

Original Article

Morphological and immunohistochemical diversity of endometrial stromal sarcoma in rats

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Abstract: To clarify the histopathological characteristics of rat endometrial stromal sarcoma (ESS), we morphologically reviewed 12 malignant uterine tumors protruding into the lumen in previous rat carcinogenicity studies. The 12 cases were classified into the following 6 types based on their morphological features: spindle cell and collagen rich type, pleomorphic/spindle cell and compact type, decidual alteration type, histiocytic and multinucleated giant cell mixture type, Antoni A-type schwannoma type, and Antoni B-type schwannoma type. Immunohistochemically, tumor cells in all cases exhibited focal or diffuse positive reactions for vimentin, and 11 of the 12 cases were positive for S-100. Interestingly, 9 cases were positive for desmin or α SMA, indicating tumor cells expressing smooth muscle properties. Both Antoni A- and B-type schwannoma types showed low reactions for both muscle markers. Positive results for estrogen receptor α in the 11 cases suggested that they were derived from endometrial stromal cells. On the basis of their immunohistochemical profiles, they were considered to be derived from endometrial stromal cells while they showed morphological variation. The detection of a basement membrane surrounding tumor cells might not be a definitive indicator for differential diagnosis of ESS from malignant schwannoma. In conclusion, ESS could exhibit wide morphological and immunohistochemical variation including features of schwannoma or smooth muscle tumor. (DOI: 10.1293/tox.2017-0056; J Toxicol Pathol 2018; 31: 95–103)

Key words: endometrial stromal sarcoma, rat, S-100, ER α , immunohistochemistry

Introduction

Tumors protruding from the endometrium into the uterus lumen are frequently observed in aging rats. Although the majority of these tumors are benign endometrial stromal polyps derived from endometrial stromal cells, a small proportion are malignant tumors known as endometrial stromal sarcomas (ESSs). Tumors diagnosed as ESS are histologically varied and are predominantly composed of spindle cells arranged in loose sheets, in interlacing fasciculi, or, in rare cases, pleomorphic polygonal cells arranged in solid sheets. In some cases, multinucleated giant cells or decidual reactions are observed within tumors^{1–5}.

Uterine sarcomas aside from ESS have also been iden-

tified, such as leiomyosarcoma and malignant schwannoma^{1, 2, 4–6}. The Hematoxylin and eosin (HE) staining-based morphological distinction of ESS from leiomyosarcoma or malignant schwannoma is often difficult due to wide histological variation and partly similarity to other sarcoma in ESS. Immunohistochemical staining or electron microscopic analysis have been accepted as useful tools for differential diagnosis of ESS from other uterine sarcomas^{1, 2, 4, 5}. Both ESS and malignant schwannoma are immunohistochemically positive for vimentin and S-100, and the presence of a basement membrane is evidence of schwannoma alone^{2, 5}. On the other hand, in routine pathological examination in carcinogenicity studies, immunohistochemical or electron microscopic analysis for definition of each uterine malignant tumor has been rarely performed due to the substantial amount of labor and time required for these analyses. Therefore, the mechanisms behind the various histological features of ESS remain unclear.

The Histological features of human ESS also vary. A number of studies on differential diagnosis have been performed, particularly to distinguish ESS from uterine smooth muscle tumors. Recently, CD10 has been identified as an effective marker of ESS in humans^{7–11}. Given that CD10 was originally observed on the cell surface of acute

Received: 21 September 2017, Accepted: 6 December 2017

Published online in J-STAGE: 2 February 2018

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Table 1. Procedure and Primary Antibodies Used in Immunohistochemical Analysis

Antibody	Clone	Dilution	Antigen retrieval	Source
Vimentin	V9	1:200	95°C for 20 min	Dako
S100	Poly	1:800	95°C for 20 min	Dako
Desmin	Poly	1:200	95°C for 20 min	Santa Cruz Biotechnology
α SMA	1A4	1:200	95°C for 20 min	Dako
CD10	56C6	1:30	95°C for 20 min	Novocastra
Inhibin α	R1	1:10	95°C for 20 min	Dako
Iba-1	Poly	1:4000	95°C for 20 min	Wako
ER α	Poly	1:50	95°C for 30 min	Santa Cruz Biotechnology
PCNA	PC10	1:400	95°C for 20 min	Dako

α SMA, α smooth muscle actin; ER α , estrogen receptor α ; PCNA, proliferating cell nuclear antigen; poly, polyclonal antibody; MW, microwave.

lymphoblastic leukemia^{7, 12, 13}, this marker is also known as common acute lymphoblastic leukemia antigen (CALLA) and has additionally been observed on normal human endometrial stromal cells and human ESS^{8, 9}. CD10 has also been characterized and investigated as another hormone-related antigen, such as the estrogen receptor (ER), progesterone receptor, and inhibin α , with utility as an ESS marker^{8, 10, 14, 15}. To our knowledge, however, no study has yet reported full details regarding the expression of these antigens in rat ESS.

