pression pattern in a large number of bone and soft tissue tumors.

Materials and methods

Tumor selection

The medical records of 297 adult patients with mesenchymal and peripheral nerve-sheath tumors, mainly of the gastrointestinal tract, mesentery, retroperitoneum, and pelvis, were selected from the pathology files of the National Cancer Center, Tokyo (Table 1). Tumors used in this study included 211 GISTs (stomach 177, small intestine 22, rectum 8, esophagus 3, omentum 1), 12 leiomyomas (stomach 9, esophagus 3), 18 leiomyosarcomas (gastrointestinal 3, retroperitoneum and pelvis 15), 14 schwannomas (stomach 13, colon 1), 17 solitary fibrous tumors (SFTs) (all from the retroperitoneum and pelvis), and 25 desmoid-type fibromatoses (DTFs) (mesentery 3, other locations 22).

We also selected 418 cases of other bone and soft tissue sarcomas, mainly of the extremities and trunk, for KIT immunostaining, which included 11 fibrosarcomas, 11 leiomyosarcomas, 52 myxoid liposarcomas, 50 well-differentiated liposarcomas, 53 myxofibrosarcomas, 18 malignant peripheral nerve-sheath tumors (MPNSTs), 44 pleomorphic malignant fibrous histiocytomas, 42 synovial sarcomas, 20 Ewing sarcoma/primitive neuroectodermal tumors (ES/PNETs), 6 desmoplastic small round cell tumors, 11 neuroblastomas, 15 clear cell sarcomas, 30 angiosarcomas, 20 osteosarcomas, and 35 rhabdomyosarcomas (Table 2).

For light microscopic review, all specimens were fixed in 10% formalin and processed routinely for paraffin embedding. Sections 4-μm thick were stained with hematoxylin and eosin. A GIST in this study was defined as a mesenchymal spindle or epithelioid cell lesion arising in the wall of the gastrointestinal tract (Fig. 1A, B) that exhibited consistent immunoreactivity for KIT. As a result, 158 (75%) GISTs were shown to be the spindle cell type, 39 (18%) the mixed (combination of spindle and epithelioid cell) type, and 14 (7%) the epithelioid cell type. Smooth-muscle tumors were classified primarily on the basis of morphological appearance and strong immunoreactivity for SMA and desmin. Leiomyomas were paucicellular and composed of elongated spindle cells with abundant eosinophilic, fibrillary cytoplasm. Leiomyosarcoma were characterized by long fascicles of eosinophilic spindle cells with elongated nuclei with rounded ends (Fig. 2A). Focal pleomorphism was common. Schwannomas were characterized microscopically by the presence of peripheral lymphoid cuffs and short fascicles of spindle cells and bizarre cells (Fig. 3A) and strong immunoreac-

Table 1 Frequency of immunostaining for each antibody in gastrointestinal stromal tumors (GISTs) and other spindle cell tumors of gastrointestinal tract. Note: 70 cases of GIST (stomach 50, non-stomach 20) were studied for neuron-specific enolase (NSE) and beta-catenin immunostaining. *KIT* c-kit/CD117, *SMA* smooth-muscle actin, *HCD* h-caldesmon

Tumor type	KIT (%)	CD34 (%)	Desmin (%)	SMA (%)	HCD (%)	S-100 (%)	NSE (%)	Beta-catenin (%)
GIST (n=211)	211 (100)	192 (91)	8 (4)	65 (31)	167 (79)	16 (8)	57 (81)	0*
Stomach (n=177)	177 (100)	173 (98)	8 (5)	42 (24)	147 (83)	6 (3)	40 (80)	0*
Non-stomach (n=34)	34 (100)	19 (56)	0	23 (68)	20 (59)	10 (29)	17 (85)	0*
Smooth-muscle tumor								
Leiomyoma (n=12)	0	0	12 (100)	12 (100)	12 (100)	0	0	0
Leiomyosarcoma (n=18)	0	0	18 (100)	18 (100)	18 (100)	0	0	0
Peripheral nerve-sheath tumor								
Schwannoma (n=14)	0	7 (50)	0	0	0	14 (100)	3 (21)	0*
Fibrous tumor								
Solitary fibrous tumor (n=17)	0	17 (100)	0	0	0	4 (24)	3 (18)	4 (24)**
Desmoid-type fibromatosis (n=25)	0*	0	5 (20)	21 (84)	0	0	25 (64)	10 (100)**

* Some of cases showed weak (1+) cytoplasmic staining (see text)
** Nuclear accumulation

Table 2 KIT (c-kit/CD117) immunoreactivity in other 418 bone and soft-tissue sarcomas. *MPNST* malignant peripheral nerve sheeth tumor, *PMFH* pleomorphic malignant fibrous histiocytoma, *ES/PNET* Ewing sarcoma/primitive neuroectodermal tumor, *DSRCT* desmoplastic small round cell tumor

Tumor type	Number	KIT/c-kit protein				Negative	Positive
		0	1+	2+	3+	(0/1+)	(2+/3+)
Fibrosarcoma	11	11	0	0	0	11 (100%)	0
Leiomyosarcoma	11	8	3	0	0	11 (100%)	0
Myxoid lipsarcoma	52	52	0	0	0	52 (100%)	0
Well-differentiated liposarcoma	50	50	0	0	0	50 (100%)	0
Myxofibrosarcoma	53	52	1	0	0	53 (100%)	0
MPNST	18	17	1	0	0	18 (100%)	0
PMFH	44	44	0	0	0	44 (100%)	0
Synovial sarcoma	42	40	0	2	0	40 (95%)	2 (5%)
ES/PNET	20	8	7	4	1	15 (75%)	5 (25%)
DSRCT	6	6	0	0	0	6 (100%)	0
Neuroblastoma	11	10	0	0	1	10 (91%)	1 (9%)
Clear cell sarcoma	15	14	0	0	1	14 (93%)	1 (7%)
Angiosarcoma	30	26	0	4	0	26 (87%)	4 (13%)
Osteosarcoma	20	20	0	0	0	20 (100%)	0
Rhabdomyosarcoma	35	35	0	0	0	35 (100%)	0
Total	418	-	-	-	-	405 (97%)	13 (3%)

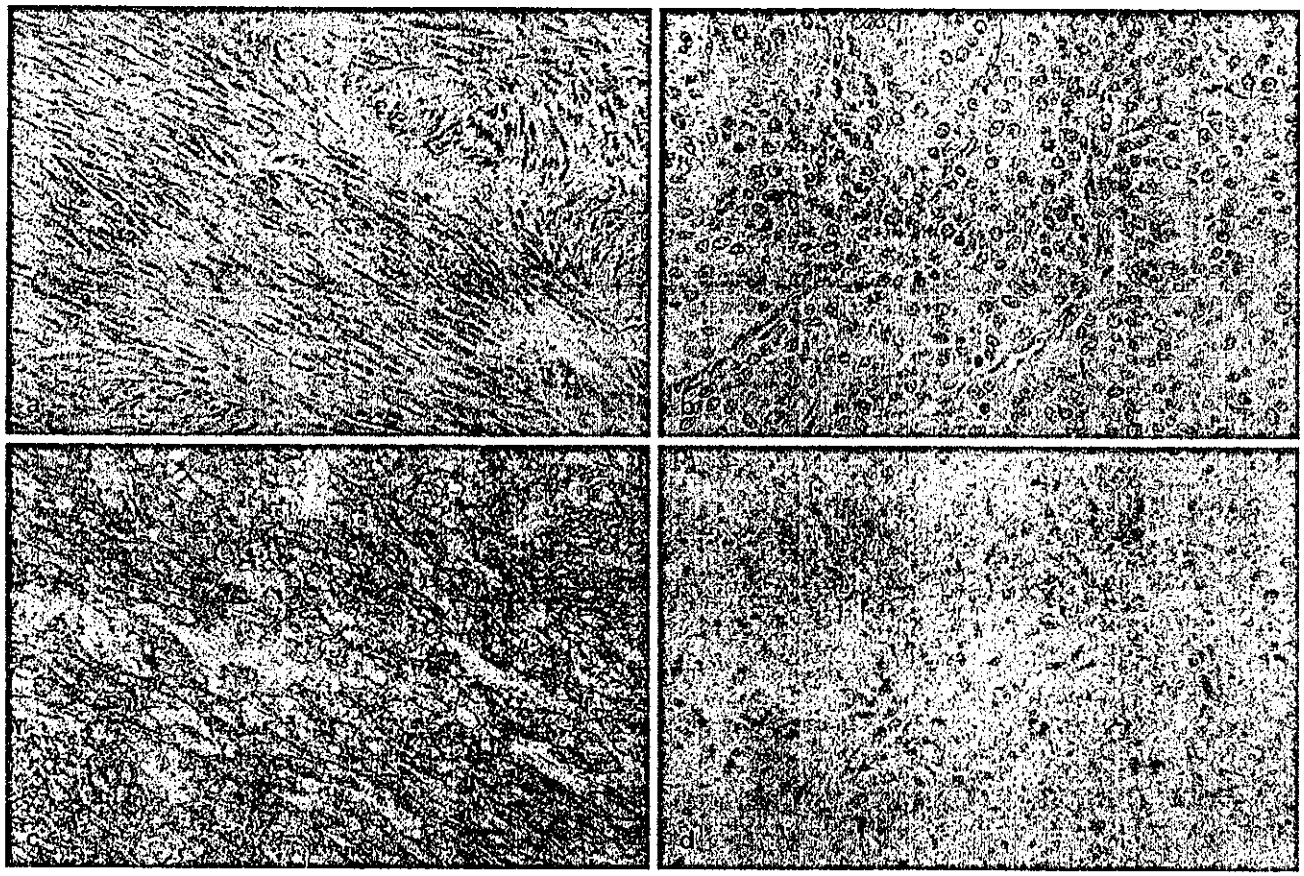

Fig. 1 Gastrointestinal stromal tumors. A gastric stromal tumor of spindle cell type is composed of uniform eosinophilic cells arranged in short fascicles (A). A small intestinal stromal tumor of epithelioid type shows a nested paraganglioma-like appearance (B). Diffuse and strong membrane and cytoplasmic staining for KIT (c-kit/CD117) in a majority of tumor cells (C). Dot-like immunoreactivity for KIT in the tumor cytoplasm (D)

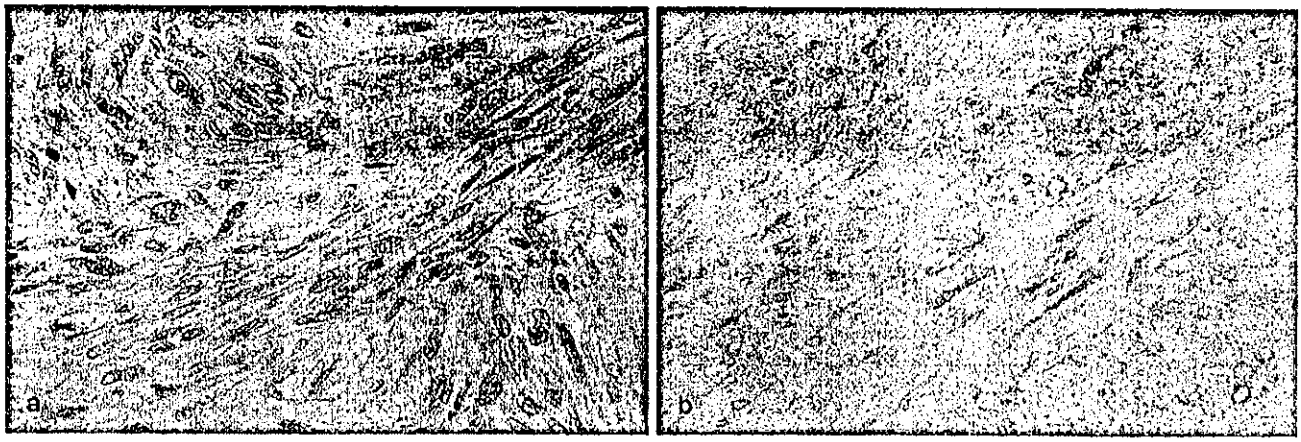

Fig. 2 Smooth-muscle tumors. A rectal leiomyosarcoma consists of long fascicles of eosinophilic spindle cells (A). Focal and strong positivity for desmin in tumor cells (B)

tivity for S-100. SFTs were microscopically defined as neoplasms showing "patternless" growth, with a haphazard arrangement of bland-looking short spindle or polygonal cells, alternating hypercellular and hypocellular sclerotic foci, keloid-like stromal hyalinization, and a prominent branching vasculature (Fig. 4A). Immunohistochemically, SFTs were always CD34 positive. DTFs were infiltrative and locally aggressive, characterized by a loose fascicular arrangement of spindle cells in a predominantly collagenous background (Fig. 4C).

