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Case Report

Malignant Peritoneal Mesothelioma with a Sarcomatoid Growth Pattern and Signet-Ring-Like Structure in a Female F344 Rat

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Abstract: We report a biphasic malignant mesothelioma in an aged female F344/DuCrlCrlj rat. Macroscopically, multiple pale brown nodules were observed in the abdominal cavity with retention of bloody ascites. Histopathologically, the tumor cells spread over the peritoneum and formed masses on the surface and underlying adipose tissues. The tumor cells dominantly proliferated in a solid, nodular or nest-like pattern with modest amount of fibrillar connective tissues, which contained hyaluronan. The tumor consisted of ovoid, polygonal or spindle-shaped cells that possessed eosinophilic cytoplasms including glycogen; some tumor cells showed a signet-ring-like structure. Multinucleated cells and mitosis were found frequently, and direct invasion to intra-abdominal organs and intravascular metastasis to the liver were observed. Immunohistochemically, keratin and mesothelin were strongly positive in most of tumor cells, while vimentin was mainly positive in spindle-shaped cells. Podoplanin was also positive, particularly in the cell membrane of tumor cells. Electron microscopically, tumor cells showed an intercellular desmosome-like structure, basement membrane and microvillus. We diagnosed the case as a malignant peritoneal mesothelioma with a sarcomatoid growth pattern and signet-ring-like structure. (DOI: 10.1293/tox.26.197; J Toxicol Pathol 2013; 26: 197–201)

Key words: rat, spontaneous, malignant mesothelioma, signet-ring-like structure

In humans, diffuse malignant mesotheliomas are rare tumors arising from the mesothelial cells on serosal surfaces such as the pleura, pericardia, peritonea and genitals; the majority of these tumors arise in the pleural cavity in association with exposure to asbestos[1, 2]. Histopathologically, a broad spectrum of the tumors is classified into three major types, the epithelioid, sarcomatoid and biphasic types. The epithelioid mesothelioma is one of the most common types; the tumors are remarkably bland, with a wide range of morphological patterns such as tubulopapillary, adenomatoid (microglandular) and sheet-like structure. The adenomatoid form shows microcystic structures with a lace-like, adenoid cystic or signet-ring appearance but does not stain for neutral mucin. More anaplastic forms are occasionally seen; undifferentiated cells are difficult to characterize as being either poorly differentiated epithelial cells or fibrosarcomatous cells[2].

In male Fischer 344 rats, mesothelioma is one of the most common spontaneous tumors mainly arising from the retroperitoneum and adipose tissue surrounding the spermatic cord[3-5]. Most of the tumors showed papillary growth of cuboidal and polygonal cells exhibiting an epithelioid phenotype. Malignant mesothelioma is relatively rare in female rats as compared with male rats[5]. Indeed, only three epithelioid types and two sarcomatous types were reported in females among 62 spontaneous mesotheliomas in F344 rats[4]. We encountered a malignant mesothelioma characterized by a sarcomatoid growth pattern and signet-ring-like structure in a female F344 rat. This report describes the gross, histopathological, immunohistochemical and electron microscopic characteristics in this case.

The affected animal was a specific pathogen-free F344/DuCrlCrlj female rat purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) that was allocated to a dose group in a carcinogenicity study. It was housed in wire-mesh stainless steel cages in a barrier-sustained animal room controlled at 22 ± 2°C with 50 ± 20% humidity, ventilation 10 times or more per hour and illumination 12 hours/day and was given a commercial diet (MF Mash, Oriental Yeast Co., Ltd., Tokyo, Japan) containing the test chemical and tap water *ad libitum*. The animal was handled during the study in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association

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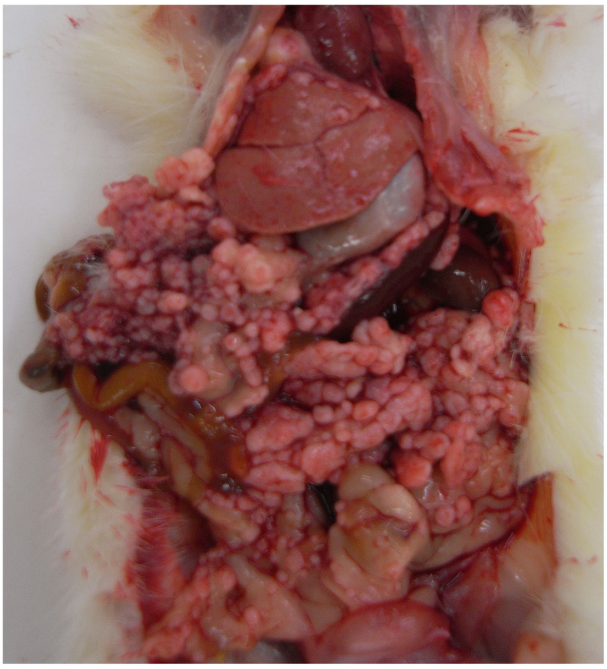

Fig. 1. Gross pathological features of the tumor. Numerous small pale brown masses were scattered on the surface of the mesenterium in the abdominal cavity.

