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CD34-positive myxoid sarcoma of the retroperitoneum: a dilemma in differential diagnosis of multiple biopsy specimens

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Summary. The author reports a case of CD34-positive malignant myxoid sarcoma in the retroperitoneum with a dilemma of differential diagnosis in multiple biopsy specimens of different locations. A 79-year-old man was diagnosed with right renal pelvic carcinoma and nephrectomy was performed. The carcinoma was urothelial carcinoma (2cm in diameter) without invasion. The patient was followed up, and a large retroperitoneal tumor was found two years after the operation. Multiple needle biopsies were performed. The patient then showed a hepatic metastasis, and died of cachexia one year after the detection of the retroperitoneal tumor. The needle biopsy specimens showed spindle cell sarcoma in the myxomatous stroma (80%) and in the non-myxomatous stroma (20%). Immunohistochemically, the tumor cells were positive for vimentin, CD34, CD99, bcl-2 and p53 protein. They were negative for cytokeratins, desmin, α-smooth muscle actin, S100 protein, melanosome, CEA, neuron specific enolase, CD68, factor VIII-related antigen, CD31, KIT, and PDGFRA. Ki67 labeling was 30%. A genetic analysis for KIT gene (exons 9, 11, 13 and 17) and PDGFRA gene (exons 12 and 18) showed no mutations. Although the differential diagnosis is problematic and difficult, the present case is probably dedifferentiated liposarcoma. The needle biopsy diagnosis of sarcomas is difficult and limited because sarcomas show heterogenous histologies with regard to locations in the same tumor.

Key words: Retroperitoneum, CD34, Liposarcoma

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Introduction

CD34, a hematopoietic progenitor cell antigen, is generally thought to be a hallmark of solitary fibrous tumor, gastrointestinal stromal tumor, and dermatofibrosarcoma protuberans for general pathologists (Rosai, 2004), However, it is not specific for these tumors; it is expressed in several other tumors, such as hematopoietic malignancies, angiogenic tumors, epithelioid sarcoma, some fibroblastic tumors, and lipomatous tumors (Miettinen and Lasota, 2005, 2006; Suster and Fisher, 1997). Therefore, CD34 is a non-specific marker, and combination with some other antigens including bcl-2 CD99, KIT, CD34, Factor VIII-related antigen, vimentin, and cytokeratins are necessary (Miettinen and Lasota, 2005, 2006). In addition, carefull histopathologic observations are mandatory for making the correct diagnosis.

Malignant myxoid tumors include myxoid malignant fibrous histiocytoma, myxofibrosarcoma, low grade fibromyxoid sarcoma, myxoid liposarcoma, and other sarcoma with focal myxoid changes (Rosai, 2004).

In the retroperitoneal areas, CD34-positive sarcomas have been reported to be solitary fibrous tumor (Vallat-Decouvelaere et al., 1998; Hasegawa et al., 2000; Kunieda et al., 2004; Rosai, 2004), liposarcoma (in particular dedifferentiated one) (Hasegawa et al., 2000), angiogenic sarcoma (Meis-Kingblom and Kingblom, 1998a), malignant solitary fibrous tumor (Tanaka et al., 2006), and gastrointestinal stromal tumor (Takizawa et al., 2006). In solitary fibrous tumor, recognition of pericytomatous and "patternless" patterns and immnohistochemicaly demonstration of CD99 and bcl-2 are necessary (Chilosi et al., 1997; Rosai, 2004). In liposarcoma, demonstration of lipobasts is essential (Hasegawa et al., 2000). In angiogenic sarcoma, vascular

channels and immunohistochemical demonstration of CD31 and factor VIII-related antigen are important (Vallat-Decouvelaere et al., 1998). In gastrointestinal stromal tumor, immunohistochemical demonstration of KIT (CD117) and molecular genetic analysis of *KIT* and *platelet-derived growth factor receptor-α* (*PDGFRA*) genes are mandatory (Miettinen and Lasota, 2005, 2006).

The author herein presents a case of CD34-positive myxoid spindle cell sarcoma in the retroperitoneum, which was difficult in differential diagnosis. The final diagnosis was probable dedifferentiated liposarcoma.

Materials and methods

A 79-year-old Japanese man was diagnosed with a right renal pelvic carcinoma, and nephrectomy was performed at the author's hospital. The pathologic diagnosis was renal pelvic papillary urothelial carcinoma (2 cm in diameter) without stromal invasion (pTa) and lymphovascular permeation. The patient was followed up, and complained of lumbago two years after the operation. CT and MRI showed a large retroperitonal tumor (8x9x7 cm) independent of the left kidney and nephrectomized right kidney (Fig. 1).