Fig. 3 Peripheral nerve-sheath tumors. A gastric schwannoma is composed of short fascicles of spindle cells and bizarre cells with peripheral lymphoid cuffs (A). Many tumor cells show diffuse and strong staining for S-100 (B)

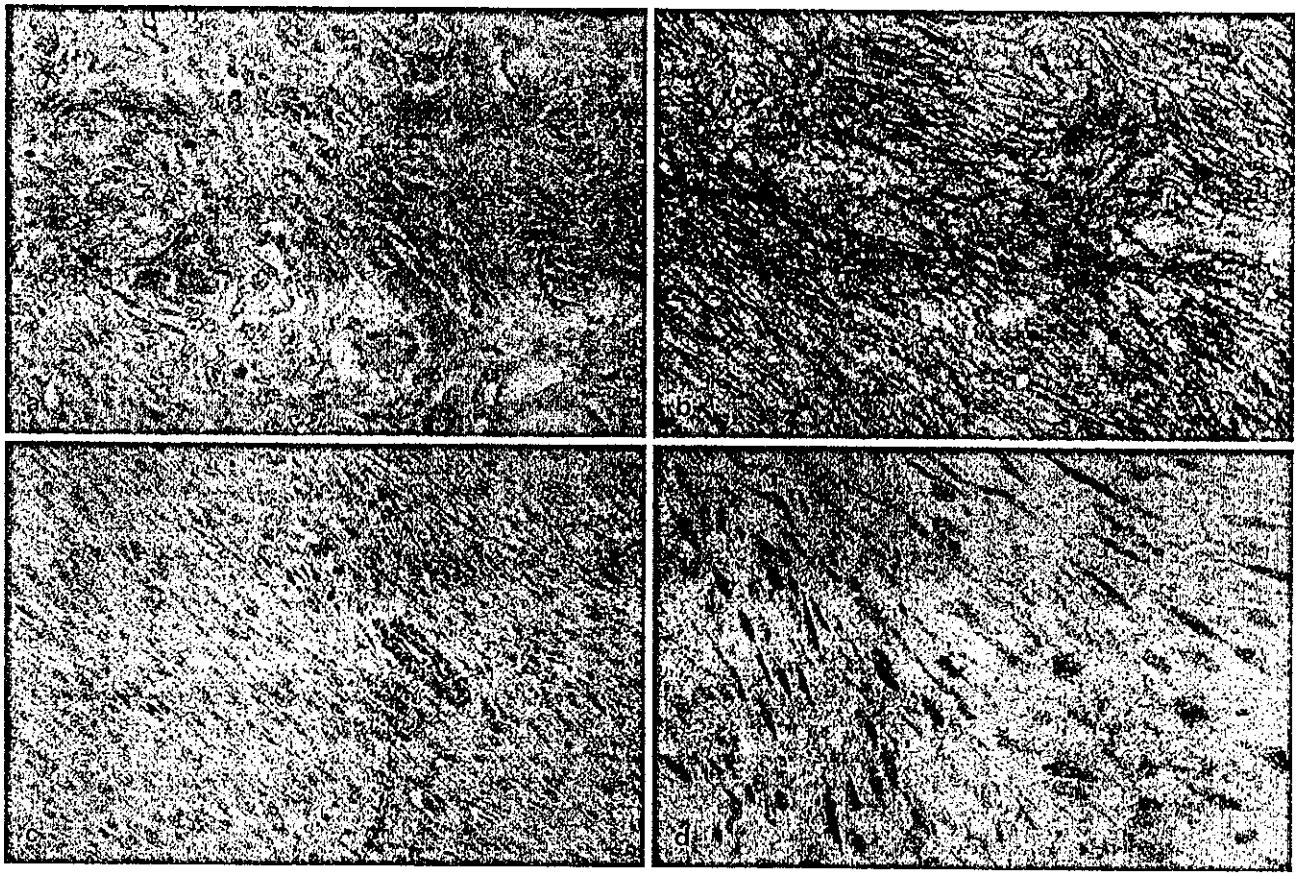

Fig. 4 Fibrous tumors. A pelvic solitary fibrous tumor shows a haphazard arrangement of spindle cells with hyalinized bands of collagen (A). Many tumor cells show strong immunoreactivity for CD34 (B). A mesenteric desmoid-type fibromatosis involving gastric wall shows a loose fascicular arrangement of spindle cells around vessels (C). Strong nuclear accumulation of beta-catenin in many spindle cells (D)

Immunohistochemical analysis

Immunohistochemical analysis was performed on tissue sections from paraffin blocks by the labeled streptavidin-biotin method. The sections were dewaxed, rehydrated, and moistened with phosphate-buffered saline (PBS; pH 7.4). They were then pretreated in an autoclave at 121°C for 10 min in 10 mmol/l citrate buffer (pH 6.0), before being incubated with antibodies against the following antigens on an automated immunostaining system (i6000; BioGenex, San Ramon, CA) for 30 min: KIT (polyclonal antibody, 1/50 dilution; DakoCytomation, Glostrup, Denmark), CD34 (clone My 10, 1/100 dilution; Becton Dickinson, San Jose, CA), desmin (clone

D33, 1/200 dilution; DakoCytomation), alpha-SMA (clone 1A4, 1/100 dilution; DakoCytomation), HCD (clone h-CD, 1/100 dilution; DakoCytomation), S-100 protein (polyclonal, 1/2000 dilution; DakoCytomation), NSE (clone BBS/NC/VI-H14, 1/200 dilution; DakoCytomation), and beta-catenin (clone 14, 1/500 dilution; Transduction Laboratories, Lexington, KY). Heat-induced epitope retrieval was not used for sections stained with antibodies against NSE and S-100 protein.

Immunohistochemical results were judged by all investigators using a multiheaded microscope. A consensus judgment was adopted as the proper immunohistochemical score of the tumor based on strength: 0, negative; 1+, weak staining; 2+, moderate staining; 3+, strong staining. Tissue mast cells, which stain 2+ or 3+, were used as internal positive controls for KIT. The distribution of positive cells was also recorded in an effort to impart the diffuse or focal nature of the positive cells: sporadic (positive cells <10%); focal (10% ≥positive cells<50%); diffuse (positive cells ≥50%). The immunohistochemical scores of 2+ and 3+ with focal to diffuse distribution were considered to be positive for all markers.

Results

The immunohistochemical results are summarized in Table 1 and Table 2.

Gastrointestinal stromal tumor

All 211 GISTs were positive for KIT, and the reactivity was typically diffuse and strong in the cytoplasm and membrane of most tumor cells (Fig. 1C). The pattern of KIT immunostaining was cytoplasmic reactivity in 113 (54%) and cytoplasmic dot-like reactivity in 98 (46%) tumors (Fig. 1D). In 16 (mixed type 9, epithelioid cell type 7) tumors, KIT immunoreactivity for epithelioid cells was weaker than in spindle cells in the same tissue sections or other tumors. CD34 positivity was observed in almost all of the tumors (98%) of the stomach and in more than half of non-gastric tumors (56%). Immunoreactivity for desmin and S-100 was usually focal (4% for desmin and 8% for S-100). Positivity for SMA and HCD was variably expressed, 65 (31%) and 167 (79%) of 211 GISTs being positive, respectively. The frequency of positive desmin tended to be higher in epithelioid cell (21%, 3/14) than that in spindle cell (2.5%, 4/158) and mixed-type GIST (2.6%, 1/39). In this study, 70 GISTs were available for immunohistochemical evaluation of NSE and beta-catenin, and 57 of 70 (81%) GISTs were found to show moderate to strong NSE immunoreactivity, whereas 59 of 70 (84%) were positive for beta-catenin, the staining showing a cytoplasmic pattern.

Smooth-muscle tumor

Immunostaining for desmin, SMA, and HCD was positive in all leiomyomas and leiomyosarcomas (Fig. 2A, B). The staining was diffuse and strong in leiomyomas but tended to be less intense and heterogeneous in leiomyosarcomas. KIT, CD34, S-100, NSE, and beta-catenin were negative in all tumors.

Peripheral nerve-sheath tumor

All schwannomas showed strong nuclear and cytoplasmic staining for S-100 (Fig. 3A, B). Of 14 schwannomas, 13 (93%) showed staining for beta-catenin, but it was weak and cytoplasmic. Of cases, 7 (50%) and 3 (21%) were focally CD34 and NSE positive, respectively. Staining for KIT, desmin, SMA, and HCD was negative.

Fibrous tumor

All SFTs stained positively for CD34 but not for KIT; the staining for CD34 was generally strong and diffuse in the cytoplasm of spindle cells (Fig. 4A, B). None of the 25 DTFs were CD34 positive; 15 (60%) DTFs showed weak (1+) coarsely granular cytoplasmic immunostaining for KIT (Fig. 5A), which was eliminated without heat-induced antigen retrieval. In all cases of DTF, immunore-

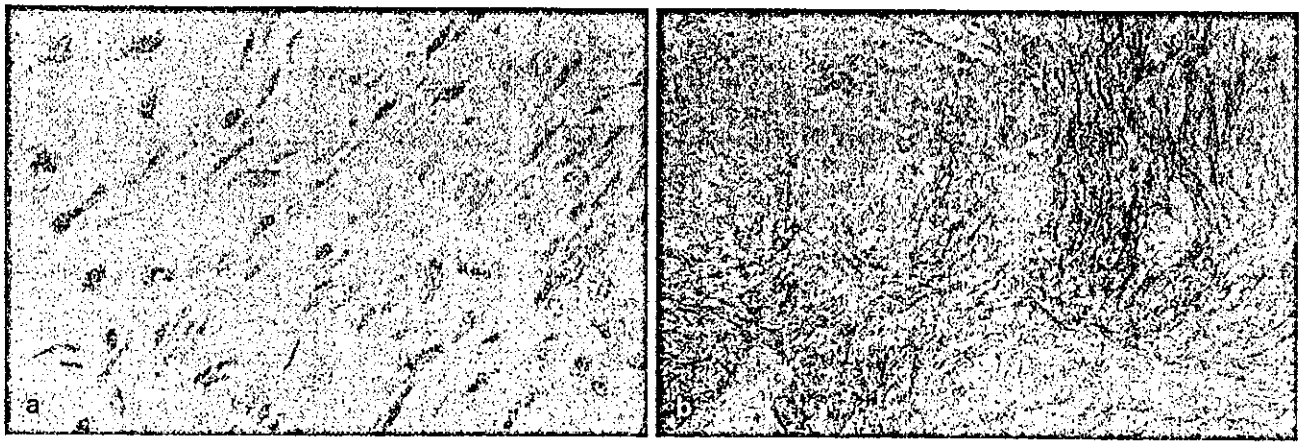

Fig. 5 A desmoid-type fibromatosis shows weak (1+) coarsely granular cytoplasmic immunostaining for KIT(c-kit/CD117) (A). A monophasic synovial sarcoma shows focal and moderate (2+) membranous immunoreactivity for KIT (B)

activity for beta-catenin was recognized in the nuclei, and the staining was uniformly distributed throughout the sections despite variable proportions of positive cells (Fig. 4C, D). As for SFT, 4 cases (24%) were noted to show nuclear accumulation of beta-catenin. Of DTF cases, 21 (84%) showed moderate to strong positivity for SMA, but all SFTs were negative. In addition, scattered desmin immunoreactivity was detected in 5 (20%) DTFs. S-100 positivity was detected in 4 SFTs (24%) but not in any of the DTFs. Immunostaining for NSE was varied. Three SFTs (18%) were NSE positive, whereas the staining tended to be more frequent in DTFs, 64% (16 cases) being moderately to strongly positive. Staining for HCD was negative in both types of tumor.

KIT immunoreactivity in other bone and soft tissue sarcomas

Staining of KIT in other bone and soft tissue sarcomas was limited (Table 2). Moderate (2+) to strong (3+) staining of KIT was observed in 2 synovial sarcomas (Fig. 5B), 5 ES/PNETs, 1 neuroblastoma, 1 clear cell sarcoma, and 4 angiosarcomas, while weak (1+) staining was identified in 3 leiomyosarcomas, 1 myxofibrosarcoma, 1 MPNST, and 7 ES/PNETs.

Discussion

GISTs have a wide spectrum of clinicopathological features, ranging from benign to evidently malignant. Most of them are predominantly spindle cell, whereas others are epithelioid and, rarely, pleomorphic cell tumors. In the earlier literature, GISTs were described as unusual tumors of smooth-muscle origin [33], designated as leiomyoblastomas [53], leiomyomas, cellular leiomyomas, and leiomyosarcomas [3]. During that time, the term stromal tumor was first used to describe tumor lacking smooth-muscle differentiation immunohistochemically and ultrastructurally [34].