Here, to clarify the morphological and immunohistochemical features of rat ESS, we performed immunohistochemical analysis on the expression of a range of antigens, including vimentin, S-100, desmin, α -smooth muscle actin (α SMA), CD10, Iba-1, ER α , and inhibin α . In addition, we assessed the utility of electron microscopic analysis in differential diagnosis of ESS from malignant schwannoma. Further, taking the findings into account, we reevaluated the diagnostic criteria of ESS.

Materials and Methods

Case selection and tissue preparation

The 12 cases used in this study had been diagnosed as ESS based on their histopathological features observed in HE-stained sections in previous two-year carcinogenicity studies. All cases were derived from control groups. All procedures were conducted with the permission of the institutional animal care and use committee at the facility.

The rat strains were as follows: Fischer344/DuCrjCrj (F344) (n=7), Wistar Hannover (RccHanTM:WIST) (WH) (n=4), and Sprague Dawley (Crj:CD) (SD) (n=1). Tissues sampled at necropsy were fixed in 10% phosphate buffer formalin, embedded in paraffin, and serially sectioned at 4 μ m. HE staining was performed according to the usual method for light microscopic examination. Sections of one tumor diagnosed as malignant schwannoma arising from a hepatodiaphragmatic nodule were used to compare with ESS.

Immunohistochemistry

Procedures for immunohistochemistry, including antibodies used as primary antibodies, are summarized in Table 1. Formalin-fixed and paraffin-embedded tissues were deparaffinized and rehydrated through graded alcohols. En-

dogenous peroxidase activity was quenched with 3% H₂O₂, followed by heat-induced antigen retrieval in a microwave at 95°C with citrate buffer at pH 6.0. After blocking endogenous peroxidase activity, sections were treated with serum-free protein block (Dako Japan, Tokyo, Japan) for 30 min at room temperature. Sections were then washed with washing buffer (Dako Japan, Tokyo, Japan) and reacted with the primary antibody overnight at 4°C. Sections were washed and treated by applying drops of Simple Stain Rat MAX PO (MUITI) (Nichirei Biosciences, Tokyo, Japan) to sections reacted with S-100, α SMA, CD10, Iba-1, and proliferating cell nuclear antigen (PCNA) primary antibodies for 10 min at room temperature or by applying drops of Simple Stain Mouse MAX-PO (G) (Nichirei Biosciences) to sections reacted with desmin for 30 min at room temperature. After washing sections with washing buffer, reactions were visualized using 3,3'-diaminobenzidine (DAB) as a chromogen. Sections were lightly counterstained with hematoxylin.

Immunohistochemical evaluation

Immunohistochemically stained sections were semi-quantitatively evaluated for the presence or absence of staining by light microscopy. The approximate rate of tumor cells showing positive reactions was classified into one of the following five grades: -, no cells positively stained in the tumor; \pm , few (<1/4) cells positively stained in the tumor; +, some (<2/4) cells positively stained in the tumor; ++, many (<3/4) cells positively stained in the tumor; +++, most (>3/4) cells positively stained in the tumor.

Electron microscopy

Electron microscopy was performed on formalin-fixed tissues from Case 3 as pleomorphic/spindle cell and compact-type ESS, and Cases 10–12 as Antoni B-type schwannoma-type ESS; these cases were selected to examine if schwannoma-type and non-schwannoma-type ESSs have a basement membrane and desmosome-like structure. Tissues were cut into 1- to 2-mm³ cubes and washed in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 30 min, postfixed in 1% osmium tetroxide, and embedded in epoxy resin. Semithin sections (1 μ m thick) were stained with 1% toluidine blue to select areas most appropriate for examination. Ultrathin sections were mounted on copper grids,

