Case Report

Spontaneous malignant myoid thymoma in an aged female Fischer 344 rat

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Abstract: A whitish mass approximately 30 mm in diameter was noted in the anterior mediastinum of a 67-week-old female Fischer 344 rat. Histopathologically, two types of tumor cells were identified on the basis of morphologic features: epithelial tumor cells with a tubular or cord-like growth pattern and rhabdomyosarcomatous tumor cells characterized by the presence of cross-striations. Immunohistochemically, the epithelial tumor cells reacted positively for cytokeratin AE1/AE3, and some reacted positively for p63, which is expressed in normal thymic epithelial cells. The rhabdomyosarcomatous tumor cells stained positively for desmin, sarcomeric actin, and S-100 protein, which coincides with the stainability of normal thymic myoid cells. Since the tumor was also found to have malignant features such as high proliferative activity, cytologic atypia, and necrotic behavior, it was diagnosed as a malignant myoid thymoma. We believe that this is the first case report of such a tumor in a rodent. (DOI: 10.1293/tox.2017-0045; J Toxicol Pathol 2018; 31: 135–139)

Key words: Fischer rat, malignant myoid thymoma, epithelial, rhabdomyosarcomatous

Malignant thymoma with neoplastic proliferation of myoid cells is an extremely rare variant in both humans and animals. It is not classified in the World Health Organization classification[1] but has been reported as rhabdomyosarcomatous thymoma[2] or thymic tumor with proliferation of myoid cells[3] in humans. The main features of these neoplastic myoid cells are large eosinophilic cytoplasm with cross-striation revealed by phosphotungstic acid-hematoxylin (PTAH) staining and a positive reaction to desmin. One case of thymoma with neoplastic myoid cells, which was diagnosed as "myoid thymoma," has been described in rodents[4], but the report contained limited detail. Here we describe the histological and immunohistochemical characteristics of a malignant myoid thymoma in an aged female Fischer 344 (F344) rat.

The animal was a specific pathogen-free F344/DuCrl-Crlj female rat purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan), that was allocated to a control group in a feeding carcinogenicity study for a period of 24 months. The animals in the study were housed in a wire-mesh stainless steel cage in a barrier-sustained animal room controlled at 22°C ± 2°C and 50% ± 20% humidity with ventilation 10 times or more per hour (all-fresh-air basis) and illumination 12 hours per day (light on at 7:00 a.m. and off at 7:00 p.m.). The animals received a commercial diet (MF Mash, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. The animals were handled during the study in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science[5] and with the Code of Ethics for Animal Experimentation of the Institute of Environmental Toxicology.

The rat that developed the mass was noted to have bradypnea at 64 weeks of age and swelling in the ventral neck region with emaciation at 65 weeks of age. It had a moribund appearance by 67 weeks of age, with decreased spontaneous motor activity and soiled fur in the external genital and perinasal regions. At this point, the animal was deeply anesthetized with isoflurane and then euthanized by exsanguination.

At necropsy, in place of the thymus, a large discrete mass (approximately 30 mm in diameter) was observed in the anterior mediastinum (Fig. 1). The mass was not adhered to any of the surrounding intrathoracic organs, such as the esophagus or thoracic wall. There were no obvious abnormal gross findings indicating circulatory failure in the heart or lung, although they were compressed by the mass.
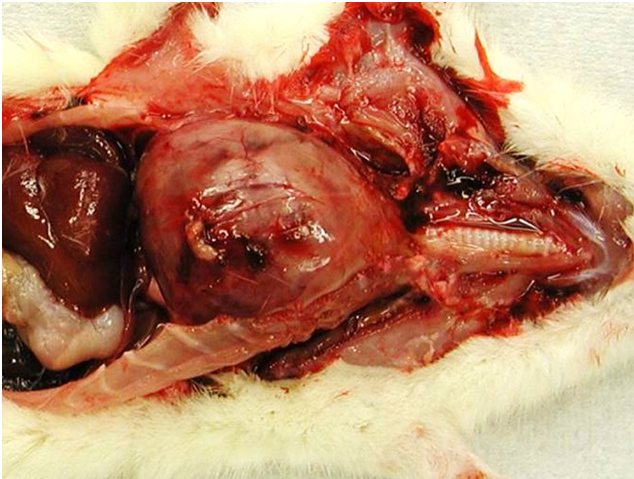
Fig. 1. Gross appearance of a mass detected in an aged female Fischer 344 rat. The mass is located in the mediastinum, corresponding to the thymus region, and measures 30 mm in diameter.

