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Case Report

Mixed Type of Malignant Mesothelioma in an Aged Male ICR Mouse

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Abstract: Multiple whitish nodules in the thoracic cavity at the site of the thymus were observed in a 101-week-old male ICR mouse. In a histopathological examination, the neoplastic cells were predominantly fusiform in shape and proliferated in sarcomatoid growth patterns. Some neoplastic cells showed epithelial growth patterns, such as the ductal structures. Mitotic figures were frequently seen, and small necrotic foci and invasion to adjacent thoracic organs were noted. In Alcian blue staining, bluish materials were observed between fusiform-shaped cells and in some of the lumens of the ductal structures. In immunohistochemistry, both fusiform-shaped and ductal structure-forming cells were positive for vimentin and weakly positive to positive for cytokeratin. Based on the aforementioned findings, the thoracic nodules were diagnosed as a mixed type of malignant mesothelioma. This case was thought to be rare because of the very low occurrence of spontaneous mesothelioma in mice. (DOI: 10.1293/tox.24.169; J Toxicol Pathol 2011; 24: 169–172)

Key words: mesothelioma, mixed type, ICR, mouse, thoracic cavity

Mesothelioma is a primary neoplasm that arises in the pleura, peritoneum and pericardium. The incidence of spontaneous mesothelioma in mice is very low, approximately 0.1% according to the historical control data from Charles River Laboratories, only 6 cases were reported in 6088 animals[1]. Maita *et al*. also reported a very low frequency[2], with only 2 mesotheliomas having occurred among 1781 mice in 2 year carcinogenicity studies. Based on the proliferation pattern, malignant mesotheliomas in rodents are classified as the epithelioid type, mesenchymal type and mixed type[3,4], with the epithelioid type predominant in rats[5]. The frequency of each type was reported in regards to asbestos and zeolite-induced mesotheliomas in 586 BALB/C male mice: there are 1 epithelioid (epithelial) type, 73 mesenchymal (fibrous) types and 9 mixed (biphasic) types[6]. However, as far as we know, the predominant type of spontaneously occurring mesothelioma in mice has not been reported. In this report, we describe a case of the mixed type of malignant mesothelioma observed in the thoracic cavity of an aged ICR mouse.

The animal was a 101-week-old male ICR mouse (Japan SLC Inc., Shizuoka, Japan). The procedures for animal care and housing were in compliance with our institutional standards for the care and use of laboratory animals. The mouse was a control group animal (0.5% carboxymethyl cellulose, gavage) in a 2 year carcinogenicity study and was individually housed in a taper-type bracket cage in a barrier-sustained room controlled at a temperature of 23 ± 2 °C, with a relative humidity of 55 ± 10%, an illumination time of 13 hours per day at an intensity of about 200 luces and 10 to 15 ventilation cycles per hour. The animal had free access to a radiosterilized pellet diet (NMF: Oriental Yeast Co., Ltd.) and tap water supplied through the nozzle of an automatic water supplying apparatus. At necropsy under anesthesia, multiple whitish nodules (approximately 2 mm in diameter) were observed in the thoracic cavity at the site of the thymus, and some of them were adhered to the thoracic wall. Enlargement in the mediastinal lymph node and clear hydrothorax (approximately 0.8 mL) were also observed. The nodules and all the tissues routinely collected were fixed in a neutral buffered 10% formalin solution and embedded in paraffin by routine procedures. Each paraffin-embedded section was stained with hematoxylin-eosin (HE). For the nodules, Alcian-blue (pH 2.5), Periodic acid-Schiff (PAS) and Masson's trichrome stains and immunohistochemistry were additionally performed. Immunohistochemistry was performed using a Dako Envision system (DAKO Japan, Tokyo, Japan), except for vimentin, which was performed using a horseradish peroxidase-conjugated rabbit anti-guinea pig antibody (DAKO Japan, Tokyo, Japan) as the second

