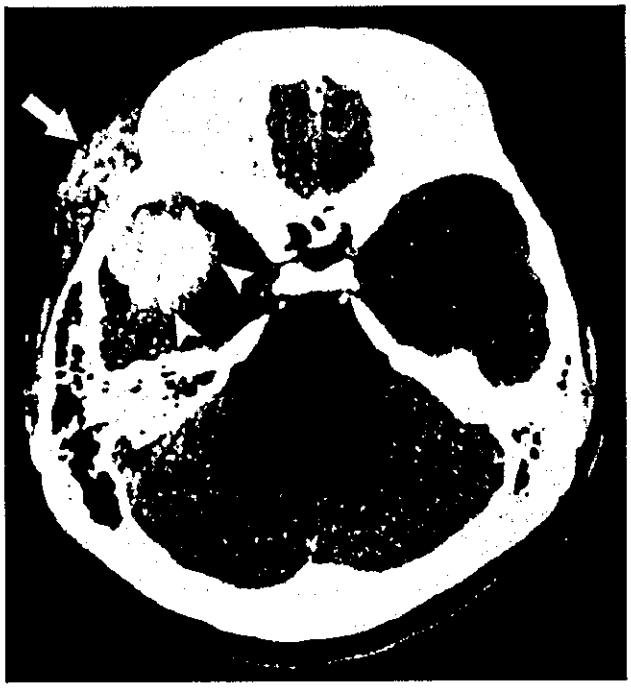

Fig. 1 Axial cranial computed tomography showing a well-mineralized tumor (*arrow*) around the right temporal bone associated with focal subarachnoid hemorrhage (*arrowheads*)

and radiological diagnosis was second primary osteosarcoma after retinoblastoma. A biopsy was performed, and the diagnosis of second primary osteosarcoma with rosette-like features was established. The patient was treated with systemic chemotherapy of high-dose methotrexate, cisplatinum, and adriamycin in March 2001, and the tumor size partially decreased. A surgery of the tumor was not performed, since the tumor was still not resectable after the chemotherapy. The patient has been alive with disease for 43 months after the treatment without any sign of regrowth of the tumor or distant metastases.

Materials and methods

The biopsy specimen was fixed in 10% formalin, embedded in paraffin, and sectioned. One of the 4-mm-thick sections was stained with hematoxylin and eosin. The other serial sections were examined using the labeled streptavidin–biotin method with appropriate use of positive and negative controls throughout, after pretreatment with heat-induced epitope unmasking in a 10-mM citrate buffer, pH 6.0, in an autoclave at 121°C for 10 min. The primary antibodies were applied as follows: cytokeratin (clone AE 1/3, 1:100, Dako, Glostrup, Denmark), CD99 (clone O-13, 1:50, Signet, Dedham, MA), epithelial membrane antigen (EMA, clone E29, 1:100, Dako) and CD56 (clone N-CAM; NCC-Lu-243, 1:200, Nihonkayaku, Tokyo, Japan). In the evaluation of immunostaining for AE1/3, CD99, EMA, and CD56, when there was only homogeneous staining, along with staining of the cell membranes or cytoplasm at the same intensity and pattern as that in the adjacent normal epithelium, the finding was assessed as 2+ positive, while heterogeneous or focal staining was assessed as 1+.

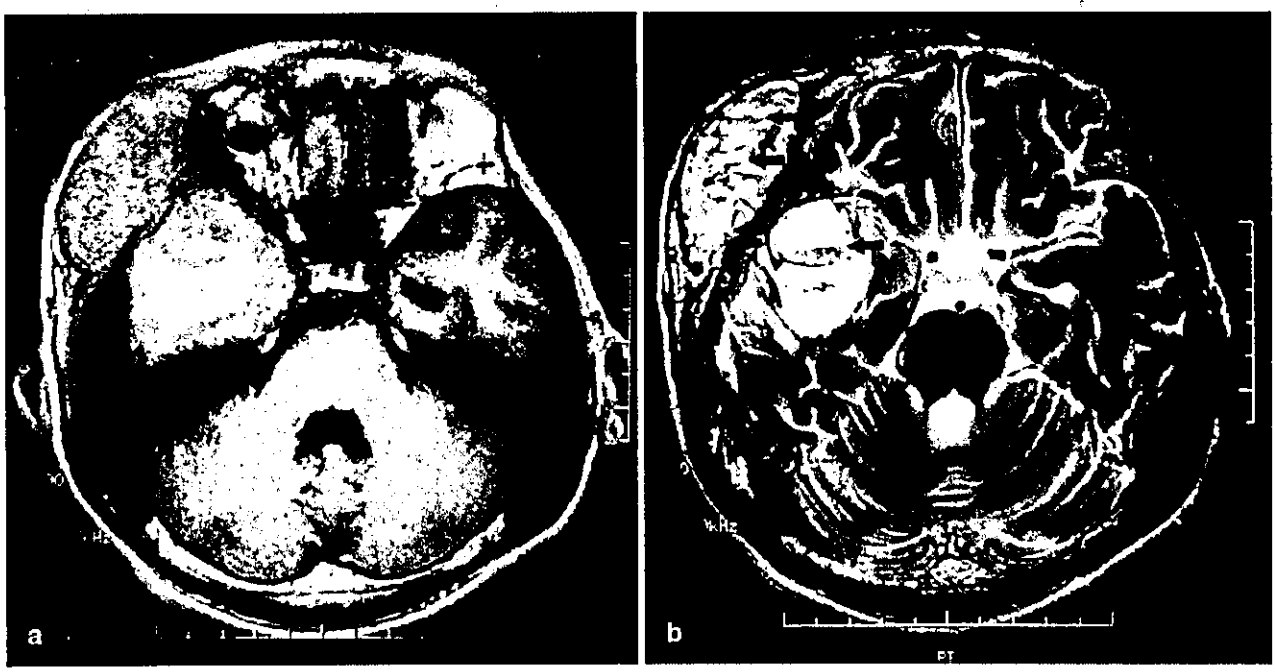

Fig. 2 a Axial T1-weighted magnetic resonance (MR) image (TR/TE: 440/7.3 ms) showed a mass of iso signal intensity relative to white matter. Focal subarachnoid hemorrhage corresponded to area of high signal intensity surrounding the tumor. b Axial T2-weighted MR image (TR/TE: 4000/118 ms) showed a mass of heterogeneous and high signal intensity relative to white matter. Also noted was the presence of multiple fluid levels within the tumor (*arrows*)

