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Alveolar soft part sarcoma

Assessment of immunohistochemical demonstration of desmin using paraffin sections and frozen sections

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Summary. The many different theories on the histogenesis of alveolar soft part sarcoma (ASPS) have caused great confusion. Owing to the recent rapid advance in immunohistochemical studies, two major hypotheses have been proposed. One group of researchers supports the idea that ASPS shows myogenic differentiation, while the other group opposes the idea. This confrontation is essentially one between a group that believes in the immunohistochemically demonstrated presence of desmin in ASPS and a group that denies it. In the present study we detected desmin in 6 of 10 formalin-fixed paraffin sections (although there were differences due to the use of five commercially available types of anti-desmin antibodies). When acetone-fixed paraffin sections and periodate-lysin-paraformaldehyde (PLP)-fixed frozen sections were used in one and three cases, respectively, they were found to be desmin positive, regardless of the type of antibody. The consistent positivity for all anti-desmin antibodies in the cases treated with acetone or PLP is very suggestive of a myogenous origin of ASPS. It is important to take into consideration the fact that formalin-fixed paraffin sections are not very suitable for immunohistochemical study of desmin.

Key words: Alveolar soft part sarcoma – Desmin

Introduction

There have been various hypotheses about the histological origin of alveolar soft part sarcoma (ASPS) in spite of its unique and characteristic histological findings. The tumor is famous for the fact that no definite opinion on its histological origin has been established. One hypothesis attributes the origin of ASPS to the paraganglia (Smetana and Scott 1951; Welsh et al. 1972), while others propose that ASPS is a malignant variant of granular cell myoblastoma (Christopherson et al. 1952; Fisher 1956), a neural neoplasm (Karnauchow and Magner 1963), a rhabdomyosarcoma (Fisher and Reidbord 1971; Marshall and Horn 1961), or a renin-producing tumour, "angioreninoma" (DeSchryver-Kecskemeti et al. 1982). With recent advances in immunohistochemistry, however, some of the grounds on which the above theories are based have been examined, and all of them have been criticized (Mukai et al. 1983). As a result of the discovery of a variety of specific proteins and the advance in their application to immunohistochemistry, various immunohistochemical studies of ASPS have been conducted by many investigators (Auerbach and Brooks 1987; Foschini et al. 1988; Denk et al. 1983; Miettinen 1988; Mukai et al. 1983; Mukai et al. 1986; Ogawa et al. 1986; Ordóñez et al. 1988a; Osborn et al. 1982; Persson et al. 1988). At present two main groups of researchers have formed concerning the histogenesis of ASPS: a group which supports the myogenic theory and a group which denies it. The confrontation between them on whether myogenic differentiation occurs in ASPS or not is essentially an antagonism as to whether desmin, an intermediate filament specific to muscle, is present in ASPS or not. Some reports support the immunohistochemical demonstration of desmin in ASPS (Denk et al. 1983; Mukai et al. 1986; Ogawa et al. 1986; Ordóñez et al. 1988a; Persson et al. 1988) and about an equal number deny the finding (Auerbach and Brooks 1987; Foschini et al. 1988; Miettinen 1988; Osborn et al. 1982) (Table 1). Close examination of these two groups of reports revealed that differ-

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Table 1. Review of immunohistochemical studies of desmin in ASPS

	Fixation	Embedding	Antibody	Staining method	Result
Denk et al. (1983)	Aceton	Frozen	Polyclonal	IF	+(1/1)
Mukai et al. (1986)	Formalin	Paraffin	Polyclonal	ABC	+(3/3)
Ogawa et al. (1986)	Formalin	Paraffin	Polyclonal	PAP, ABC	+(3/5)
Ordóñez et al. (1988)	Formalin, ethanol, Methacarn, B-5, Bouin's solution	Paraffin	Monoclonal	ABC	+(2/2)
Persson et al. (1988)	Formalin	Paraffin	Monoclonal	ABC	+(8/10)
Osborn et al. (1982)	Ethanol	Paraffin	Polyclonal	IF	−(0/1)
Auerbach et al. (1987)	Buffered formalin, Bouin's solution	Paraffin	Polyclonal Monoclonal	ABC	−(0/9)
Miettinen (1988)	Formalin	Paraffin	Monoclonal	ABC	−(0/1)
Foschini et al. (1988)	Buffered formalin	Paraffin	Polyclonal	BSA	−(0/2)