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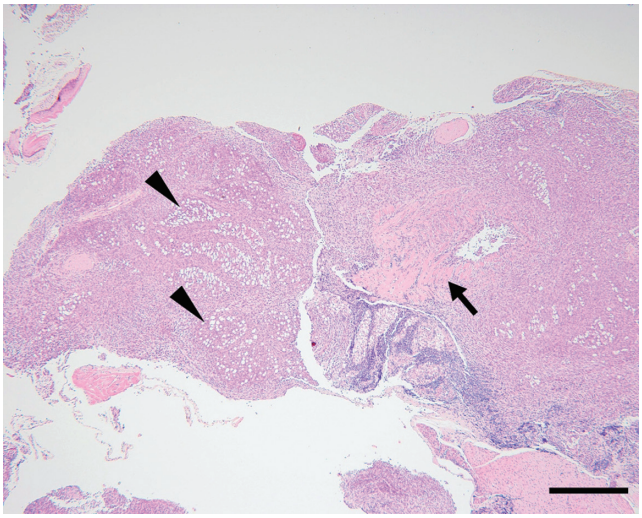


Fig. 1.

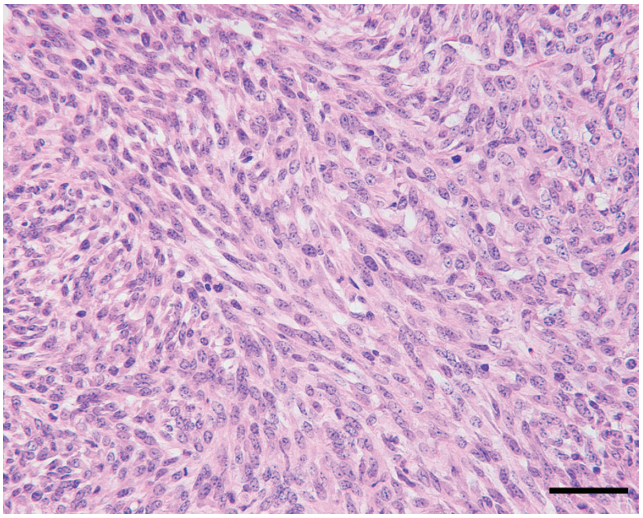
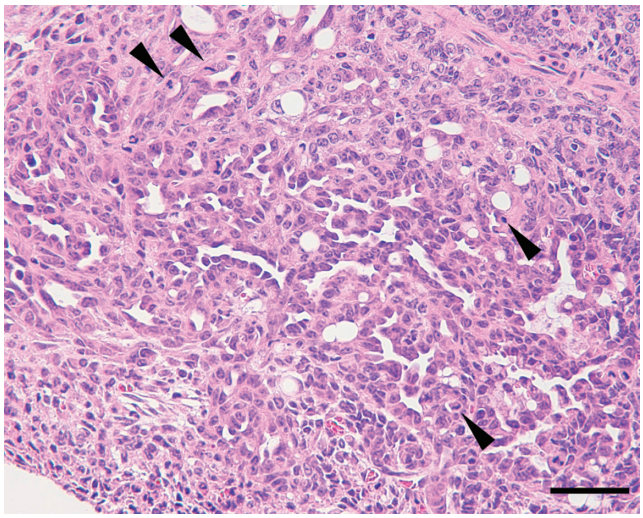


Fig. 2.



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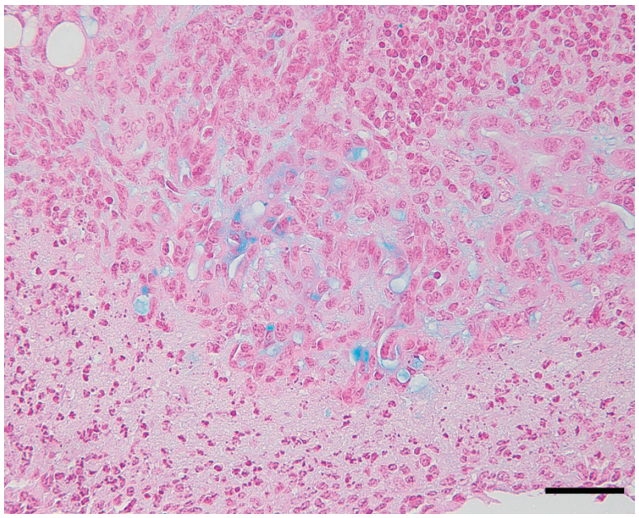

Fig. 4.

