



NOTE

Pathology

Histological comparison of malignant epithelioid mesothelioma in young and adult cattle

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ABSTRACT. The histology and immunohistochemistry of pleomorphic and conventional epithelioid mesotheliomas were examined. The former was detected in two young calves aged 2 and 4 months and was characterized by pleomorphic and atypical cells with decreased expression of cytokeratin 7 (CK7). In contrast, the latter was found in a 31-month-old heifer, consisting of tumor cells uniform in size and shape with CK7 expression in nearly all cells. Production of collagen by tumor cells was demonstrated in both histological types, and was considered to be characteristic of bovine epithelioid mesothelioma. Pleomorphic mesothelioma is far more pleomorphic and mitotically active than conventional mesothelioma, and its normal counterpart may be immature mesothelial cells with high proliferation potential, which exist in fetal life and early calthood.

KEY WORDS: cattle, collagen production, cytokeratin 7, epithelioid mesothelioma, pleomorphic mesothelioma

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Human malignant mesothelioma is histologically characterized into three major types: epithelioid, biphasic, and sarcomatoid, the latter two being most common in children [1, 2]. As in most adult cases, the epithelioid type shows a papillary, tubuloglandular, or solid growth pattern in children and adolescents [3]. Pleomorphic and deciduoid mesotheliomas are rare cytological variants of epithelioid mesothelioma, and are accompanied with large epithelioid cells [19, 20]. The former occurs mainly in the pleura of aged men with a history of asbestos exposure [20]. The latter is detected in patients variable in age, and may affect children or adolescents with no asbestos exposure [13]. These variants are diagnosed based on cytomorphological characteristics [23]. Several immunohistochemical markers are available for the diagnosis of mesothelioma, but there are no specific markers [23].

In cattle, mesothelioma is more frequently observed in calves than in adults and is considered to be congenital in young animals [6]. As in humans, this neoplasm is characterized into three histological types [5, 7, 21]. In the epithelioid type, tumor cells in calves [22] are more pleomorphic than in adult cattle [6]. Coexpression of cytokeratin (CK) and vimentin was detected in a case of epithelioid mesothelioma [6], and epithelioid cells producing collagen have been observed in the biphasic type [7]. Desmin is used to distinguish between reactive mesothelial hyperplasia and malignant mesothelioma in human medicine, but is expressed in some malignant cases [8]. Here, we report pleomorphic mesotheliomas in two young calves and a conventional epithelioid mesothelioma in a heifer, both of which were characterized by collagen production by tumor cells. Unusual expression of CK7 and CK20 is discussed.

Case 1 was a 2-month-old Holstein male calf with a history of depression and diarrhea. On clinical examination, the calf exhibited abdominal distension, enophthalmos, yellowish-white diarrhea, and a rectal temperature of 40.4°C. Two days later, the animal developed astasia and exploratory laparotomy revealed the presence of multiple tumor nodules. The animal was euthanized because of poor prognosis. Case 2 was a 4-month-old Holstein female calf showing anorexia, severe abdominal distension, and yellowish-brown muddy stools. A defoaming agent was administered. The next day, exploratory laparotomy revealed massive ascitic fluid, adhesion of the stomachs, and multiple nodular lesions throughout the visceral and parietal peritoneum, which were partially removed for histological examination. The animal died within the day, and was necropsied two days later. Case 3 was a 31-month-old crossbred (Japanese Black × Holstein) heifer that appeared healthy just before slaughter.

In case 1, 30 liters of bloody ascitic fluid with fibrin clots were collected from the abdominal cavity, and many grayish white to

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reddish brown tumor nodules up to 1 cm in diameter were diffusely distributed on the ruminal serosa, splenic capsule, and parietal peritoneum. In case 2, many firm, whitish tumor nodules up to 5 cm in diameter were scattered over the peritoneum, and a few were on the pulmonary and pericardial pleura. Because of postmortem changes, no tissues were collected for histology. In case 3, many milky white tumor nodules or masses were observed on the costal pleura, and fewer numbers were on the diaphragmatic and pericardial pleura. These measured 0.5 to 10 cm in diameter, had irregular surfaces, and were hard on cut section. Similar nodules, 2 to 3 cm in diameter, were abundantly present on the caudal lobes and sparsely on the other lobes, without invasion to the lung parenchyma.