All organs and tissues, including the mass, were routinely collected and fixed in 10% neutral-buffered formalin, embedded in paraffin wax, and sectioned at a thickness of approximately 5 μm. The sections were stained with hematoxylin and eosin. The sections of the mass were also used for PTAH staining and immunohistochemical investigations[6]. The primary antibodies used for immunohistochemistry were as follows: mouse anti-cytokeratin (AE1/AE3, monoclonal, prediluted), anti-vimentin (V9, monoclonal, 1:50), anti-α-smooth muscle actin (SMA) (1A4, monoclonal, 1:100), anti-p63 (4A4, monoclonal, 1:10), anti-sarcomeric actin (Alpha-Sr-1, monoclonal, 1:50), and anti-proliferating cell nuclear antigen (PCNA) (PC10, monoclonal, 1:1,000) and rabbit anti-desmin (polyclonal, prediluted) and anti-S-100 protein (polyclonal, 1:400). All primary antibodies were purchased from Dako (Glostrup, Denmark). Antigen retrieval was performed in 0.1 M citrate buffer (pH 6.0) using an autoclave at 121°C for 5 min. After treatment with 4% Block Ace (DS Pharma Biomedical, Osaka, Japan) at room temperature for 20 min, the sections were incubated with the primary antibodies at 4°C overnight, after which the secondary antibody reactions were performed at 37°C for 30 min using EnVision+ System-HRP anti-mouse or anti-rabbit (Dako, Glostrup, Denmark). Finally, positive reactions were visualized with 3,3-diaminobenzidine (DAB) solution consisting of one DAB tablet (Wako Pure Chemical Industries, Osaka, Japan) in 20 mL phosphate-buffered saline and 10 μL of 30% hydrogen peroxide, and counterstaining was performed with Gill's hematoxylin.

Histopathologically, the mass was well encapsulated by thin fibrous connective tissue. It mainly consisted of two different tumor cell components: one consisting of epithelial tumor cells (Fig. 2a) and another consisting of rhabdomyosarcomatous tumor cells (Fig. 2b). The former was characterized by a proliferation of epithelial cuboidal cells with

oval nuclei and basophilic cytoplasm and showed a tubular (Fig. 2c) or cord-like (Fig. 2d) growth pattern. Mitotic figures were observed, and they were particularly found in the tumor cells with a tubular growth pattern. The latter component was characterized by a proliferation of large pleomorphic cells and spindle cells. The large pleomorphic tumor cells contained multiple giant round to bizarre nuclei with prominent nucleoli and abundant eosinophilic cytoplasm (Fig. 2e). Mitotic figures were often observed. The spindle tumor cells contained one or a few oval nuclei with eosinophilic cytoplasm, and PTAH staining revealed cross-striations in the cytoplasm (Fig. 2f). Mitotic figures were rare. The rhabdomyosarcomatous tumor cells proliferated in the central area of the mass, and the epithelial tumor cells proliferated mainly at the margins of the mass. Although the areas of these two tumor components were relatively well demarcated, there were some areas in which both components were intermingled. Necrotic and hemorrhagic rhabdomyosarcomatous lesions were evident. In addition to the abovementioned two types of tumor cell components, undifferentiated tumor cells were observed partially between the area of proliferating epithelial tumor cells and that of rhabdomyosarcomatous tumor cells (Fig. 2b). The undifferentiated tumor cell population was much smaller than the other two tumor cell populations. The tumor cells had polygonal shaped cytoplasm that possessed oval nuclei and proliferated with a solid growth pattern (Fig. 2g). Mitotic figures were frequently found. No invasion of any types of tumor cells into the surrounding connective tissue or blood vessels was observed. Aggregations of normal lymphocytes were occasionally found at peripheral sites in the mass. No obvious histopathological findings due to compression by the tumor mass were found in any intrathoracic organs.