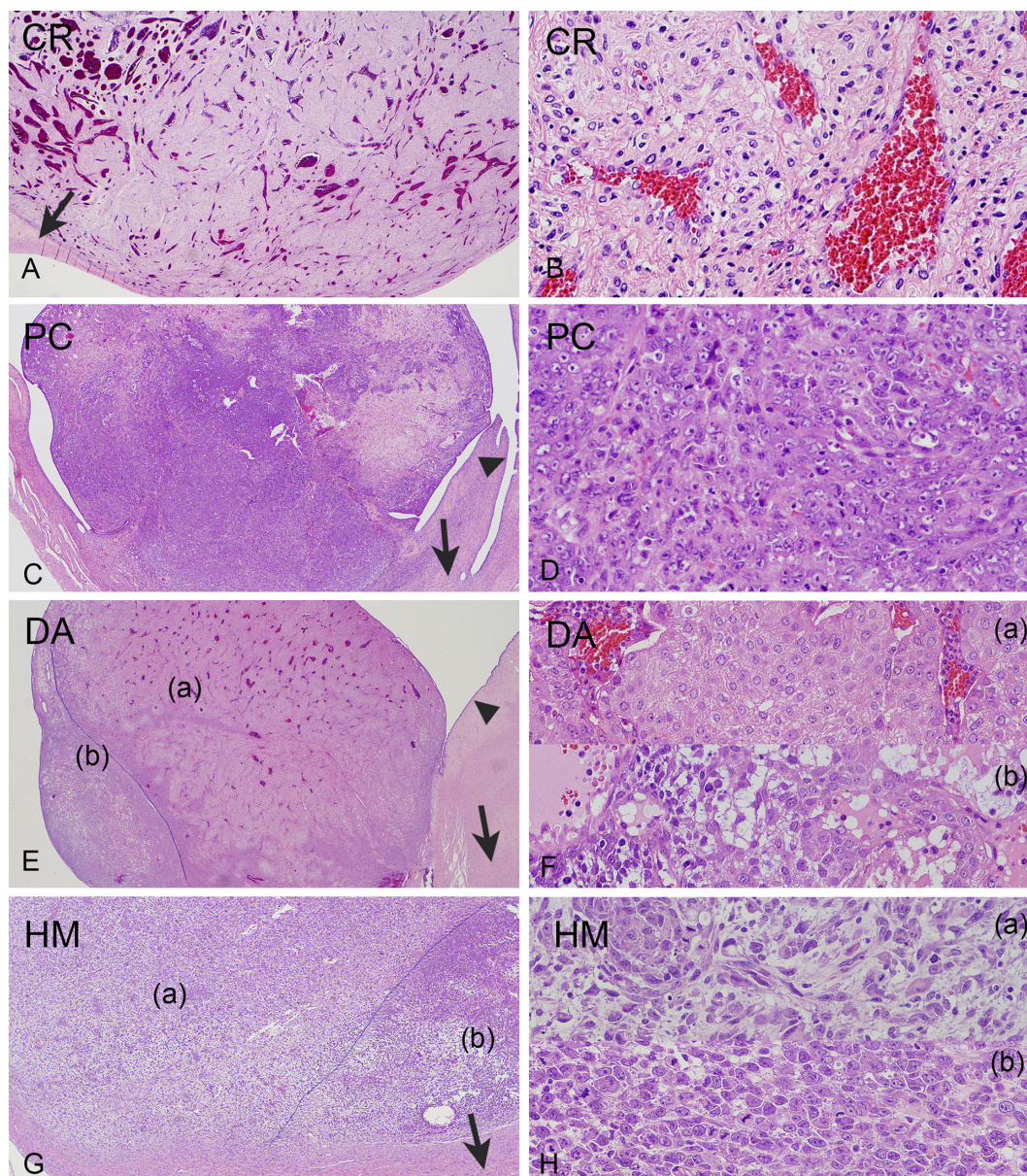


Fig. 1. Histological features of the CR, PC, DA, and HM types of ESS. (A, B) CR type, Case 2. Spindle tumor cells with round nuclei and abundant eosinophilic cytoplasm are visible in the collagenous stroma. (C, D) PC type, Case 3. Pleomorphic tumor cells with large nuclei are present compactly. (E, F) DA type, Case 6. Two different areas, (a) an epithelioid decidual cell proliferation area and (b) a spindle cell proliferation area, are recognizable. (G, H) HM type, Case 7. Two different areas, (a) a histiocytic sarcoma-like cell proliferation area and (b) a densely proliferated round nuclei tumor cell area, are distinctive. HE stain. Arrows = muscle layer; arrowheads = endometrial epithelium. Magnification: A, C, E, and G = 4 \times ; B, D, F, and H = 40 \times .

stained with uranyl acetate and lead citrate, and examined using an H-7600 transmission electron microscope (Hitachi High-Tech Fielding Corporation, Tokyo, Japan).

Results

Histological and immunohistochemical evaluation

The 12 cases were classified into 6 types based on morphological characteristics, as follows: spindle cell and collagen-rich (CR) type (n=2), pleomorphic/spindle cell

and compact (PC) type (n=3), decidual alteration (DA) type (n=1), histiocytic/multinucleated giant cell (HM) type (n=1), Antoni A-type schwannoma (SA) type (n=2), and Antoni B-type schwannoma (SB) type (n=3) (Fig. 1 and 2, Table 2).

CR type: Two cases were classified as the CR type (Cases 1 and 2). The histological features of the CR type were the proliferation of spindle tumor cells arranged in a loose sheet with abundant collagenous stroma. Tumor cells had round nuclei and abundant eosinophilic cytoplasm (Fig. 1B). Various sizes of blood vessels were frequently observed within