Neoplastic tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, processed for histopathology, and sections 4 µm in thickness were stained with hematoxylin and eosin (HE), trichrome of Masson for collagen, Alcian blue, pH 2.5 (AB), mucicarmine and periodic acid-Schiff (PAS) with or without diastase digestion. Immunohistochemistry was performed by the streptavidin-biotin complex/horseradish peroxidase (SAB) method on paraffin sections using a commercially available Histofine kit (Nichirei, Tokyo, Japan). Details of the primary antibodies used are presented in Table 1.

Histologically, in case 1, the neoplastic tissue was composed mainly of large or very large pleomorphic cells with well- or moderately-differentiated monomorphic cells admixed (Fig. 1A, 1B). Pleomorphic cells showed an irregular tubulopapillary, solid or diffuse growth pattern. In the cytoplasm, there were intracytoplasmic lumina, vacuoles, lipid droplets, or endoplasmic reticulum-like tubular structures. Collagen fibrils were sometimes present within the former two (Fig. 1C, 1D), and did not stain for AB and mucicarmine. Intracytoplasmic eosinophilic hyaline globules were rare. Well-developed connective tissues composed of myofibroblasts surrounded neoplastic tissues, and pleomorphic cells invading the connective tissues tended to show severe atypia. The well- and moderately-differentiated cells arranged in tubules or papillae were cuboidal and columnar in shape, respectively. In cuboidal cells, the cytoplasm was less abundant and eosinophilic than in pleomorphic cells. On the other hand, columnar cells frequently had abundant, vacuolated, and clear cytoplasm (Fig. 1A). PAS-positive granules were present mainly in this type of cells, and were digested with diastase. AB-positive material, which was flocculent, filamentous, granular or vesicular, was detected on the apical surface of some cuboidal or columnar tumor cells, and was rarely observed within intracytoplasmic lumina of these cells. Microvilli on apical surface were seen in all cell types, and some neoplastic cells had pale green cytoplasm in sections stained with trichrome. Connective tissue stroma was scarcely developed, but acellular homogeneous or slightly fibrous collagen deposits existed intercellularly (Fig. 1E), and stained negatively or faintly with AB. The histological characteristics in case 2 were similar to those in case 1 (Fig. 1F). However, pleomorphic cells showing a tubulopapillary growth pattern predominated, and the vast majority of more differentiated cells were columnar in shape. In cases 1 and 2, normal-appearing mesothelium exhibited continuous transition to monomorphic mesothelioma cells, which was also continuous with pleomorphic mesothelioma cells.

In case 3, the neoplastic cells were arranged in tubules, cords, or small clusters with abundant collagenous stroma, which was relatively poor in cellular components and stained negatively or very faintly with AB. Tubular structures, which predominated at the periphery, were continuous with normal appearing mesothelium, and were not invasive into underlying fatty tissue. Only a very small quantity of AB-positive material was present on the apical surface of tumor cells forming tubules. Papillary projections of tumor cells on the adluminal surface of monolayered tubules showed transition to inner tubules of double-layered ones, which appeared inverted and frequently contained an acellular homogeneous or faintly fibrous core of collagen (Fig. 1G, 1H). The cores were negative for AB and mucicarmine. Some larger cores or deposits, which were in contact with surrounding stroma through the basal portion of papillary projections, showed a shift to bundled collagen fibers (Fig. 1I). Cytologically, the neoplastic cells resembled well-differentiated cells of cuboidal shape in case 1. Intracytoplasmic lumina or vacuoles were seen in occasional cells, some of which contained either AB-positive material or collagen fibrils. A summary of cytological features in cases 1–3 is given in Table 2.