Multiple needle biopsies from different locations were performed. The patient then showed hepatic metastasis, and died of cachexia one year after the detection of the peritoneal tumor. No tumors other than the renal pelvic tumor and retroperitoneal tumor were recognized in the clinical course. Skin tumors were absent. Autopsy was not performed.

The materials were the multiple needle biopsy specimens taken from the tumor from different locations. The biopsies were fixed in 10% formalin and embedded in paraffin. Multiple 3-μm sections were made and two of them were stained with hematoxylin and eosin and with alcian blue. Immunohistochemical study was performed by the Dako's Envision method, as previously described (Terada et al., 2002; Terada and Kawaguchi, 2005). The antibodies used and results in tumor cells are shown in Table 1.

Genetic analyses for *KIT* gene (exons 9, 11, 13 and 17) and the *PDGFRA* gene (exons 12 and 18) were performed by the PCR-direct sequencing method, as described previously (Terada, 2008, 2009). Exons of both genes were selected because they are frequent mutation sites. The primers are shown in Table 2.

Results

The multiple needle biopsy specimens showed proliferation of atypical spindle cells in the myxomatous stroma (Fig. 2A) positive with alcian blue (80% in area). Non-myxomatous areas were also recognized (20% in area) (Fig. 2B). No fat elements or collagenous areas were recognized. The tumor cells show hyperchromatic nuclei, and were regarded as spindle cell sarcoma. Some large tumor cells with hyperchromatic nuclei were recognized (Fig. 2A,B). Mitotic figures were present in 3 per 10 high power fields. No lipoblasts were recognized. No storiform, pericytomaous, or "patternless pattern"

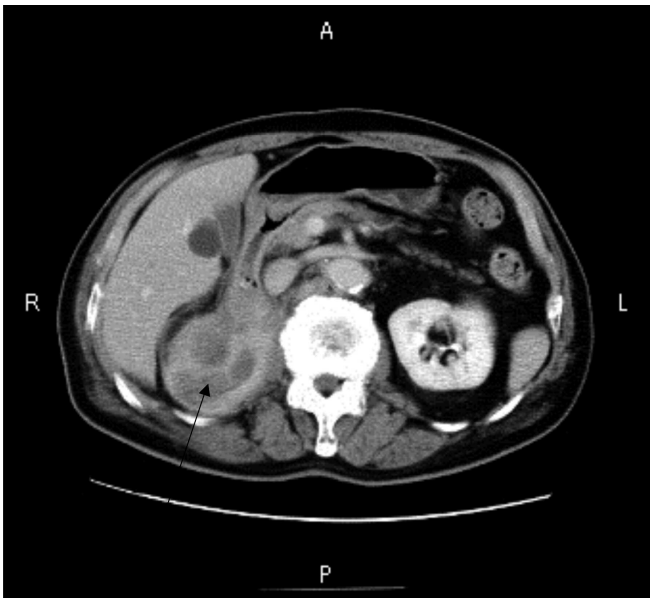

Fig. 1. CT finding of the retroperitoneal tumor (arrow). The tumor is large.

Table 1. Immunohistochemical agents and results in tumor cells.

Antigen	Clone	Source	Results
Vimentin	Vim 3B4	Dako Glostrup, Demmark	+
CD34	QBEND10	Dako	+
p53 protein	DO7	Dako	+
CD99	12E7	Dako	+
bcl-2	124	Dako	+
Cytokeratin	AE1/AE3	Dako	-
Cytokeratin	polyclonal	Dako	-
Desmin	D33	Dako	-
α-SMA	1A4	Dako	-
S100 protein	polyclonal	Dako	-
melanosome	HMB45	Dako	-
CEA	polyclonal	Kyowa, Japan	-
Chromogranin	DAK-A3	Dako	-
Synaptophysin	polyclonal	Dako	-
NSE	N1557	Dako	-
CD68	KP-1	Dako	-
Factor VIII-RA	polyclonal	Dako	-
CD31	JC70A	Dako	-
KIT	polyclonal	Dako	-
PDGFRA	polyclonal	Santa Cruz, CA, USA	-
Ki67	MIB-1	Dako	30%