Recent molecular pathology studies have revealed that most GISTs are immunoreactive for KIT and CD34 [13, 17, 20, 32, 35, 38, 49, 55]. The 211 GISTs selected for the present study were all KIT positive. We carefully evaluated the variability in the subcellular localization of KIT staining and found that over half of the GISTs had cytoplasmic immunostaining, whereas the others showed a combination of both cytoplasmic and dot-like immunostaining. No example of a pure dot-like staining pattern was observed among our materials. In 16 tumors (8%), however, immunoreactivity in epithelioid cells was weaker than that in spindle cells in the same tissue sections or other tumors. The significance of this heterogeneity of KIT staining in GISTs remains to be investigated, but it is necessary to be aware of these patterns when carrying out immunostaining in suspected cases of GIST. There is a possibility that these varied staining patterns are associated with different KIT mutations [8].

It is known that a small number of GISTs are KIT negative. Over the past year, mutations of platelet-derived growth factor alpha (PDGFRA) gene encoding the PDGFRA have been reported in some of these tumors, suggesting that instead of KIT, PDGFRA appears to play an important role in development of GISTs without KIT mutations [14, 18]. We analyzed PDGFRA gene mutation using polymerase chain reaction (PCR) techniques in 27 cases of KIT-weak or -negative GIST, including the above 16 tumors. As a result, the PDGFRA gene mutation was observed in 17 of 27 cases (63%) (unpublished data).

Although the results of KIT immunostaining in other bone and soft tissue sarcomas are at times conflicting [4, 19, 48, 52], at least a small number of these tumors are KIT positive from weak, focal staining to occasionally strong staining. These findings suggest the possibility that KIT immunostaining would lead to diagnostic confusion because KIT positivity was detected in other morphological similar tumors, including intra-abdominal desmoid fibromatoses [30, 59] and other mesenchymal tumors, such as leiomyosarcoma, fibrosarcoma, and synovial sarcoma [48, 52]. In our 25 cases of DTF, KIT staining was frequent (60%) using heat-induced antigen retrieval, but it was eliminated without the antigen retrieval. Further PCR analysis revealed that neither mutations of c-kit exon 11 nor PDGRA exon 12 or 18 were detected in these tumor samples (data not shown). False positives for KIT in other non-GIST tumors may occur due to the inappropriate staining technique used. Moreover, KIT-positive non-GIST tumors, in the absence of any accompanying mutation such as KIT and PDGFRA gene mutation, have no therapeutic significance with Glivec.

The present study revealed variable immunoreactivity for desmin, SMA, HCD, and S-100 in the GISTs we examined. As discussed in a previous study [13], a large percentage of GISTs are HCD positive, and SMA positivity was present in approximately 30% of the present cases, indicating possible traits of smooth-muscle differentiation in these tumors. Although staining for desmin was very rare in GISTs, the frequency of positive desmin tended to be higher in epithelioid cell (21%) than that in spindle cell (2.5%) and mixed-type GIST (2.6%). There was no significant difference observed among these three types of GIST in immunostaining pattern for the remaining markers (data not shown).

Information on NSE reactivity in GISTs is limited [7]. We found that over 80% of GISTs were NSE positive and that approximately 30% of non-gastric tumors were S-100 positive, suggesting the possibility of neural differentiation. Gastrointestinal autonomic nerve tumors (GANTs) are described as distinctive entities that differ from other mesenchymal tumors of the gastrointestinal tract [2, 57], and most have been reported as immunoreactive for vimentin, NSE, and occasionally S-100 [7]. However, recent studies have suggested that GANTs are not a separate entity because they share a molecular genetic identity with conventional spindle and epithelioid cell forms of GIST [28].

Leiomyomas and leiomyosarcomas are the main tumors of the gastrointestinal tract that are often confused with GISTs. Their well-differentiated smooth-muscle cells are negative for KIT and CD34 and positive for SMA and, usually, for desmin [25, 36, 37, 38, 39, 41, 58]. Our findings are similar to those of previous studies and confirm that all leiomyomas and leiomyosarcomas were immunoreactive for desmin, SMA, and HCD.

Schwannomas rarely occur in the gastrointestinal tract and are characterized microscopically by the presence of peripheral lymphoid cuffs and short fascicles of spindle cells and bizarre cells. Unlike conventional soft tissue schwannomas, they usually have regular whorls or a storiform pattern and lack distinct palisading [6]. Positive CD34 immunostaining was detected not only in tumors located in the stomach, as in our findings, but also detected in tumors that occurred in other locations [16]. Positivity for S-100 and negativity for smooth-muscle markers and KIT can separate schwannomas from GISTs.

SFTs may occur in the gastrointestinal tract, mesentery, or retroperitoneum and become a diagnostic problem because they mimic GISTs to some extent histologically. Microscopically, SFTs have been described as showing "patternless" growth, with a haphazard arrangement of bland-looking short spindle or polygonal cells, alternating hypercellular and hypocellular sclerotic foci, keloid-like stromal hyalinization, and a prominent branching vasculature. Immunohistochemically, all of the SFTs were CD34 positive and KIT negative, in agreement with previous studies [38, 49, 50], and none of the SFTs showed immunostaining for smooth-muscle markers (desmin, SMA, HCD). These findings suggest that the combination of immunostaining for KIT, CD34, and smooth-muscle markers might be helpful for differentiating GISTs from SFTs.

Beta-catenin is an important multifunctional protein involved in the Wingless/Wnt signal transduction pathway and also acts as a cell–cell adhesion regulator when binding to E-cadherin adhesion molecules [5, 15]. Constitutional activation of the Wingless/Wnt signaling pathway by stabilization and accumulation of beta-catenin in the nucleus and cytoplasm, caused mainly by inactivating mutations in the adenomatous polyposis coli (APC) gene, has been revealed to be important in the development of human colon cancers and other carcinomas [45] and also in deep fibromatosis [1, 54] and some sarcomas [12, 24]. Reports on the expression of beta-catenin in GISTs, however, are very limited. Previous authors reported that no nuclear accumulation was detected in GISTs [43]. Our present study showed that none of 70 GISTs had nuclear immunostaining, although more than 80% of them had weak cytoplasmic immunostaining. A large percentage of schwannomas also showed weak cytoplasmic immunoreactivity for beta-catenin, the extent and pattern of the staining being quite similar as those in GISTs.

Intra-abdominal DTFs are uncommon tumors that primarily affect the mesentery or retroperitoneum and often invade the wall of the gastrointestinal tract. They are infiltrative and locally aggressive, characterized by florid fibroblastic proliferation. It is well known that DTFs typically have APC gene and beta-catenin gene mutations [9, 42, 51, 54] and that APC-truncating mutations confer a proliferative advantage on aggressive fibromatosis cells through beta-catenin [29]. In our series, nuclear accumulation was detected in all DTFs, suggesting the usefulness of beta-catenin for distinguishing this tumor from GISTs, as reported previously [43]. However, careful evaluation should be done, because there is an overlap in the nuclear accumulation of beta-catenin in DTFs and SFTs. In this situation, additional CD34 immunostaining might be helpful for separating SFTs from DTFs.

Metastatic melanoma and primary clear cell sarcoma may occur in the walls of the intestines or stomach and should be separated from GIST due to the histological resemblance. Positivity for melanocytic markers (tyrosinase, melan-A, and HMB-45) with the combinations of other markers such as KIT, CD34, and S-100, or using molecular cytogenetic methods to detect the chromosomal translocation t(12;22)(q13;q12) or the EWS-ATF1 fusion transcript are diagnostic [60].

The extent and patterns of KIT immunostaining in GISTs are varied, and KIT immunostaining, although in a limited number, is also detected in other mesenchymal tumors that may involve the gastrointestinal tract and abdominal cavity. Thus, it is inevitable for the diagnosis of GISTs to use an immunohistochemical panel along with appropriate morphological evaluation. In this context, the findings that consistent (100%) immunoreactivity for KIT, CD34, desmin, and S-100 and nuclear accumulation of beta-catenin in GISTs, SFTs, smooth-muscle tumors, schwannomas, and DTF each suggest that these are key markers for clinical diagnosis of GISTs and other spindle cell tumors that can arise in the gastrointestinal tract; whereas, SMA, HCD, and NSE are of only limited value, because immunoreactivity for these tumors was observed in a wide range of these tumors.

References

1. Alman BA, Li C, Pajerski ME, Diaz-Cano S, Wolfe HJ (1997) Increased beta-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumor). Am J Pathol 151:329–334
2. Antonioli DA (1989) Gastrointestinal autonomic nerve tumors. Expanding the spectrum of gastrointestinal tumors. Arch Pathol Lab Med 113:831–833
3. Appelman HD (1990) Mesenchymal tumors of the gut: histological perspectives, new approaches, new results, and does it make any difference. Monogr Pathol 31:220–246
4. Barisella M, Andreola S, Rosai J (2002) CD117 in soft tissue sarcomas. Am J Clin Pathol 118:470–471
5. Brabletz T, Herrmann K, Jung A, Faller G, Kirchner T (2000) Expression of nuclear beta-catenin and c-myc is correlated with tumor size but not with proliferative activity of colorectal adenomas. Am J Pathol 156:865–870
6. Daimaru Y, Kido H, Hashimoto H, Enjoji M (1988) Benign schwannoma of the gastrointestinal tract: a clinicopathologic and immunohistochemical study. Hum Pathol 19:257–264

