An Ultrastructural Study of Malignant Mesotheliomas in Two Cows

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(Received 25 August 1988/Accepted 10 November 1988)

ABSTRACT. Malignant mesotheliomas of two Holstein cows were examined by light and electron microscopy. These tumours were derived from peritoneal surfaces and were of biphasic type composed of mesothelial lining cells and submesothelial mesenchymal cells. Ultrastructurally, the neoplastic mesothelial cells were characterized by intermediate filaments, microvilli, desmosomes, tight junctions and basal laminae. The neoplastic submesothelial cells were closely associated with collagen filaments and some cells had several resemblance to the neoplastic mesothelial cells. The ultrastructural features of the mesotheliomas are discussed in comparison with those of adenocarcinomas and fibroblastic neoplasms. In one case a malignant granulosa cell tumour involved the left ovary and its morphology was apparently different from that of the mesothelioma. Multiple primary tumours are uncommon in cattle.—KEY WORDS: cow, granulosa cell tumour, mesothelioma, multiple primary tumours, ultrastructure.


Mesothelioma is a rare mesodermal neoplasm that arises from mesothelial cells and supporting tissues covering the pleural, pericardial and peritoneal cavities. Both mesothelial cells and connective tissue elements proliferate together, though in varying degrees, to form these neoplasms [17].

The ultrastructure of mesothelioma has been investigated in man [5], dogs [7, 24] and rabbit [15] and the association between malignant mesothelioma and asbestos exposure has been reported in man [2] and dogs [7]. Mesothelioma is found most commonly as a congenital tumour in calves or young adult cattle [1]. This tumour, however, occurs also in adult cattle [18] and may have relation to asbestos [3, 20].

Multiple primaries have been defined as multiple tumours of different kinds in the same or different organ systems [22]. Although many multiple primary tumours have been described in domestic animals, they occurred most frequently in dogs [4] and rarely in goats [14] and cattle [19].

The purposes of this study is to elucidate the ultrastructural features of bovine mesothelioma and to describe multiple primary malignancies (mesothelioma and granulosa cell tumour) in one case.

MATERIALS AND METHODS

Animals. Case 1 was a 6-year-old Holstein cow and Case 2 was a 4-year-old Holstein cow. They were brought to abattoirs in Saitama Prefecture as normal animals.

Microscopical examination. Tissues were fixed in 10% buffered formalin and processed in the usual way for paraffin embedding. Sections were stained with periodic acid-Schiff (PAS), Alcian blue and Azan by standard methods. Formalin-fixed tissues were also prepared for electron microscopy by rinsing in phosphate buffer, post-fixing in osmium tetroxide and embedding in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEM-100CX electron microscope. Similar treatment for electron mic-
Fig. 1. Case 1. Many cystic or solid nodules with elongated peduncles are suspended from the abdominal wall like icicles.

Light microscopy was applied to the specimens of the pleura and peritoneum from a normal cow as a control.

RESULTS

**Macroscopic findings.** In Case 1, multiple, one to 10 cm diameter, white or reddish-white, cystic or solid nodules which had fibrous peduncles up to 25 cm in length were present throughout the peritoneum (Fig. 1). The cysts contained clear serous fluid, colourless, reddish-brown or yellowish-brown in colour. The uterus revealed caseous necrosis and atrophy presumably owing to torsion and its wall became thin. Watery or gelatinous substances were seen in the lumen. The ovaries were atrophic.

In Case 2, the left ovary was enlarged to approximately 20 cm in diameter and its surface was reddish-white and coarsely nodular. On the cut surface, the mass had several cysts, ranging from 3 to 6 cm in diameter. The cysts contained reddish-yellow, lucent, serous fluid and their walls were fibrous and hard with haemorrhage and necrosis. The right ovary showed a normal appearance. There were many homogeneous, fleshy, cream-coloured nodules, 0.5 to 1.5 cm in diameter, throughout the peritoneum and on the parietal and diaphragmatic pleura. A few nodules were observed on the visceral pleura.

**Light microscopy.** In Case 1, the neoplastic tissues protruded from the serous membrane and contained complicated lumina (Fig. 2). Most of the lumina looked like clefts, but tubular or cystic structures were also present. These lumina were lined by a single layer of flattened mesothelial cells, which were also present on the surfaces of the neoplastic nodules. In some lumina there were free-floating mesothelial cells and bodies composed of central collagen fibre cores and a few surrounding mesothelial cells. The neoplastic cells frequently were cuboidal and at times showed papillary or tubulopapillary proliferations. The underlying tissues were made up of abundant collagen fibres and scattering spindle-shaped cells. The boundary lines between the neoplastic tissues and surrounding nor-
mal serosa are not well-defined. Rough surfaced serosa or cuboidal mesothelial cells were not infrequent even in the normal sites.