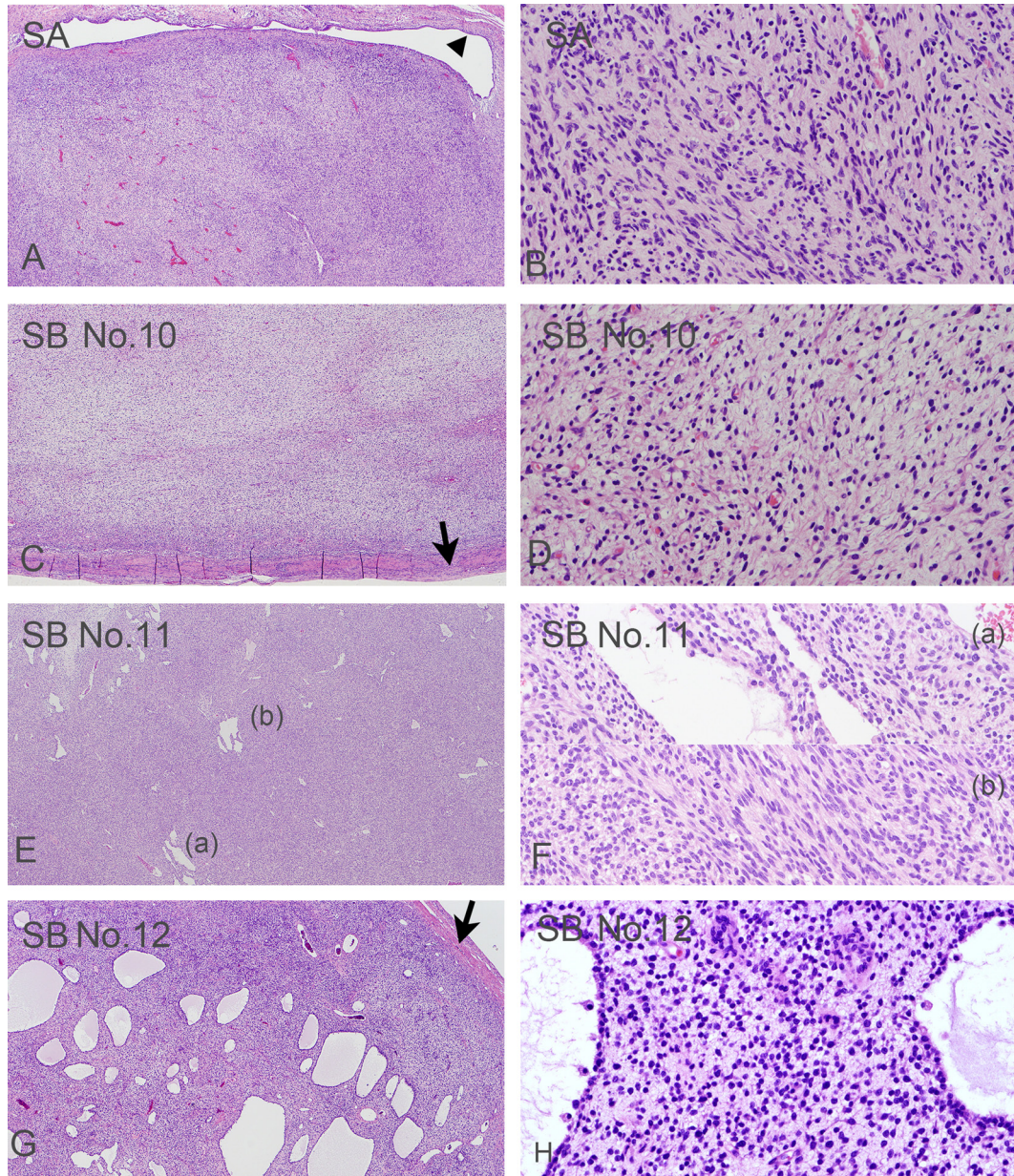


Fig. 2. Histological features of the SA and SB types of ESS. (A, B) SA type, Case 8. Spindle cells are arranged in an interlacing fasciculus with a nuclear palisading pattern. (C, D) SB type, Case 10. Spindle cells are sparsely proliferated with an edematous stroma, but there are no cystic structure in this field. (E, F) SB type, Case 11. Two different morphological areas, (a) an area with spindle cells forming cystic structure and (b) an area with spindle cells proliferating in interlacing fasciculi, are evident. (G, H) SB type, Case 12. Many cystic structures are present in this case. HE stain. Arrows = muscle layer; arrowhead = endometrial epithelium. Magnification: A, C, E, and G = 4 \times ; B, D, F, and H = 40 \times .

the tumors (Fig. 1A and B). Cases 1 and 2 were positive on immunohistochemical analysis for vimentin, S-100, and either or both desmin or α SMA (Table 2, Fig. 3) and were positive for CD10 and ER α (Table 2). Only Case 2 was positive for inhibin α (Table 2). The CR type cases required a differential diagnosis from fibrosarcoma due to the morphological commonality between the two in terms of “abundant collagen”. However, CR-type cases expressed S-100, which is expressed on rat ESS but not expressed on fibrosarcoma, and either or both α SMA or desmin. Further, CR types also

expressed ER α (expressed on rat endometrial stromal cells). Two CR type cases were therefore diagnosed as ESS.

PC type: Three cases were classified as the PC type (Cases 3–5). The morphological characteristics of the PC type were dense and solid proliferation of spindles to pleomorphic cells (Fig. 1C and D). Tumor cells had large nuclei and abundant cytoplasm with an indistinct cell border and some necrotic areas observed within the tumors. Cases 4 and 5 showed areas of tumor cells arranged in interlacing fasciculi. Cases 3–5 were immunohistochemically positive

Table 2. Summary of the Cases Examined, Immunohistochemistry, and Electron Microscopic Analysis