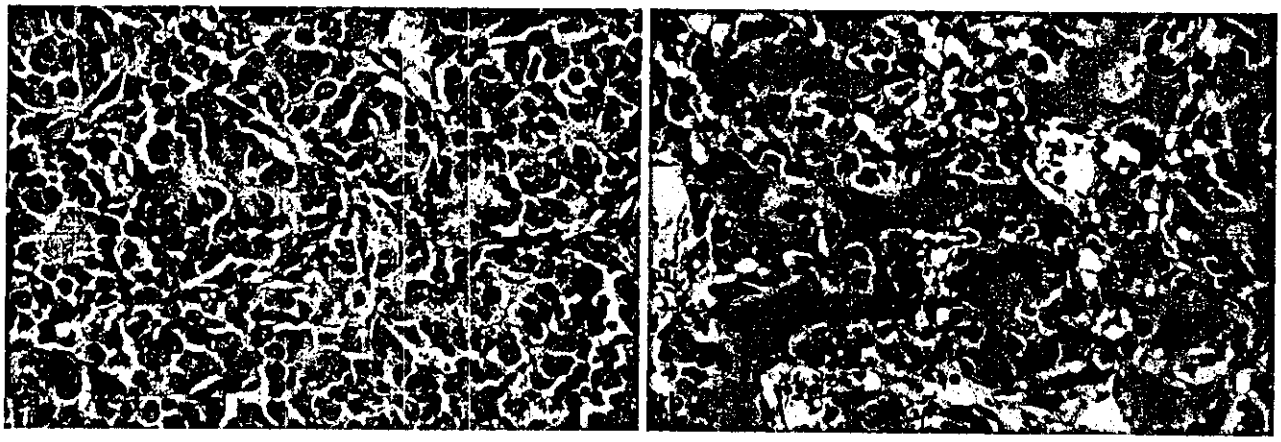

Fig. 3 a Microphotograph of the biopsy specimen showed a proliferation of tumor cells with prominent rosette-like structures. The tumor cells were generally small and round with abundant mitoses. Eosinophilic material was seen in the center of the rosette (×400). b Microphotograph showed conventional lace-like osteoid formation with partial mineralization (×400)

Results

Light microscopic findings

The biopsy showed a small multi-nodular proliferation of the tumor cells. These tumor cells formed a rosette-like structure, and eosinophilic material was seen in the center of the rosette. Focal mineralization was apparent in the eosinophilic material. Between the rosette-like structures, many small vessels proliferated, showing a hemangiopericytoma-like pattern. Under high power, tumor cells were generally small and round with abundant mitoses. In several areas, conventional lace-like osteoid was seen. The rosette-like structures were also observed around the conventional osteoid area. There was no typical Flexner-Wintersteiner rosette in the biopsied specimen (Fig. 3A, B).

Fig. 4 Immunostaining of the tumor cells with EMA and CD56. The tumor cells showed a characteristic membrane-positive appearance for EMA (left, ×200) and for CD56 (right, ×200)

Immunostainings

In immunohistochemical features, CD56 was 2+ positive, CD 99 and EMA were 1+ positive (Fig. 4), but cytokeratin was negative.

Discussion

Osteosarcoma is the most common type of second malignancy associated with hereditary retinoblastoma [11, 14]. Hawkins et al. reported that the incidence of osteosarcoma after heritable retinoblastoma is 300 times greater than the risk in the general population [8]. More than 100 cases of second primary osteosarcoma in the irradiated fields in patients with retinoblastoma have been reported in the literature [2, 3, 7, 14]. However, to our knowledge, there has not been any report describing second primary osteosarcoma having rosette-like structures. The rosette-like feature of osteosarcoma was first described in the textbook by Unni in 1996 [23]. In 2002, we reported that several primary osteosarcomas in the extremities showed a rosette-like structure with production of osteoid in the center. This special type of osteosarcoma usually showed a positive immunoreaction for EMA, CD56, and CD99 [15]. The histological and immunohistochemical features described in the literature were identical to those of the current case.

Second primary sarcomas often demonstrate both high-grade and undifferentiated features, making them difficult to distinguish from small, undifferentiated round cell tumors [19]. In addition, the rosette-like structure raises the possibility of recurrent retinoblastoma. However, the long interval (24 years) in the current case between the primary retinoblastoma and development of the second tumor suggests that a recurrence of retinoblastoma is less likely [22]. Furthermore, a definite tumor osteoid

in the center of a rosette-like structure and scattered typical lace-like osteoid, indicating osteosarcoma, and the absence of a typical Flexner-Wintersteiner rosette should be emphasized in the differential diagnosis between osteosarcoma with rosette-like features and recurrent retinoblastoma.

Careful immunohistochemical examinations were useful for distinguishing the current tumor from recurrent retinoblastoma or other small round cell tumors such as rhabdomyosarcoma, Ewing sarcoma/Primitive neuroectodermal tumor (ES/PNET), and metastatic cancer [9]. Positive immunoreactivity for EMA, CD56, CD99, but negativity for cytokeratin in the current case were consistent with those of osteosarcoma with rosette-like features [15]. Positive immunoreaction for EMA would suggest metastatic cancer, but the patient had no cancer history or visceral lesion. Furthermore, negative reaction for cytokeratin indicates that metastatic cancer would be an unlikely diagnosis. It is well known that CD 56 (N-CAM) and CD99 are expressed not only in neural neoplasms or ES/PNET but also in other several kinds of tumors [13, 18, 20]. Definite osteoid formation by tumor cells strongly favored the diagnosis of osteosarcoma.

Little is known in the literature regarding the relationship between treatment of the second primary osteosarcoma and prognosis [2, 4, 10, 16, 19]. Recently, encouraging results have been achieved with adjuvant chemotherapy and/or aggressive surgery [2, 21]. The current patient has remained alive with disease after effective treatment by systemic chemotherapy without any regrowth of the tumor or distant metastasis for 43 months. Similarly, in the review of the other four cases registered in the National Cancer Center in Tokyo (data not shown), three died of disease, but one patient was alive with disease for 99 months after systemic chemotherapy. However, our previous study showed that rosette-like feature in primary osteosarcoma is an adverse prognostic factor [15]. Further study is required with regard to prognosis of osteosarcoma with this specific background and histology.

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CASE REPORT

Extramedullary myeloid tumour (EMMT) of the gallbladder

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This report describes a rare case of an extramedullary myeloid tumour (EMMT) of the gallbladder in a patient without leukaemia. A 33 year old man visited a local hospital because of jaundice. Abdominal computed tomography revealed a tumorous mass measuring 6.0 × 4.5 cm and involving the entire gallbladder. A percutaneous needle biopsy was attempted, but because adenocarcinoma could not be completely ruled out, the use of undue force was considered dangerous. Under a preoperative diagnosis of gallbladder carcinoma, a hepatopancreatoduodenectomy was performed. The tumour cells exhibited various amounts of eosinophilic cytoplasm, had medium sized round nuclei with indentation and grooving, and were strongly immunoreactive for myeloperoxidase, CD43, and c-kit protein (CD117). After surgery, the patient underwent combination chemotherapy as prescribed for cases of acute myeloblastic leukaemia. The patient did not develop acute leukaemia during a follow up period of four years. In conclusion, a correct diagnosis of EMMT can be made using appropriate immunohistochemical staining.

Extramedullary myeloid tumour (EMMT), otherwise termed granulocytic sarcoma or chloroma, is a rare extramedullary tumour composed of immature cells of the myelomonocytic series. Males and females are equally affected, with a mean age of 48 years (range, 2–81).[1] About 70% of reported cases are in patients with acute myelogenous leukaemia, chronic myelogenous leukaemia, or other myeloproliferative diseases, but in the remaining 30% no known underlying disease has been noted at the time of diagnosis.[1] The most common sites of involvement are the skin, lymph nodes, and bone, although other organs have been implicated. A large proportion (75–86%) of EMMTs in non-leukaemic patients are initially misdiagnosed.[2] An EMMT developing in the gallbladder of a patient without leukaemia is extremely rare. Geddy and Wedgwood reported a case of myelofibrosis of the gallbladder,[3] but unlike our case, lesions outside the gallbladder were recognised in the bone marrow. Here, we report a rare case of EMMT of the gallbladder, detailing the clinicopathological and immunohistochemical features of this entity, which was accurately diagnosed and has been followed up for a long period.