7. Dhimes P, Lopez-Carreira M, Ortega-Serrano MP, Garcia-Munoz H, Martinez-Gonzalez MA, Ballestin C (1995) Gastrointestinal autonomic nerve tumors and their separation from other gastrointestinal stromal tumors: an ultrastructural and immunohistochemical study of seven cases. Virchows Arch 426:27–35
8. Fletcher CDM, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW (2002) Diagnosis of gastrointestinal stromal tumors: a consensus approach. Hum Pathol 33:459–465
9. Giarola M, Wells D, Mondini P, Pilotti S, Sala P, Azzarelli A, Bertario L, Pierotti MA, Delhanty JD, Radice P (1998) Mutations of adenomatous polyposis coli (APC) gene are uncommon in sporadic desmoid tumors. Br J Cancer 78:582–587
10. Graadt van Roggen JF, van Velthuysen MLF, Hogendoorn PCW (2001) The histopathological differential diagnosis of gastrointestinal stromal tumors. J Clin Pathol 54:96–103
11. Greenson JK (2003) Gastrointestinal stromal tumors and other mesenchymal lesions of the gut. Mod Pathol 16:366–375
12. Hasegawa T, Yokoyama R, Matsuno Y, Shimoda T, Hirohashi S (2001) Prognostic significance of histologic grade and nuclear expression of beta-catenin in synovial sarcoma. Hum Pathol 32:257–263
13. Hasegawa T, Matsuno Y, Shimoda T, Hirohashi S (2002) Gastrointestinal stromal tumor: consistent CD117 immunostaining for diagnosis, and prognostic classification based on tumor size and MIB-1 grade. Hum Pathol 33:669–676
14. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA (2003) PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299:708–710
15. Hirohashi S (1998) Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. Am J Pathol 153:333–339
16. Hirose T, Tani T, Shimada T, Ishizawa K, Shimada S, Sano T (2003) Immunohistochemical demonstration of EMA/Glut1-positive perineurial cells and CD34-positive fibroblastic cells in peripheral nerve sheath tumors. Mod Pathol 16:293–298
17. Hirota S, Isozaki k, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279:577–580
18. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y (2003) Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology 125:660–667
19. Hornick JL, Fletcher CDM (2002) Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. Am J Clin Pathol 117:188–193
20. Hurlimann J, Gardiol D (1991) Gastrointestinal stromal tumors. An immunohistochemical study of 165 cases. Histopathology 19:311–320
21. Joensuu H, Roberts PJ, Sarlomo-Rikara M, Andersson LC, Tervahartiala P, Tuveson D, Silberman SL, Capdeville R, Dimitrijevic S, Druker B, Demetri GD (2001) Effect of tyrosine kinase inhibitor STI-571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 344:1052–1056
22. Kindblom L, Remotti HE, Aldenborg F, Meis-Kindblom JM (1998) Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 152:1259–1269
23. Kitamura Y, Hirota S, Nishita T (1998) Molecular pathology of c-kit proto-oncogene and development of gastrointestinal stromal tumors. Ann Chir Gynaecol 87:282–286
24. Kuhnen C, Herter P, Muller O, Muehlberger T, Krause L, Homann H, Steinau HU, Muller KM (2000) Beta-catenin in soft tissue sarcomas: expression is related to proliferative activity in high-grade sarcomas. Mod Pathol 13:1005–1013
25. Lasota J, Jasinski M, Sarlomo-Rikara M, Miettinen M (1999) C-kit mutations occur preferentially in malignant versus benign GISTs and do not occur in leiomyomas and leiomyosarcomas. Am J Pathol 154:53–60
26. Lasota J, Wozniak A, Sarlomo-Rikara M, Rys J, Kordek R, Nassar A, Sobin LH, Miettinen M (2000) Mutations in exons 9 and 13 of KIT gene are rare events in gastrointestinal stromal tumors: a study of two hundred cases. Am J Pathol 157:1091–1095
27. Lauwers GY, Erlandson RA, Casper ES, Brennan MF, Woodruff JM (1993) Gastrointestinal autonomic nerve tumors. A clinicopathological, immunohistochemical, and ultrastructural study of 12 cases. Am J Surg Pathol 17:887–897
28. Lee JR, Joshi V, Griffin JW Jr, Lasota J, Miettinen M (2001) Gastrointestinal autonomic tumors: immunohistochemical and molecular identity with gastrointestinal stromal tumor. Am J Surg Pathol 25:979–987
29. Li C, Bapat B, Alman BA (1998) Adenomatous polyposis coli gene mutation alters proliferation through its beta-catenin-regulatory function in aggressive fibromatosis (desmoid tumor). Am J Pathol 153:709–714
30. Lucas DR, Al-Abbadi M, Tabaczka P, Hamre MR, Weaver DW, Mott MJ (2003) c-Kit expression in desmoid fibromatosis. Am J Clin Pathol 119:339–345
31. Lux M, Rubin BP, Biase TL, Chen CJ, Maclure T, Demetri G, Xiao S, Singer S, Fletcher CD, Fletcher JA (2000) KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. Am J Pathol 156:791–795
32. Ma CK, Amin MB, Kintanar E, Linden MD, Zarbo RJ (1993) Immunohistologic characterization of gastrointestinal stromal tumors: a study of 82 cases compared with 11 cases of leiomyomas. Mod Pathol 6:139–144
33. Martin JF, Bazin P, Feroldi J (1960) Tumeurs myoides intramurales de lestomac; consideration microscopiques a propos de 6 cas. Ann Anat Pathol 5:484–497
34. Mazur MT, Clark HB (1983) Gastric stromal tumors: reappraisal of histogenesis. Am J Surg Pathol 7:507–519
35. Miettinen M, Lasota J (2001) Gastrointestinal stromal tumors: definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. Virchows Arch 438:1–12
36. Miettinen M, Virolainen M, Sarlomo-Rikara M (1995) Gastrointestinal stromal tumors: value of CD34 antigen in their identification and separation from true leiomyomas and schwannomas. Am J Surg Pathol 19:207–216
37. Miettinen M, Sarlomo-Rikara M, Sobin LH, Lasota J (2000) Gastrointestinal stromal tumors and leiomyosarcomas in the colon: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases. Am J Surg Pathol 24:1339–1352
38. Miettinen M, Sobin LH, Sarlomo-Rikara M (2000) Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with a reference to CD117 (KIT). Mod Pathol 13:1134–1142
39. Miettinen M, Furlong M, Sarlomo-Rikala M, Burke A, Sobin LH, Lasota J (2001) Gastrointestinal tumors, intramural leiomyomas, and leiomyosarcomas in the rectum and anus: a clinicopathologic, immunohistochemical, and molecular genetic study of 144 cases. Am J Surg Pathol 25:1121–1133
40. Miettinen M, Majidi M, Lasota J (2002) Pathology and diagnostic criteria of gastrointestinal stromal tumors (GISTs): a review. Eur J Cancer 38[Suppl]:S39–S51
41. Miettinen M, Kopczynski J, Makhiouf HR, Sarlomo-Rkala M, Gyorffy H, Burke A, Sobin LH, Lasota J (2003) Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the duodenum: a clinicopathologic, immunohistochemical, and molecular genetic study of 167 cases. Am J Surg Pathol 27:625–641
42. Miyoshi K, Iwao K, Nawa G, Yoshikawa H, Ochi T, Nakamura Y (1998) Frequent mutation in the beta-catenin gene in desmoid tumors from patients without familial adenomatous polyposis. Oncol Res 10:591–594
43. Montgomery E, Torbenson MS, Kaushal M, Fisher C, Abraham SC (2002) Beta-catenin immunohistochemistry separates me-

senteric fibromatosis from gastrointestinal stromal tumor and sclerosing mesenteritis. Am J Surg Pathol 26:1296–1301
44. Moskaluk CR, Tian Q, Marshall CR, Rumpel CA, Franquemont DW, Frierson HF Jr (1999) Mutation of c-kit JM domain are found in a minority of human gastrointestinal stromal tumors. Oncogene 18:1897–1902
45. Polakis P (1999) The oncogenetic activation of beta-catenin. Curr Opin Genet Dev 9:15–21
46. Rubin BP, Fletcher JA, Fletcher CD (2000) Molecular insights into the histogenesis and pathogenesis of gastrointestinal tumors. Int J Surg Pathol 8:5–10
47. Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R, Hibbard MK, Chen CJ, Xiao S, Tuveson DA, Demetri GD, Fletcher CD, Fletcher JA (2001) KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 61:8118–8121
48. Sabah M, Leader M, Kay E (2003) The problem with KIT: clinical implications and practical difficulties with CD117 immunostaining. Appl Immunohistochem Mol Morphol 11:56–61
49. Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, Miettinen M (1998) CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. Mod Pathol 11:728–734
50. Shidham VB, Chivukula M, Gupta D, Rao RN, Komorowski R (2002) Immunohistochemical comparison of gastrointestinal stromal tumor and solitary fibrous tumor. Arch Pathol Lab Med 126:1189–1192
51. Shitoh K, Konishi F, Iijima T, Ohdaira T, Sakai K, Kanazawa K, Miyaki M (1999) A novel case of a sporadic desmoid tumor with mutation of the beta-catenin gene. J Clin Pathol 52:695–696
52. Smithey BE, Pappo AS, Hill DA (2002) c-kit expression in pediatric solid tumor: a comparative immunohistochemical study. Am J Surg Pathol 26:486–492
53. Stout AP (1962) Bizarre smooth muscle tumors of the stomach. Cancer 15:400–409
54. Tejpar S, Nollet F, Li C, Wunder JS, Michils G, dal Cin P, Van Cutsem E, Bapat B, van Roy F, Cassiman JJ, Alman BA (1999) Predominance of beta-catenin mutations and beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor). Oncogene 18:6615–6620
55. Ueyama T, Guo KJ, Hashimoto H, Daimaru Y, Enjoji M (1992) A clinicopathologic and immunohistochemical study of gastrointestinal stromal tumors. Cancer 69:947–955
56. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciot R, Van Glabbeke M, Silverman S, Nielsen OS (2001) Safety and efficacy of imatinib (STI-571) in metastatic gastrointestinal stromal tumors: a phase I study. Lancet 358:1421–1423
57. Walker P, Dvorak AM (1986) Gastrointestinal autonomic nerve (GAN) tumor: ultrastructural evidence for a newly recognized entity. Arch Pathol Lab Med 110:309–316
58. Wieczorek TJ, Faquin WC, Rubin BP, Cibas ES (2001) Cytologic diagnosis of gastrointestinal stromal tumor with emphasis on the differential diagnosis with leiomyosarcoma. Cancer (Cancer Cytopathol) 93:276–287
59. Yantiss RK, Spiro IJ, Compton CC, Rosenberg AE (2000) Gastrointestinal stromal tumor versus intra-abdominal fibromatosis of the bowel wall: a clinically important differential diagnosis. Am J Surg Pathol 24:947–957
60. Zambrano E, Reyes-Mugica M, Franchi A, Rosai J (2003) An osteoclast-rich tumor of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: reports of 6 cases of a GIST simulator. Int J Surg Pathol 11:75–81

Expression of Epidermal Growth Factor Receptor, *ERBB2* and *KIT* in Adult Soft Tissue Sarcomas

A Clinicopathologic Study of 281 Cases

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BACKGROUND. Little is known about the expression of receptor tyrosine kinases in adult soft tissue sarcomas (STS). In the current study, the authors analyzed the expression of epidermal growth factor receptor (EGFR), *ERBB2*, and *KIT* in 281 patients with STS who were treated in a single institution. Verification of the presence of an association with prognosis was performed.

METHODS. The current study included 281 adult patients with STS of the extremity and trunk who were diagnosed and treated in the National Cancer Center, Tokyo. Expression was assessed using immunohistochemical stains for EGFR, *ERBB2*, and *KIT* on formalin-fixed, paraffin-embedded tissue sections by standard avidin-biotin peroxidase complex technique and EGFR detection system.

RESULTS. Positive staining of EGFR was observed in 168 of 281 (60%) patients. Positive staining was common in pleomorphic malignant fibrous histiocytomas (89%), myxofibrosarcomas (89%), synovial sarcomas (76%), malignant peripheral nerve sheath tumors (89%), and leiomyosarcomas (73%). It was less common in well differentiated liposarcomas (38%), fibrosarcomas (36%), and myxoid liposarcomas (6%). In contrast, positive staining of *ERBB2* and *KIT* was very limited. Increased levels of EGFR were significantly associated with a decreased probability of overall survival ($P = 0.01$), although by univariate analysis; probability of overall survival at 5 years was 64% in patients with increased levels of EGFR and 79% in patients without such overexpression. The overexpression of EGFR was significantly associated with histologic grade ($P < 0.001$). Moreover, stratified log-rank test revealed that there is an interrelation between EGFR overexpression and histologic grade.

CONCLUSIONS. EGFR overexpression was found to be a negative prognostic factor of adult STS, which is strongly associated with histologic grade. STS patients with EGFR overexpression may benefit from treatment with currently available biospecific inhibitors for EGFR. *Cancer* 2005;103:1881–90.

KEYWORDS: epidermal growth factor receptor (EGFR), soft tissue sarcoma, adult, *ERBB2*, *KIT*.

Soft tissue sarcomas (STS) currently represent 1% of adult malignancies, and their treatment is controversial.[1] Although local control can be obtained through surgery and radiation, up to 30% of patients with extremity and/or trunk STS will eventually experience recurrence at distant sites, and the overwhelming majority of these patients will ultimately die from this cause.[2] Obstacles to success of contemporary treatments include development of drug resistance in tumor cells and insufficient tumor-selective treatments.

Receptor tyrosine kinases (RTK) are gaining attention as prognos-

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tic markers and as possible therapeutic targets as the number of trials grows for biospecific inhibitors. The *ERBB2* (also called HER-2) protooncogene, which is located on human chromosome 17, encodes a 185-kilodalton transmembrane glycoprotein that shows significant structural similarity[3] and functional association to the epidermal growth factor receptor (EGFR).[4–6] *ERBB2* has been found to be overexpressed in many different types of human malignancies, notably, lung,[7] breast,[8] ovarian,[9] pancreatic,[10] and osteosarcoma.[11,12] Overexpression of *ERBB2* in breast carcinoma has been associated with poor overall survival and has been shown to enhance malignancy and metastatic potential. Trastuzumab (Herceptin; Genentech, South San Francisco, CA), a monoclonal antibody that targets *ERBB2*, demonstrated therapeutic benefit in advanced breast carcinoma patients.[13] Aberrant expression and activation of EGFR is characteristic of many human cancers and is often associated with poor clinical outcome and chemoresistance.[14] Gefitinib (Iressa; AstraZeneca Plc., London, UK), a small molecule inhibitor of EGFR tyrosine kinase activity, has already been approved for cancer treatment.[15]

KIT, an RTK for the stem cell factor, has been implicated in the pathophysiologic mechanisms of a variety of human tumors.[16–19] Activating mutations of *KIT* have been described in gastrointestinal stromal tumors (GIST). STI571 (Glivec; Novartis Ag, Basel, Switzerland), a specific inhibitor of tyrosine kinases, has shown promise in the management of patients with GIST.[20]

Relatively little is known about expression of these RTKs in STS and their clinical significance to STS has been poorly investigated. Our group studied expression patterns of EGFR, *ERBB2*, and *KIT* on 281 patients with common adult STS by standard immunohistochemical staining and, then, verified whether a significant association with prognosis was present.

