchi K.: Ultrastructural characterization of the interstitial cells of Cajal. Arch Histol Cytol 62: 295-316, 1999.
9) Hirota S.: Gain of function mutation of c-kit gene and molecular target therapy in GISTs. Nippon Shokakibyo Gakkai Zasshi 100: 13-20, 2003.