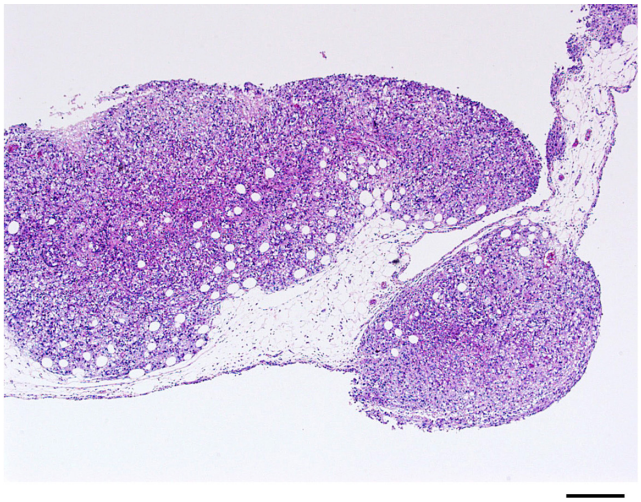

Fig. 2. Histopathological features of the tumor cell masses spreading over the peritoneum. The serosal surface was partially thickened by the proliferation of tumor cells, which invaded into underlying adipose tissues. Bar = 200 μm.

for Laboratory Animal Science[6] and the Code of Ethics for Animal Experimentation of this institute.

The animal was found dead at 92 weeks of age. At necropsy, multiple pale brown nodules (less than 5 mm in diameter) were observed in the abdominal cavity with retention of bloody ascites (Fig. 1). The nodules were scattered on the surface of the mesenterium and several intra-abdominal organs such as the liver, spleen, gastrointestinal tracts, uterus and ovaries. An enlarged, opaque eye and a hepatodiaphragmatic nodule of the liver were also observed spontaneously. Systemic organs and tissues including nodules were fixed in 10% neutral-buffered formalin, routinely processed, and embedded in paraffin. Paraffin sections (5 μm) were stained with hematoxylin and eosin (H&E). The sections of nodules and liver were additionally stained with periodic acid-Schiff (PAS) and Alcian blue (pH 2.5) with or without diastase and hyaluronidase treatment, respectively. Eight-micrometer frozen sections of formalin-fixed nodules and liver were stained with Oil Red O. Immunohistochemistry was conducted using a Dako EnVision kit (Dako, Glostrup, Denmark). The sections were incubated with the following primary antibodies: anti-cow keratin and S-100 protein polyclonal antibody, anti-swine vimentin monoclonal antibody (V9), anti-rat mesothelin polyclonal antibody, anti-rat podoplanin monoclonal antibody, anti-mouse calretinin polyclonal antibody, anti-human α-smooth muscle actin (αSMA) monoclonal antibody (1A4) and anti-chicken desmin polyclonal antibody. The primary antibodies of mesothelin, podoplanin and calretinin were obtained from Immuno-Biological Laboratories Co., Ltd. (Gunma, Japan), AngioBio, Inc. (Del Mar, CA, USA) and Acris Antibodies GmbH (Herford, Germany), respectively, while others were from Dako. The antibodies of mesothelin, podoplanin and calretinin were raised against cell surface glycoprotein, transmembrane mucoprotein and calcium-binding protein, respectively, and these antibodies are commonly-used markers for the diagnosis of mesothelioma in humans[7]. For electron microscopic examination, a part of the formalin-fixed nodules was refixed in 2% osmium, routinely processed and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under an electron microscope (JEOL Ltd., Tokyo, Japan).