"A large proportion (75–86%) of extramedullary myeloid tumours in non-leukaemic patients are initially misdiagnosed"

CASE REPORT

A 33 year old man visited a local hospital because of jaundice. He was diagnosed as having gallbladder carcinoma based on a radiographic examination, and was referred to the National Cancer Centre, Tokyo, Japan. Laboratory data indicated that carcinoembryonic antigen, CA19-9, and elastase concentrations were within normal limits, and that T-bilirubin (51 mg/litre), D-bilirubin (37 mg/litre), alkaline phosphatase (688 IU/litre), glutamic oxaloacetic transaminase (84 U/litre), glutamic pyruvate transaminase (317 U/litre), lactate dehydrogenase (431U/litre), and the white blood cell count (9.6×10^9/litre) were slightly raised.

Abdominal computed tomography imaging showed partial infiltration of the tumour into the gallbladder wall. We tried to perform a percutaneous needle biopsy, but because adenocarcinoma could not be completely ruled out the use of undue force was considered dangerous. We performed a cytological examination which was unable to provide definitive information on the lesion. The preoperative diagnosis was a malignant neoplasm that probably originated from the neck of the gallbladder, cystic duct, or common bile duct. However, it is unusual for a carcinoma to grow so large without showing signs of invasion of the liver and portal vein (fig 1A). The differential diagnosis was an extranodal malignant lymphoma, and a hepatopancreatoduodenectomy was performed.

Macroscopically, the gallbladder lumen was filled with blood and degenerative tissue, and the cut surface of the tumour had a nodular, well circumscribed, glistening appearance (fig 1B). The tumour measured 6.0 × 4.5 cm at its maximum diameter.

Microscopically, the tumour cells had various amounts of eosinophilic cytoplasm and medium sized round nuclei with indentation and grooving. They were arranged in a trabecular to sheet-like pattern within the thin fibrous septa (fig 2A), and did not show a cohesive growth pattern. The tumour cells had invaded the muscular layer of the gallbladder, but most of the gallbladder epithelium was intact (fig 2B). Tumour invasion was seen in the cystic duct, common bile duct, portal vein, part of the liver parenchyma, hepatoduodenal ligament, omentum, part of the muscular layer of the transverse colon, and duodenum. The histological differential diagnosis for such small round cell tumours included undifferentiated carcinoma, small cell carcinoma, Ewing's sarcoma, rhabdomyosarcoma, monophasic synovial sarcoma, nephroblastoma, and haemopoietic tumour.

Upon immunohistochemical examination, the tumour cells showed diffuse and strong reactivity for myeloperoxidase (MPO), CD43, and c-kit protein (CD117) (fig 2A–C), and weak reactivity for CD45 (LCA) and CD99. The cells were negative for CD20, CD79a, CD3, CD34, CD45RO (UCHL-1), CD68, CD56, terminal deoxynucleotidyl transferase, WT1, desmin, vimentin, and cytokeratins (CAM 5.2, AE 1/3, and KL1). Based on these results, we made a final diagnosis of EMMT.

After surgery, the patient underwent combination chemotherapy as prescribed for cases of acute myeloblastic leukaemia, but clinical investigations—including computerised tomography and a bone marrow trephine biopsy

Abbreviations: EMMET, extramedullary myeloid tumour; MPO, myeloperoxidase

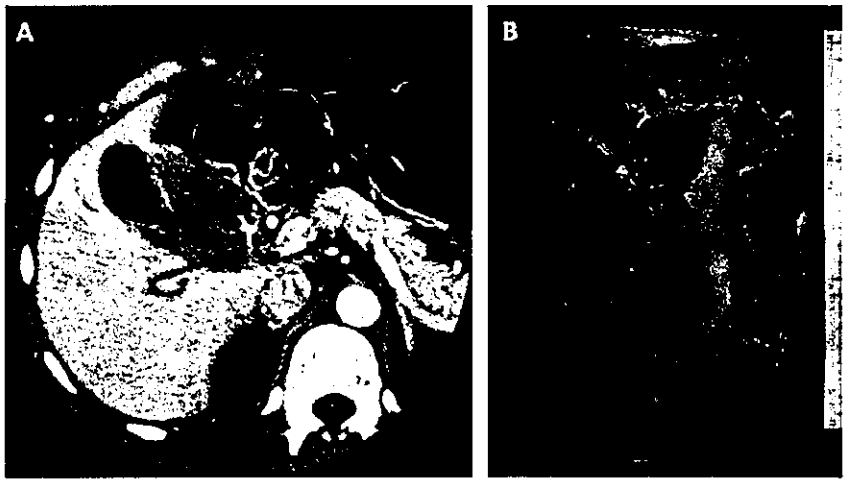

Figure 1 (A) Abdominal computerised tomography image showing partial infiltration of the tumour into the gallbladder wall but no sign of invasion of the liver or portal vein. (B) Macroscopically, the gallbladder lumen is filled with blood and degenerative tissue, and the cut surface of the tumour has a nodular, well circumscribed, glistening appearance.

specimen—did not detect more lesions. The patient did not develop acute leukaemia during a follow up period of four years.

DISCUSSION

A large proportion (75–86%) of EMMTs in non-leukaemic patients are initially misdiagnosed[3] because of their morphological and immunohistochemical similarity to other small round cell tumours. Most of them are diagnosed as malignant lymphoma, and occasionally as Ewing's sarcoma or eosinophilic granuloma.[4]

> "In cases of suspected extramedullary myeloid tumour, antibodies to myeloperoxidase and CD43 should be used, along with other B cell and T cell specific antibodies"

Some small round cell tumours, such as undifferentiated carcinoma and small cell carcinoma, usually show aggressive invasion, but in our case the lesion had not invaded the portal vein or the gallbladder epithelium, and staining for cytokeratins was negative. Other small round cell tumours, such as Ewing's sarcoma, rhabdomyosarcoma, monophasic synovial sarcoma, and nephroblastoma, were also eliminated because of negative immunostaining for desmin, cytokeratins, and WT1, respectively. Malignant lymphomas may be positive for CD45 (LCA) along with either pan B cell (CD79a, L-26) or T cell (CD3) markers, but are negative for MPO, which was positive in our case.

Immunohistochemical markers, such as CD43 and MPO, may be helpful in the diagnosis of EMMT. However, a few cases of EMMT show reactivity for pan B cell (CD79a, L-26) or T cell (CD3) markers. Furthermore, CD43 is an excellent