MATERIALS AND METHODS

Patients

Records of 281 patients with primary localized STSs of the extremity and trunk wall, who were diagnosed and treated between January, 1975 and December, 2002 were retrieved from the pathology files of the National Cancer Center (NCC), Tokyo, Japan. Common histologic categories such as liposarcoma, myxofibrosarcoma, malignant fibrous histiocytoma (MFH), synovial sarcoma, and spindle cell sarcoma (fibrosarcoma, leiomyosarcoma, and malignant peripheral nerve sheath tumor: MPNST) were included in the current study. MFH lesions were subdivided into pleomorphic MFH (PMFH) and myxofibrosarcoma. Liposarcoma was subclassified into well differentiated liposarcoma and myxoid liposarcoma. Dedifferentiated liposarcoma and pleomorphic liposarcoma were not included in the current study. Between the two dates, 745 newly diagnosed STS patients were seen. Among them, 379 were excluded because of tumor histology: rhabdomyosarcoma (194), alveolar soft part sarcoma (20), angiosarcoma (30), epithelioid sarcoma (25), extraskeletal myxoid chondrosarcoma (20), extraskeletal Ewing sarcoma (21), extraskeletal osteosarcoma (13), clear cell sarcoma (16), dedifferentiated liposarcoma (34) and pleomorphic liposarcoma (6). Our group excluded rhabdomyosarcoma and Ewing sarcoma, as their treatment is different. The other histologic categories were excluded because of rarity. Because tumors having the same histology will exhibit different behavior patterns depending on anatomical site,[2] 42 patients with retroperitoneal, head and neck, visceral, and intrathoracic tumors were excluded. Patients with Stage IV sarcomas (43) were also excluded.

There were 152 females and 129 males, whose ages ranged from 10 to 85 years (median 50 yrs). The clinical details, including follow-up information, were obtained by reviewing all medical charts. All of the patients underwent tumor resection. Adjuvant treatment in the form of radiotherapy (25 patients), chemotherapy (37), or both (23) was administered as part of standard care or as part of clinical trials. Preoperative adjuvant treatment was given to 43 patients. No patients were lost to follow up. Follow-up data began on the date of diagnosis. Median follow up was 60 months. Overall survival was recorded as the time to death due to cause related with disease.

The protocol of this study was reviewed and approved by the Institutional Review Board of the NCC.

Pathology Reviewing, Grade, and Staging

Histologic slides of primary tumors from all patients were reviewed for diagnosis by an NCC pathologist (T. H.) who had developed the grading system.[21] Whenever necessary, immunohistochemistry was used for confirming the diagnosis or tumor typing according to the World Health Organization Classification of Soft Tissue Tumors (2002).[22] The histologic grade is a three-grade system (using scores 0, 1, 2, 3) that is obtained by adding scores for tumor differentiation, tumor necrosis, and the MIB-1 labeling index. MIB-1 scores were determined by staining sections with an antibody for MIB-1 (1:100, Immunotech, Marseille, France). An MIB-1 score of 1 was assigned to lesions with an MIB-1 labeling index (LI) of 0–9%, an MIB-1 score of 2 was assigned to lesions with an MIB-1 LI of 10–29%, and an MIB-1 score of 3 was assigned to lesions with an MIB-1 LI $\geq$ 30%.[23] Our

TABLE 1
Primary Antibodies.

Antibody	Source	Clone	Dilution	Positive control
EGFR, mouse monoclonal	DakoCytomation[a]	2-18C9	Prediluted	Breast carcinoma
ERBB2, rabbit polyclonal	DakoCytomation		1:500	Breast carcinoma
KIT, rabbit polyclonal	DakoCytomation		1:50	Mast cells

[a] Manufacturer is located in Glostrup, Denmark.

group has previously shown that validity and reproducibility of the MIB-1 score are superior to those of the mitotic score.[24]

For TNM staging classification,[22] Grade 1 tumors that were assessed by using the above-mentioned grading system were grouped as low grade, and Grade 2 and 3 tumors as high grade. To define depth, superficial lesions did not involve the superficial fascia, and deep lesions had reached to or invaded the superficial fascia.

Immunohistochemical Analysis of EGFR, *ERBB2*, and *KIT* in Tissue Samples

Immunohistochemical analysis of *ERBB2* and *KIT* was performed on tissue sections from paraffin blocks by the labeled streptavidin-biotin method. Sections were dewaxed, rehydrated, and moistened with phosphate-buffered saline (PBS; pH 7.4). They were pretreated in an autoclave at 121 °C for 10 minutes in 10 mmol/L citrate buffer (pH 6.0), before being incubated either with antihuman *ERBB2* or *KIT* antibody on an automated immunostaining system (i6000™; BioGenex, San Ramon, CA) for 30 minutes. Immunohistochemical staining of EGFR on sections from paraffin blocks was performed according to manufacturer's instructions included with the EGFR detection system (EGFR pharmDx *kit*, DakoCytomation, Glostrup, Denmark). Specifications of primary antibodies are listed in Table 1. Anti-EGFR monoclonal antibody, clone 2-18C9, which binds to an epitope located near the ligand binding domain on the extracellular domain of EGFR, is specific for EGFR and does not crossreact with *ERBB2* or the other receptors of the *ERBB* family.[25]

In an attempt to elucidate whether the overexpressed EGFR was really functioning, immunohistochemical analysis of phosphorylated EGFR was performed on formalin-fixed, paraffin-embedded tissue sections from 20 synovial sarcomas in which an increased level of EGFR was detected. Our group used a mouse monoclonal anti-HER1pY1092 (1068) antibody, kindly provided by DakoCytomation A/S (Glostrup, Denmark), which detects specifically the levels of phosphorylated EGFR at tyrosine 1068. It does not detect other phosphorylated EGFR families.

A multiheaded microscope was used to read immunohistochemical results, which were judged by investigators, all of whom were blinded to the clinical status of patients. A consensus judgment was adopted as to the proper immunohistochemical score of a tumor based on strength: 0 = negative; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining. Negative controls, in which the primary antibody was omitted, were included with each run. Breast carcinomas with stain results of 3+ were used as positive controls for anti-EGFR and anti-*ERBB2* antibodies. Tissue mast cells, which stained a 3+ score, were used as internal positive controls for the anti-*KIT* antibody. The distribution of positive cells was also recorded to impart the diffuse or focal nature of positive cells; sporadic (positive cells $< 10\%$); focal ($11\% <$ positive cells $< 50\%$); diffuse (positive cells $\geq 50\%$). The immunohistochemical scores of 2+ and 3+ with focal to diffuse distribution were considered to be positive for all three antibodies.

Statistical Methods

The chi-square test was used to evaluate the association between two dichotomous variables. Kaplan-Meier plots and the log-rank test were used to evaluate the association of overexpression of EGFR with overall survival. Cox proportional-hazards regression analysis with forward selection of variables was performed to estimate the rate ratios for possible risk factors for the occurrence of adverse events. Data analysis was performed with an SAS software statistical package (version 6.0, SAS Institute, Cary, NC).

RESULTS

The study group comprised 281 primary STSs, which included 52 myxoid liposarcomas, 50 well differentiated liposarcomas, 53 myxofibrosarcomas, 44 PMFHs, 42 synovial sarcomas, 18 MPNTs, 11 fibrosarcomas, and 11 leiomyosarcomas.

Results are summarized in Table 2. Moderate (2+) to strong (3+) staining of EGFR was observed 168 of 281 (60%) patients, which included 39 of 44 (89%) PMFH, 47 of 53 (89%) myxofibrosarcomas, 32 of 42 (76%) synovial sarcomas, 16 of 18 (94%) MPNST, and 8 of 11 (73%) leiomyosarcomas (Figs. 1 and 2). The incidence of positive staining was lower in fibrosarcomas (4 of 11, 36%), myxoid liposarcomas (3 of 52, 6%), and well differentiated liposarcomas (19 of 50, 38%).

Overexpression of both *ERBB2* and *KIT* was limited. Moderate (2+) staining of *ERBB2* was observed in 3 synovial sarcomas, whereas weak (1+) staining was

TABLE 2
Immunohistochemical Pattern of EGFR, *ERBB2*, and *KIT* in 281 STS Patients

Histologic type	No. Patients	EGFR				*ERBB2*				*KIT*			
		0	1+	2+	3+	0	1+	2+	3+	0	1+	2+	3+
Fibrosarcoma	11	7	0	4	0	11	0	0	0	11	0	0	0
Leiomyosarcoma	11	3	0	8	0	11	0	0	0	8	3	0	0
Myxoid lipsarcoma	52	48	1	3	0	52	0	0	0	52	0	0	0
Well differentiated liposarcoma	50	28	3	11	8	50	0	0	0	50	0	0	0
Myxofibrosarcoma	53	5	0	26[a]	22	53	0	0	0	52	1	0	0
MPNST	18	1	0	6[a]	11	17	1	0	0	17	1	0	0
PMFH	44	4	0	22[a]	18	44	0	0	0	44	0	0	0
Synovial sarcoma	42	4	6	15	17	23	16	3	0	40	0	2	0
Biphasic type	11												
Spindle cells		0	2	4	5	2	9	0	0	11	0	0	0
Epithelioid cells		5	6	0	0	2	6	3	0	11	0	0	0
Monophasic type	31												
Spindle cells		4	4	11	12	21	10	0	0	29	0	2	0

MPNST: malignant peripheral nerve sheath tumor, PMFH: pleomorphic MFH.
[a] Each of these groups includes a case with sporadic distribution. They were evaluated as negative staining.

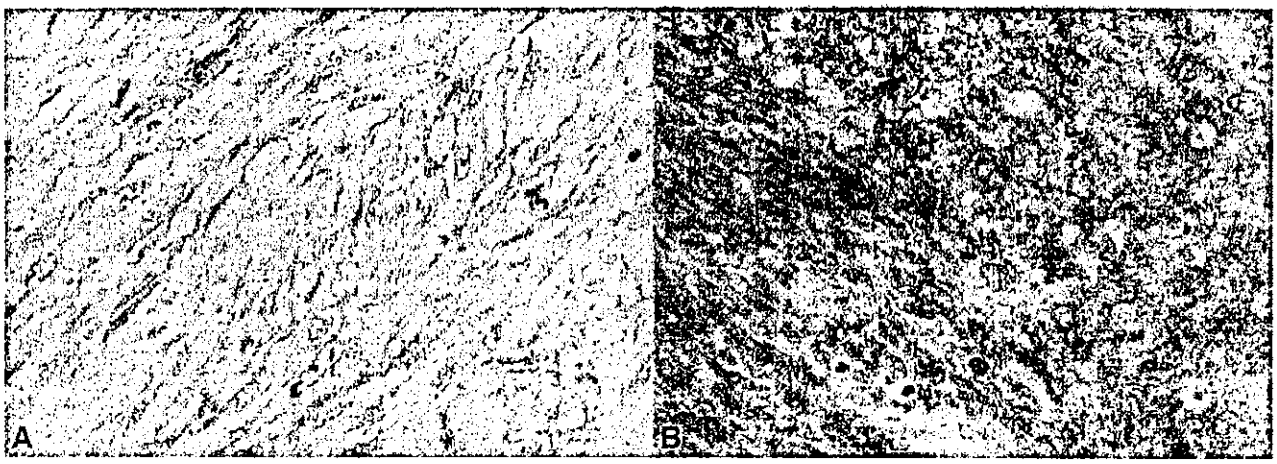

FIGURE 1. Immunohistochemical pattern of EGFR overexpression in adult soft tissue sarcoma. Moderate (2+) and diffuse staining in a leiomyosarcoma (A), and strong (3+) and diffuse staining in a malignant peripheral nerve sheath tumor. (Magnification × 400).

noted in 16 synovial sarcomas and 1 MPNST. In synovial sarcomas, *ERBB2* was expressed in the glandular epithelial component of 9 biphasic tumors and in solid spindle cell areas of 10 monophasic tumors. In contrast, moderate (2+) to strong (3+) staining of EGFR was found only in spindle cells of either biphasic or monophasic tumors (Fig. 2). In *KIT* expression, moderate (2+) staining was identified in 2 monophasic synovial sarcomas. Weak (1+) staining was observed in 3 leiomyosarcomas, 1 myxofibrosarcoma, and 1 MPNST

Noteworthy is that overexpressed EGFR may actually be functioning in tumors. Immunohistochemical analysis of phosphorylated EGFR was performed in 20 synovial sarcomas in which overexpression of EGFR was identified. Positive membranous staining of phosphorylated EGFR was found in 10 of 20 (50%) tumors (Fig. 3). Phosphorylated EGFR was expressed in either spindle cells or epithelioid cells of monophasic and biphasic tumors.