Histopathologically, the tumor cells spread over the peritoneum in the abdominal cavity (Fig. 2). The serosal surface was partially thickened by the proliferation of tumor cells, forming variable-sized masses. The tumor cells dominantly proliferated in a solid or nodular pattern with a modest amount of fibrillar connective tissues (Fig. 3A). Nest-like proliferation or a signet-ring-like structure was also observed (Fig. 3B and 3C). The tumor consisted of oval or polygonal cells that possessed a notable atypical nucleus and eosinophilic cytoplasm including granules and vacuoles. The tumor cells with a signet-ring-like structure contained large cytoplasmic vacuoles. The vacuoles in the tumor cells were completely negative for Oil Red O stain. Granules observed in some tumor cells were positive for the PAS reaction and negative for the reaction after pretreatment with diastase; the granules were identified as glycogen. Spindle-shaped cells were dominantly proliferated in some parts of the tumor and invaded into adipose tissues. Multinucleated cells, pleomorphic cells and mitosis were frequently seen (Fig. 3D). Fibrillar connective tissues contained hyaluronan, because hyaluronidase pretreatment markedly diminished the Alcian-blue-positive reaction. Variably sized necrotic

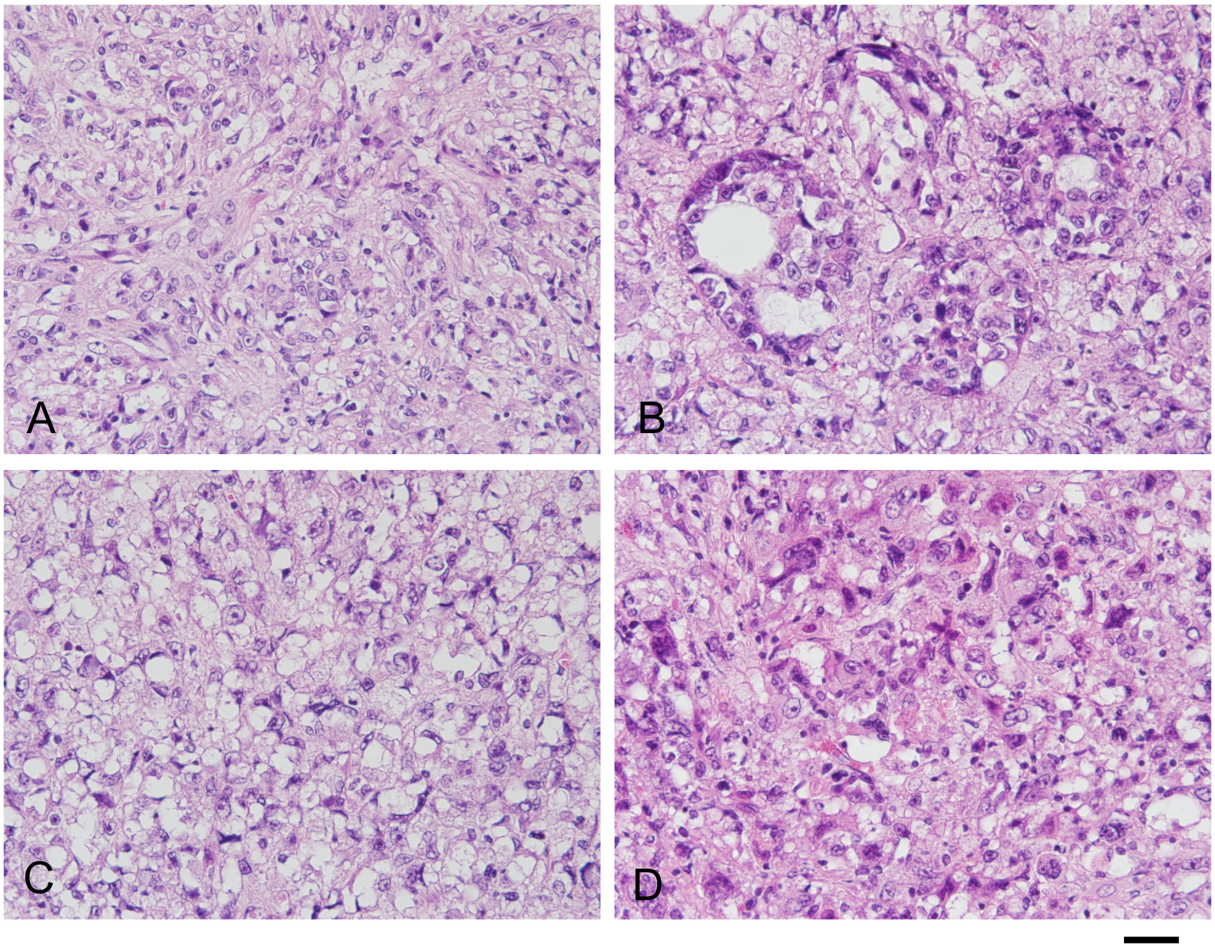

Fig. 3. Histopathological features of the tumor. (A) Proliferation of spindle-shaped cells with oval nuclei, cytoplasmic vacuoles and a modest amount of fibrillar connective tissues. (B) Nest-like proliferation of tumor cells with oval nuclei and eosinophilic cytoplasms. (C) Signet-ring-like structure of tumor cells with bizarre nuclei and prominent cytoplasmic vacuoles. (D) Pleomorphic and multinucleated cells with cytoplasmic vacuoles. Bar = 20 μm.

foci with surrounding inflammatory cell infiltrate and slight calcification were also observed within the nodule. Disseminated metastasis was observed in the liver, pancreas, ovary and diaphragm; tumor cells appeared to directly invade these parenchymal tissues from the serosa. Intravascular metastasis was also identified in the liver, as small nests of tumor cells were scattered in the portal area, particularly in the portal veins.