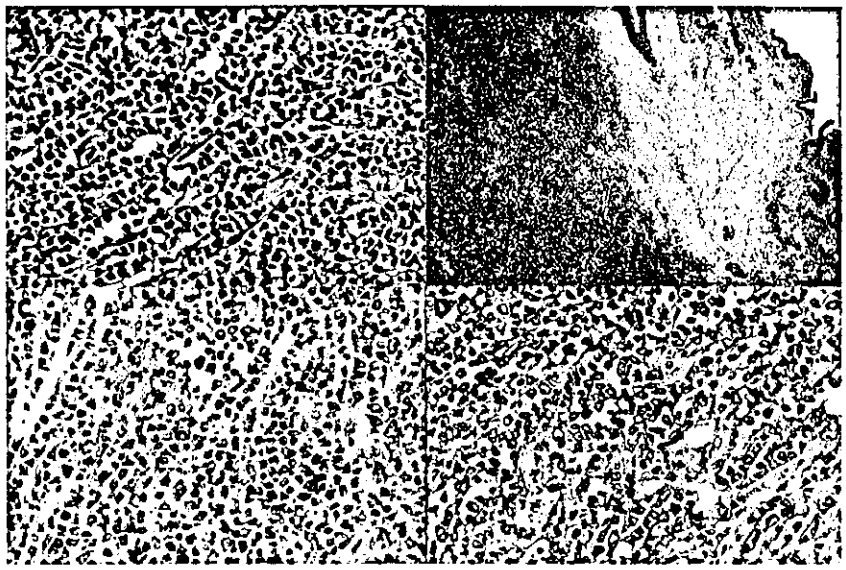

Figure 2 (A) The tumour cells have various amounts of eosinophilic cytoplasm and are arranged in a trabecular to sheet like pattern (haematoxylin and eosin (H&E) stain; original magnification, ×400). (B) Most of the gallbladder epithelium is not involved (H&E stain; original magnification, ×40). The tumour cells show diffuse and strong reactivity for (C) myeloperoxidase and (D) CD43 (original magnification, ×400).

Take home messages

- We present a rare case of extramedullary myeloid tumour of the gallbladder in a non-leukaemic patient that was diagnosed successfully using an appropriate panel of immunohistochemical stains
- The patient underwent combination chemotherapy after diagnosis and has not developed leukaemia after four years of follow up
- It is important not to misdiagnose such cases as malignant lymphoma, because the pathological diagnosis influences the prognosis of the patient

marker for myeloid cells and is also used as a T cell marker.[2] If myeloid associated antigens are not investigated, a misdiagnosis of malignant lymphoma is likely. Therefore, in cases of suspected EMMT, antibodies to MPO and CD43 should be used, along with other B cell and T cell specific antibodies.

Recently, c-kit tyrosine kinase inhibitors (such as Glivec) have been studied as possible treatments for haemopoietic malignancies, so that c-kit detection may have important implications for treatment. Jian *et al* reported c-kit reactivity in 87% of EMMT cases,[5] and many tumours have been shown to express CD117/KIT as assessed by the anti-KIT polyclonal antibody (DakoCytomation, A4502; Glostrup, Denmark) used in our laboratory, which has low specificity. Although tumours carrying mutations of either c-kit or the platelet derived growth factor α gene (for example, gastrointestinal stromal tumours) are sensitive to tyrosine kinase inhibitors, in the absence of an accompanying mutation other KIT positive tumours show no therapeutic benefit with Glivec.[6] We were unable to isolate and sequence the c-kit gene from formalin fixed, paraffin wax embedded tissue sections of our present case.

Most non-leukaemic patients with EMMT develop acute leukaemia one to 49 months after the diagnosis (mean, 10.5).[1] If the case is correctly diagnosed as EMMT at the initial presentation, and the patient receives intensive systemic chemotherapy with or without radiotherapy, longterm survival is good.[7] Aggressive forms of treatment are necessary for patients who present with acute myeloid leukaemia after being treated with chemotherapy for non-Hodgkin lymphoma as a result of initial misdiagnosis. These patients frequently fail to attain durable remission and have a poor prognosis.[3]

In summary, we present a rare case of EMMT of the gallbladder in a non-leukaemic patient that was diagnosed successfully using an appropriate panel of immunohistochemical markers. Care should be taken not to misdiagnose such cases as malignant lymphoma, because the pathological diagnosis influences the prognosis of the patient.

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Case Reports

CD56-positive Small Round Cell Tumor: Osseous Plasmacytoma Manifested in Osteolytic Tumors of the Iliac Bone and Femora

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Monoclonal gammopathy of undetermined significance does not overexpress cluster of differentiation (CD) 56, but plasma cell myeloma frequently overexpressed it. However, plasma cell leukemia and extramedullary plasmacytoma usually down-regulate CD56 expression. Plasmacytoma, especially 'solitary plasmacytoma of bone', is difficult to diagnose as plasma cell neoplasm, because it occasionally appears similar to other bone tumors, both clinically and pathologically, and is rarely accompanied by monoclonal protein in the serum or urine. The present case was a patient with an osteolytic 'small round cell tumor' of the iliac bone, which also invaded the femora. An immunohistopathological finding of CD56 expression played a key role in making a diagnosis. The definitive diagnosis of plasmacytoma was made based on the electron microscopic findings. The plasma cells which infiltrated her sternum showed the same restriction to kappa light chain expression in their cytoplasms as that of the iliac bone tumor cells, but did not express CD56. Locally infiltrating osteolytic bone tumors should be examined for surface immunoglobulin light chains as well as CD56 expression when plasmacytoma is suspected.

Key words: CD56 – osseous plasmacytoma – osteolytic bone tumor – plasmacytoma – SBP

INTRODUCTION

'Plasmacytoma' is one of the plasma cell neoplasms locally infiltrating bones or spreading to extramedullary sites from the bones (1). The new World Health Organization (WHO) criteria defines solitary plasmacytoma of bone (SBP) as 'a localized bone tumor consisting of plasma cells identical to those seen in plasma cell myeloma, which appears as a solitary lytic lesion on radiological examination' (1). Electrophoreses of serum and urine samples in patients with SBP tend to reveal monoclonal proteins less frequently than plasma cell myeloma, and clinical diagnosis of SBP is generally difficult in such cases without monoclonal proteins (2). Previous reports showed that 65–78% of cases with plasma cell myeloma strongly expressed cluster of differentiation (CD) 56, but patients with monoclonal gammopathy of undetermined significance (MGUS) seldom showed CD56 expression (3,4). On the other hand, plasma cells obtained from patients with plasma cell leukemia (PCL) were reported to lack or barely express CD56 in either the peripheral blood (PB) or the bone marrow (BM) (5). Myeloma cells obtained from extramedullary sites revealed absence of CD56, although those of BM expressed CD56 in the same patients (6). Furthermore, CD56-negative multiple myeloma had a higher incidence of extramedullary disease, a plasmablastic morphology, and poor prognosis (7). The present case was one in which the CD56 expression played a key role in reaching a definitive diagnosis of plasma cell neoplasm.

CASE REPORT

In February 2000, a 57-year-old female suffered from severe pain in her buttocks. She had been to a chiropractor but her pain had not been improved. In December 2000, she was referred to a hospital, where an iliac bone biopsy was performed. The histopathological diagnosis was a 'small round cell tumor'. In January, 2001, she was referred to and admitted to our hospital. Computerized tomography (CT) revealed an extensive osteolytic tumor in her right iliac bone (Fig. 1). Magnetic resonance imaging (MRI) also showed a bone

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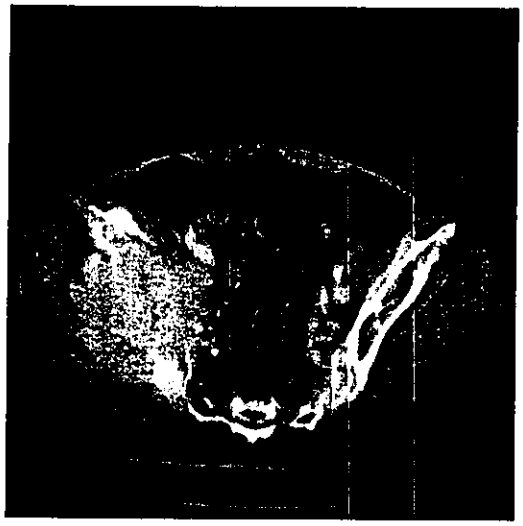

Figure 1. CT showing the osteolytic iliac bone tumor.