Immunohistochemical results of CKs and vimentin were almost the same in cases 1 and 2 (Table 3). Most neoplastic cells stained positive for CK18 and CK20 (Fig. 2A), and pan-CK in cases 1 and 2. CK20 expression was also observed in normal-appearing mesothelium. In case 1, CK7 was expressed chiefly in well-differentiated cuboidal cells, and only occasionally or rarely in the other cell types (Fig. 2B). In case 2, tubules or papillae of columnar cells were CK7 positive in some places (Fig. 2C), and pleomorphic cells expressing CK7 were rare. In cases 1 and 2, serial sectioning showed that vimentin was expressed in a small number of cells, with or without positive reactivity to CK18 and pan-CK (Fig. 2D–F). Many pleomorphic cells showed nuclear positivity for PCNA and Ki-67 with varying intensity in cases 1 and 2, and more differentiated cells were less frequently positive and

Table 1. Primary antibodies utilized for immunohistochemistry

Antibody	Clone	Dilution	Manufacturer	Antigen retrieval
CK7	Ks7.18	1:10	Acris Antibodies, Herford, Germany	Mh (pH6)
CK18	Ks18.04	Pd	Progen Biotechnik, Heidelberg, Germany	0.05% pepsin
CK20	SPM140	1:40	Santa Cruz Biotechnology, Dallas, TX, USA	Mh (pH6)
Pan-CK	MNF116	1:25	Dako Corporation, Carpinteria, CA, USA	0.05% pepsin
Vimentin	V9	Pd	Dako Corporation, Carpinteria, CA, USA	No treatment
SMA	1A4	1:50	Dako A/S, Glostrup, Denmark	No treatment
Desmin	D9	1:10	Bio-Science Products, Emmenbrücke, Switzerland	No treatment
PCNA	PC10	1:25	BioGenex, Carpinteria, CA, USA	Mh (pH6)
Ki-67	pAb	1:200	Abcam, Cambridge, UK	Mh (pH6)

CK, cytokeratin; SMA, α -smooth muscle actin; PCNA, proliferating cell nuclear antigen; pAb, rabbit polyclonal antibody; Pd, prediluted; Mh, microwave heating.