IF: indirect immunofluorescence
ABC: Avidin-biotin complex method
PAP: Peroxidase-antiperoxidase method
BSA: Biotin-streptavidin method

Table 2. Case reports

Case no.	Age	Sex	Location	Size (cm)	Treatment	Metastasis	Follow-up (years)	Survival
1	27	M	Lumbosacral region	5 × 8	Wide excision + chemotherapy	Lung	7	Dead
2	31	M	R. axilla	6 × 6	Enucleation + chemotherapy	Lung, bone	2	Dead
3	42	M	R. thigh	7 × 5	Wide excision + chemotherapy	–	2	Living
4	20	F	R. thigh	12 × 10	Wide excision + chemotherapy	Lung, bone	3	Dead
5	33	M	L. leg	4 × 6	Wide excision + chemotherapy	Lung, brain	6	Dead
6	10	M	Nuchal region	3 × 3	Enucleation + chemotherapy	–	6	Living
7	38	M	L. thigh	5 × 6	Wide excision + chemotherapy	–	1	Living
8	41	F	R. Knee	11 × 10	Enucleation + chemotherapy	Lung	4	Dead
9	22	F	Back	3 × 7	Wide excision + chemotherapy	Lung	1	Living
10	39	M	R. leg	4 × 4	Wide excision + chemotherapy	Lung, brain	2	Dead

ent antibodies – polyclonal and monoclonal – were used in individual studies, and that different methods of specimen fixation, embedding and immunostaining were used (Table 1).

In the present investigation, we attempted to demonstrate desmin by various immunostaining methods using various commercially available antibodies – polyclonal and monoclonal – in formalin-fixed and paraffin-embedded specimens from 10 cases of ASPS. Some of them were also used for a comparative study by simultaneous use of other methods for fixation and embedding.

