

## A Histologic, Immunohistochemical and Ultrastructural Study of Fibroma, Myofibroblastoma, Leiomyoma and Hemangiopericytoma in Cattle

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### Abstract

The immunohistochemical features of tumors from 7 Holstein cows showing fibroblastic, myofibroblastic, smooth muscle or pericytic differentiation are described. In cases 1 and 2, the tumors were characterized by neoplastic fibroblasts immunostained with vimentin alone, but some cells appeared to transiently express alpha smooth muscle actin (SMA) during tumor development. Cutaneous (case 3) and nasal (case 4) myofibroblastomas, which were characterized by SMA and fibronectin positivity, with a minority of cells showing positive reactivity for desmin, were readily distinguishable from tumors of fibroblasts, smooth muscle cells and pericytes by fibronectin staining. The same staining pattern was seen in a vulvar myofibroblastoma (case 5), which differed from a vaginal leiomyoma (case 6) coexpressing SMA and desmin in nearly all neoplastic cells. Both neoplasms, however, had estrogen receptor (ER), and may be derived from immature stromal cells distributed in the vulvovaginal region. A hemangiopericytoma (case 7) resembled myofibroblastomas in cytoskeletal immunophenotypes, but lacked fibronectin. Benign or locally invasive soft tissue tumors with spindle cell morphology and expression of SMA could be divided into several types by immunostaining for SMA, desmin, fibronectin and ER.

**Discipline:** Animal health

**Additional key words:** alpha smooth muscle actin, desmin, estrogen receptor, fibronectin

### Introduction

In humans, soft tissue fibrous tumors are classified into various types, including some myofibroblastic tumors<sup>3</sup>. In contrast, they are divided into only few types in the veterinary literature, and the majority are diagnosed as fibroma or fibrosarcoma in cattle<sup>4</sup>. Tumors of myofibroblasts, however, have been reported in cattle, and had immunohistochemical and ultrastructural features intermediate between smooth muscle cells and fibroblasts<sup>1,7</sup>.

Hemangiopericytoma has been described in the inguinal regions of castrated male calves<sup>9,15</sup>. The tumor

was characterized by a perivascular whorled arrangement of tumor cells, and showed an immunolabeling pattern similar to that of myofibroblastic tumors<sup>9,15</sup>. In this paper, we describe the immunohistochemical features of bovine fibrous and myofibroblastic tumors. A leiomyoma and a hemangiopericytoma were also examined for comparison.

### Materials and methods

Seven benign or locally invasive tumors composed principally of spindle-shaped cells were studied retrospectively. The animals were all Holstein cows, and their clinical and macroscopic findings are presented in Table 1.

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**Table 1. Clinical data and gross pathology**

Case	Age (year)	Clinical findings and gross pathology	Diagnosis
1	5	Inability to stand. Euthanized. A multilobulated mass, 10 cm in diameter, on the mucosal surface of the rumen. A smaller mass in the adjacent mucosa.	Fibroma
2	10	Inability to stand. Euthanized. A sessile polyp, 1.5 cm in diameter, on the mucosal surface of the rumen.	Fibroma
3	5	A raised mass, 3 cm in diameter, on the abdominal skin. Enlarged to 15 cm diameter 2 months later. Surgically removed.	Cutaneous myofibroblastoma
4	7	One month after removal of a 5 cm-diameter mass in the left nasal cavity, a recurrent tumor mass was surgically removed. 25 × 10 × 8 cm in size. Grayish white and soft with widespread areas of hemorrhage.	Nasal myofibroblastoma
5	2	A mass in the perianal region had been surgically excised. A 5 cm-diameter vulvar mass was removed. Cauliflower-like in shape and very hard.	Vulvar myofibroblastoma
6	4	Vaginal tumor mass. Surgically removed. 9 × 6 × 5 cm in size. Grayish white and hard.	Vaginal leiomyoma
7	2	Multiple tumor nodules in the perianal region. Surgically removed.	Hemangiopericytoma

Tissue samples from the tumor masses were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (HE) and Gomori's trichrome stains. Some paraffin sections were selected and were immunostained with the avidin-biotin-peroxidase complex (ABC) method. The following were utilized as primary antibodies: mouse monoclonal antibodies to vimentin (Dako Corporation, Carpinteria, USA), alpha smooth muscle actin (SMA) (Dako A/S, Glostrup, Denmark) and desmin (Bio-Science Products, Emmenbrücke, Switzerland), and rabbit polyclonal antibody to fibronectin (NeoMarkers, Fremont, USA). Subsequent procedures were performed by an ABC kit (BioGenex Laboratories, San Ramon, USA). Prior to staining for fibronectin, the tissue sections were treated by microwave heating with citrate buffer (pH 6.0).