SMA: smooth muscle actin; NSE: neuron specific enolase; Factor-VIII-RA: Factor VIII-related antigen; PDGFRA: platelet growth factor receptor-alpha.

features were recognized. Vessels in the tumor were relatively abundant. Inflammatory cell infiltrations were little or none. Immunohistochemically, the tumor cells were positive for vimentin, CD34 (Fig. 2C), CD99, bcl-2, and p53 protein (Table 1). The tumor cells were negative for cytokeratins, desmin, α-smooth muscle actin, S100 protein, melanosome, CEA, chromogranin, synaptophysin, neuron specific enolase, factor VIII-related antigen, CD31, KIT, and PDGFRA (Table 1). Ki67 labeling (MIB-1, Dako) was 30%. The genetic

Table 2. Primer sequence.

	Forward	Reverse
KIT exon 9	5'-TCC TAG AGT AAG CCA GGG CTT-3'	5'-TGG TAG ACA GAG CCT AAA CAT CC-3'
KIT exon11	5'-GAT CTA TTT TTC CCT TTC TC-3'	5'AGC CCC TGT TTC ATA CTG AC-3'
KIT exon 13	5'-GCT TGA CAT CAG TTT GCC AG -3'	5'-AAA GGC AGC TTG GAC ACG GCT TTA-3'
KIT exon 17	5'-CTC CTC CAA CCT AAT AGT GT-3'	5'-GTC AAG CAG AGA ATG GGT AC-3'
PDGFRA exon12	5'-TTG GAT ATT CAC CAG TTA CCT GTC-3'	5'-CAA GGG AAA AGC TCT TGG-3'
PDGFRA exon 18	5'-ACC ATG GAT CAG CCA GTC TT-3'	5'-TGA AGG AGG ATG AGC CTG ACC-3'