Immunohistochemically, the majority of tumor cells reacted positively for keratin; a population of tumor cells with nest-like proliferation was strongly positive (Fig. 4A). Most tumor cells also reacted positively for mesothelin (Fig. 4B). Podoplanin immunoreactivity was restricted to the cell membrane of the tumor cells (Fig. 4C). Meanwhile, vimentin was mainly positive in spindle-shaped cells (Fig. 4D). A small population of tumor cells showed immunoreactivity for desmin and S-100 protein. The tumor cells showed no immunoreactivity for calretinin and αSMA.

Electron microscopically, tumor cells contained small or large intracytoplasmic vacuoles, a prominent microvillus on their surfaces, an intercellular desmosome-like structure and a basement membrane. The stroma was composed mainly of compactly arranged collagen fibers.

In the present case, the macroscopic nature with intraabdominal spread suggested the tumor was a malignant mesothelioma, whereas the tumor was histopathologically characterized by sarcomatous proliferation of undifferentiated lipomatous tumor cells with no evidence of a typical epithelioid form. The following morphological findings, however, supported the diagnosis of malignant mesothelioma: 1) the tumor cells spread over the peritoneum, forming multiple masses on the surface and underlying adipose tissues; 2) immunohistochemically, the tumor cells reacted positively for both keratin and vimentin as epithelial and mesenchymal cell markers, respectively; 3) the tumor cells contained glycogen and produced hyaluronic acid on their stroma; and 4) electron microscopy showed that the tumor cells had an intercellular desmosome-like structure, basement membrane, and microvillus. Collectively, these findings are consistent with the observations in malignant mesothelioma as reported in humans[2, 7-10].