Small blocks taken from formalin-fixed neoplastic tissues were post-fixed with 1% osmium tetroxide, and embedded in epoxy resin. Ultra-thin sections were cut, stained with uranyl acetate and lead citrate, and examined with an electron microscope (EM).

## Results

### 1. Histology

In case 1, widespread areas of the tumor tissue were composed of short or long fusiform fibroblasts with slender nuclei which showed no mitotic activity. They were mostly small in size and were sparsely distributed in collagenous stroma. In parts, especially just beneath the epidermis, there were larger spindle- or stellate-shaped cells with oval or irregular nuclei. Mitoses were rarely seen in such cells. In case 2, the tumor tissue was composed of a

mixture of small and larger cells resembling those in case 1, with relatively delicate collagen fibers in the stroma (Fig. 1).

Neoplastic cells of varied size, which were present in the neoplastic tissue of case 3, were fusiform, triangular, stellate or spider-like in shape, with fibrous and often hyalinized stroma. The nuclei were oval to spindle-shaped, and binuclear or multinuclear profiles were detected (Fig. 2). In parts, perivascular tumor cell accumulations were detected. Mitotic figures were rare. Though similar cytologic features were detected in case 4, the stroma was edematous and occasionally hemorrhagic. In case 5, tumor cells were slender spindle-shaped or plump fibroblastoid, with elongated to oval nuclei (Fig. 3). A few mitoses were present. The stroma was fibrous and relatively abundant. In cases 3–5, a few isolated stress fibers demonstrable by Gomori's trichrome stain were present in occasional cells.

In the neoplastic tissue of case 6, there were abundant fibrous tissue and neoplastic cells, most of which were slender spindle-shaped with fusiform nuclei (Fig. 4). Some areas consisted of smaller cells and edematous mucoid tissue. Mitotic figures were found rarely. In the tumor tissue of case 7, slender spindle-shaped cells predominated in number, and in places, there were large fibroblastoid cells with large ovoid nuclei. Some neoplastic cells were arranged concentrically around blood vessels (Fig. 5). Small numbers of mitotic figures were present. The stroma was fibrous or myxoid. The cytoplasm of the neoplastic cells in cases 6 and 7 appeared to be evenly red with trichrome stain, while cells containing several myofibrils were occasionally seen in the former.