Association of EGFR Overexpression with Clinical and Pathologic Features

Immunostaining for EGFR was positive more frequently in superficial tumors ($P = 0.001$) and in smaller tumors ($P = 0.002$). Overexpression of EGFR was significantly associated with tumor stage and histologic grade ($P \leq 0.001$). No significant association

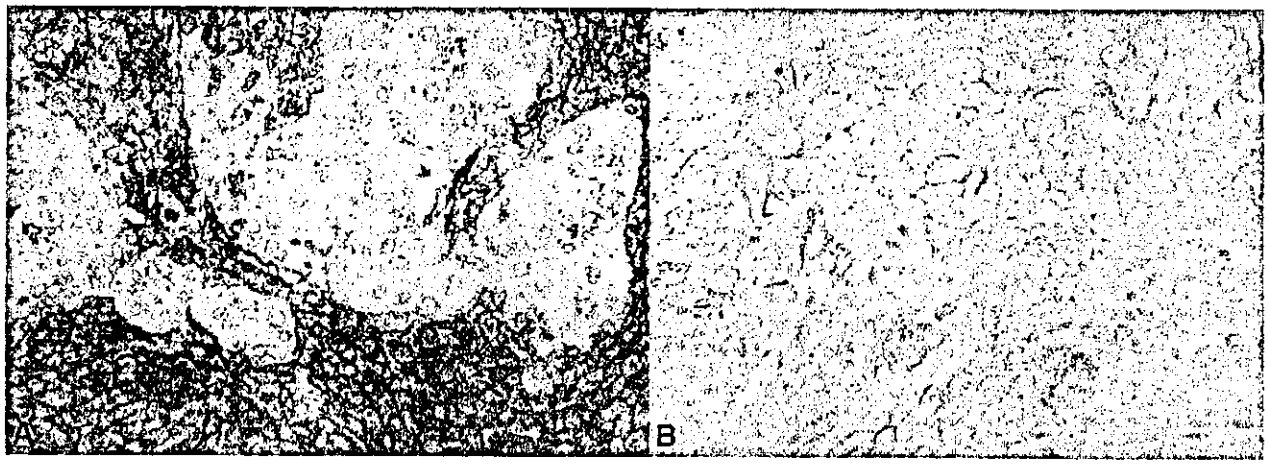

FIGURE 2. Immunohistochemical pattern of EGFR and *ERBB2* expression in a biphasic synovial sarcoma. Strong (3+) staining of EGFR in spindle cells (A) and moderate (2+) staining of *ERBB2* in epithelioid cells (B). (Magnification × 400).

FIGURE 3. Immunohistochemical pattern of phosphorylated EGFR in synovial sarcoma in which increased levels of EGFR were detected. Positive membranous staining of phosphorylated EGFR in both epithelioid and spindle cells of a biphasic synovial sarcoma. (Magnification × 400).

was found between the incidence of EGFR positivity and age, gender, or anatomic site (Table 3).

In 281 patients, increased levels of EGFR were significantly associated with a decreased probability of overall survival ($P = 0.01$); probability of overall survival at 5 years was 63.6% in patients with increased levels of EGFR and 79.1% in patients without such overexpression (Fig. 4). The survival of these patients within each histologic subgroup was no different between EGFR positive and negative tumors (data not shown).

Several variables were tested to assess whether they had an impact on survival. Univariate analysis showed that tumor stage, histologic grade, tumor depth, tumor size, and positive EGFR staining were predictors of survival (Table 4). Multivariate analysis revealed that histologic grade and tumor size persisted as independent risk factors for poor outcome. The association between EGFR overexpression and decreased survival of patients was no longer statistically significant (Table 5). This allowed comparison between EGFR positive and negative tumors stratified either by histologic grade or tumor size (Figs. 5 and 6). There was no statistical difference in survival of patients between EGFR positive and negative tumors within each histologic grade subgroup. A significant association between EGFR overexpression and decreased survival was observed only in the > 5 cm to 10cm tumor size subgroup.

DISCUSSION

The current study provides the first immunohistochemical evidence based on large series that EGFR overexpression occurs in 60% of STSs. Increased levels of EGFR were significantly associated with a decreased overall survival on univariate analysis and correlated with histologic grade, which is an independent risk factor for a poor outcome. Moreover, phosphorylation of EGFR at tyrosine 1068 was detected in at least 50% of synovial sarcomas that overexpressed EGFR. Tyrosine 1068 within the cytoplasmic domain of the receptor is an autophosphorylation site used as a marker of receptor activation.[26] Our results indicate that this parameter may provide prognostic information and, therefore, the authors of the current study suggest that specific therapy with humanized monoclonal antibodies against EGFR or small molecular inhibitors for tyrosine kinase of EGFR be considered in a significant number of STSs. In contrast, the distribution of *ERBB2* and *KIT* overexpression is limited,

TABLE 3
Association of EGFR and Clinical Variables

Variable	No. of patients	EGFR overexpression			
		Negative	Positive	%[a]	*P* value
Age (yrs)					
< 50	123	54	69	56	0.3
≥ 50	158	59	99	63	
Gender					
Male	129	47	82	64	0.3
Female	152	66	86	57	
Site					
Extremity	202	87	115	57	0.1
Trunk	79	26	53	67	
Size					
≤ 5 cm	69	17	52	75	0.002
5–10 cm	135	55	80	59	
> 10 cm	77	41	36	47	
Tumor depth					
Superficial	65	15	50	77	0.001
Deep	216	98	118	55	
Histologic type					
Well differentiated liposarcoma	50	31	19	38	< 0.001
Myxoid liposarcoma	52	49	3	6	
Myxofibrosarcoma	53	6	47	89	
PMFH	44	5	39	89	
Synovial sarcoma	42	10	32	76	
MPNST	18	2	16	89	
Leiomyosarcoma	11	3	8	73	
Fibrosarcoma	11	7	4	36	
Histologic grade					
I	96	65	31	32	< 0.001
II	74	22	52	70	
III	111	26	85	77	
Stage					
I	72	23	49	68	< 0.001
II	129	68	61	47	
III	80	22	58	73	

PMFH: pleomorphic MFH; MPNST: malignant peripheral nerve sheath tumor.
[a] Percentage of positive cases

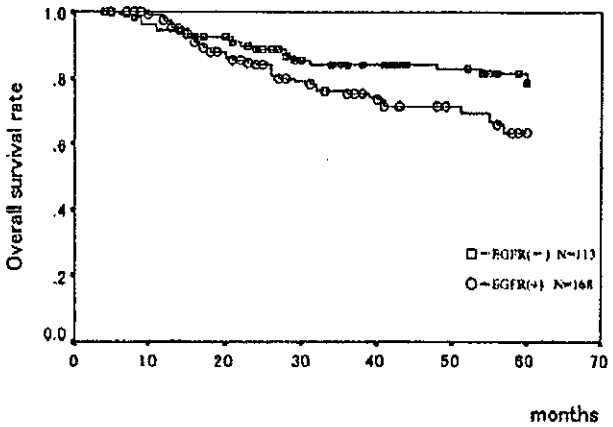

FIGURE 4. Kaplan–Meier plots for overall survival for EGFR positive cases compared with negative cases.

TABLE 4
Variables Associated with Patient Survival: Univariate Analysis

Variables	No. patie	5-yr survival rate (95% CI)	Log rank *P* value	Relative risk
Age (yrs)				
[a]< 50	123	71.0 (61.6–80.3)	0.5	(—)
≥ 50	158	69.8 (61.2–78.3)		
Gender				
Male	129	71.7 (63.4–80.1)	0.9	(—)
Female	152	71.4 (62.3–80.5)		
Site				
Extremity	202	72.1 (64.7–79.6)	0.5	(—)
Trunk	79	66.3 (54.6–78.1)		
Size				
< 5 cm	69	81.5 (70.3–91.8)	0.02	1
5–10 cm	135	71.2 (62.0–80.3)		1.8 (0.9–3.7)
> 10 cm	77	58.5 (45.4–71.5)		2.7 (1.3–5.7)
Tumor depth				
Superficial	65	81.8 (70.7–92.9)	0.04	1
Deep	216	66.8 (59.4–74.2)		2.0 (1.0–4.1)
Histologic type				
Well differentiated liposarcoma	50	100	< 0.001	(—)
Myxoid liposarcoma	52	76.7 (63.8–89.6)		
Myxofibrosarcoma	53	84.7 (73.0–96.4)		
PMFH	44	44.9 (27.5–62.3)		
Synovial sarcoma	42	58.5 (41.9–74.9)		
MPNST	18	48.2 (22.8–73.6)		
Leiomyosarcoma	11	45.5 (8.1–82.8)		
Fibrosarcoma	11	68.6 (32.1–100)		
Histologic grade				
I	96	97.8 (93.6–100)	< 0.001	1
II	74	79.7 (69.3–90.1)		13 (1.8–104)
III	111	41.9 (30.9–53.0)		57 (8.0–414)
Stage				
I	72	94.0 (87.2–100)	< 0.001	1
II	129	74.6 (65.5–83.8)		5.3 (1.6–17)
III	80	41.2 (28.2–54.2)		18 (5.4–57)
EGFR overexpression				
Negative	113	79.1 (70.5–87.6)	0.01	1
Positive	168	63.6 (54.7–72.4)		1.8 (1.1–3.0)

CI: confidence interval; PMFH: pleomorphic MFH; MPNST: malignant peripheral nerve sheath tumor.

suggesting that their therapeutic use does not appear promising.

Immunohistochemistry (IHC) is the most common method for detection of RTK overexpression, but it is significantly affected by the sensitivity and specificity of the antibodies used, the type of tissue (frozen versus formalin-fixed, paraffin-embedded), and various interpretative criteria and scoring systems used to evaluate cases. For these reasons, our group used an EGFR IHC test developed by DakoCytomation (Glostrup, Denmark), the pharm Dx EGFR IHC assay, to evaluate EGFR immunoreactivity and two polyclonal antibodies to evaluate *ERBB2* and *KIT*, respectively. These antibodies are arguably the most diffuse

TABLE 5
Factors Relevant for Survival: Cox Proportional Hazards Regression Model

Variable	Hazard ratio	95% CI	*P* value
Tumor size			
≤ 5 cm	1		
5–10 cm	1.4	0.68–3.0	0.35
> 10 cm	2.4	1.1–5.2	0.029
Depth			
Superficial	1		
Deep	1.1	0.53–2.4	0.78
Grade			
I	1		
II	16	2.2–130	0.007
III	64	8.7–480	< 0.001
EGFR overexpression			
Negative	1		
Positive	0.83	0.48–1.4	0.51

CI: confidence interval.

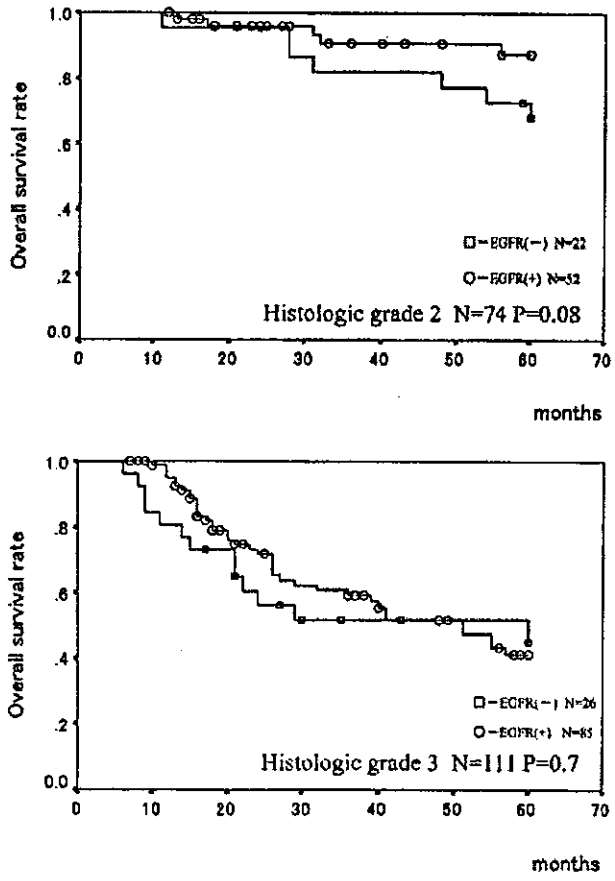

FIGURE 5. Kaplan–Meier survival curves stratified by EGFR status and by histologic grade. A: histologic Grade 2, B: histologic Grade 3