Case number	Hist type	Rat strain	Immunohistochemistry										EM	
			Area	Vimentin	S-100	Desmin	α SMA	CD10	Inhibin α	ER α	Iba1	PCNA	BM	D
1	CR	F344		+++	+++	+++	-	+	-	+++	NE	+++	NE	
2		F344		+	+++	++	+++	+++	++	\pm	NE	+++	NE	
3		WH		+++	\pm	-	+	-	-	+++	NE	+++	P ND	
4	PC	F344		+++	+	++	++	\pm	++ (weak)	++	NE	+++	NE	
5		F344		+++	\pm	+++	+++	+	-	++	NE	+++	NE	
6	DA	F344	a	++ (weak)	+++	+++	-	-	-	+++	NE	+++	NE	
			b	++	+	+	-	-	-	+++	NE	+++	NE	
7	HM	WH	a	+++	+++	-	+++	-	-	+++	-	+++	NE	
			b	+++	+++	-	-	-	-	+++	-	+++	NE	
8	SA	F344		++	++	-	-	++	++	++	NE	++	NE	
9		SD		+++	-	-	-	-	-	-	NE	\pm	NE	
10		F344		+++	+++	++	-	+++	-	+++	NE	+++	P P	
11	SB	WH	a	+++	+++	-	\pm	\pm	-	+++	NE	++	P ND	
			b	+++	++	-	++	\pm	-	+++	NE	++	P ND	
12		WH		+++	+++	-	-	-	-	+++	NE	+++	P ND	

ESS, endometrial stromal sarcoma; CR, spindle cell and collagen-rich type; PC, pleomorphic/spindle cell and compact type; DA, decidual alteration type; HM, histiocytic sarcoma type; SA, Antoni A-type schwannoma; SB, Antoni B-type schwannoma; F344, Fischer 344; WH, Wistar Hannover; α SMA, α smooth muscle actin; ER α , estrogen receptor α ; PCNA, proliferating cell nuclear antigen; EM, electron microscopy; BM, basement membrane; D, desmosome; NE, not examined; -, no cells positively stained in the tumor; \pm , few (<1/4) cells positively stained in the tumor; +, some (<2/4) cells positively stained in the tumor; ++, many (<3/4) cells positively stained in the tumor; +++, most (>3/4) cells positively stained in the tumor. Weak staining reactions were observed for inhibin α in sections from Case 4 and for vimentin in sections from case 6. Strong staining intensities were observed in all sections except for the two mentioned above. P, present; ND, not detected.

for vimentin and S-100 and any or all of α SMA, desmin, and ER α (Table 2, Fig. 3). Only Case 4 was positive for CD10 and inhibin α (Table 2). The PC-type cases required a differential diagnosis from leiomyosarcoma. However, the cases expressed S-100, and leiomyosarcoma was therefore excluded. The cases were therefore diagnosed as ESS.

DA type: One case was classified as the DA type (Case 6). Two areas with different morphological features were present within the tumor (Fig. 1E and F). Almost all areas of the tumor exhibited proliferation of epithelioid decidual cells (area a in Fig. 1E and F), and another area (area b in Fig. 1E and F) exhibited proliferation of spindle to and pleomorphic cells. The area of spindle cell proliferation resembled the PC type. Transition between both areas was observed in the tumor. Both areas were positive on immunohistochemical analysis for vimentin, S-100, and desmin (Table 2, Fig. 3). In addition, both areas were positive for ER α and negative for CD10 and inhibin α (Table 2). Tumor cells of this type were derived from endometrial stromal cells, as the cells of origin of decidual reaction are uterine stromal cells and uterine metrial gland cells. The decidual reaction is sometimes associated with endometrial stromal polyp. The DA type was diagnosed as ESS due to mixed features of a decidual reaction and PC-type lesions and due to positive reaction for S-100 and ER α of the tumor cells.

HM type: One case was classified as the HM type (Case 7). Two areas with different morphological features were present within the tumor. In areas in the Fig. 1G and H, histiocytic sarcoma-like cells with an abun-

dant pleomorphic cytoplasm and a round-to-wedge-shaped nucleus proliferated with multinucleated giant cells. In area b in Fig. 1G and H, tumor cells having abundant cytoplasm and round nuclear densely proliferated. In both areas, most tumor cells were positive on immunohistochemical analysis for vimentin and S-100, but tumor cells only in area a were positive for α SMA. Cells in both areas were positive for ER α and negative for Iba-1 (histiocyte marker), CD10, and inhibin α (Table 2). The HM type was morphologically similar to histiocytic sarcoma. However, the HM-type case examined in this study was completely negative for Iba1 and positive for α SMA, S-100, and ER α . The HM-type case was therefore diagnosed as ESS.

SA type: Two cases were classified as the SA type (Cases 8 and 9). Spindle tumor cells with an indistinct cell border arranged in an interlacing fasciculus and nuclear palisading pattern were observed, with substantial morphological similarity to schwannoma Antoni A (Fig. 2A and B). Case 8 was positive for vimentin and S-100, negative for muscle markers, and positive for ER α , CD10, and inhibin α (Table 2, Fig. 4). Case 9 was negative for all antigens except vimentin (Table 2, Fig. 4). The SA type was difficult to diagnose as ESS due to its morphological and immunohistochemical similarity to malignant schwannoma. However, Case 8 was diagnosed as ESS due to positivity for ER α , CD10, and inhibin α . Case 9 could not be distinctly diagnosed due to negative reactions in the immunohistochemical examination.