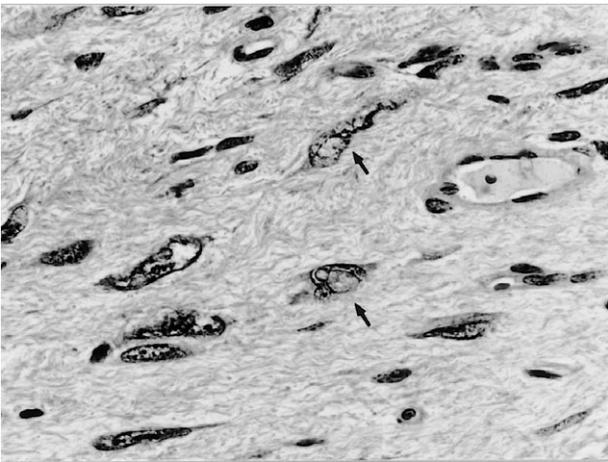


Fig. 1

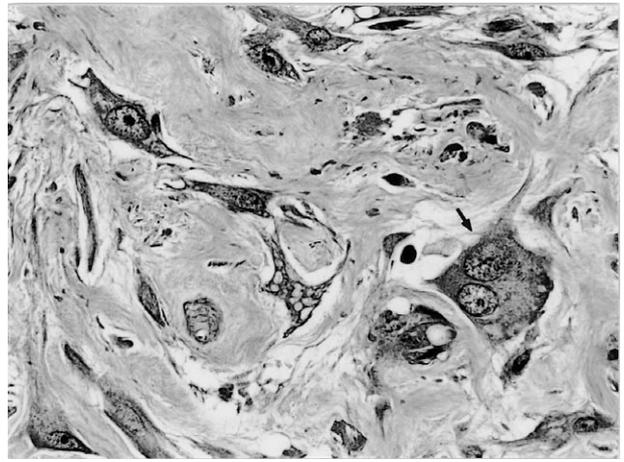


Fig. 2

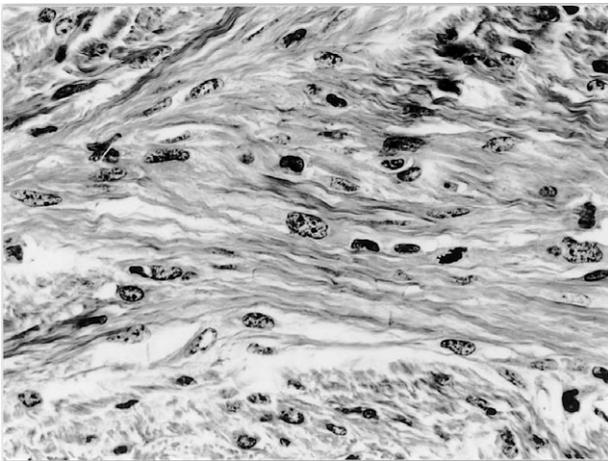


Fig. 3

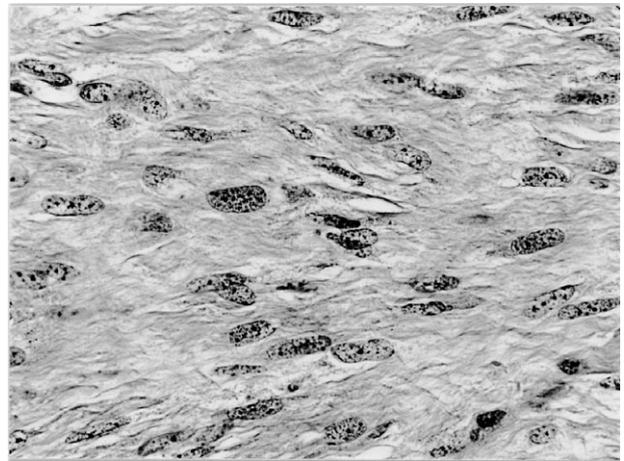


Fig. 4

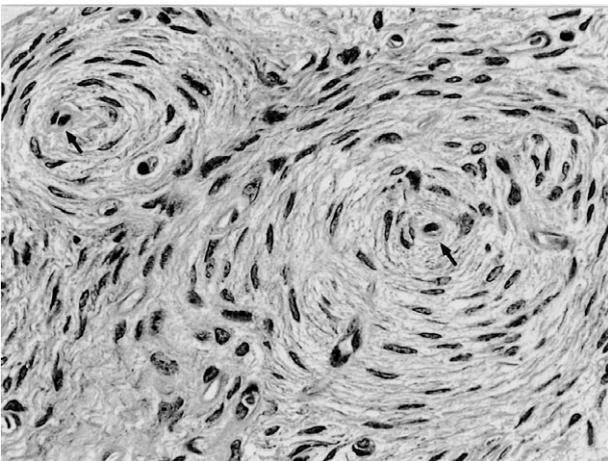


Fig. 5

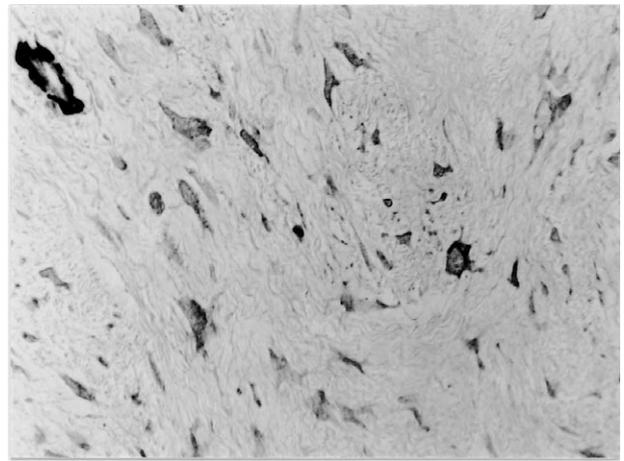


Fig. 6

- Fig. 1. Case 2: Larger cells with irregular nuclei (arrows) are visible in this field (HE  $\times 400$ )  
Fig. 2. Case 3: There are relatively large neoplastic cells, one of which has two nuclei (arrow) (HE  $\times 400$ )  
Fig. 3. Case 5: The tissue consisting of sparsely distributed neoplastic cells and bundles of collagen fibers gives the tumor the appearance of a fibroblastic tumor (HE  $\times 400$ )  
Fig. 4. Case 6: There is a considerable amount of connective tissue between tumor cells, whose nuclei have blunted ends (HE  $\times 400$ )  
Fig. 5. Case 7: Spindled cells are arranged around capillaries (arrows) in a concentric fashion (HE  $\times 400$ )  
Fig. 6. Case 1: Tumor cells displaying weak cytoplasmic reactivity for SMA are scattered in this area (ABC  $\times 400$ )