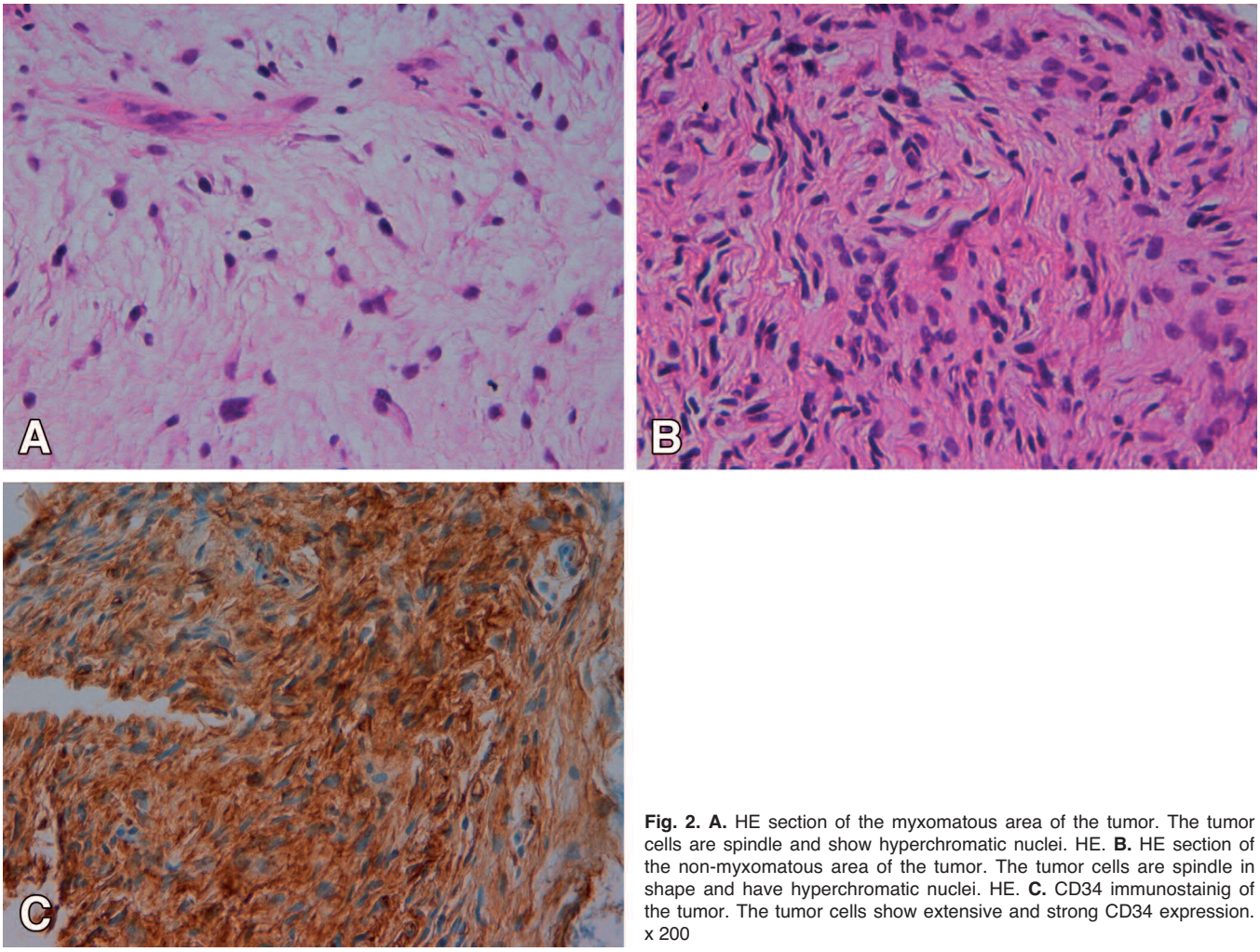

Fig. 2. A. HE section of the myxomatous area of the tumor. The tumor cells are spindle and show hyperchromatic nuclei. HE. **B.** HE section of the non-myxomatous area of the tumor. The tumor cells are spindle in shape and have hyperchromatic nuclei. HE. **C.** CD34 immunostainig of the tumor. The tumor cells show extensive and strong CD34 expression. x 200

analysis for *KIT* gene (exons 9, 11, 13 and 17) and *PDGFRA* gene (exons 12 and 18) showed no mutations.

Discussion

The present case showed a renal pelvic papillary urothelial carcinoma (2 cm in diameter) and a spindle cell sarcoma of the retroperitoneum two years after the urothelial carcinoma. The urothelial carcinoma showed no invasion (pTa) and no lymphovascular invasions. The spindle cell sarcoma of the retroperitoneum was entirely different from the urothelial carcinoma. Therefore, the renal pelvic urothelial carcinoma and the spindle cell sarcoma were different, independent tumors.

CD34, a hematopoietic progenitor cell antigen, is a hallmark of solitary fibrous tumor (Rosai, 2004), However, it is not specific for solitary fibrous tumor; it is expressed in several other tumors, such as gastrointestinal stromal tumor, dermatofibrosarcoma protuberans, hematopoietic malignancies, angiogenic tumors, epithelioid sarcoma, some fibroblastic tumors, and lipomatous tumors (Suster and Fisher, 1997; Miettinen and Lasota, 2005, 2006). Several kinds of CD34-positive sarcomas occur in the retroperitoneum. They include liposarcoma (in particular dedifferentiated one) (Hasegawa et al., 2000), angiogenic sarcoma (Meis-Kingblom and Kingblom, 1998a), malignant solitary fibrous tumor (Tanaka et al., 2006), and gastrointestinal stromal tumor (Takizawa et al., 2006).

The malignant nature of the present case is supported by the histology, p53 expression, high Ki67 labeling index, and liver metastasis. Therefore, the present case is a malignant myxoid sarcoma. The present case is not angiogenic sarcoma because no vascular channels were recognized and endothelial markers (CD31 and Factor VIII-related antigen) were negative. The present case is not extragastrointestinal stromal tumor because KIT immunoreactivity and mutations of *KIT* and *PDGFRA* genes were not recognized.

The remaining diagnoses were liposarcoma and solitary fibrous tumor. The differential diagnosis is difficult, because the biopsy specimens do not show the overall histologies of the tumor and is limited in the definite diagnosis. In the present case, neither lipoblasts characteristic of liposarcoma nor pericytomatus pattern characteristic of solitary fibrous tumor was recognized. Nevertheless, the HE specimens are in favor of dedifferentiated liposarcoma. However, there is a dilemma in differential diagnosis in HE sections.

With regard to immunohistochemistry, the present tumor was stained with CD99 and bcl-2, well known markers of solitary fibrous tumor (Rosai, 2004). However, these antigens are not specific for solitary fibrous tumor (Rosai, 2004). Therefore, the immunohistochemical data cannot differentiate between dedifferentiated liposarcoma and solitary fibrous tumor.