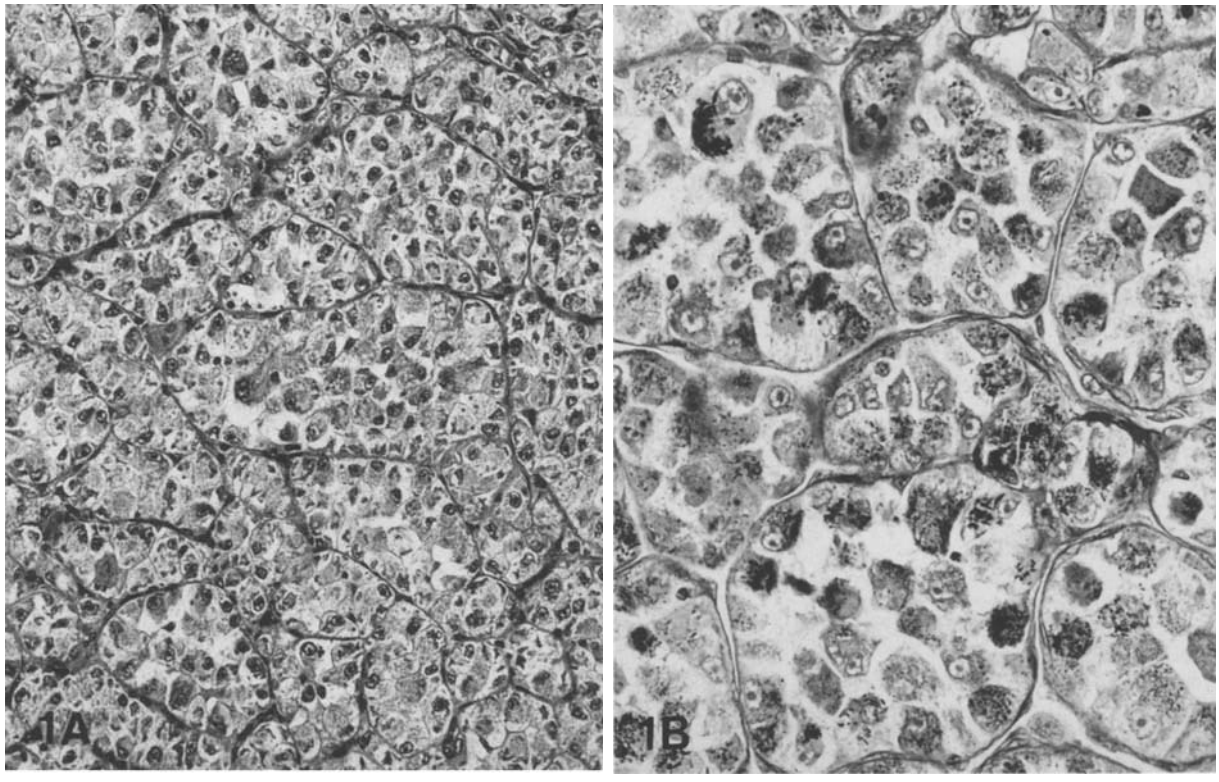
Materials and methods

Table 2 summarizes the 10 cases of ASPS. The surgical specimens were fixed in 10% formalin, and paraffin-embedded sections were prepared. They were stained with haematoxylin and eosin, and subjected to the periodic acid-Schiff (PAS) reaction with and without diastase pretreatment. Cases 1, 2 and 5, whose immunohistochemical data were reported previously, were positive for desmin (although the antibody used were not commercially available one) (Mukai et al. 1986).

The following commercially available antibodies used for desmin:

Rabbit polyclonal antibody to chicken gizzard desmin (1), DAKOPATTS, Denmark; 1:200 dilution), Rabbit polyclonal antibody to chicken gizzard desmin (2), BioGenex Kit, BioGenex Laboratories, California, U.S.A.; 1:1 dilution), Mouse monoclonal antibody to porcine stomach desmin (3), DAKOPATTS, Denmark; 1:30 dilution). Mouse monoclonal antibody to human leiomyoma desmin (4), BioGenex Kit, 1:1 dilution), Mouse monoclonal antibody to porcine stomach desmin (5), Amersham International Plc., Buckinghamshire, UK; 1:80 dilution).

Sections of formalin-fixed paraffin-embedded tissue blocks from 10 cases were available. From Case 7, sections from the blocks prepared by fixation with acetone at 4° C for 3 h and paraffin embedding of a part of the surgical specimens were also used. Fresh tissue samples were available from Cases 1, 2 and 5. They were fixed in periodate-lysin-paraformaladehyde (PLP) overnight at 4° C according to the method of McLean and Nakane (1974). Subsequently, the tissue samples were washed in graded sucrose solutions up to 20% in 0.01 M phosphate buffered saline and rapidly frozen in dry ice-alcohol.



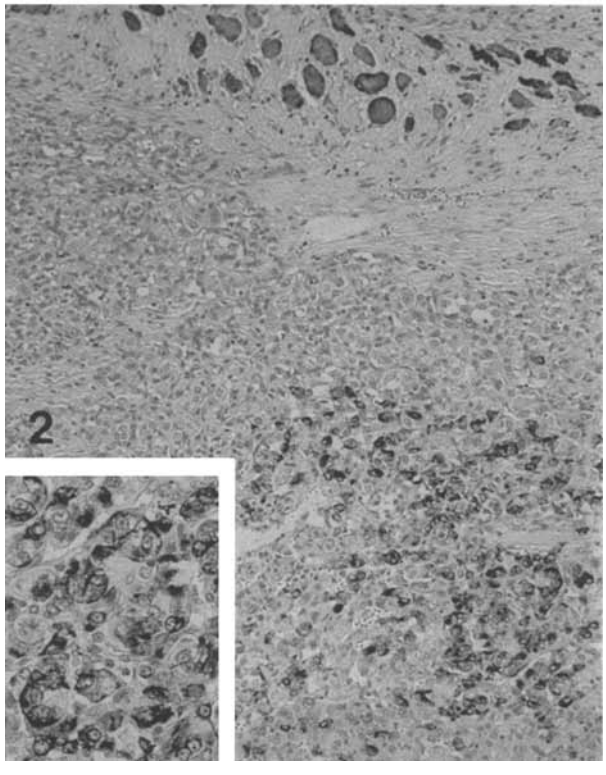


Fig. 1. (**A**) The tumour is composed of characteristic alveolar nests of round and polygonal cells with abundant eosinophilic cytoplasm (Case 9). (H-E, ×200). (**B**) PAS-positive, diastase-resistant cytoplasmic granules and rods are seen in many cells (Case 9). (PAS with diastase pretreatment, ×400)