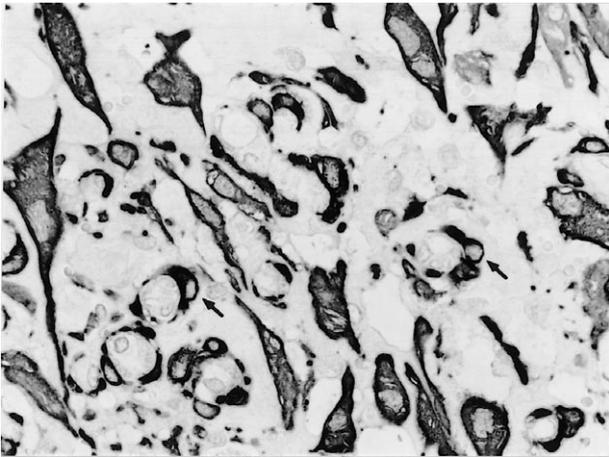


Fig. 7

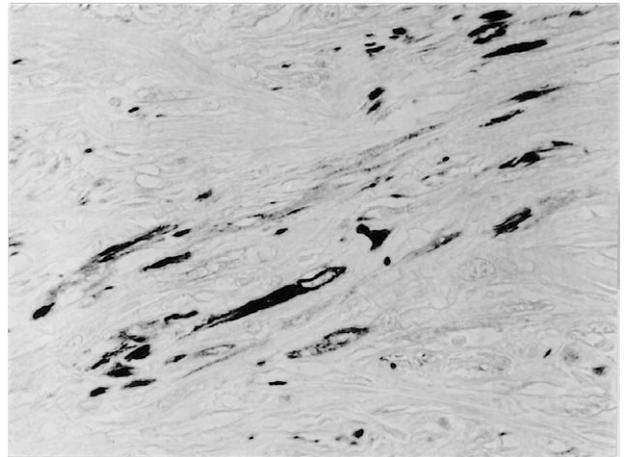


Fig. 8

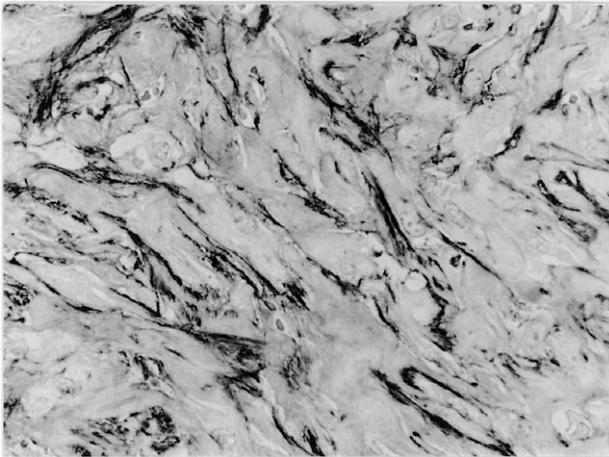


Fig. 9

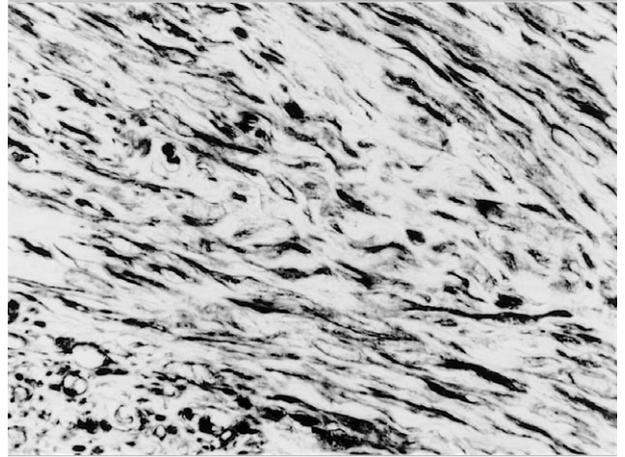


Fig. 10

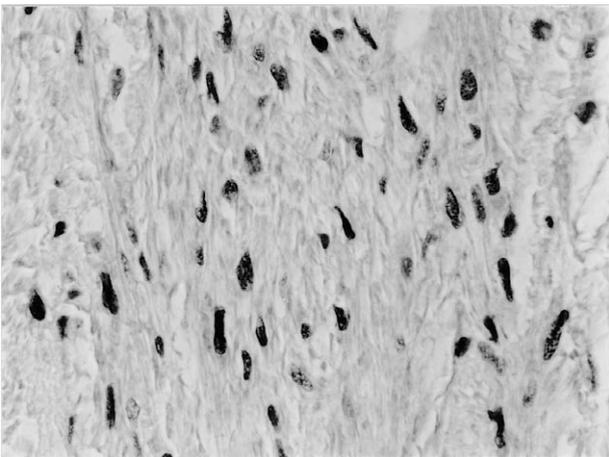


Fig. 11

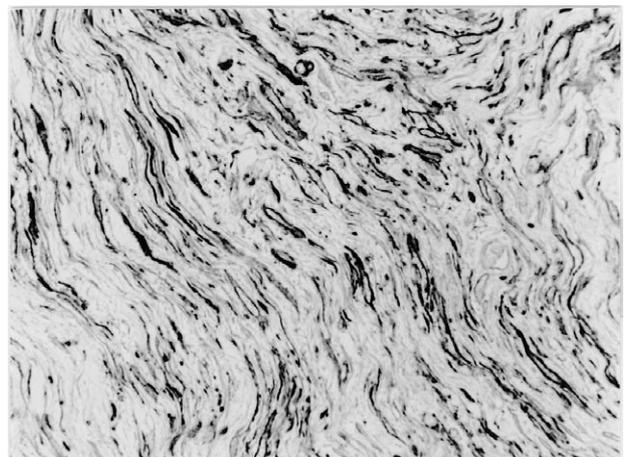


Fig. 12

**Fig. 7.** Case 4: Large tumor cells show moderate cytoplasmic reactivity for SMA, with more intense staining along the cell membrane, and smaller cells with diffuse strong cytoplasmic staining are vascular smooth muscle cells (arrows) (ABC  $\times 400$ )

**Fig. 8.** Case 5: Several desmin-positive neoplastic cells are visible in this area (ABC  $\times 400$ )

**Fig. 9.** Case 3: Positive reactivity for fibronectin is observed at tumor cell surfaces (ABC  $\times 400$ )

**Fig. 10.** Case 6: Almost all tumor cells are desmin positive (ABC  $\times 400$ )

**Fig. 11.** Case 5: Distinct nuclear positivity for ER is detected in great numbers of neoplastic cells (ABC  $\times 400$ )