The flattened mesothelial cells possessed ovoid to fusiform nuclei and inappositive nucleoli and their cytoplasm was fairly sparse. The cuboidal epithelial cells were relatively large with round to ovoid nuclei and sometimes had abundant cytoplasm. In both types of epithelial cells irregular nuclear outlines were frequently seen and mitotic figures were rare. The submesothelial cells were fibroblastoid with ovoid to spindle-shaped nuclei, small nucleoli and scanty cytoplasm. The neoplastic tissues stained negatively with PAS and Alcian blue.

Although the characteristics of the mesotheliomas were similar in Case 1 and Case 2, some differences were present. In the neoplastic tissues of Case 2, papillary and tubular structures were well developed and cuboidal or columnar mesothelial cells with abundant cytoplasm predominated (Fig. 3). There were loose collagen fibres and spindle-shaped cells in the supporting connective tissues and there were sometimes ovoid or cuboidal cells. Metastasis was found in the subcapsular and trabecular sinuses of a lateral iliac lymph node and consisted of various sized bodies. These bodies had a central core of collagen fibres which was surrounded by cuboidal or flat epithelioid cells of varied number (Fig. 4). Most of the bodies contained no fibroblastoid cells in the cores, but some bodies had a few cells.

The left ovary was completely replaced by the neoplastic growths showing insular and follicular patterns (Figs 5, 6). In the insular pattern, the neoplastic cells formed clusters separated by connective tissue septa and small cavities were often seen among the neoplastic cells. Microfollicular pattern with Call-Exner bodies was rare. A large cavity surrounded by stratified neoplastic cells was sometimes seen and resembled Graafian follicles (macrofollicular pattern). The neoplastic cells were polyhedral and uniform
with round to ovoid nuclei and moderately prominent nucleoli. The cytoplasm was moderate to relatively abundant and mitoses occasionally occurred. Extensive areas of necrosis and haemorrhage were present. Slight invasion of the mesothelioma cells was observed on the surface of the right ovary, but there were no mesothelioma cells in the left ovary and uterus.

**Electron microscopy.** In Case 1, the neoplastic mesothelial cells had irregularly contoured nuclei with evenly dispersed or slightly condensed chromatin. Intracytoplasmic organelles were slightly to moderately developed and intermediate filaments, lysosomes and pinocytotic vesicles were present in the cytoplasm. It was extremely rare to find cilia which projected into the lumen or invaginating vacuoles (Fig. 7). A few short microvilli lined the lumen and were sometimes coated with a glycocalyx (Fig. 8). Cell membranes were irregular and membrane infolding resulted in interdigitation of adjacent cells and on rare occasion, intercellular cavities with
were poorly developed, multiple Golgi apparatus sometimes occurred. Intermediate filaments, lysosomes, pinocytotic vesicles, a few microvillous projections (Fig. 9) and rare cilia (Fig. 10) were detected in the cytoplasm. Hemidesmosomes were easily found, while desmosomes were few (Figs 11, 12).

The mesothelial cells of Case 2 were rich in intermediate filaments and displayed various developments of microvilli (Fig. 13). These microvilli were present not only on the luminal side but also on the basal side and were covered with no glycocalyx. Inter-cellular cavities or intracytoplasmic crypts
with or without microvilli were observed. Floating bodies within the lumina were composed of cores of collagen filaments and surrounding epithelioid cells (Fig. 14). Other features were almost identical to those of Case 1 (Fig. 15).

The submesothelial cells of Case 2 were often connected to each other (Fig. 16). Desmosomes and tight junctions were distinctly observed between two cells and hemidesmosomes were also present. Cytoplasmic organelles were slightly to relatively well developed with dilated cisternae of rough endoplasmic reticulum (RER) and multiple Golgi apparatus. Intermediate filaments were abundant with occasional bundle formation and intracytoplasmic crypts were extremely rare (Fig. 17). Some cells
had numerous microvilli and were suggestive of a transitional form into epithelioid cells (Fig. 18). There were fragments of basal lamina-like structures around the neoplastic cells and collagen filaments were not so abundant as in Case 1, but closely associated with the neoplastic cells (Fig. 19). Cilia could not be found. Other features were the same as in Case 1.

Normal mesothelial and submesothelial cells had similar structures to their malignant counterparts. Cilia were extremely rare in the normal submesothelial cells.