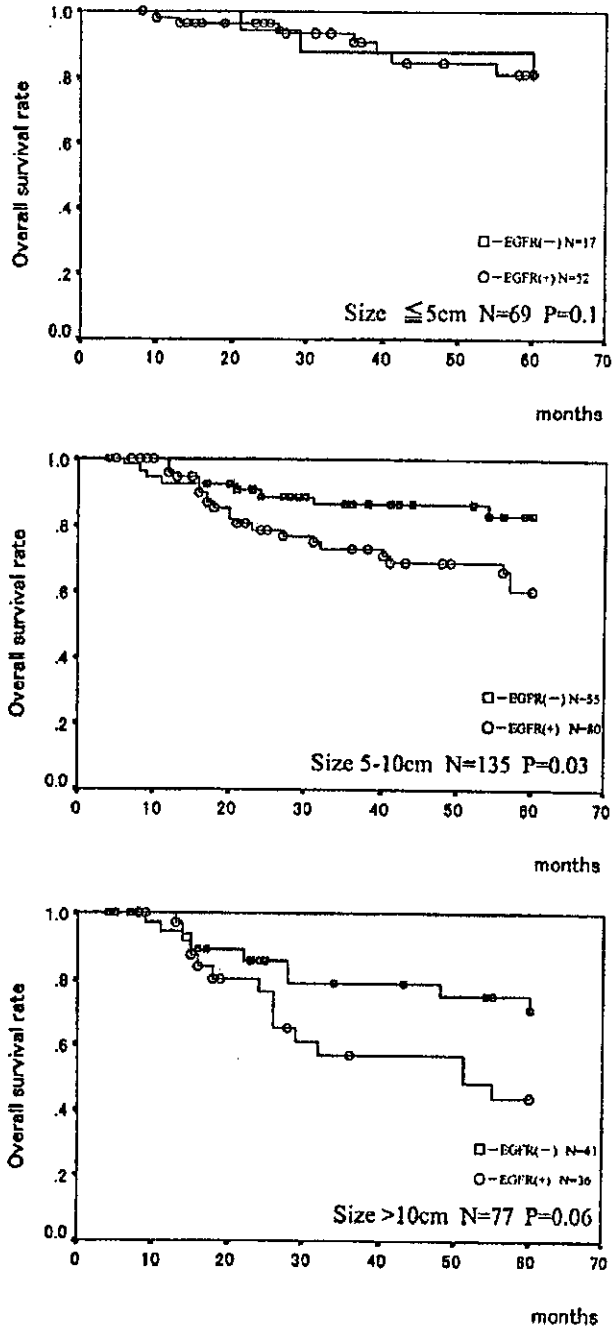

FIGURE 6. Kaplan–Meier survival curves stratified by EGFR status and by tumor size. A: size ≤ 5 cm, B: 5–10 cm, C: > 10 cm.

and thoroughly tested antibodies for each of the three RTKs. Positivity was determined by evaluating both intensity and distribution of immunostaining. The scoring system was adopted from tests of *ERBB2* expression. Breast carcinomas with stain 3+ were used as a positive control of EGFR, and *ERBB2* expression and tissue mast cells with stain 3+ were

used as an internal positive control of *KIT* expression.

The overexpression of EGFR in STSs was first documented by two teams of investigators in the 1980s. Guesterson et al.[27] identified overexpressed EGFR in 18 of 35 sarcoma specimens by IHC. Strong immunostaining was noted in MFH, epithelioid sarcoma, and synovial sarcoma. Perosio et al.[28] demonstrated that 20 of 40 STSs exhibited positive immunoreactivity. Afterward, Duda et al.[29] determined gene amplification and expression of EGFR in sarcomas. Amplification of EGFR was identified in 2 of 117 (1.7%) sarcomas. Overexpression of EGFR was identified in 21 of 43 (49%) sarcomas. The overexpression was frequently observed in MFH and leiomyosarcoma. A recent investigation reported gene expression profiles of 41 soft tissue tumors with cDNA microarray analysis. Among these sarcomas, 6 monophasic synovial sarcomas were characterized by a unique expression pattern of a cluster of 104 genes, including EGFR.[30] Barbashina et al.[31] reported that 13 of 19 (68%) synovial sarcomas were immunoreactive to EGFR. Nielsen et al.[32] showed that 52% of synovial sarcomas were strongly immunoreactive to the same monoclonal antibody, 2-18C9, used in this study. The current study expands upon previous work by demonstrating, in a larger cohort of patients, that positive staining of EGFR was found in 60% of 281 adult STSs, and that PMFH, myxofibrosarcoma, synovial sarcoma, MPNST, and leiomyosarcoma had a substantial proportion of tumors with strong, diffuse positivity for EGFR. FISH analysis, used in the current study, has also disclosed that this overexpression is not associated with gene amplification in most of the EGFR 3+ tumors (data not shown).

To date, there has been no report showing the association of EGFR overexpression and clinical outcome in STSs. In our STS patients, increased levels of EGFR were significantly associated with decreased overall survival rate, although at least by univariate analysis. When histologic grade and tumor size variables were analyzed by the Cox proportional hazards model, this association was no longer statistically significant. The stratified log-rank test failed to show statistical difference in survival of patients between EGFR positive and negative tumors in each grade–size subgroup, with the exception of the > 5–10 cm tumor size subgroup. These observations suggest that EGFR overexpression is an unfavorable prognostic factor that may have a strong association with histologic grade. The initial univariate association may be a reflection of differences in histologic grade.

Whether *ERBB2* and *KIT* overexpression occur in STSs remains controversial. Reports demonstrated that *ERBB2* is overexpressed in 30–50% of STSs.[29,31,33,34] However, Merimsky et al.[35] found no overexpression of *ERBB2* in 230 cases of STS. There have been several reports of *KIT* immunostaining in a limited number of STSs, other than GISTs, including clear cell sarcoma, synovial sarcoma, dermatofibrosarcoma protuberans, and Ewing sarcoma.[36] Hornick et al.[37] evaluated 365 specimens of STSs and found *KIT* overexpression in a very limited number of tumors. Our results are consistent with studies based on large series showing limited expression of *ERBB2* and *KIT* in STS.

There was a significant difference in EGFR expression between well differentiated and myxoid liposarcomas. It is suggested that well differentiated liposarcomas contain exclusively fibroblastic spindle and satellite cells.[38,39] In contrast, myxoid liposarcomas are neoplasms composed primarily of a mixture of cell types, varying from undifferentiated mesenchymal cells to late lipoblasts, and, occasionally, mature adipocytes.[40] The distinct tumor cell components may explain the differences in EGFR expression between these two subgroups of liposarcoma.

The overexpression of EGFR was more frequent in small and/or superficial tumors compared to large/deep tumors. There was also a significant correlation with decreased overall survival. This counterintuitive result may be explained by the heterogeneity of these groups and does not appear biologically significant. A number of small/superficial tumors included PMFH, synovial sarcomas, and MPNST, which were of high grade and expressed EGFR frequently. Conversely, a number of large/deep tumors included well differentiated liposarcomas and myxoid liposarcomas, which were of low grade and expressed EGFR less frequently.

It is unknown why tumors associated with Stage II disease were less likely to demonstrate EGFR overexpression than Stage I or III tumors. Stages are determined by histologic grade, tumor size, and tumor depth. The impact of EGFR overexpression varies according to each of the three factors, and, overall, this averaged difference is not so great. Therefore, this may be an example of random variation.

An interesting observation is reciprocal expression of EGFR and *ERBB2* in synovial sarcoma. EGFR was predominantly expressed in spindle cells, whereas *ERBB2* was expressed in epithelioid cells, which is concordant with recent studies.[31,41,42] It is likely that biphasic synovial sarcomas coexpress EGFR and *ERBB2*. *ERBB2* forms heterodimers with EGFR and modulates EGFR function.[4,5] The heterodimerization of EGFR and *ERBB2* may play a role in the mesenchymal to epithelial differentiation of synovial sarcoma. Synovial sarcoma consistently shows a specific t(X;18; p11;q11), which usually represents either of two gene fusions, SYT-SSX1 or SYT-SSX2, encoding putative

transcriptional protein differing at the 13 amino acid position. There is a strong association of fusion type and morphology, with almost all SYT-SSX2 tumors showing absence of glandular differentiation (monophasic histology) and almost all biphasic tumors containing SYT-SSX1.[43,44] It is unknown whether these translocation-derived chimeric transcription factors are associated with the *ERBB2* gene expression profile.

Therapeutic approaches targeting the EGFR signaling pathway, either alone or in combination with radiation or cytotoxic agents, are being intensively investigated. Strategies that are in various stages of development include blockade of the extracellular receptor domain,[45,46] inhibition of the intracellular tyrosine kinase activity, inhibition of receptor production by antisense approaches, expression of a truncated dominant-negative EGFR mutant, and so on. For example, anti-EGFR monoclonal antibody, IMC-C225/cetuximab, in combination with chemotherapy or radiation, is being addressed in Phase III clinical trials, and many small-molecule tyrosine kinase inhibitors are in Phase I–II clinical testing (See Grunwald.[15]). These results, along with the determination of the high frequency of EGFR expression in STS and its association with a negative prognosis, may open the door to a clinical trial of currently available biospecific inhibitors of EGFR in STS.

REFERENCES

1. Antman KH. Adjuvant therapy of sarcomas of soft tissue. *Semin Oncol.* 1997;24:556–560.
2. Stojadinovic A, Leung DH, Allen P, Lewis JJ, Jaques DP, Brennan MF. Primary adult soft tissue sarcoma: time-dependent influence of prognostic variables. *J Clin Oncol.* 2002;20:4344–4352.
3. Bargmann CI, Hung MC, Weinberg RA. The neu oncogene encodes an epidermal growth factor receptor-related protein. *Nature.* 1986;319:226–230.
4. Kokai Y, Myers JN, Wada T, Brown VI, LeVea CM, Davis JG, et al. Synergistic interaction of p185c-neu and the EGF receptor leads to transformation of rodent fibroblasts. *Cell.* 1989;58:287–292.
5. Wada T, Qian XL, Greene MI. Intermolecular association of the p185neu protein and EGF receptor modulates EGF receptor function. *Cell.* 1990;61:1339–1347.
6. Wada T, Myers JN, Kokai Y, Brown VI, Hamuro J, LeVea CM, et al. Anti-receptor antibodies reverse the phenotype of cells transformed by two interacting proto-oncogene encoded receptor proteins. *Oncogene.* 1990;5:489–495.
7. Potti A, Willardson J, Forseen C, Kishor Ganti A, Koch M, Hebert B, et al. Predictive role of HER-2/neu overexpression and clinical features at initial presentation in patients with extensive stage small cell lung carcinoma. *Lung Cancer.* 2002;36:257–261.
8. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science.* 1987;235:177–182.
9. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science.* 1989;244:707–712.
10. Dugan MC, Dergham ST, Kucway R, Singh K, Biernat L, Du W, et al. HER-2/neu expression in pancreatic adenocarcinoma: relation to tumor differentiation and survival. *Pancreas.* 1997;14:229–236.
11. Kilpatrick SE, Geisinger KR, King TS, Sciarrotta J, Ward WG, Gold SH, et al. Clinicopathologic analysis of HER-2/neu immunoexpression among various histologic subtypes and grades of osteosarcoma. *Mod Pathol.* 2001;14:1277–1283.
12. Akatsuka T, Wada T, Kokai Y, Kawaguchi S, Isu K, Yamashiro K, et al. ErbB2 expression is correlated with increased survival of patients with osteosarcoma. *Cancer* 2002;94:1397–1404.
13. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER-2 for metastatic breast cancer that overexpresses HER-2. *N Engl J Med.* 2001;344:783–792.
14. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol.* 1995;19:183–232.
15. Grunwald V, Hidalgo M. Developing inhibitors of the epidermal growth factor receptor for cancer treatment. *J Natl Cancer Inst.* 2003;95:851–867.
16. Turner AM, Zsebo KM, Martin F, Jacobsen FW, Bennett LG, Broudy VC. Nonhematopoietic tumor cell lines express stem cell factor and display c-*kit* receptors. *Blood.* 1992;80:374–381.
17. DiPaola RS, Kuczynski WI, Onodera K, Ratajczak MZ, Hijiya N, Moore J, et al. Evidence for a functional kit receptor in melanoma, breast, and lung carcinoma cells. *Cancer Gene Ther.* 1997;4:176–182.
18. Tian Q, Frierson HF, Jr., Krystal GW, Moskaluk CA. Activating c-kit gene mutations in human germ cell tumors. *Am J Pathol.* 1999;154:1643–1647.
19. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science.* 1998;279:577–580.
20. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med.* 2001;344:1052–1056.
21. Hasegawa T, Yamamoto S, Yokoyama R, Umeda T, Matsuno Y, Hirohashi S. Prognostic significance of grading and staging systems using MIB-1 score in adult patients with soft tissue sarcoma of the extremities and trunk. *Cancer.* 2002;95:843–851.
22. Fletcher CD, Sundaram M, Rydholm A, Coindre JM, Singer S. Soft tissue tumours: epidemiology, clinical features, histological typing and grading. In: Fletcher CD, Unni KK, Mertens F. World Health Organization classification of tumours, pathology and genitics: tumors of soft tissue and bone. Lyon: IARCPress, 2002:12–18.
23. Hasegawa T, Yokoyama R, Lee YH, Shimoda T, Beppu Y, Hirohashi S. Prognostic relevance of a histological grading system using MIB-1 for adult soft-tissue sarcoma. *Oncology.* 2000;58:66–74.
24. Hasegawa T, Yamamoto S, Nojima T, Hirose T, Nikaido T, Yamashiro K, et al. Validity and reproducibility of histologic diagnosis and grading for adult soft-tissue sarcomas. *Hum Pathol.* 2002;33:111–115.