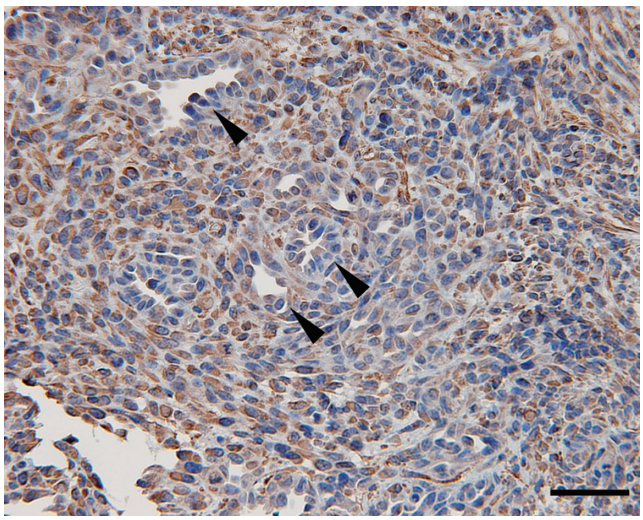

Fig. 5.

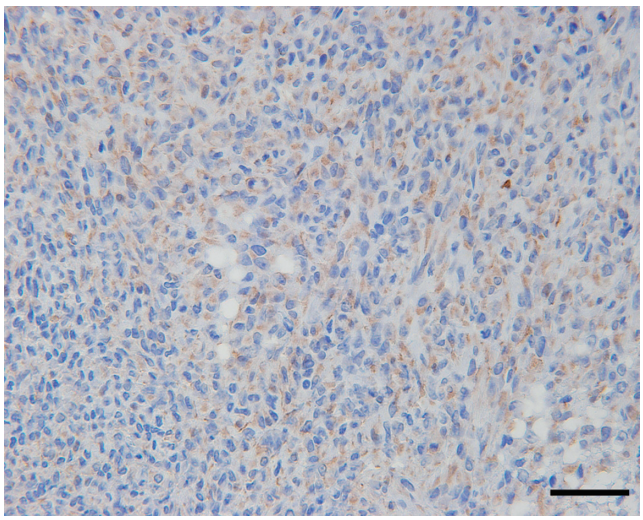

Fig. 6.

antibody. A section was incubated with antibodies against cytokeratin AE1/AE3 (Dako Japan), vimentin (PROGEN Biotechnik, Heidelberg, Germany), S-100 (Dako Japan), alpha-SMA (Covance, Emeryville, CA, USA) and desmin (Spring Bioscience, Pleasanton, CA, USA). For the lung, immunohistochemical examination using antibodies for cytokeratin AE1/AE3 and vimentin was performed. The reaction products were visualized with 3,3′-diaminobenzidine tetrahydrochloride.

In the histopathological examination, the neoplastic cells were disseminated and invaded thoracic tissues including the bronchus, atrial auricle, adipose tissue, thoracic aorta and mediastinal lymph node through serosa (Fig. 1). The neoplastic cells showed predominantly sarcomatoid proliferation. These cells had fusiform-shaped cytoplasms, round to oval nuclei and small nucleoli and proliferated in bundle to wave-like patterns (Fig. 2). In part, an epithelial growth pattern was also observed. Some neoplastic cells had a small amount of cuboidal- to polygonal-shaped cytoplasms, round nuclei that stained darkly, and ductal patterns of proliferation (Fig. 3). Oval- to polygonal-shaped cells connecting densely to loosely to make clusters were also observed. Mitotic figures were frequently seen, and there were small necrotic foci. There were no similar lesions in any other extrathoracic organs. In the lung, a bronchioloalveolar carcinoma proliferating in papillary patterns was observed in the cranial lobe of the right lung. The bronchioloalveolar carcinoma had tall columnar cytoplasms, oval nuclei that exhibited pale staining and a lack of continuity with the tumor mentioned above.

In Alcian blue-stained sections, material that stained pale blue between the fusiform-shaped cells and material that stained blue in some of the lumens of the ductal structures were observed (Fig. 4). In the Masson's trichrome-stained sections, a few fibrous materials stained blue were observed between the fusiform-shaped cells. In the PAS-stained sections, only a few neoplastic cells growing in ductal patterns had reddish granules in their cytoplasm.

In the immunohistochemical examinations, the neoplastic cells including the cells showing epithelial growth patterns were positive for vimentin (Fig. 5) and weakly positive to positive for cytokeratin (Fig. 6), while they were negative for S-100, alpha-SMA and desmin. The neoplastic cells of the bronchioloalveolar carcinoma were positive for cytokeratin but negative for vimentin.