Figure 2. MRI showing the iliac bone tumor involving the femoral bones.

tumor in her right iliac bone and osteolytic lesions at the necks of her bilateral femora (Fig. 2). She had been lying in bed with such severe pain on her right hip joint that she required a lumbar subdural morphine infusion. Re-biopsy of her iliac bone tumor was performed and hematoxylin–eosin staining of the specimen showed the diffuse proliferation of condensed small round cells (Fig. 3). Although the tumor cells had scant cytoplasms, their nuclei appeared rather eccentric. Her blood tests were almost normal except for a low hemoglobin level of 10.2 g/dl and a slightly high calcium concentration of 10.9 mg/dl. As for the levels of immunoglobulins, only IgA was abnormal and slightly declined to 92 mg/dl [normal range (NR), 107–390 mg/dl]. A bone marrow aspiration of her sternum revealed 4% of plasma cells. Her findings did not meet any

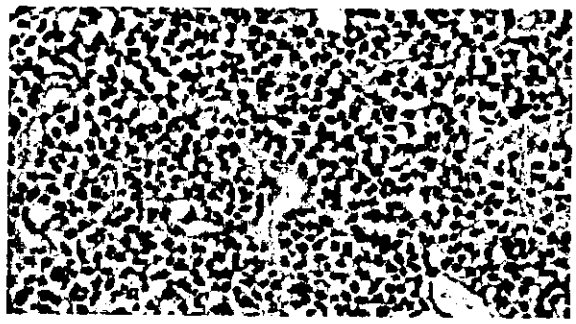

Figure 3. Hematoxylin–eosin staining of the iliac bone tumor specimen which shows a small round cell tumor (600×).

major diagnostic criteria of the Southwest Oncology Group for multiple myeloma (8). Immunohistochemical analysis of her iliac bone tumor specimen showed neither epithelial markers such as cytokeratin or epithelial membrane antigen nor the markers for neural differentiation such as neuron-specific enolase, CD57 (Leu7), synaptophysin or neurofilament. Hematopoietic cell markers such as leukocyte common antigen (CD45), CD34, terminal deoxynuceotidyl transferase, myeloperoxidase, CD3 and CD79a were not expressed, but CD56 was expressed (Fig. 4A). In February 2001, the pain worsened, because the head of her right femur was dislocated into the acetabular fossa necessitating the towing of her right femur. She had become progressively worse and it was suggested that there was a strong possibility of either malignant lymphoma or plasma cell neoplasm. Therefore, we started chemotherapy with 750 mg/m^2 cyclophosphamide, 50 mg/m^2 doxorubicin, and 1.4 mg/m^2 vincristine i.v., and 100 mg prednisolone orally for five consecutive days.

The study by Van Camp et al. (3), which concluded that strong CD56 expression was common in multiple myeloma, suggested that there was a great likelihood of plasma cell neoplasms. We therefore performed additional immunohistochemical stainings of the specimens, and plasmacytoma was confirmed by the positive immunostaining in the cytoplasms of tumor cells with an anti-kappa light chain antibody (Fig. 4B), but negative with an anti-lambda antibody (data not shown). We re-performed bone marrow aspiration and plasma cells from her sternum were clearly distinguished from other BM cells or lymphoid cells of her sternum on two-color cytogram (performed with EPICS XL-MCL flow cytometer, Beckman Coulter Inc., Miami, FL) with a fluorescein isothiocyanate (FITC)-anti-CD38 antibody staining (Immunotech, a Beckman Coulter Company, Marseille, France). The result revealed an increased ratio of the cytoplasmic kappa/lambda chains [32.2 (61.1%/1.9%)]. Furthermore, the electron microscopic examination of her iliac bone tumor cells showed abundant rough endoplasmic reticula with regular parallel arrays, which were consistent with plasma cells (Fig. 5). The immunohistochemical analysis of the plasma cells in the clot section of her sternal BM showed the same pattern of the restricted kappa chain expression as that of the tumor specimen obtained from

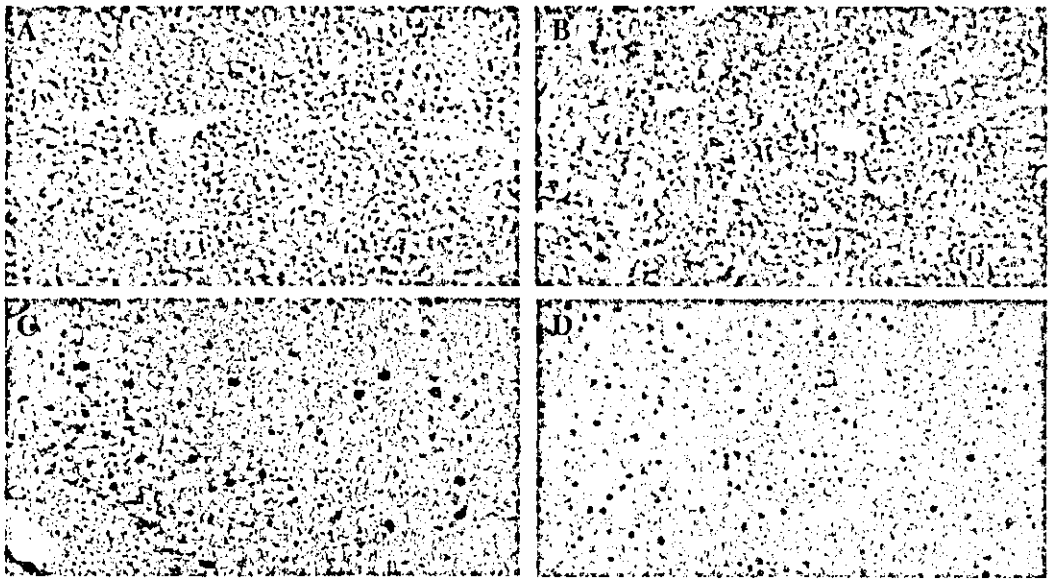

Figure 4. Immunohistochemical findings of the iliac plasmacytoma and the plasma cells in the sternal bone marrow (600×). Plasma cells in the iliac tumor showed CD56 expression (A). Plasma cells in the tumor specimen obtained from the iliac bone showed the restricted kappa chain expression (B). The clot section of the sternum also showed the same pattern of restricted kappa chain expression (C). Interestingly, plasma cells in the sternum lacked CD56 expression (D).

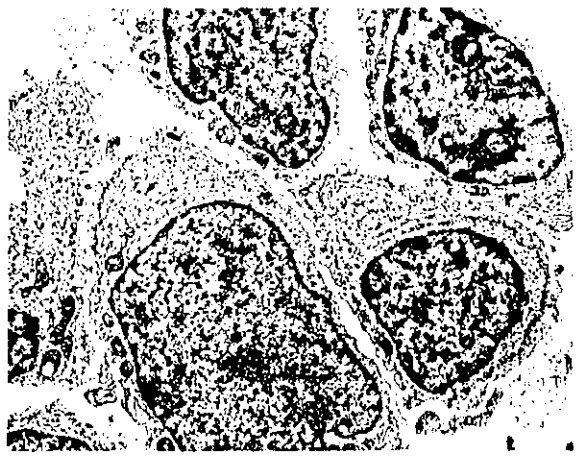

Figure 5. Electron micrograph showing the infiltration of "granular endoplasmic reticulum"-rich cells that were consistent with plasma cells.

her iliac bone (Fig. 4C). However, the plasma cells in her sternum lacked CD56 expression (Fig. 4D). Additional serum examination showed 2.58 mg/l of β2-microglobulin (NR 0.92–1.40 mg/l). Although ordinary urine examination showed no protein, immunoelectrophoresis of her concentrated urine disclosed kappa type of Bence–Jones protein (BJP).