SB type: Three cases were classified as the SB type

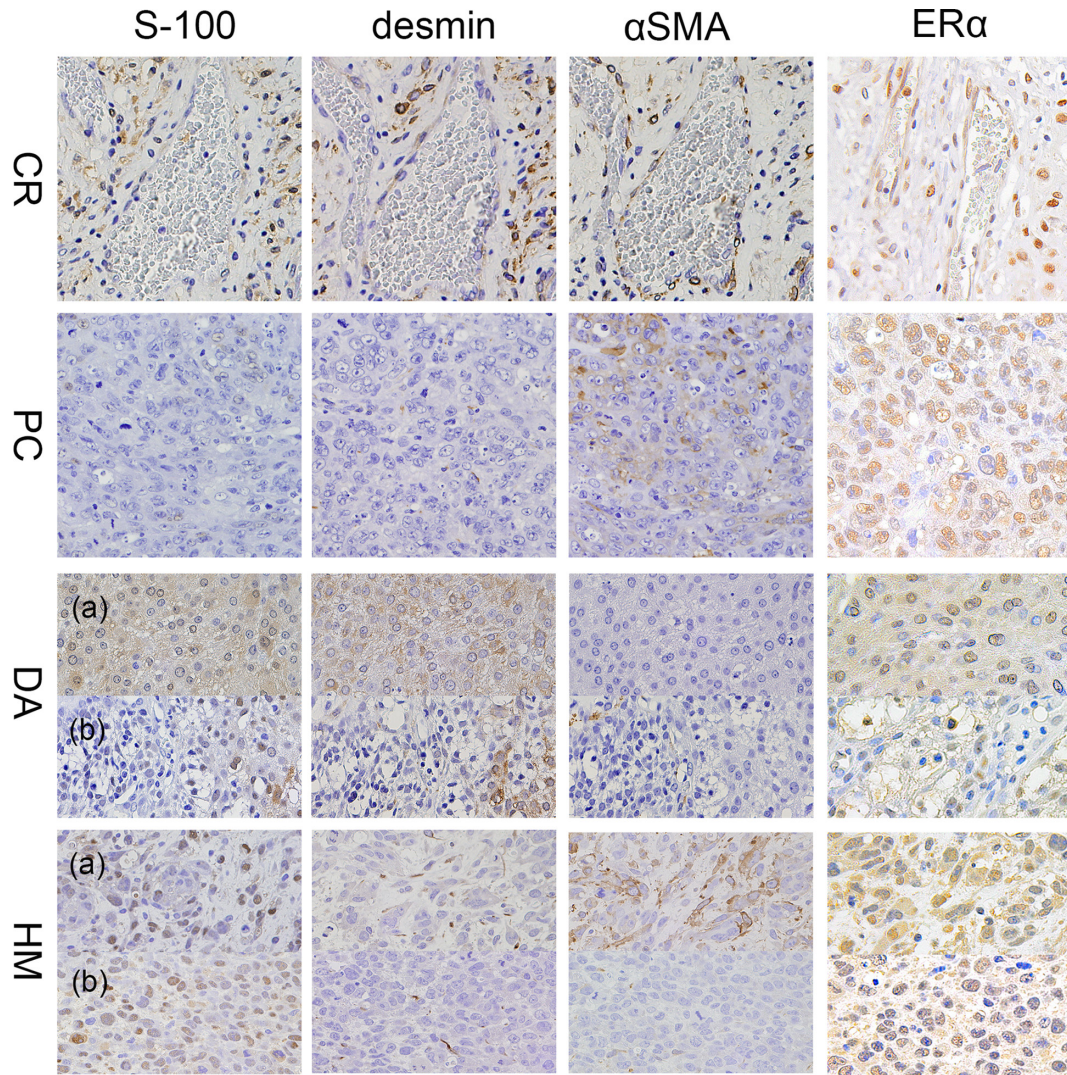


Fig. 3. Immunohistochemical images of cases from Figure 1. Top to bottom: CR type (Case 2), PC type (Case 3), DA type (Case 6), and HM type (Case 7). Left to right: S-100, desmin, α SMA, and ER α . Magnification: 40 \times .

(Cases 10–12). Spindle cells sparsely proliferated with an edematous stroma and formed cystic structures (Fig. 2C–H). The number of cystic structures in the tumors differed between the three cases: few structures were observed in Case 10, while many were observed in most areas of Case 12. In Case 11, two different morphological areas (areas a and b in Fig. 2E and F) were observed. In area a, spindle cells formed cystic structures, while in area b, spindle cells proliferated in interlacing fasciculi. All three cases were positive on immunohistochemical analysis for vimentin and S-100 (Table 2, Fig. 4). The reactivity for muscle markers was related to the presence of cystic structures. Areas without cystic structures in Cases 10 and 11 were positive for either desmin or α SMA, while areas with cystic structures in Cases 11 and 12 were only slightly positive or negative for these markers (Table 2, Fig. 4). All three cases were positive for ER α and negative for inhibin α , and Cases 10 and 11 were positive for CD10 (Table 2, Fig. 4). The SB type was also difficult to

diagnose as ESS; however, cases 10 and 11 could be diagnosed as ESS, as they were positive for ER α and positive for muscle markers which suggested the same muscular marker manifestation potential as the CR, PC, DA, and HM types. Case 12 was assumed to be also ESS based on the positive reaction for ER α , but a distinct diagnose could not be made.