Based on the above-mentioned findings, the intrathoracic lesion consisting of multiple nodules was diagnosed as malignant mesothelioma. The histopathological features in this case were compatible with those of malignant mesothelioma described in the *International Classification of Rodent Tumors: The mouse*[4]. Moreover, according to a textbook[7], the immunohistochemical appearance as both vimentin and cytokeratin positive and Alcian blue (pH 2.5) positive are important clues for diagnosis of a tumor as mesothelioma, and these were observed in this case. The malignant mesotheliomas in mice are classified as the epithelioid type, mesenchymal type and the mixed type[4]. The types are composed of an epithelial structure, sarcomatoid structure and both epithelial and sarcomatoid structures, respectively. This case was considered to be the mixed type because there were regions showing both epithelial and sarcomatoid growth patterns.

As a differential diagnosis, bronchioloalveolar carcinoma is important because the neoplastic cells frequently become anaplastic and resemble mesothelioma of the mesenchymal type in metastatic lesions of bronchioloalveolar carcinoma in the thoracic cavity[4]. In this case, the bronchioloalveolar carcinoma was observed in the cranial lobe of the right lung, but direct continuity was not identified between intrathoracic lesions and the bronchiole-alveolar carcinoma. In addition, there was no metastasis to any examined extrathoracic tissue nor was there clear invasion to pleura. Moreover, the bronchioloalveolar carcinoma showed different cytologic features and a different growth pattern from those of intrathoracic lesions, even in the epithelial growth pattern, and was vimentin negative in the immunohistochemistry. These pathological features showed that the bronchioloalveolar carcinoma had no association with the intrathoracic lesion, the malignant mesothelioma. Malignant thymoma should also be a differential diagnosis because the nodules were observed at the site of the thymus. Malignant thymoma originates in the epithelial component of the thymus, which features a high proportion of epithelial cells and wherein squamous differentiation is often observed[8]. In this case, the tumor was mainly composed of sarcomatoid cells which were positive for vimentin, and there was no squamous

Fig. 1. Histopathological appearance of the nodules within thoracic organs. Neoplastic cells were disseminated and invaded adjacent atrial auricle (arrowhead) and adipose tissues (arrow). HE stain. Bar = 500 μm.

Fig. 2. Histopathological appearance of sarcomatoid neoplastic cells. The neoplastic cells were predominantly fusiform-shaped and proliferated in bundle to wave-like patterns. HE stain. Bar = 50 μm.

Fig. 3. Histopathological appearance of neoplastic cells with epithelial growth patterns. Some neoplastic cells were cuboidal to polygonal in shape and proliferated in ductal patterns. Mitotic figures (arrowhead) were frequently observed. HE stain. Bar = 50 μm.

Fig. 4. Histopathological features of neoplastic cells with ductal structures stained with Alcian blue. Materials that stained blue were observed in the lumens of the ductal structures. Alcian blue-stain. Bar = 50 μm.

Fig. 5. Immunohistochemistry of neoplastic cells for vimentin. The neoplastic cells including the cells showing epithelial growth patterns (arrowhead) were positive for vimentin. Bar = 50 μm.

Fig. 6. Immunohistochemistry of neoplastic cells for cytokeratin. The neoplastic cells were weakly positive to positive for cytokeratin. Bar = 50 μm.

differentiation. In regard to other differential diagnoses, sarcomas composed of spindle-shaped cells, such as leiomyosarcoma, malignant peripheral nerve sheath tumor, rhabdomyosarcoma, are possible. These diagnoses were rejected because the neoplastic cells showed epithelial growth patterns in HE-stained sections and were negative for S-100, alpha-SMA and desmin.

In conclusion, multiple whitish nodules observed in the thoracic cavity of a 101-week-old male ICR mouse were diagnosed as a mixed type of malignant mesothelioma. It was reported that chemical-induced murine mesothelioma was mostly the mesenchymal type[6], but spontaneous occurrence of malignant mesothelioma in mice is very low[1,2], and there have been no reports regarding the incidences of each type of spontaneous mesothelioma. Hence, the predominant type of mesothelioma in mice is unknown, and this case is a rare spontaneous mesothelioma classified as the mixed type.

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