Fig. 2. The findings obtained by staining by the ABC method in a formalin-fixed paraffin section from Case 8 when antibody 2) was used. Many positive cells were observed in the ASPS tissue, together with the peripheral skeletal muscle (*upper portion*). The positive finding was observed in the cytoplasm of tumour cells of ASPS (*Inset*). (Immunoperoxidase with haematoxylin, ×100, Inset: ×200)

Four-micron frozen sections were cut with a DAMON microtome.

The sections were immunostained with the above antibodies by the following two procedures. The avidin-biotin complex (ABC) method was performed essentially according to the method which we have conventionally used (Mukai et al. 1984a; Mukai et al. 1984b; Mukai et al. 1985; Mukai et al. 1986). For paraffin sections, sections which were incubated with 0.1% trypsin in phosphate buffer (pH 7.4) at room temperature for 30 min as pretreatment and sections without pretreatment were prepared. There was a difference in the efficacy of this pretreatment according to the type of antibody. The results will be described later. For paraffin sections, endogenous peroxidase was blocked by incubation of the slides in methanol/0.3% H_2O_2 solution for 30 min. The above treatment was not, of course, used for frozen sections, but the subsequent procedures were the same as those for the paraffin sections. After being washed, the slides were incubated with the primary antibodies at room temperature for 2 h. They were then exposed to a biotinylated antirabbit or antimouse immunoglobulin antiserum (dilution, 1:500), avidin (dilution, 1:1000), and biotinylated horseradish-peroxidase complex. The reagents were purchased from Vector Laboratories (Vectastain, Burlingame, CA, U.S.A.). We developed the peroxidase reaction by incubating the slides in 0.005% H_2O_2 and 0.02% 3,3′-diaminobenzidine tetrahydrochloride for 10 min.

In the Biotin-streptavidin (BSA) method the procedure up to development of the reaction with primary antibodies was the same as that described above. Subsequently, the reaction was developed by using a kit for the Biotin-streptavidin method (Kit Stravigen: BioGenex Laboratories). The procedure for the final peroxidase reaction is the same as that in the ABC method. In both ABC and BSA, normal rabbit or mouse serum was used as a control for the reaction instead of primary antibodies.

Results

All of the 10 cases presenting histological findings of typical alveolar soft part sarcoma showed characteristic diastase-resistant PAS-positive granules and rod structures (Fig. 1).

Immunohistochemical data are summarized in Table 3. Paraffin sections treated by formalin fixation are discussed first. There was a difference in the efficacy of the pretreatment with trypsin according to the type of antibody. Specimens not pretreated with trypsin showed good staining when antibody 1) was used; they showed good staining regardless of the presence or absence of the pretreatment when antibody 2) or 4) was used; and the specimens pretreated with trypsin showed good staining when antibody 3) or 5) was used. Table 3 shows the results obtained under the conditions in which good staining was observed when each antibody was used. Desmin was definitely detected in many tumour cells in 6 of the 10 cases (Fig. 2), but there was a slight difference in the location according to the type of antibody (Table 3). Even in the same case, both positive and negative findings were obtained according to the antibody used (Table 3) (Fig. 3). Although there was a slight difference in staining intensity between the ABC method and the BSA method, there were no significant differences due to a difference in procedure. The cases with positive findings included one with many positive tumour cells (Fig. 2), and one in which groups of positive cells were observed in an island-like pattern (Fig. 3A).