25. Spaulding DC, Spaulding BO. Epidermal growth factor receptor expression and measurement in solid tumors. *Semin Oncol.* 2002;29:45–54.
26. Nielsen UB, Cardone MH, Sinskey AJ, MacBeath G, Sorger PK. Profiling receptor tyrosine kinase activation by using Ab microarrays. *Proc Natl Acad Sci U S A.* 2003;100:9330–9335.
27. Gusterson B, Cowley G, McIlhinney J, Ozanne B, Fisher C, Reeves B. Evidence for increased epidermal growth factor receptors in human sarcomas. *Int J Cancer.* 1985;36:689–693.
28. Perosio PM, Brooks JJ. Expression of growth factors and growth factor receptors in soft tissue tumors. Implications for the autocrine hypothesis. *Lab Invest.* 1989;60:245–253.
29. Duda RB, Cundiff D, August CZ, Wagman LD, Bauer KD. Growth factor receptor and related oncogene determination in mesenchymal tumors. *Cancer.* 1993;71:3526–3530.
30. Nielsen TO, West RB, Linn SC, Alter O, Knowling MA, O'Connell JX, et al. Molecular characterisation of soft tissue tumours: a gene expression study. *Lancet.* 2002;359:1301–1307.
31. Barbashina V, Benevenia J, Aviv H, Tsai J, Patterson F, Aisner S, et al. Oncoproteins and proliferation markers in synovial sarcomas: a clinicopathologic study of 19 cases. *J Cancer Res Clin Oncol.* 2002;128:610–616.
32. Nielsen TO, Hsu FD, O'Connell JX, Gilks CB, Sorensen PH, Linn S, et al. Tissue microarray validation of epidermal growth factor receptor and SALL2 in synovial sarcoma with comparison to tumors of similar histology. *Am J Pathol.* 2003;163:1449–1456.
33. Sato T, Peiper M, Heinecke A, Zurakowski D, Eisenberger CF, Hosch S, et al. Expression of HER-2/neu Does not Correlate with Survival in Soft Tissue Sarcoma. *Onkologie.* 2003; 26:268–271.
34. Foster H, Knox S, Ganti AK, Hebert BJ, Koch M, Tendulkar K, et al. HER-2/neu overexpression detected by immunohistochemistry in soft tissue sarcomas. *Am J Clin Oncol.* 2003;26: 188–191.
35. Merimsky O, Issakov J, Schwartz I, Dadia S, Kollender Y, Bickels J, et al. Lack of ErbB-2 oncogene product overexpression in soft tissue sarcomas. *Acta Oncol.* 2002;41:366–368.
36. Smithey BE, Pappo AS, Hill DA. C-kit expression in pediatric solid tumors: a comparative immunohistochemical study. *Am J Surg Pathol.* 2002;26:486–492.
37. Hornick JL, Fletcher CD. Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. *Am J Clin Pathol.* 2002;117:188–193.
38. Bolen JW, Thorning D. Liposarcomas. A histogenetic approach to the classification of adipose tissue neoplasms. *Am J Surg Pathol.* 1984;8:3–17.
39. Chorneyko K. The ultrastructure of liposarcomas with attention to "dedifferentiation." *Ultrastruct Pathol.* 1997;21:545–557.
40. Hasegawa T, Seki K, Hasegawa F, Matsuno Y, Shimodo T, Hirose T, et al. Dedifferentiated liposarcoma of retroperitoneum and mesentery: varied growth patterns and histological grades:a clinicopathologic study of 32 cases. *Hum Pathol.* 2000;31:717–727.
41. Allander SV, Illei PB, Chen Y, Antonescu CR, Bittner M, Ladanyi M, et al. Expression profiling of synovial sarcoma by cDNA microarrays: association of ERBB2, IGFBP2, and ELF3 with epithelial differentiation. *Am J Pathol.* 2002;161:1587–1595.
42. Nuciforo PG, Pellegrini C, Fasani R, Maggioni M, Coggi G, Parafioriti A, et al. Molecular and immunohistochemical analysis of HER-2/neu oncogene in synovial sarcoma. *Hum Pathol.* 2003;34:639–645.
43. Kawai A, Woodruff J, Healey JH, Brennan MF, Antonescu CR, Ladanyi M. SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. *N Engl J Med.* 1998;338:153–160.
44. Ladanyi M, Antonescu CR, Leung DH, Woodruff JM, Kawai A, Healey JH, et al. Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. *Cancer Res.* 2002;62:135–140.
45. Huang SM, Harari PM. Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. *Clin Cancer Res.* 2000; 6:2166–2174.
46. Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res.* 1995;1:1311–1318.

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Inflammatory myofibroblastic tumor of the lung

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Abstract

Objective: Inflammatory myofibroblastic tumor (IMT) is a rare disease that usually occurs in the lung. Recently, several reports have suggested that IMT is a true neoplasm rather than a reactive lesion. In this retrospective study, we reviewed clinicopathological characteristics and prognoses for all patients with surgically resected IMT of the lung at our institute. **Methods:** From January 1985 to December 2002, nine patients had surgical intervention for IMT of the lung at the National Cancer Center Hospital, Tokyo. The resected lesions were studied histologically, immunohistochemically, and ultrastructurally. Follow-up was complete in all patients and varied from 3 months to 16 years 2 months (median, 6 years 2 months). **Results:** These nine patients included five men and four women. They ranged in age from 25 to 66 years. Seven patients were asymptomatic. The two symptomatic patients had problems including cough, hemoptysis, and dyspnea. For all these patients, the diagnostic procedure was surgical excision. The resected tumor size ranged from 1.0 to 4.0 cm in diameter. Histologically, a variety of inflammatory and spindle cells were observed. The spindle cells corresponded ultrastructurally to myofibroblasts or fibroblasts. With the exception of one patient who had spontaneous resolution of a recurrent tumor, there was no recurrence in these patients, and all of them are in good health. **Conclusions:** Histopathologically, IMT is characterized by myofibroblasts that are mixed with chronic inflammatory cells, including plasma cells, lymphocytes, and histiocytes. Surgical resection, when possible, can be chosen as the treatment. Complete resection leads to excellent survival.

Keywords: Lung pathology; Surgery; Survival; Inflammatory pseudotumor; Pulmonary neoplasm

1. Introduction

Inflammatory myofibroblastic tumor (IMT) is a rare disease that usually occurs in the lung. IMT has been described by various terms because of its variable cellular components, which includes plasma cell granuloma, inflammatory pseudotumor, xanthogranuloma, and fibrous histiocytoma [1–18]. The notion of IMT being a reactive lesion or a neoplasm was controversial [18]. However, this entity has been characterized by not variable chronic inflammatory cells but myofibroblasts, and the recent cytogenetic studies have suggested that IMT is a true neoplasm [14–16]. There is little information on the clinicopathological features because IMT is rare and its terminology was confusing.

To examine the clinicopathological characteristics and prognosis, we reviewed a set of patients with surgically resected IMT of the lung.

2. Material and methods

2.1. Patients

Between January 1985 and December 2002, nine patients had surgical intervention for IMT of the lung at the National Cancer Center Hospital, Tokyo. These patients comprised 0.18% of 4893 patients who had thoracic surgical procedures at our institute during the same period. The clinical characteristics of these patients are shown in Table 1. Preoperative work-up included laboratory examinations, fiberoptic bronchoscopy, chest radiograph, and computed tomographic (CT) scans. Follow-up was complete in all

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Table 1
Clinical characteristics of patients with inflammatory myofibroblastic tumor

Case	Sex/age	Symptom	Location	Tumor size (cm)	Mode of operation	Prognosis after surgery
1	F/59	None	HLN	2.5	Extirpation	16 years 2 months, alive
2	F/41	None	LLL	1.0	Lobectomy	13 years 9 months, alive
3	F/58	Cough/ Hemoptysis	RIB	2.0	Bilobectomy	1 year 4 months, alive
4	M/25	None	LUL	2.2	Segmentectomy	2 years 3 months, alive
5	M/49	None	LUL	3.6	Segmentectomy	9 years 6 months, alive
6	M/66	None	LUL	3.5	Segmentectomy	6 years 6 months, alive
7	M/47	Cough/ Dyspnea	LMB	4.0	Segmental bronchial resection	6 months, alive
8	M/26	None	LLL	3.0	Segmentectomy	5 years 2 months, alive
9	F/30	None	RUL	3.2	Lobectomy	3 months, alive

HLN, hilar lymph node; LLL, left lower lobe; RIB, right intermediate bronchus; LUL, left upper lobe; LMB, left main bronchus; RUL, right upper lobe.

patients and ranged from 3 months to 16 years 2 months (median, 6 years 2 months).

2.2. *Pathological and ultrastructural evaluations*

In each case, the tissue was fixed in 10% buffered formalin, processed routinely, and embedded in paraffin. Sections 4 μm thick, were cut and then stained with hematoxylin and eosin. Each section was also evaluated immunohistochemically. Immunohistochemical staining was accomplished by the labeled streptavidin-biotin method using an LSAB kit (Dako Corporation, Carpinteria, CA). Primary antibodies against various antigens were used in this study: vimentin (V10 clone; Dako; 1:200), cytokeratin (CAM5.2 clone; Becton Dickinson, San Jose, CA; 1:100), cytokeratin (AE1/AE3 clone; Dako; 1:125), desmin (Dako; 1:500), smooth muscle actin (1A4 clone; Dako; 1:100), CD34 (My10 clone; Becton Dickinson; 1:100), S100 protein (Dako; 1:2000), and epithelial membrane antigen (Dako; 1:100).

Small fresh fragments of tumor tissue in four cases (cases 3, 4, 7 and 9) were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. After contrasting with uranyl acetate and lead citrate, ultrathin sections were examined with a transmission electron microscope.

3. Results

3.1. *Clinical findings*

These nine patients included five men and four women. They ranged in age from 25 to 66 years, with a mean age of 44.6 years. Seven patients were asymptomatic and were found to have pulmonary nodules on routine chest radiography (Fig. 1). One of these patients (case 6) was clinically suspected of pulmonary metastasis. This was pointed out during postoperative follow-up of a right nephrectomy for renal cell carcinoma that the patient had undergone 5 years before. The two symptomatic patients had problems including cough, hemoptysis, and dyspnea. The preoperative laboratory results were within normal limits for eight patients, but one patient (case 5) had a C-reactive protein (CRP) rate of 9.4 mg/dl and a white blood cell (WBC) count of 10,000/μl. These findings returned to normal within 10 days after operation. All patients underwent a fiberoptic bronchoscopy preoperatively. Six patients did not have any bronchial abnormality. One (case 1) had a stenosis of the right basal bronchus. The other two had an endobronchial tumor. One patient (case 7) with an endobronchial tumor had complete atelectasis of the left lung (Fig. 2). Chest CT showed a solitary, well-circumscribed nodule or mass in all patients. A definitive diagnosis of IMT was not made in any of the patients, although all patients had undergone transbronchial biopsy or transthoracic needle biopsy for diagnosis preoperatively. The spindle cells and inflammatory cells in small biopsied specimen, even if they were taken by biopsy, were useless for

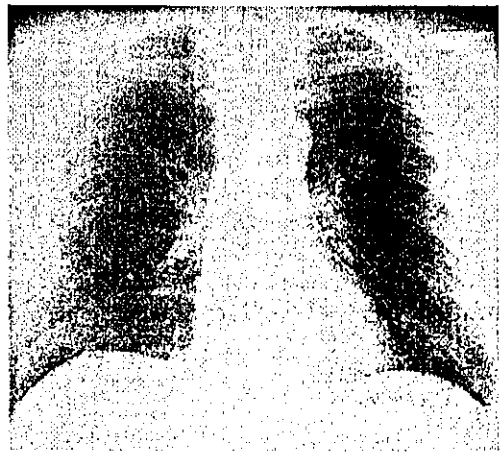

Fig. 1. Chest radiograph shows a well-defined mass in the left lung (case 5).