After six courses of the described chemotherapy, partial remission was obtained. She underwent surgery for residual tumor resection in the iliac bone and artificial hip-femur joint replacement. The resected specimen revealed fibrotic and bleeding tissue without any residual tumor. The postoperative recovery was uneventful and she recovered mobility with the use of a wheelchair through rehabilitation.

DISCUSSION

When a bone tumor is encountered which is histopathologically diagnosed as 'small round cell tumor', the differential diagnosis includes Ewing sarcoma, osteosarcoma of small cell type, malignant lymphoma, plasmacytoma, metastatic small cell lung cancer and so on. To make a definitive diagnosis, immunohistochemistry is usually necessary in addition to hematoxylin–eosin staining. As for non-Hodgkin's lymphoma (NHL), some anaplastic large cell lymphomas (9) as well as NK/T-cell lymphomas have been reported to express CD56 (10). However, CD30 was negative and the sites frequently involved in NK/T-cell lymphoma were not affected in the present case. Although some tumor cells of osteosarcoma also show positive immunoreactivity for CD56 (11), the present case had CD56-positive cells in her iliac bone tumor and this was a clue to carrying the investigation forward to reach a definitive diagnosis. In plasma cell neoplasms, it has been reported that CD56 expression is common in plasma cell myeloma, but normal plasma cells of healthy volunteers and benign plasma cells of MGUS were negative for CD56 (3,4). On the other hand, expression of CD56 was down-regulated in PCL (5) and extramedullary plasmacytoma (6). CD56-negative myeloma is characterized by higher levels of β2-microglobulin, BJP, renal insufficiency, thrombocytopenia, plasmablastic morphology and high incidence of extramedullary disease (7). The level of serum β2-microglobulin in this case was not so high, and the tumor cells showed very small round shapes without blastic appearance. Renal function was intact and platelet counts were within normal limits. CD56 is a neural cell adhesion molecule (NCAM), one of the recognition molecules which operate via both homophilic (NCAM

to NCAM) and heterophilic (NCAM to heparan sulfate proteoglycan and various other collagens) binding mechanisms. During embryogenesis, this molecule is down-regulated during migrating events but re-expression usually occurs when target organs are reached (12).

Furthermore, comparing the presence of lytic bone lesions on radiography, Pellat-Deceunynck et al. (5) reported that 80% of patients with plasma cell myeloma which expressed CD56 had one or more lytic bone lesions but that they were found only in 44% of patients who lacked or weakly expressed CD56. Ely and Knowles (13) also reported that strong expression of CD56 by plasma cells correlated with the presence of lytic bone regions and strong CD56 expression by osteoblasts in BM. Although the levels of CD56 expression on plasma cells of SBP have not been reported, they were supposed to be high according to the above-mentioned reports. Although the present case with femoral invasion does not fit with the strict definition of SBP as described in the new WHO classification (1), it is suggested that such an 'osseous plasmacytoma' as an osteolytic, relatively localized plasmacyotma with CD56 expression is the opposite manifestation of plasma cell neoplasms to PCL or extramedullary myeloma. Plasma cells in the osseous plasmacytoma that overexpress CD56 might adhere to and proliferate in BM. Homophilic interactions among myeloma cells through CD56 might facilitate a mass formation of plasma cells. Moreover, the destruction of bone trabeculae can be attributable to heterophilic interactions between plasma cells and osteoblasts through CD56 (13). CD56 seemed to be up-regulated and down-regulated during development of plasma cell dyscrasia and changed its character according to the relationship of myeloma cells to BM.

CONCLUSION

"Osseous plasmacytoma" might present itself histopathologically as "small round cell tumor" and often remains undiagnosed if only hematoxylin–eosin staining is performed. In addition, low levels of the serum or urine monoclonal protein make its clinical diagnosis difficult. From previous observations and the present finding, the level of CD56 expression is suggested to be high in "osseous plasmacytoma". Therefore, locally infiltrating osteolytic bone tumors should be examined for surface immunoglobulin kappa and lamda light chains as well as CD56 expression when "osseous plasmacytoma" is suspected.

Acknowledgments

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ORIGINAL ARTICLE

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Differential diagnosis of gastrointestinal stromal tumor and other spindle cell tumors in the gastrointestinal tract based on immunohistochemical analysis

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Abstract To confirm the usefulness of an immunohistochemical panel of antibodies for KIT (c-kit/CD117), CD34, desmin, smooth-muscle actin (SMA), h-caldesmon (HCD), S-100 protein, neuron-specific enolase (NSE), and beta-catenin, 297 mesenchymal and peripheral nerve-sheath tumors of the gastrointestinal tract and intra-abdominal locations including 211 gastrointestinal stromal tumors (GISTs), 12 leiomyomas, 18 leiomyosarcomas, 17 solitary fibrous tumors (SFTs), 14 schwannomas, and 25 desmoid-type fibromatoses (DTFs) were analyzed immunohistochemically. Consistent (100%) immunoreactivity for KIT, CD34, desmin and S-100, and nuclear accumulation of beta-catenin were detected in GISTs, SFTs, smooth-muscle tumors, schwannomas, and DTFs, respectively. Immunoreactivity for SMA, HCD, and NSE was observed in a wide range of these tumors. In addition, 418 bone and soft tissue tumors were enrolled in this study for KIT immunostaining. As a result, a limited number of these tumors were KIT positive, including synovial sarcoma that showed morphological similarity to GISTs. These findings suggest that KIT, CD34, desmin, S-100, and beta-catenin are key markers for clinical diagnosis of GISTs and other spindle cell tumors that may involve the gastrointestinal tract, whereas SMA, HCD, and NSE have only limited value.

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Keywords Gastrointestinal stromal tumor (GIST) · Spindle cell tumor · KIT · Beta-catenin · S-100 · Soft tissue sarcoma

Introduction

The primary mesenchymal and peripheral nerve sheath tumors arising in the gastrointestinal tract of adults form a large, diverse group that differ in their histological features and clinical behavior, but sometimes pose diagnostic problems. Gastrointestinal stromal tumors (GISTs) comprise the majority of this tumor group. GIST is an entity that was defined recently on the basis of its ultrastructural and immunohistochemical similarity to the interstitial cells of Cajal [22, 27, 46]. Immunohistochemically, most GISTs are KIT (c-kit proto-oncogene protein) positive, and c-kit tyrosine kinase is rendered constitutively active by mutations [17, 23, 25, 26, 31, 44, 47]. Application of c-kit tyrosine kinase inhibitor STI-571 (Glivec, imatinib) to the treatment of GISTs has proved to be promising for clinical management [21, 56]. Accordingly, it has become more important for pathologists to diagnose GISTs accurately.