The immunohistochemical staining results are summarized in Table 1. The epithelial tumor cells with tubular and cord-like growth patterns reacted positively for cytokeratin AE1/AE3 (Fig. 3a, b), which was strongly expressed in the cells with a cord-like growth pattern. The tumor cells with the tubular growth pattern reacted negatively for p63, but the nuclei of some cells with the cord-like growth pattern reacted positively for p63 (Fig. 3c). The epithelial tumor cells were negative for desmin, sarcomeric actin, and S-100. Both spindle and large pleomorphic rhabdomyosarcomatous tumor cells showed the same degree of stainability: these tumor cells were strongly positive for desmin and sarcomeric actin (Fig. 3d) and weakly positive for S-100. The undifferentiated tumor cells were moderately positive for desmin (Fig. 3e) but negative for cytokeratin AE1/AE3, p63, S-100, and sarcomeric actin. PCNA immunostaining revealed high growth activity for all types of tumor cells. Particularly, it revealed strong positive staining in the undifferentiated tumor cells. Vimentin and SMA did not react with any types of tumor cells.

The tumor described here was characterized by proliferation of both epithelial and rhabdomyosarcomatous tumor cells. Undifferentiated tumor cells were also present in the tumor. The epithelial tumor cells were immunopositive for
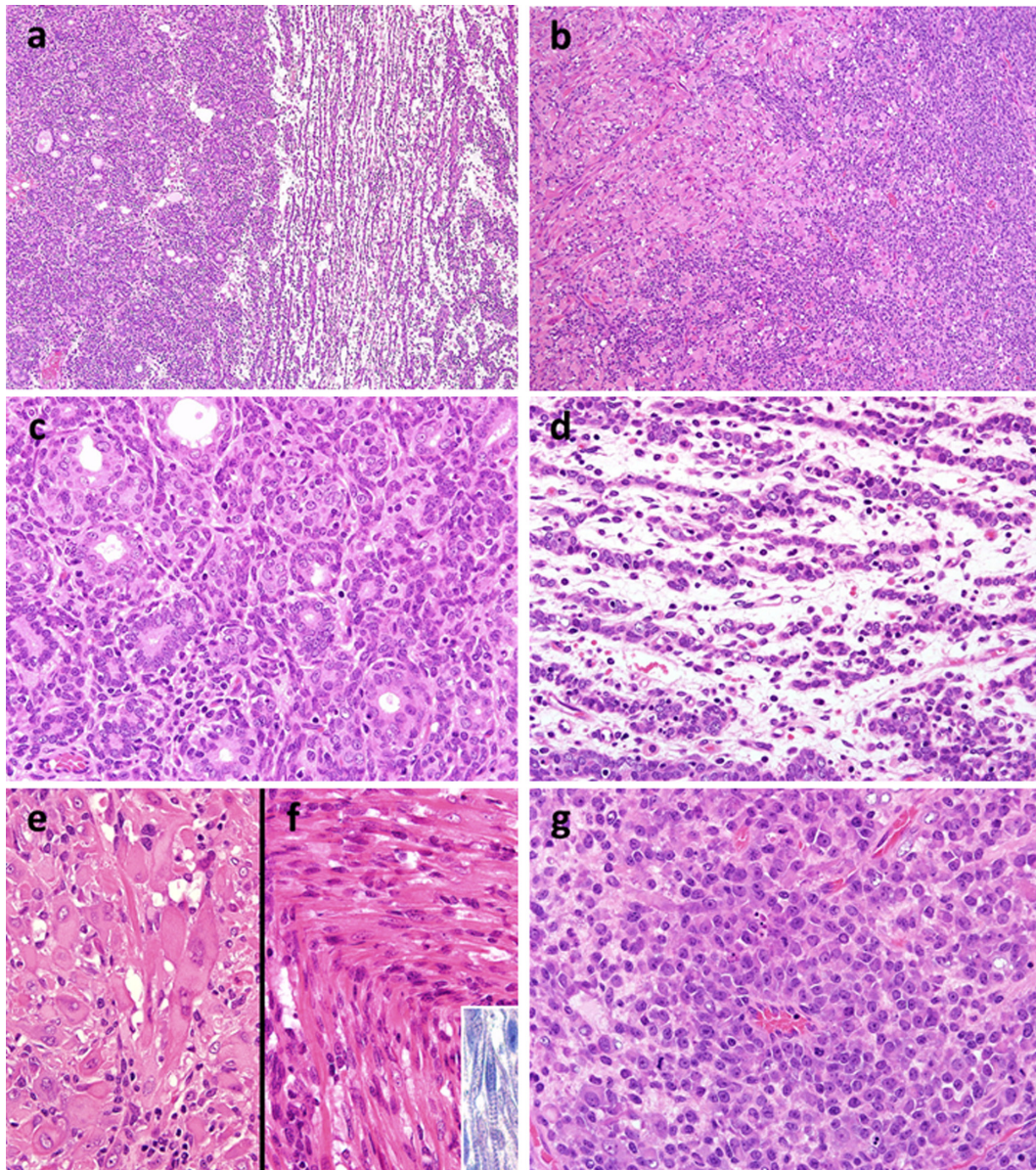
Fig. 2. Hematoxylin and eosin-stained photomicrographs of the tumor. (a) Epithelial tumor cells showing a tubular or cord-like growth pattern. The cell density is high in the area of the tubular growth pattern (left) and low in the area of the cord-like growth pattern (right). Both components are