**Fig. 12.** Case 7: Most slender spindled cells stain positively for SMA (ABC  $\times 400$ )

## 2. Immunohistochemistry

Immunohistochemical findings are shown in Table 2. In cases 1 and 2, weak SMA positivity was seen in larger neoplastic cells alone (Fig. 6). Myofibroblastomas (cases 3–5), in contrast, showed positive reactivity for SMA in almost all cells (Fig. 7), with positive staining for desmin in a minority (Fig. 8). Fibronectin was also present in these cases (Fig. 9). By such stainings, myofibroblastomas could be distinguished from a leiomyoma (Fig. 10), but the neoplasms arising from the vulvovaginal region (cases 5 and 6) showed positive staining for ER in many neoplastic cells (Fig. 11). In case 7, the tumor cells were similar in cytoskeletal immunophenotype to those in myofibroblastic tumors (Fig. 12), but fibronectin was absent.

## 3. Electron microscopy

The tumor tissue in case 5 was inadequately preserved for ultrastructural examination. The neoplastic cells in cases 1 and 2 had oval, spindle or irregular nuclei, with inconspicuous nucleoli and small quantities of marginated heterochromatin. There were small to modest amounts of rough endoplasmic reticulum (RER) in the cytoplasm, and accumulations of microfilaments were not recognized (Fig. 13-a). In cases 3 and 4, most tumor cells were spindle, and showed mild nuclear indentation and evenly dispersed chromatin. The nucleoli were inconspicuous or moderately prominent. In addition to moderately well developed RER, the cells possessed tracts of microfilaments bearing focal densities (Fig. 13-b). Slender spindle-shaped tumor cells predominated in case 6, and the elongated or elliptical nuclei were indented or partially notched, with little heterochromatin and small nucleoli. In some cells, the cytoplasm was occupied by many microfilaments with focal densities, and a few strands of RER were seen (Fig. 13-c). However, others contained fair amounts of RER and smaller numbers of microfilaments. In case 7, the tumor cells

were mostly spindle, frequently with branching cell processes. The nuclei were ovoid to fusiform with little heterochromatin and small to moderately prominent nucleoli. Like in myofibroblastic and smooth muscle cell tumors, areas of microfilaments were detectable, but focal densities were absent (Fig. 13-d). The RER was moderately well developed, and glycogen granules were seen in occasional cells. Collagen fibers closely attached to tumor cells were detected in all cases examined.

## Discussion

Since the myofibroblast is a precisely defined cell at the ultrastructural level, its definition at the light and immunohistochemical levels is decisively less precise<sup>14</sup>. Although there were many reports of benign myofibroblastomas in humans, the majority were not evaluated ultrastructurally<sup>14</sup>. The ultrastructural features of vulvar myofibroblastoma, uterine leiomyoma, soft tissue myofibroblastoma and hemangiopericytoma have been reported in cattle<sup>1,7,9,15</sup>. In all of the present cases except case 5, it was ascertained that the tumor cells had ultrastructures corresponding to those of fibroblasts, myofibroblasts, smooth muscle cells, or pericytes.