Table 3. Immunohistochemical data

Case no.	Polyclonal antibodies		Monoclonal antibodies		
	1	2	3	4	5
Formalin-fixed Paraffin sections					
1	−	+	−	+	−
2	+	+	+	+	−
3	−	−	−	−	−
4	−	+	−	+	−
5	−	−	−	−	−
6	−	+	−	+	−
7	+	+	−	+	−
8	+	+	+	+	−
9	−	−	−	−	−
10	−	−	−	−	−
Acetone-fixed Paraffin sections					
7	+	+	+	+	+
PLP-fixed frozen sections					
1	+	+	+	+	+
2	+	+	+	+	+
5	+	+	+	+	+

No desmin was detected in tumour cells of 4 of the 10 cases by any antibody, in spite of the fact that desmin was observed in the skeletal muscle and the vascular smooth muscle in the same section in these 4 cases. Case 5, which had previously shown positive reactions with the antibodies that were not commercially available, showed negative findings with the commercially-available antibodies in the present study.

For acetone-fixed paraffin sections (Case 7) and PLP-fixed frozen sections (Case 1, 2, 5), on the other hand, desmin was detected regardless of the type of antibody (Table 3) (Figs. 4 and 5). Particularly in Case 5, the PLP-fixed frozen sections were positive to all antibodies (Fig. 5), despite the fact that the formalin-fixed paraffin sections were negative to all of them.

Discussion

Desmin, a kind of intermediate filament, has become well known as a sensitive marker of myogenic differentiation, and many immunohistochemical studies of desmin have been performed with myogenic tumours (Denk et al. 1983; Gab-