adjacent to each other with a relatively clear border (×80). (b) Rhabdomyosarcomatous tumor cells (eosinophilic area) and undifferentiated tumor cells (basophilic area) are intermingled (×80). (c, d) Higher magnification of tubular (c) and cord-like (d) growth patterns. Both types of tumor cells are cuboidal and have oval nuclei and basophilic cytoplasm. The stroma of the cells with the cord-like growth pattern is edematous, and scattered lymphocytes and macrophages are present (×320). (e) Large pleomorphic rhabdomyosarcomatous cells have a few round to bizarre nuclei with a few nucleoli and eosinophilic cytoplasm (×320). (f) Spindle rhabdomyosarcomatous cells have oval nuclei and eosinophilic cytoplasm. Inset: Cross-striations visible on PTAH staining (×320). (g) Higher magnification of undifferentiated tumor cells. The tumor cells are polygonal with oval nuclei and show a solid proliferation pattern. Mitotic figures are frequently observed (×320).

cytokeratin AE1/AE3 but not for vimentin. Cells with p63 immunopositivity were also seen. There is some research showing that p63 is expressed in normal and neoplastic thymic epithelial cells in rats[7]. In our case, the location of the tumor in the mediastinum as well as the histomorphological and immunohistochemical characteristics of the epithelial tumor cell component suggested that the origin of the tumor was the thymus. Since there have been reports suggesting that expression of p63 decreases depending on the maturity of the cells[8], it was considered that the difference in p63 expression between cells with a cord-like growth pattern and those with a tubular growth pattern may depend on the degree of differentiation.