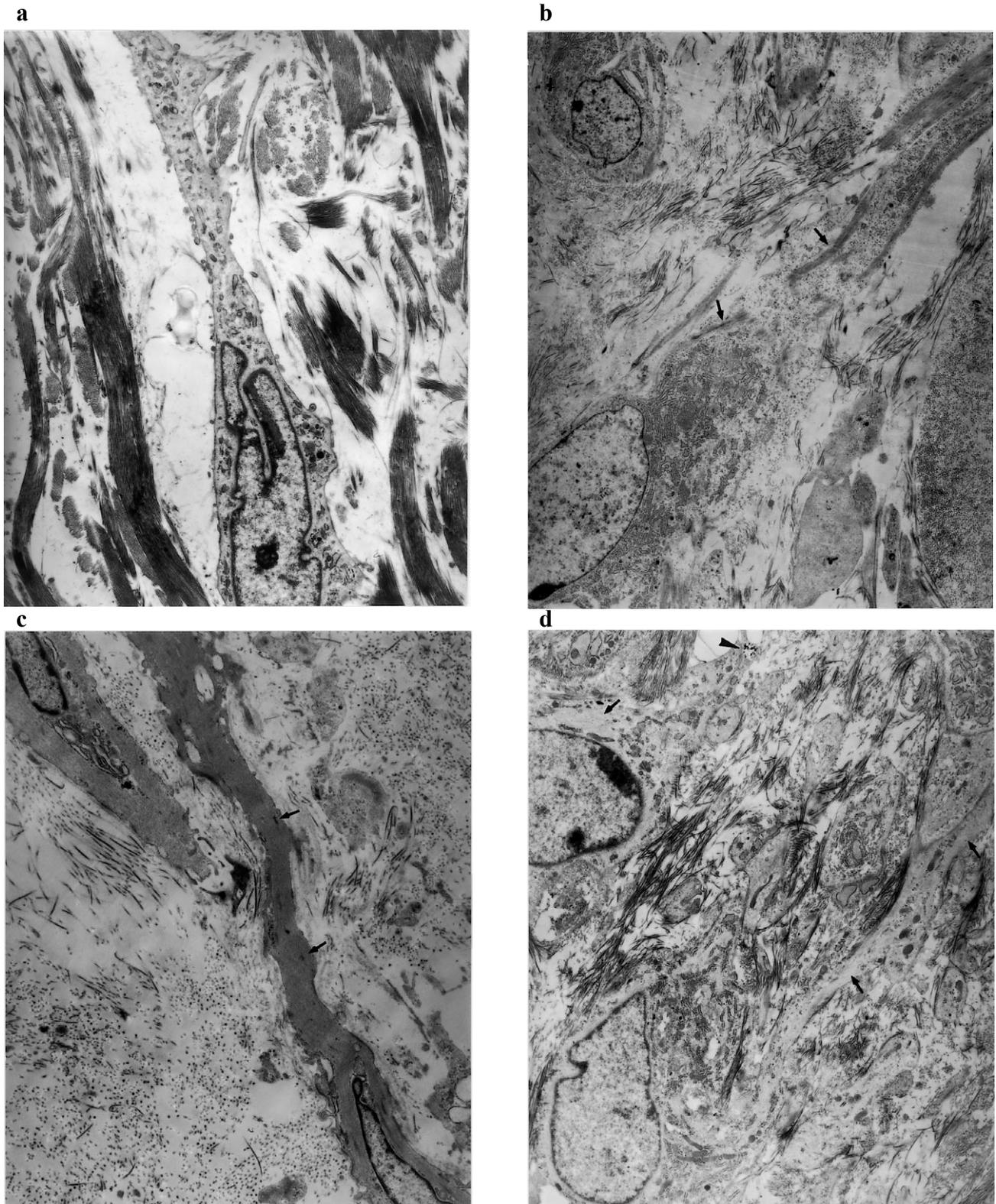
In case 1, most neoplastic cells were labeled positively for vimentin alone, and the tumor was diagnosed as fibroma. Larger and pleomorphic fibroblastoid cells showing weak SMA positivity in parts were considered to be immature cells and to be at the stage of proliferation. The lesion in case 2 showing a similar labeling pattern was thought to be at an early stage of tumor development. These findings indicate that the proliferating cells mature with conversion from a myofibroblastic phenotype to a fibroblastic/fibrocytic phenotype, as has been suggested by Valentine et al.<sup>16</sup> in equine desmoid tumors. A similar phenomenon can be observed in normal wound healing<sup>13</sup>.

In contrast to cases 1 and 2, positive reactivity for

**Table 2. Immunohistochemical findings**

Case No.	Vimentin	SMA	Desmin	Fibronectin	ER
1	++++	+	–	–	–
2	++++	+	–	–	–
3	++++	++++	+	+++	–
4	++++	++++	+	+++	–
5	++++	++++	+	+++	+++
6	++++	++++	++++	–	+++
7	++++	++++	–	–	+

–: negative, +: <10% of tumor cells staining, ++: 10–49% of tumor cells staining, +++: 50–90% of tumor cells staining, ++++: >90% of tumor cells staining.