There are additional immunohistochemical mark-

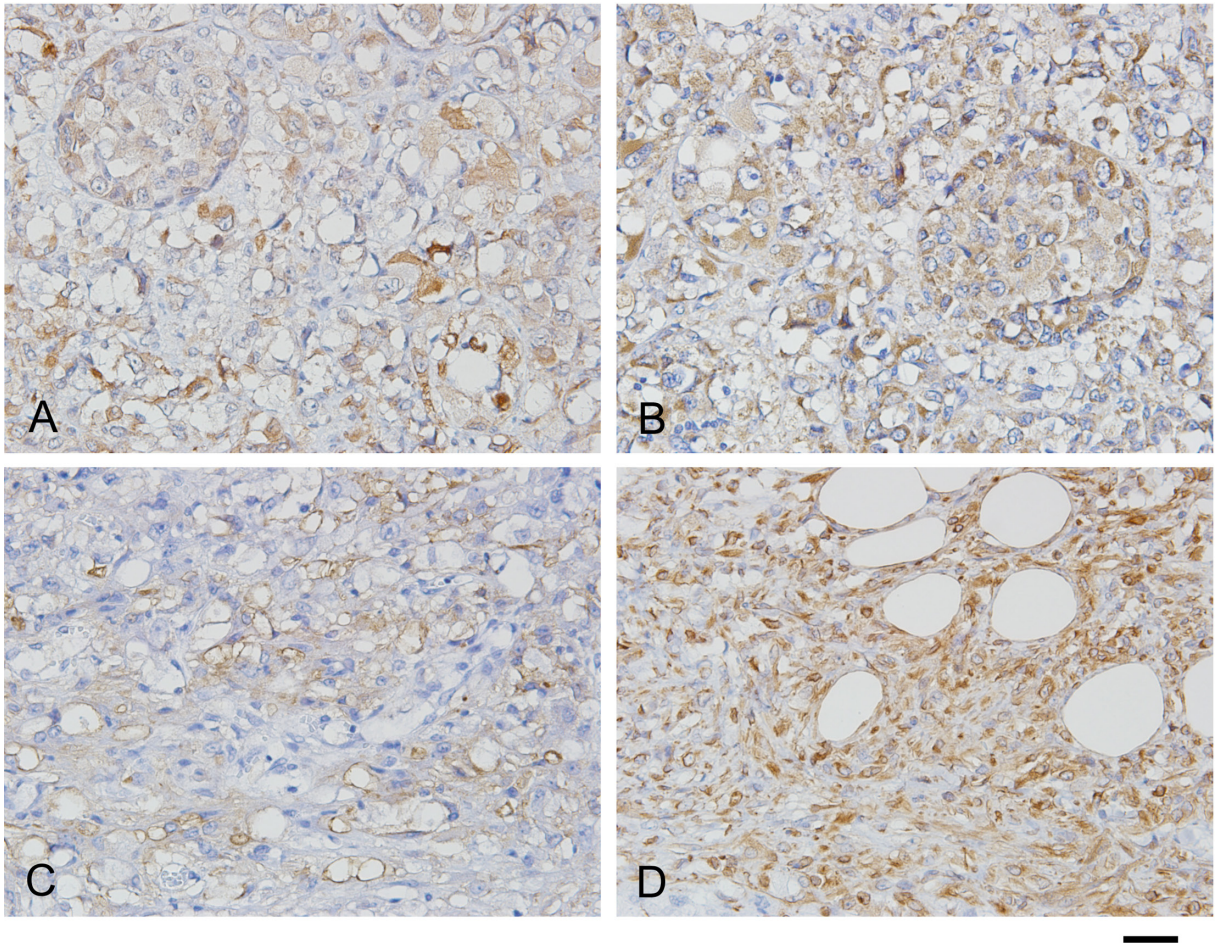

Fig. 4. Immunohistochemical staining for the tumor. The most tumor cells reacted positively for keratin (A) and mesothelin (B). The cell membrane of the tumor cells showed immunoreactivity for podoplanin (C). Vimentin was mainly positive in spindle-shaped cells (D). Bar = 20 µm.

ers for diagnosis of malignant mesothelioma in humans[8,9]. Recently, Sandeck *et al*. re-evaluated malignant mesothelioma-specific markers including mesothelin, podoplanin and calretinin; as a result, these markers were positive in nearly all cases of malignant mesothelioma (mesothelin, 44/48 (91.7%); podoplanin, 44/46 (95.6%); calretinin, 49/49 (100%))[7]. We tested the reliability of these markers in the rat peritoneum, resulting in clear positive reactions in normal mesothelial cells except for calretinin. As expected, the tumor cells were intensely positive for mesothelin and podoplanin in the present case. Consistent with our findings, several researchers demonstrated that mesothelin and podoplanin were strongly positive in mesothelial cells and/or typical mesothelioma cells in rats[11–13]. The negativity demonstrated for calretinin in our case suggested a species difference in terms of specification of this calcium-binding protein between human and rat mesothelial/mesothelioma cells.

The differential diagnosis in the present case includes lipomatous tumors. Intra-abdominal pleomorphic liposarcoma was reported in an aged Wistar rat, which exhibited a tumor consisting of two types of cells, round cells with abundant cytoplasm and spindle-shaped cells with scant cytoplasm, both of which contained variably sized lipid droplets[14]. Interestingly, lipid-rich pleural mesothelioma was reported in a male golden retriver cross dog; the tumors were composed of solid sheets and papillary aggregates of medium-sized polygonal cells that contained Oil Red O-positive vacuoles[15]. Liposarcomatous differentiation was also observed in a diffuse pleural mesothelioma in a woman[16]. In our case, however, the results of Oil Red O staining indicated that the cytoplasmic vacuoles by which the present tumor was characterized were not lipid droplets. In humans, microcystic (adenomatoid) and signet-ring-like tumor cells are infrequently observed in the epithelioid form of malignant mesothelioma[1]. A microcystic (adenomatoid) configuration is characterized by cyst-like spaces lined by attenuated tumor cells and a loculated structure with indistinct borders[8]. Mesothelial vacuoles (such as the vacuoles of signet-ring cells) are usually interpreted as cell degeneration[8]. This interpretation might be consistent with our finding, because we could not demonstrate the presence of even neutral mucin in the vacuoles of the cytoplasm. Thus, we concluded that the intracytoplasmic vacuoles had a mi-

crocystic configuration of malignant mesothelioma but were not lipid droplets resulting from lipogenic differentiation of mesothelial cells.

The present case of mesothelial tumor was the only one detected within the group of treated animals in the study. Therefore, this tumor was considered to be spontaneous and not related to the treatment. In conclusion, the tumor is believed to have been a biphasic malignant mesothelioma with 1) a sarcomatous growth pattern and 2) a signet-ring-like structure and nest-like proliferation, which might mimic a microcystic phenotype of epithelioid mesothelioma in humans.

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