The neoplastic cells of the granulosa cell tumour had round or slightly indented nuclei with small to moderate amounts of heterochromatin. The organelles were developed in varying degrees ranging from mildly to well and intermediate filaments were distributed evenly throughout the cytoplasm. Few cells had one or several lipid droplets or lysosomal dense bodies. While some cells inclined to be non-cohesive, others were attached by desmosomes and basal cells rested on the thin basal lamina.

DISCUSSION

There are two views to explain the differences of the submesothelial cells between Case 1 and Case 2. One is that the submesothelial cells in Case 2 have a stronger tendency to differentiate into epithelioid cells and Case 2 may be an intermediate form between the epithelial type and the typical biphasic type such as Case 1. The other is that Case 1 is later than Case 2 in the stage of neoplastic growth, because the tumour masses in Case 1 were far larger than those in Case 2 and collagen fibres in Case 1 were more abundant than those in Case 2. Well developed RER and close contact with collagen filaments in the submesothelial cells of Case 2 suggest active production of collagen filaments by these
cells [6]. Although cell to cell attachment and desmosomes are also prominent, abundant collagen filaments may finally separate the cellular contact between adjacent cells, and desmosomes may become hemidesmosomes. Such cells resemble the submesothelial cells of Case 1. Well developed microvilli were seen in some submesothelial cells of Case 2 and surrounding collagen filaments were relatively scarce. On the other hand, in Case 1 microvilli were poorly developed and collagen filaments were plentiful. The development of microvilli may be associated with the quantity of the fibrillar matrix.

Suzuki, Chahinian and Ohnuna [23] consider that the neoplastic submesothelial mesenchymal cell has a limited capacity to differentiate into epithelioid cells. Most of the ultrastructural features observed in the mesothelial cells of our cases were also present in the submesothelial cells in spite of quantitative differences of each organelle. Large bodies found in the metastatic lesion of Case 2 were composed of collagen fibre cores surrounded by epithelioid cells. Since most of the cores were acellular, such cores may be produced by the lining mesothelial cells. These findings suggest that the neoplastic mesothelial cells and submesothelial cells are substantially the same.

In the problem most frequently encountered is distinguishing mesothelioma from adenocarcinoma. Adenocarcinoma often contain characteristic secretory granules and mesotheliomas do not contain such granules [6]. Low-density granules and lamellar bodies in a bovine bronchoalveolar neoplasm [21] and mucous granules in bovine pulmonary adenocarcinomas [9] have been described. These granules were absent in our cases and they may be useful in differential diagnosis. Furthermore, our cases lacked core filaments and glycocalyceal bodies, which were characteristic of some adenocarcinomas [9, 11]. Cilia found in the present neoplasms and normal tissues were considered to be 'oligocilia' [8], whereas the neoplastic cells of a bovine pulmonary adenocarcinoma had multiple surface cilia. In undifferentiated adenocarcinomas [9, 11], anaplastic cells showed sarcomatous growths and had no relation to collagen filaments unlike the neoplastic submesothelial cells of our cases.

The fibrous mesothelioma may be difficult to distinguish from fibroblastic neoplasms. Ghadially [6] claimed that desmosome-like structures were quite different from true desmosomes. The former and hemidesmosome-like structures are markers for many mesenchymal cells and their tumours and have been observed in a bovine mesenchymal chondrosarcoma [25], while true desmosomes and hemidesmosomes are principally markers for epithelia (also mesothelium and meninges) and their tumours. According to this view the submesothelial cells bearing desmosomes and hemidesmosomes are distinguishable from fibroblastic cells, and other features suggestive of epithelioid cell nature may be of help to the differential diagnosis.

For the accurate diagnosis of multiple primary tumours, the possibility that each tumour may be a metastatic lesion from the other must be excluded. In Case 2, the ovarian tumour characterized by insular or multilayered growths with non-neoplastic connective tissue stroma was distinctly different from the biphasic mesothelioma in which epithelioid cells showed monolayered arrangements with underlying neoplastic fibroblastoid cells. Ultrastructurally, there were many differences between the mesothelioma and the granulosa cell tumour. Lipid droplets observed in the granulosa cells were absent in the mesothelioma cells, but on the other hand tight junctions, cilia and variously developed microvilli were seen only in the mesothelioma. The mesothelioma cells had abundant intermediate filaments and well-
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differentiated desmosomes, while inter-
mediate filaments were less developed
and desmosomes were fewer and attenuated
in the granulosa cells. Multiple primary
tumours are rare in cattle and its reasonable
explanations have been presented by Pri-
est [19]. Multiple primary tumours may be
related to hereditary or iatrogenic diseases
in man [13].