Although numerous studies have evaluated various pathological and immunohistochemical parameters in an attempt to identify GISTs specifically [10, 11, 35, 36, 37, 40, 41, 43, 58, 59], there have been no systematic studies that attempt to distinguish GISTs from other spindle cell tumors arising in the gastrointestinal tract and intra-abdominal locations. The present study was conducted to confirm the usefulness of an immunohistochemical panel of antibodies for KIT, CD34, desmin, smooth-muscle actin (SMA), h-caldesmon (HCD), S-100 protein, neuron-specific enolase (NSE), and beta-catenin, which might be used to clinically diagnose GISTs and other spindle cell tumors that can arise in the gastrointestinal tract. In addition, as relatively little is known about expression of KIT in other mesenchymal tumors, we studied its ex-

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 liposarcoma	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%)

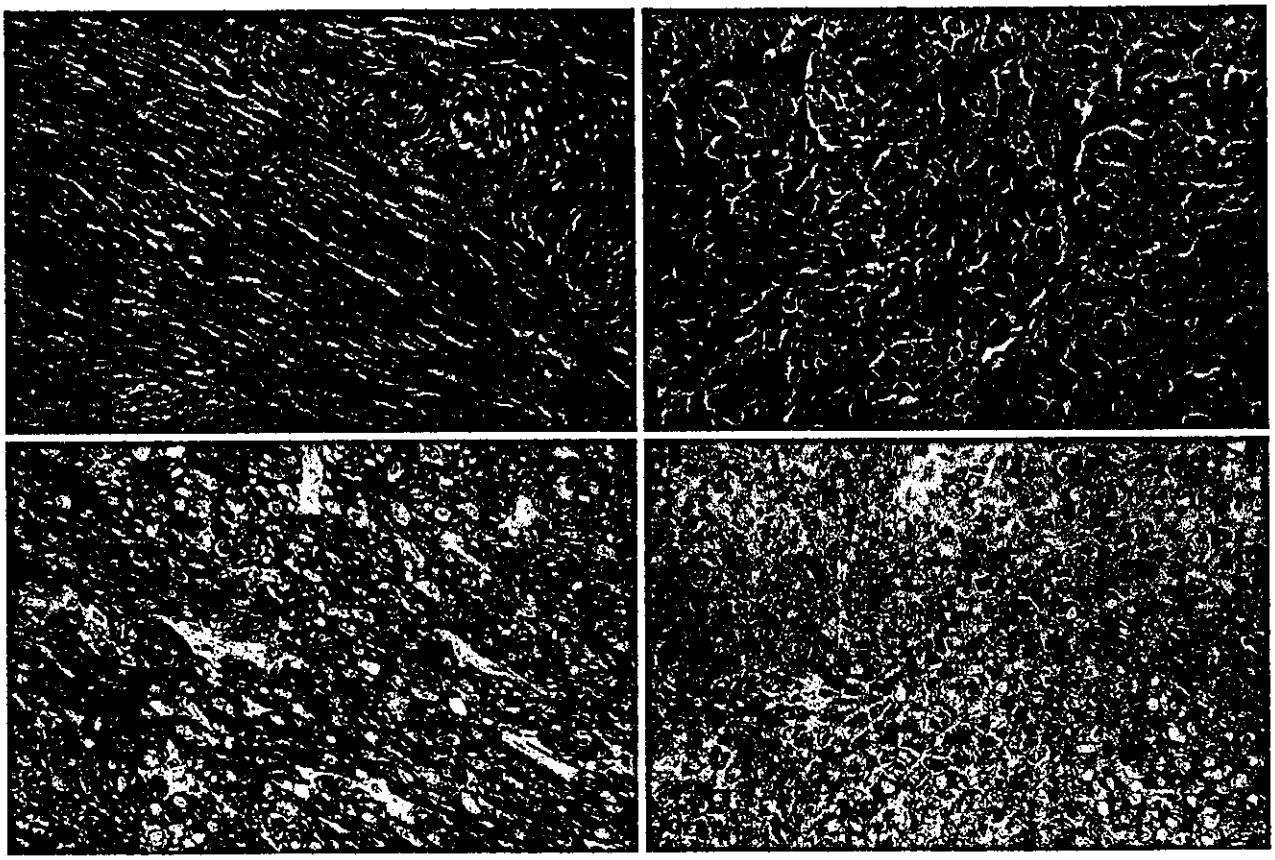

Fig. 1 Gastrointestinal stromal tumors. A gastric stromal tumor of spindle cell type is composed of uniform eosinophilic cells arranged in short fascicles (A). A small intestinal stromal tumor of epithelioid type shows a nested paraganglioma-like appearance (B). Diffuse and strong membrane and cytoplasmic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

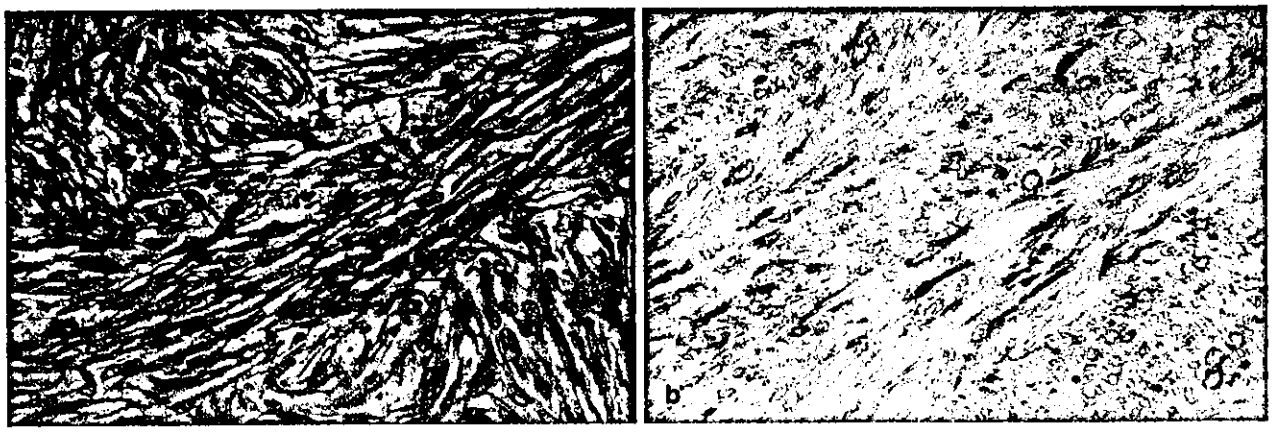

Fig. 2 Smooth-muscle tumors. A rectal leiomyosarcoma consists of long fascicles of eosinophilic spindle cells (A). Focal and strong positivity for desmin in tumor cells (B)

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).