**Fig. 13. Electron microscopy (EM) of tumor cells**

- a: Case 1; This fibroblastoid cell has an irregular nucleus, and organelles are sparsely and evenly distributed throughout the cytoplasm (EM  $\times 3,750$ ).
- b: Case 3; The neoplastic cell illustrated contains abundant RER in the perinuclear region (lower left), and bundles of microfilaments are visible (arrows) though focal densities are difficult to see at this power (EM  $\times 3,000$ ).
- c: Case 6; In the neoplastic cells illustrated, many microfilaments with associated focal densities (arrows) occupy the cytoplasm (EM  $\times 6,000$ ).
- d: Case 7; There are clear areas of microfilaments (arrows) in the cytoplasm of neoplastic cells, and glycogen granules (arrowhead) are seen (EM  $\times 3,750$ ).

both SMA and desmin, which is good evidence supporting that a neoplasm is of smooth muscle origin<sup>5,7</sup>, was detected in case 6. The other 4 tumors were SMA positive but desmin-positive cells were few or absent, and were thought to be myofibroblastic or pericytic tumors. Additionally, a few isolated stress fibers, which are not shown by smooth muscle cells<sup>14</sup> and pericytes, could be demonstrated in 3 cases of myofibroblastoma (cases 3–5).

Although human soft tissue myofibroblastomas are well-circumscribed and apparently benign tumors<sup>6</sup>, a bovine myofibroblastoma of the neck appeared locally invasive<sup>7</sup>. In cases 3 and 4, the tumors were large in size, and recurrence occurred in case 4. They grew in a manner similar to aggressive fibromatosis<sup>10</sup>, but consisted exclusively of myofibroblasts. Like a swine myofibroblastic sarcoma<sup>8</sup>, the tumors in cases 3 and 4 showed neoplastic cells accumulating around blood vessels, and may be closely associated with vascular smooth muscle cells or vascular leiomyoma. As stated above, fibrous tumors such as fibroma and desmoid fibromatosis are composed partially of myofibroblastoid cells, which are thought to appear temporarily<sup>14</sup>. The present 2 myofibroblastomas, consisting entirely of neoplastic myofibroblasts, are completely independent of such fibroblastic tumors.

Human angiomyofibroblastoma is an uncommon tumor which occurs principally in the vulvovaginal region of women in their reproductive years, and is a well-circumscribed lesion, usually less than 5 cm in diameter, composed of plump round, ovoid or spindle-shaped cells<sup>3</sup>. The neoplastic cells have a desmin-positive, actin-negative immunophenotype in the majority of cases<sup>3</sup>. The tumor cells in case 5, which were positive for SMA and predominantly negative for desmin, differed in cytoskeletal immunophenotype from the cells in human angiomyofibroblastoma, but estrogen receptors were detected in both neoplasms<sup>3</sup>. In a case of human angiomyofibroblastoma, the immunostains for desmin and SMA were negative, and the tumor cells were considered to be derived from primitive mesenchymal cells which occur normally in the vulva and which show the potential for diverse lines of myoid differentiation<sup>11</sup>. Taking into account this view, the neoplastic cells in cases 5 and 6 may have originated from stromal cells existing in the bovine genitalia, and may have shown myofibroblastic and smooth muscle differentiation, respectively. Thus, these two neoplasms seemed similar in histogenesis, but could be separated by immunohistochemistry for desmin and fibronectin<sup>2</sup>.

In humans, infantile myofibromatosis was thought to be a true pericytic tumor and was designated as

myopericytoma<sup>3</sup>, though it had been interpreted to be composed of myofibroblasts<sup>12</sup>. Bovine hemangiopericytoma was characterized by a perivascular whorled arrangement of tumor cells<sup>9</sup>, but it may be inconspicuous in some cases<sup>15</sup>, where distinction from fibrous, myofibroblastic or smooth muscle tumors is needed. Electron microscopy made it possible to discriminate between this tumor and myofibroblastic tumors<sup>9,15</sup>. In addition, the negative reactivity for fibronectin, which was recognized in case 7, may be helpful in finding out a pericytic tumor among tumors showing cytoskeletal immunophenotypes of myofibroblasts in cattle.

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