In human patients, the association be-
tween asbestos exposure and the develop-
ment of mesothelioma of the pleural and
peritoneal surfaces is well known [2, 16].
Recently the incidence of bovine meso-
thehlioma increases in some countries [12, 16]
and also in Japan, and the association
between bovine mesothelioma and environ-
mental asbestos has been recognized
epidemiologically [3]. Although the number
of asbestos fibres examined in the lung of a
cow with peritoneal mesothelioma by using
low-temperature ashing was greater than
that of a normal animal [20], identical fibres
were not found in the neoplastic tissues of
the present cases by common histological
methods.

ACKNOWLEDGEMENTS. We thank Dr S. Ishino,
Messrs. M. Ito and Y. Ando their help with this work.

REFERENCES

Pathol. Vet. 4: 149–156.
of the lung and other organs: their epidemiology
3. Croft, W. 1983. Environmental asbestos and
Cancer Res. 24: 188.
Hibbard, H. H. 1968. Survey of animal neoplasms
in Alameda and Contra Costa Countries, Califor-
nia. I. Methodology and description of cases. J.
Mesothelioma, histological and electron micros-
ocopic study of human cases. Acta Pathol. Jpn. 34:
1411–1424.
of diagnostic problems. pp. 63–316. In: Diagnostic
Electron Microscopy of Tumours, 2nd ed., But-
terworths, London.
Malignant mesothelioma in urban dogs. Vet.
Pathol. 20: 531–540.
8. Henderson, D. W., Papadimitriou, J. M., and
Coleman, M. 1986. The neoplastic cell II: cyto-
plasmic ultrastructure. pp. 34–56. In: Ultrastruc-
tural Appearances of Tumours, 2nd ed., Churchill
Livingstone, Edinburgh.
Ultrastructure and origin of adenocarcinomas
detected in the lungs of three cows. J. Comp.
10. Kadota, K., Nei, T., Niizeki, H., and Kita, M.
1985. Identification of two cases of bovine un-
differentiated tumours (squamous cell carcinoma
and adenocarcinoma) by electron microscopy. J.
adenocarcinoma with stromal cells containing
12. Klopfer, U., Brenner, G., Nobel, T. A., Perl, S.,
and Yakobson, B. 1983. Mesothelioma in cattle, a
rare or an unidentified tumour. Zentralbl. Veter-
primary neoplasms. Metabolism and Disease,
Supplement, Progress in Cancer Research 83 20:
259–267 (In Japanese).
14. Lairmore, M. D., Knight, A. P., and DeMartini,
J. C. 1987. Three primary neoplasms in a goat:
hepatocellular carcinoma, phaeochromocytoma
Tumors of the respiratory tract and thorax. pp.
535–565. In: Veterinary Cancer Medicine, 2nd ed.
(Theilen, G. H., and Madewell, B. R. eds), Lea
& Feiger, Philadelphia.
17. Moulton, J. E. 1978. Tumors of the pancreas,
In: Tumors in Domestic Animals, 2nd ed. (Moul-
ton, J. E. ed.), University of California Press,
Berkeley and Los Angeles, California.
18. Pearson, G. R., Bryson, D. G., and McCracken,
R. M. 1982. Mesothelioma in an abattoir survey
of cattle in Northern Ireland. Irish Vet. J. 36:
13–14.
19. Priester, W. A. 1977. Multiple primary tumors in
domestic animals, a preliminary view with par-
ticular emphasis on tumors in dogs. Cancer 40:
1845–1848.

要約

牛悪性中皮腫2例の超微形態学的研究：橋本夏美・織田利昭・門田耕一（埼玉県食肉衛生検査センター熊谷支所，1）農林水産省家畜衛生試験場）——2頭のホルスタイン種、雄成牛の腹膜原発中皮腫を光顕的、電顕的に観察した。腫瘍は二相性で、腺腔を形成する腫瘍性中皮細胞と膠原線維中に散在する腫瘍性中皮下細胞より構成されていた。中皮細胞は中間径フィラメント、微細毛、基底膜、接着斑、閉鎖帯を特徴とし、まれに綿毛が認められた。中皮下細胞は膠原線維維と密着しており、一部の細胞はいくつかの中皮細胞の特徴と考えられる構造を持っていた。1例では左卵巢に顆粒膜細胞腫があり、牛ではまれな重複腫瘍と考えられた。