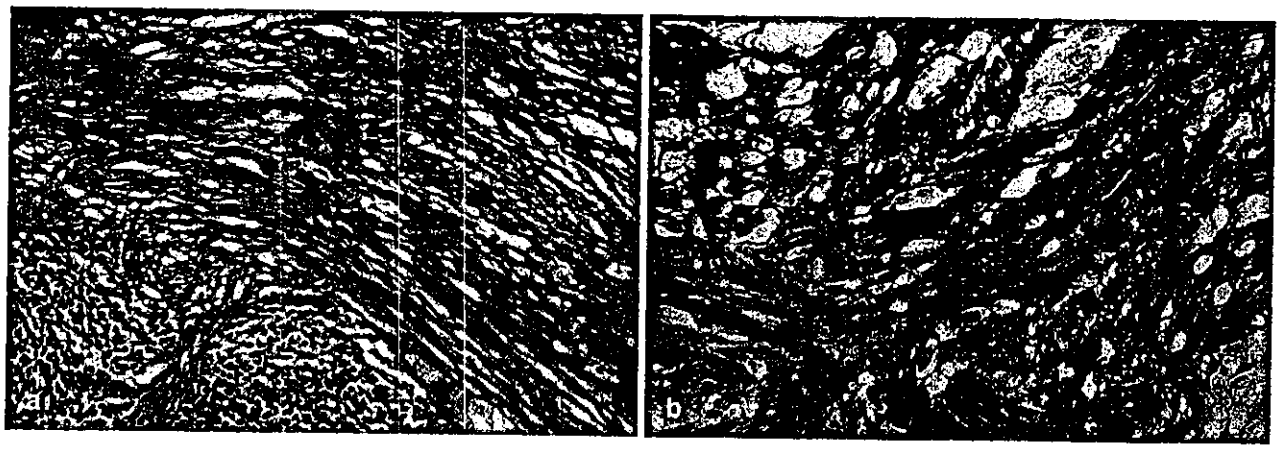

Fig. 3 Peripheral nerve-sheath tumors. A gastric schwannoma is composed of short fascicles of spindle cells and bizarre cells with peripheral lymphoid cuffs (**A**). Many tumor cells show diffuse and strong staining for S-100 (**B**)

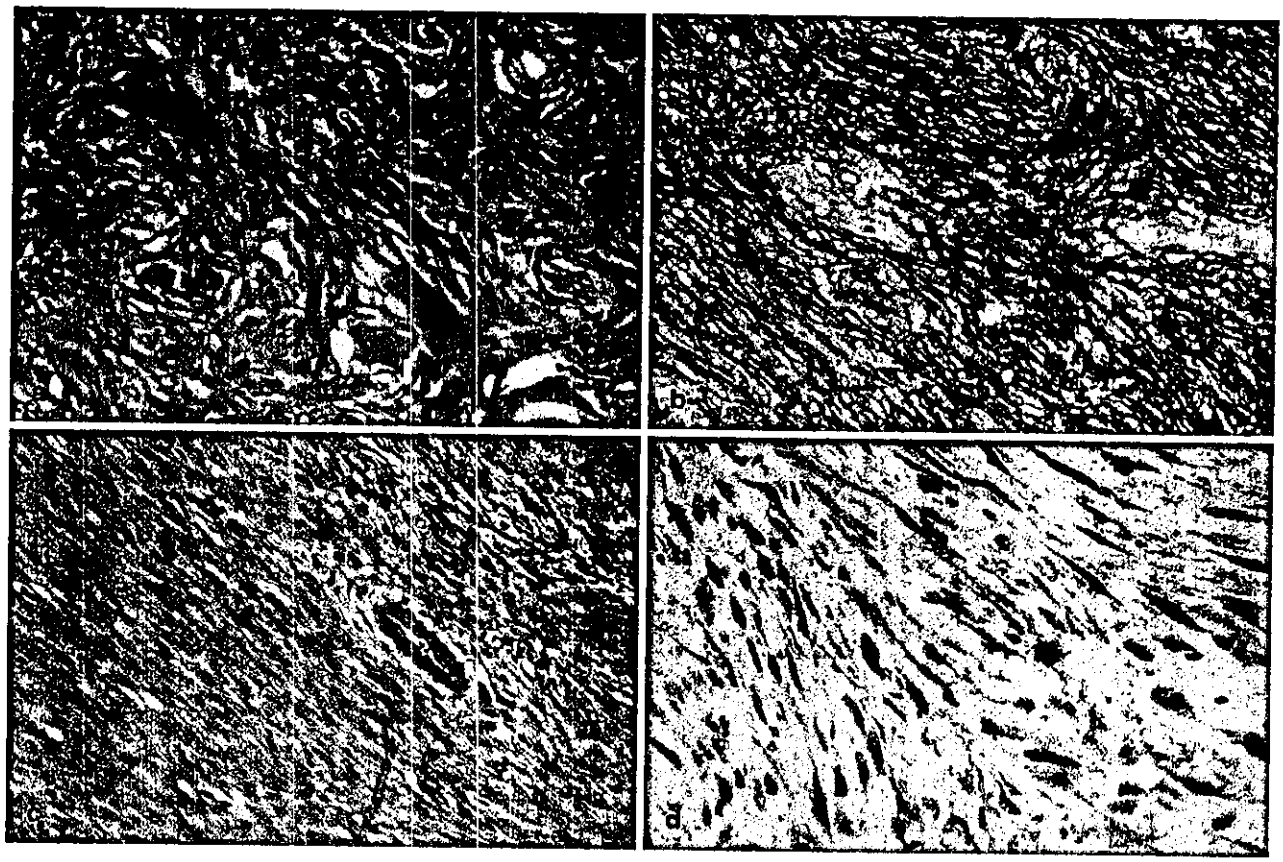

Fig. 4 Fibrous tumors. A pelvic solitary fibrous tumor shows a haphazard arrangement of spindle cells with hyalinized bands of collagen (**A**). Many tumor cells show strong immunoreactivity for CD34 (**B**). A mesenteric desmoid-type fibromatosis involving gastric wall shows a loose fascicular arrangement 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-

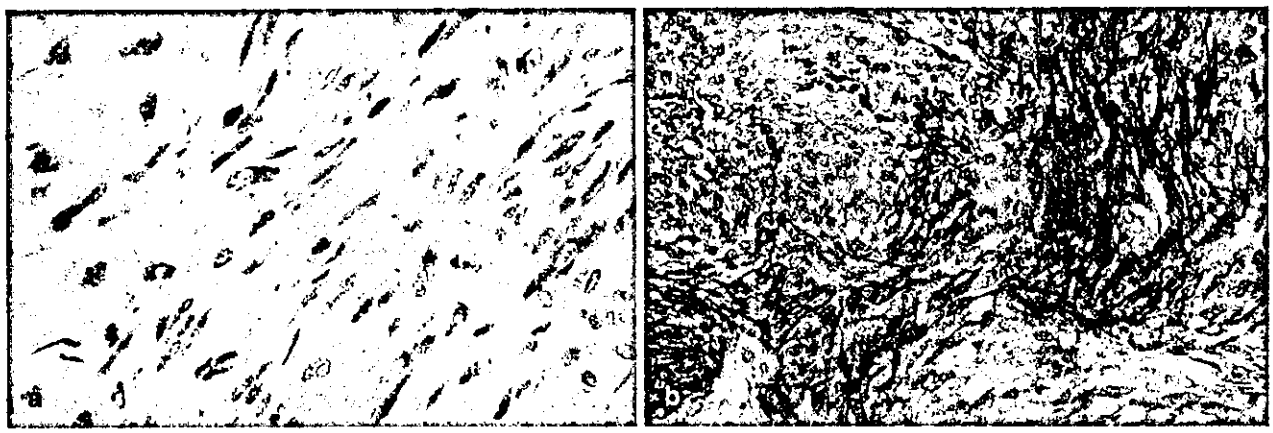

Fig. 5 A desmoid-type fibromatosis shows weak (1+) coarsely granular cytoplasmic immunostaining for KIT(c-kit/CD117) (A). A monophasic synovial sarcoma shows focal and moderate (2+) membranous immunoreactivity for KIT (B)

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 GIST's 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.

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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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