Of note, comparative sections diagnosed as malignant schwannoma arising from the hepatodiaphragmatic nodule were positive for S-100 and negative for ER α .

Electron microscopy

Electron microscopic analysis was performed in three SB-type cases and one PC-type case. A basement membrane was detected in tumor cells of all four cases, and a desmosome-like structure was also observed in one of the SB-type cases (Table 2, Fig. 5).

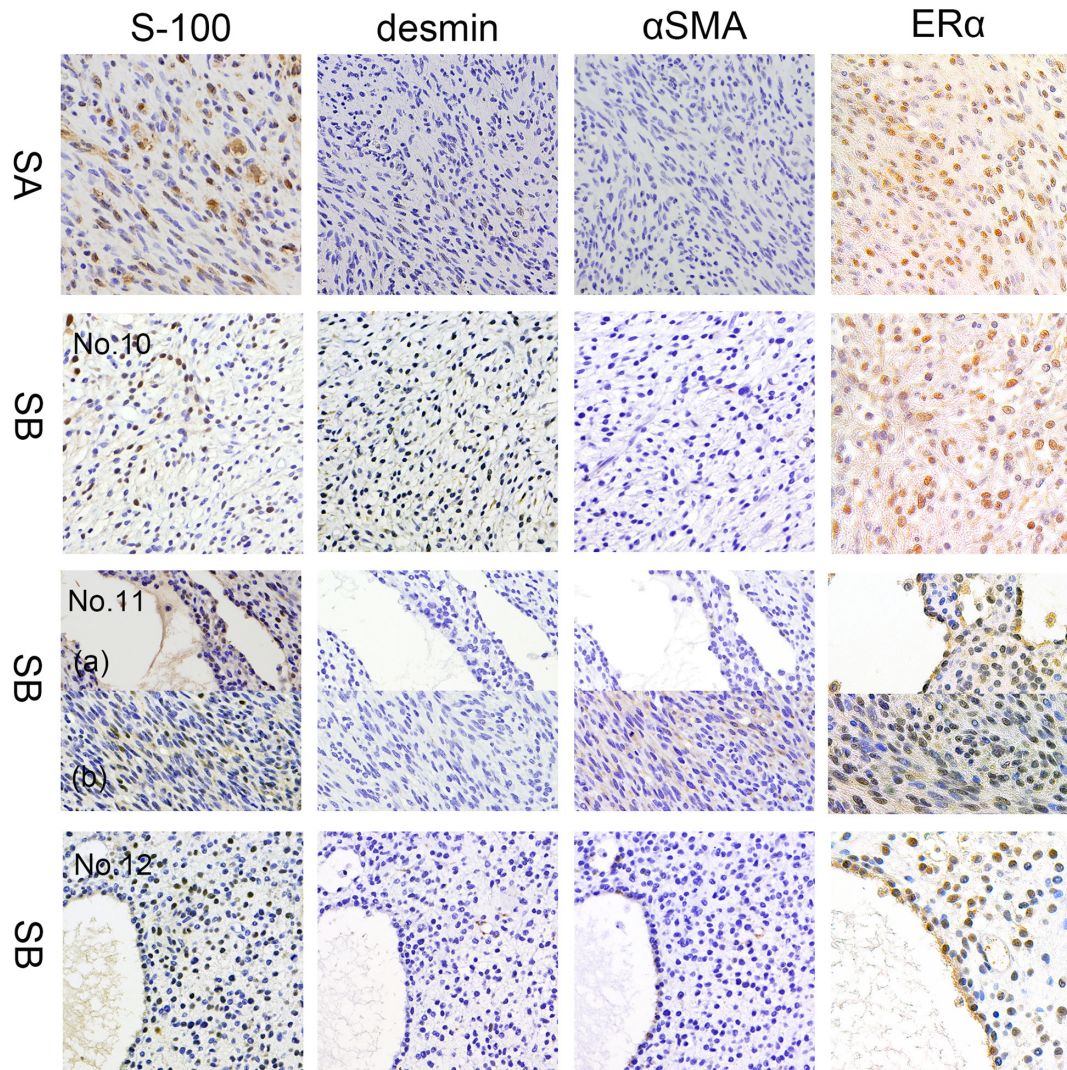


Fig. 4. Immunohistochemical images of cases from Figure 2. Top to bottom: SA type (Case 8) and SB type (Cases 10–12). Left to right: S-100, desmin, α SMA, and ER α . Magnification: 40 \times .