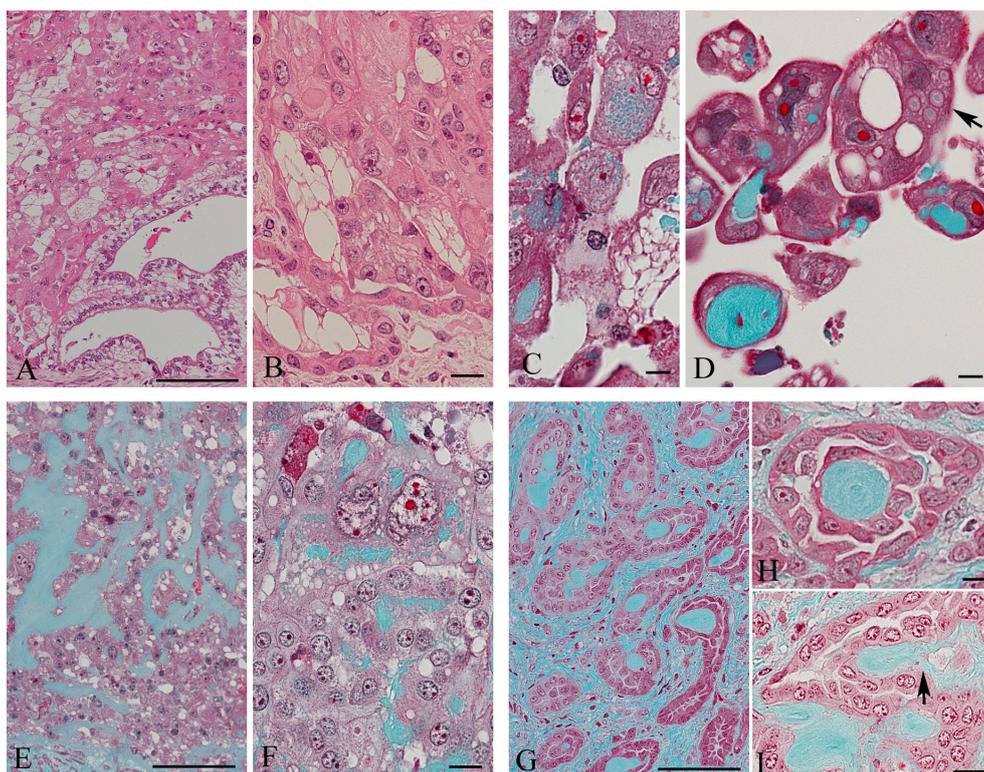


Fig. 1. Histology. (A) Case 1. Pleomorphic cells and more differentiated columnar cells (lower right) are visible. Hematoxylin and eosin (HE). Bar=100 μ m. (B) Case 1. Pleomorphic cells are different in nuclear and nucleolar size from well-differentiated neoplastic cells forming tubules (lower left). HE. Bar=10 μ m. (C) Case 1. Pleomorphic cells possess various amounts of intravacuolar collagen fibrils in the cytoplasm. Trichrome. Bar=5 μ m. (D) Case 1. A giant epithelioid cell has intracytoplasmic lumina. Some of them are enlarged, and others are lined by microvilli and contain collagen fibrils (arrow). Collagenous cores are also visible in other cells, and a larger one is fibrous and contains cytoplasmic fragments (lower left). Trichrome. Bar=5 μ m. (E) Case 1. Unlike in the connective tissue (lower right), collagen deposits in the neoplastic tissue are homogeneous and contain only a few blood vessels as cellular components. Trichrome. Bar=50 μ m. (F) Case 2. Neoplastic cells with intravacuolar collagen fibrils (top) are much larger in size than surrounding neoplastic cells. Trichrome. Bar=10 μ m. (G) Case 3. Acellular collagenous cores are relatively homogeneous and lie within neoplastic tubular structures. Trichrome. Bar=50 μ m. (H) Case 3. A collagenous core, which appears faintly concentric, is encircled by double layered tubules. Trichrome. Bar=5 μ m. (I) Case 3. A collagenous core is continuous with oriented connective tissue stroma at the base of a papillary projection (arrow). Trichrome. Bar=10 μ m.

Table 2. Cytological features of mesothelioma cells

	Case 1			Case 2		Case 3
	Pleo	Col	Cub	Pleo	Col	
Nuclei	One or multiple, round, oval or irregular	Round or oval	Round or oval	One or multiple, round, oval or irregular	Round or oval	Round or oval
Nucleoli	Variably sized	Inconspicuous	Inconspicuous	Variably sized	Inconspicuous	Inconspicuous
Chromatin	Slightly condensed	Moderately condensed	Moderately condensed	Slightly condensed	Moderately condensed	Moderately condensed
Cytoplasm	Abundant and eosinophilic	Abundant, vacuolated and clear	Less abundant and eosinophilic	Abundant and eosinophilic	Abundant, vacuolated and clear	Less abundant and eosinophilic
Production of collagen	Prominent	Not prominent	Not prominent	Prominent	Not prominent	Prominent
Mitosis	Occasional	Rare	Rare	Occasional	Rare	Rare

Pleo, pleomorphic cells; Col, columnar cells; Cub, cuboidal cells.

tended to show weaker staining. Myofibroblasts in surrounding connective tissues were SMA and desmin positive in case 1, whereas SMA-positive, desmin-negative slender stromal myofibroblasts were detected at the periphery of the neoplastic tissue in case 2. In case 3, most neoplastic cells were positive for the CK markers examined (Fig. 2G, Table 3), and also for PCNA and Ki-67.