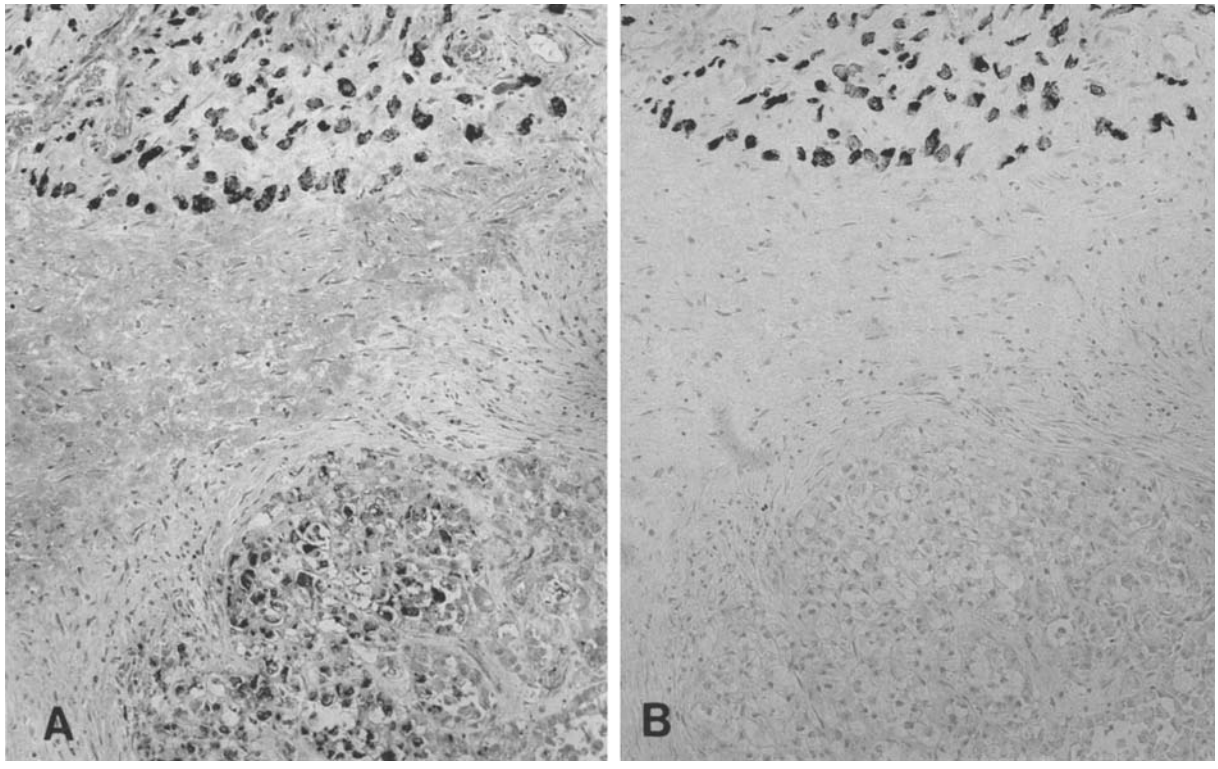


Fig. 3. The findings obtained by staining by the ABC method in formalin-fixed paraffin sections from Case 6. (**A**) A section in which antibody 4) was used. Groups of positive cells were observed in an island-like pattern in the tumour tissues, together with the peripheral skeletal muscle (*upper portion*). (**B**) A section in which antibody 1) was used. Desmin was observed in the skeletal muscle, but not in the tumour cells. (Immunoperoxidase with haematoxylin; A: ×100, B: ×100)

biani et al. 1981; Miettinen et al. 1982; Schürch et al. 1987).

A state of confusion remains concerning the histogenesis of ASPS, because there are considerable differences in the data from immunohistochemical studies of this sarcoma among the investigators. Some authors suggest myogenic differentiation of ASPS because of the presence of desmin (Denk et al. 1983; Mukai et al. 1986; Ogawa et al. 1986; Ordóñez et al. 1988a; Persson et al. 1988). Other studies have led to the conclusion that ASPS is negative for desmin (Auerbach and Brooks 1987; Foschini et al. 1988; Miettinen 1988; Osborn et al. 1982), although some authors (Foschini et al. 1988) support the concept of myogenic differentiation of ASPS because of the presence of striated muscle actin.

It should be kept in mind that formalin-fixed paraffin sections are not very suitable for immunohistochemical demonstration of desmin (Altmannsberger et al. 1981; Schürch et al. 1987). Desmin can often be demonstrated in formalin-fixed paraffin sections, but it is true that other methods of fixation and embedding are superior to this type of processing.

In our present study, desmin was detected in 6 cases even when formalin-fixed paraffin sections were used. However, there were distinct differences according to the antibody used. Case 5, which had shown positive findings with the antibodies which were not commercially available, showed negative findings with the commercially-available antibodies in the present study, suggesting that results vary with the antibodies used. When acetone-fixed paraffin sections or PLP-fixed frozen sections were used, all the specimens were positive regardless of the type of antibody.

In the present study, formalin-fixed paraffin sections of 4 cases were desmin negative. We interpret this finding as follows: in these cases, the antigenicity of the small amount of desmin in ASPS tumour cells was completely destroyed in the unsuitable processing-using formalin fixation and paraffin embedding.

A survey of the reports on the presence of desmin in ASPS which have appeared so far reveals that some investigators found positive cases even when formalin-fixed paraffin sections were used (Mukai et al. 1986; Ogawa et al. 1986; Ordóñez et al. 1988a; Persson et al. 1988) and others found