A review of the literature revealed 20 cases of solitary fibrous tumor in the retroperitoneum (Nielsen et al., 1997; Vallat-Decouveleare et al., 1998; Hasegawa et al., 1999; Monimitsu et al., 2000; Clayton et al., 2001; Nakatani et al., 20002; Nagasako et al., 2004). Most of them are benign except for four malignant cases reported by Vallat-Decouvelaere et al. (1998) and Tanaka et al. (2006). Solitary fibrous tumor occasionally shows focal areas of myxoid stroma (de Saint Aubain Somerhausen et al. 1999). de Saint Aubain Somerhausen et al. (1999) advocated myxoid solitary fibrous tumor when the myxoid stroma occupies more than 50% of solitary fibrous tumor. A review of the literature showed only eight cases of myxoid solitary fibrous tumor (Wei et al., 1997; de Saint Aubain Somerhausen et al., 1999). All of them are benign SFTs (Wei et al., 1997; de Saint Aubain Somerhausen et al., 1999), and malignant counterpart of the myxoid solitary fibrous tumor has not been reported, to the best of the author's knowledge. The present case may be malignant solitary fibrous tumor, although this possibility is low.

On the other hand, liposarcoma is much more common in the retroperitoneal area than solitary fibrous tumor. As is well known, liposarcomas are classified into well differentiated, round cell, myxoid, pleomorphic and dedifferentiated ones. Detection of lipoblasts is an important hallmark in making the diagnosis of liposarcoma. Dedifferentiated liposarcoma may show CD34 and bcl-2 (Nakanishi et al., 1997; Hasegawa et al., 2000), as in the present case. The present case is probably dedifferentiated liposarcoma from overall histological and immunohistochemical appearances.

In the present case, some large tumor cells with hyperchromatic nuclei were recognized in the retroperitoneal tumor. Such large cells or giant cells may occur in many sarcomas. The most representative tumor is malignant fibrous histiocytoma. Such large cells are also present in pleomorphic liposarcoma, dedifferentiated liposarcoma, malignant peripheral nerve sheath tumors, pleomorphic rhabdomyosarcoma, osteosarcoma, chodrosarcoma, and other sarcomas (Rosai, 2004). In general, solitary fibrous tumor and spindle cell-type gastrointestinal stromal tumor usually does not show such large cells with hyperchromatic nuclei (Rosai, 2004; Miettinen and Lasota, 2005, 2006). In contrast, dedifferentiated liposarcoma frequently shows large cells or giant cells with hyperchromatic nuclei (Hasegawa et al., 2000). Therefore, the presence of large cells with hyperchromatic nuclei is in favor of the diagnosis of dedifferentiated liposarcoma in the present tumor.

The present case should be differentiated from malignant myxoid tumors such as metastatic myxoinflammatory fibroblastic sarcoma of the skin, low-grade fibromyxoid sarcoma (Evans tumor), myxofibrosarcoma and myxoid malignant fibrous histiocytoma. In metastatic myxoinflammatory fibroblastic tumor, this tumor occurs almost always in the acral skin, and shows CD34 positivity, numerous inflammatory cells, and fibrosis (Meis-Kindblom and Kindblom, 1998b; Hassanein et al., 2008). The present case lacked acral skin tumor, and the retroperitoneal

myxoid tumor lacked inflammatory cells. Therefore, the present retroperitoneal tumor appears different from metastatic myoinflammatory fibroblastic tumor. In low-grade fibromyxoid sarcoma, CD34 is negative (Billings et al., 2005) and histologic features are somewhat different from the present case. In myxoid malignant fibrous histiocytoma, CD34 is negative (Coindre et al., 2003) and typical pleomorphic-storiform pattern is present. However, in the present case, CD34 is positive and the pleomorphic-striform pattern was not recognized. In addition, it is reported that most malignant fibrous hystiocytomas in the retroperitoneum are actually dedifferentiated liposarcoma (Coindre et al., 2003). In myxofibrosarcoma, the author extensitively reviewed the literature but could not find the CD34 positivity in myxofibrosarcoma. Therefore, it appears that there is no CD34 in myxofibrosarcoma. In addition, the histology of the present case is different from this tumor because no fibrosarcomatous areas were present in the present case.

In summary, the author reported a probable dedifferentiated liposarcoma in the retroperitoneum in multiple needle biopsy specimens obtained from different sites of the tumor. The biopsy diagnosis of soft tissue sarcoma is difficult and limited because of tumor histology heterogeneity.

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