Mesothelioma is classified roughly into epithelioid, biphasic, and sarcomatous histological types. The present three cases were included in the first type because of the presence of papillary, tubular, or solid growth pattern, but there were cytological differences between the adult and calf cases. In case 3, the epithelial neoplastic cells were relatively uniform in size and shape

(monomorphic), resembling those in a peritoneal epithelioid mesothelioma in a 14-year-old cow [6], and in biphasic mesotheliomas in cows aged 4 and 6 years [7]. In contrast, as in the previously reported cases of calf mesothelioma of the peritoneal cavity origin [22], the tumor cells in cases 1 and 2 showed pleomorphism and atypia. In humans, pleomorphic and deciduoid mesotheliomas have rarely been reported. The former is characterized by multinuclear giant tumor cells [20], whereas the latter is composed of large, polygonal or ovoid tumor cells, with tubulopapillary areas in some cases [19]. Since the tumor cells in cases 1 and 2 were more pleomorphic than in these human cases, a diagnosis of pleomorphic mesothelioma was made.

Epithelial cells in malignant epithelioid or biphasic mesothelioma of adult cattle is monomorphic with rare mitoses, and lymph node metastasis is observed in some cases [6, 7]. Although no metastasis was observed in case 3, the view that this case is malignant was supported by widespread distribution of multiple tumors and positivity for markers of proliferation in most tumor cells. It is probable that the body cavity is occupied by large tumor masses at later stages of tumor development in adult mesotheliomas with multiple lesions [6, 7].

In general, collagen production is characteristic of sarcomatous mesothelioma cells, but it is not infrequent to see collagen cores or balls encircled by tumor cells within the body cavity fluid in human malignant mesothelioma [4, 9]. The presence of cross-striated microfibrils within collagenous cores has been ultrastructurally observed in calf or congenital mesotheliomas (unpublished data) and in epithelioid cells in bovine biphasic mesotheliomas [7]. In the current cases, similar matrix stained green with trichrome but negative with AB stain was judged to be collagenous in nature [4, 9]. The presence of collagen within intracytoplasmic lumina or vacuoles (called intracellular collagen) [5, 10] and the presence of acellular collagen deposits or cores encircled by tumor cells were thought to be indisputable evidence of collagen synthesis by tumor cells. Considering the fact that mesothelial cells can show transition to or from submesothelial cells [14] and their malignant counterparts may have similar ultrastructural features in cattle [7], it is quite likely that collagen production is observed in bovine epithelioid mesothelioma. Malignant epithelioid mesothelioma needs to be distinguished from adenocarcinomas, and the production of collagen by epithelioid mesothelioma cells was considered to be helpful for the differential diagnosis in cattle. In the previous [7] and current cases, collagen cores or deposits were homogeneous or faintly fibrous, and collagen fibrils may be less apt to become tightly bundled collagen fibers, owing to the lack of tension or compression [15]. This view is supported by the following findings: homogeneous collagen fibrils appeared to have become bundled after contact with firm fibrous stroma in case 3, whereas tumor cells appeared floating in homogeneous collagen matrix in cases 1 and 2.

As in human malignant epithelioid mesothelioma [12, 16], pan-CK and CK18 were expressed in the cases described here. Positive CK7 immunostaining was observed in well- or moderately-differentiated cells, but seldom in pleomorphic cells. The loss of this marker, which is presumably associated with histological transformation to highly malignant or dedifferentiated cells, was considered to be characteristic of calf mesothelioma, and to be available for distinguishing from adult epithelioid mesothelioma. In humans, however, positive CK7 staining is detected in pleomorphic and deciduoid mesotheliomas as well as in classic epithelioid mesothelioma [19, 20], and

Table 3. Immunohistochemical results

Antibody	Case 1			Case 2		Case 3
	Pleo	Col	Cub	Pleo	Col	
CK7	+	+	+++	+	++	+++
CK18	+++	+++	+++	+++	+++	+++
CK20	+++	+++	+++	+++	++	+++
Pan-CK	+++	+++	+++	+++	+++	+++
Vimentin	+	+	+	+	+	+
Desmin	+	+	+	+	+	+

CK, cytokeratin; Pleo, pleomorphic cells; Col, columnar cells; Cub, cuboidal cells. +++=mostly positive; ++=occasionally positive; +=rarely positive.