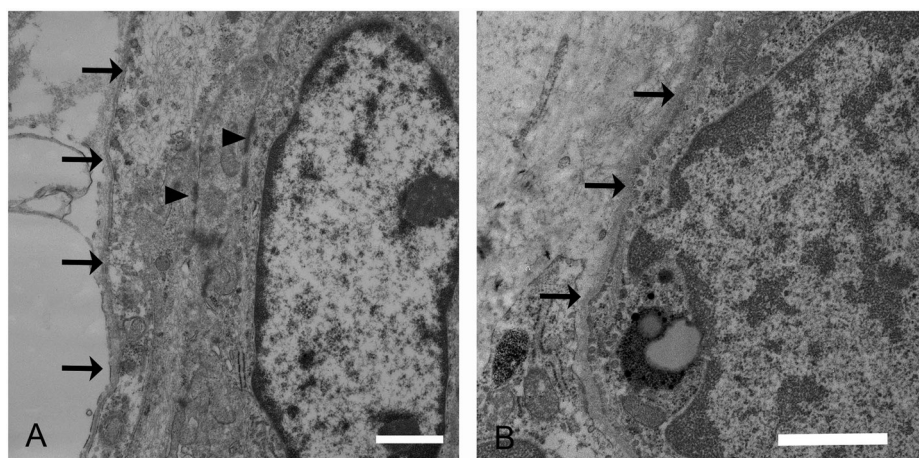


Fig. 5. ESS with basement membrane. (A) SA type, Case 10. (B) PC type, Case 3. Arrows = basement membrane; arrowheads = desmosome-like structure. Scale bar = 1 μ m.

Discussion

This study clarified the morphological and immunohistochemical features of rat ESS using human ESS markers. Our results demonstrated that most tumors protruding into the uterine lumen were derived from endometrial stromal cells. Additionally, the immunohistochemical results indicated that S-100 and ER α are effective immunohistochemical markers for identifying ESS in rats.

A number of reports have cited a positive reaction for S-100 in ESSs^{1, 2, 4, 5}. S-100 is not expressed on normal endometrial stromal cells but is expressed on decidual cells of the placenta in pregnant rats¹⁶. S-100 might therefore be useful for differential diagnosis from leiomyosarcoma. Further, ER α is widely distributed throughout the uterus of healthy rats¹⁷; given that most cases in the present study reacted positively for ER α , the origin of these tumors might be uterine components. In addition, ER α is not expressed on Schwann cells of the peripheral nerve of the rat uterus or malignant schwannomas occurring in other tissues in rats. S-100 and ER α might therefore be useful in differentiating ESS from leiomyosarcoma and malignant schwannoma, respectively.

Positive reaction to S-100 and presence of a basement membrane in electron microscopy have been accepted as useful markers for diagnosis of malignant schwannoma. However, based on the following findings the positive expression of S-100 in most ESSs and basement membrane surrounding tumor cells in several ESSs, indicated the both markers are not sufficient or useful to determine that tumor cells originated from schwann cells. A previous study reported that ESSs have the potential to produce a basement membrane and desmosome-like structures, because human endometrial stromal cells producing basement protein laminin around cells during the secretory phase¹⁸. Another also reported that stromal cells have the potential to produce a basement membrane and desmosome-like structures in rabbit decidosarcoma¹⁹. ESS is considered to have potential to form a basement membrane and desmosome-like structure due to endometrial stromal cells having the ability to differentiate to decidual cells.

The conventional criteria for immunohistochemical reaction in rat ESS state that muscle markers such as desmin and actin are negative in rat ESSs^{1, 2, 4, 5}. In human ESSs, a positive reaction for muscular markers in immunohistochemical reactions indicate that ESS tumor cells have differentiated to smooth muscle^{8, 10, 20}. In a previous report about endometrial stromal sarcoma emphasizing fibroblastic and smooth muscle differentiation, Yilmans et al. thought that the presence of even focal endometrial stromal differentiation in an invasive uterine mesenchymal lesion with a predominant low-grade smooth muscle, fibroblastic, and/or myxoid phenotype should permit classification as low-grade sarcoma and that the lesions should be considered endometrial stromal sarcomas¹¹. Similar results in almost cases in the present study, particularly in the area of spindle cells arranged in interlacing fasciculi resembling muscle tumors, suggest a variation of ESS with smooth muscle differentia-

tion. A positive reaction for muscle markers might not be definitive for crucially distinguishing ESS from leiomyosarcoma.

While CD10, an established marker of ESS in humans^{8–10} is expressed on human endometrial stromal cells, it is not expressed on normal rat endometrial stromal cells²¹. Although positive results for this marker in 5 of the 12 cases were obtained in the present study, CD10 is not considered to be an effective marker for diagnosing rat ESS.

We concluded that almost all cases of the six types of uterine malignant tumor protruding into the lumen observed in this study were ESS. While differentiation of ESS from other sarcomas can be difficult due to its varying morphological features, the generalization that tumors should be diagnosed based on their cellular origin suggests that most uterine sarcomas protruding into the lumen may be diagnosed as ESSs, regardless of the morphological features and degree of differentiation. However, in cases in which a tumor is similar to other sarcomas, immunohistochemical analysis may be necessary to obtain a correct diagnosis.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest.

Acknowledgments: We would like to thank Ms. Chie Shiroumaru, Ms. Masayo Onozawa, Ms. Misako Morino, Ms. Kiyoe Otobe, Ms. Miki Kudo, and Ms. Keika Kinoshita for their technical support.

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