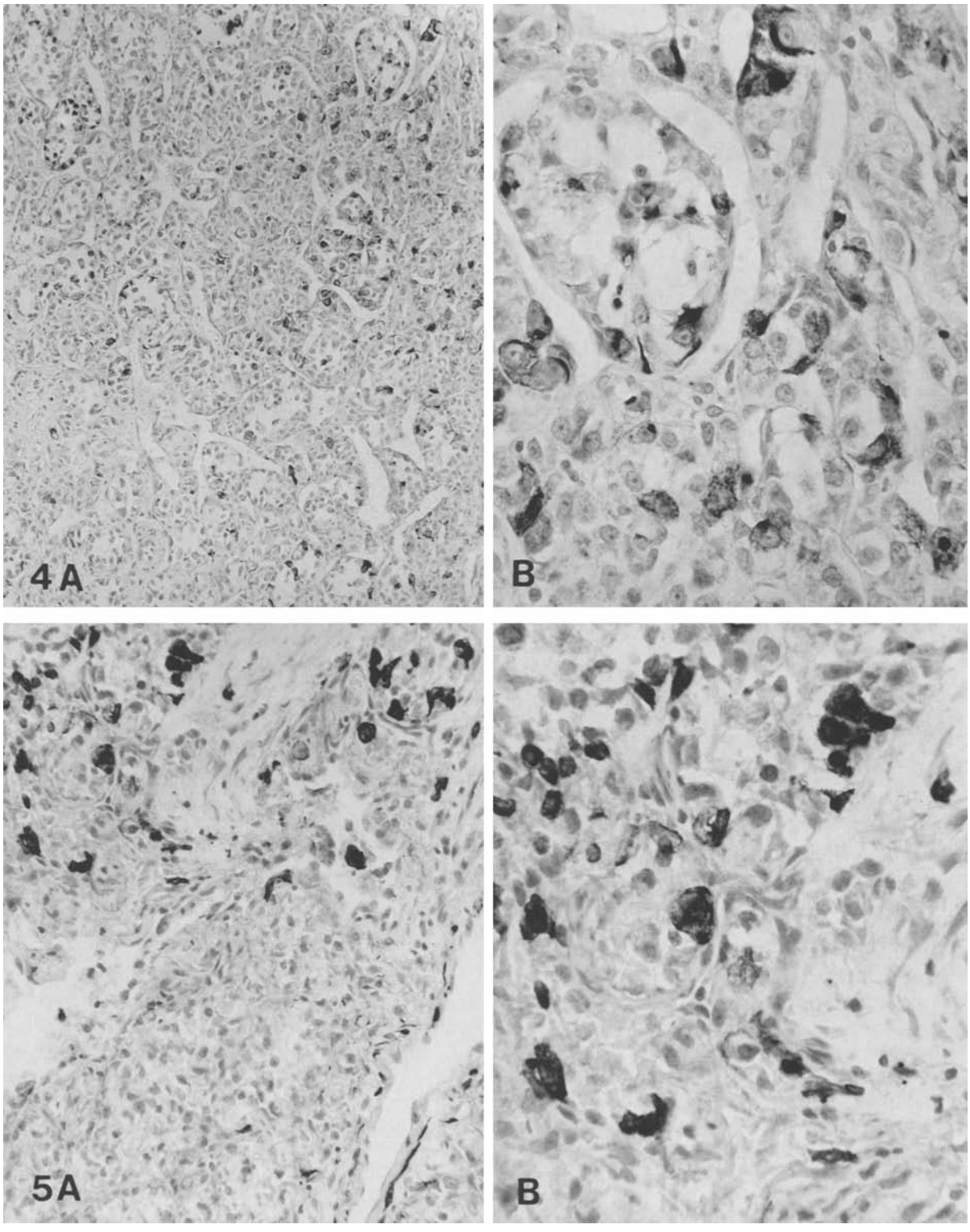


Fig. 4. The findings obtained by staining by the BSA method in an acetone-fixed paraffin section from Case 7 when antibody 1) was used. (**A**) Many positive tumour cells are present. (**B**) High-power view of (**A**). Desmin is definitely present in the cytoplasm of the tumour cells. (Immunoperoxidase with hematoxylin; A: ×100, B: ×400)

Fig. 5. The findings obtained by staining according to the BSA method in a PLP-fixed frozen section from Case 5 when antibody 5) was used.
(**A**) Groups of positive tumour cells are seen in an island-like pattern together and the vascular smooth muscle shows positive findings (*lower right*). (**B**) High-power view of (**A**). The cytoplasm of the tumour cells in the nests are definitely positive for desmin. (Immunoperoxidase with haematoxylin; A: ×200, B: ×400)

no positive cases (Auerbach and Brooks 1987). When frozen sections were used, one team of investigators found a positive case (Denk et al. 1983). Furthermore, there was a report showing that better results of immunostaining could be obtained with alcohol- or methacarn-fixed paraffin sections or alcohol-fixed cytological preparations than with formalin-fixed paraffin sections (Ordóñez et al. 1988).

All of our cases in which acetone-fixed paraffin sections and PLP-fixed frozen sections were used were positive regardless of the type of antibody used. Taking our data into consideration along with the reports by the above investigators, we believe the presence of desmin in ASPS is confirmed. Although there are still various opinions about the histogenesis of ASPS, the presence of desmin is very suggestive of a myogenous origin for this sarcoma.

In the future, a larger series of specimens treated more appropriately (by PLP treatment) will have to be studied to substantiate the expression of desmin in ASPS. Such studies should establish a broad consensus regarding the presence of desmin in ASPS and, in turn, the myogenic differentiation of ASPS.

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