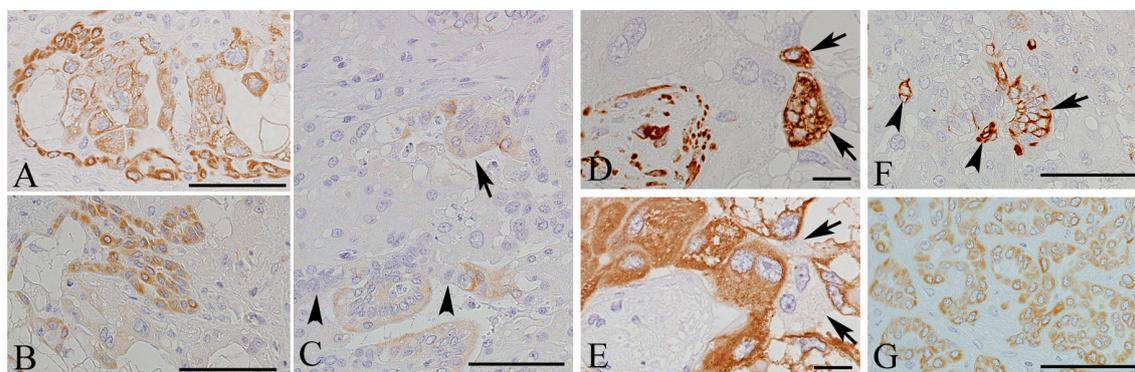


Fig. 2. Immunohistochemistry. (A) Case 1. Both well-differentiated cuboidal cells and pleomorphic cells show positivity for cytokeratin 20 (CK20), with the former staining more intensely in this field. Streptavidin-biotin complex/horseradish peroxidase (SAB) method. Bar=50 µm. (B) Case 1. Well-differentiated cuboidal cells show distinct positivity for CK7, whereas pleomorphic cells are faintly positive or negative. SAB. Bar=5 µm. (C) Case 2. Papillae or tubules of columnar cells are positive (arrow) or negative (arrowheads) for CK7. (D) Case 1. Arrows indicate vimentin-positive pleomorphic cells, whereas other pleomorphic cells are negative. Connective tissue stromal cells are also positive for this marker (lower left). SAB. Bar=10 µm. (E) Case 1. Adjacent section shows the same cells as those depicted in D. Contrary to the vimentin staining, only pleomorphic cells indicated by arrows and stromal cells (lower left) are CK18 negative. SAB. Bar=10 µm. (F) Case 2. Some of moderately differentiated columnar cells show positivity for vimentin (arrow). Smaller positive cells are blood capillary cells (arrowheads). (G) Case 3. CK7 is expressed in well-differentiated epithelioid cells. SAB. Bar=50 µm.

these mesotheliomas are less pleomorphic than calf mesothelioma. Conversely, CK20 was expressed in all of the present cases, but not in human malignant mesotheliomas [24]. Obvious differences in CK18 and CK19 expression have been observed between bovine and human hepatic carcinomas [11], and species differences should be taken into account in the diagnosis of bovine neoplasms, based on CK immunohistochemistry.

In mice, the proliferation potential of hepatic mesothelial cells is high in the fetus and declines after birth. In addition, fetal mesothelial cells express far more various growth factors than differentiated mesothelial cells [18]. Taking into account the rapid development of the rumen and reticulum after suckling and concomitant proliferation of the surrounding visceral and parietal peritoneum, immature mesothelial cells with high proliferation potential presumably still exist in young calves, and may be the normal counterpart of mesothelioma cells in cases 1 and 2. In humans, in contrast, pleomorphic mesothelioma is an extremely rare tumor occurring mainly in the pleura of aged men with a history of asbestos exposure or smoking [20]. In general, human mesothelial cells undergoing a series of chronic injury, inflammation, and proliferation show malignant transformation after a long latency period [17]. These facts imply that the pathogenesis of pleomorphic mesothelioma is different in humans and calf.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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