It is known that thymic tumors contain a small popula-

Table 1. Results of Immunohistochemical Staining

Types of tumor cells	Cytokeratin AE1/AE3	p63	Desmin	Sarcomeric actin	S−100	PCNA	Vimentin	SMA
Epithelial tumor cell								
tubular growth pattern	+	−	−	−	−	++	−	−
cord-like growth pattern	+++	+	−	−	−	+	−	−
Rhabdomyosarcomatous tumor cell	−	−	+++	+++	+	++	−	−
Undifferentiated tumor cell	−	−	++	−	−	+++	−	−

 Criteria for grading: −, negative; +, weak; ++, moderate; +++,strong.
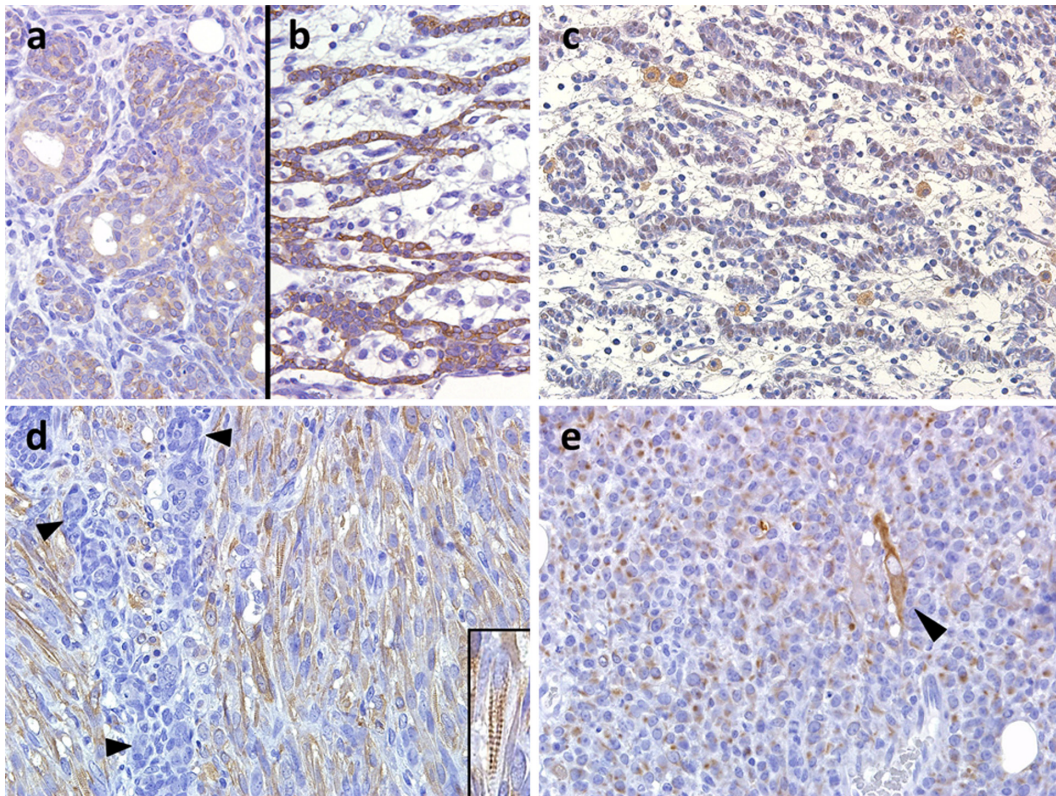
Fig. 3. Immunohistochemical photomicrographs of the tumor (×320). (a, b) Cytokeratin AE1/AE3 immunostaining. Epithelial tumor cells with tubular (a) and cord-like (b) growth patterns react positively for cytokeratin AE1/AE3, but the reactivity is stronger in the cord-like growth pattern. (c) p63 immunostaining. Some epithelial tumor cells with the cord-like growth pattern react positively for p63. (d) Sarcomeric actin immunostaining. Spindle rhabdomyosarcomatous tumor cells stain positively for sarcomeric actin, and cross-striations in the cytoplasm are clearly seen. Epithelial tumor cells (arrowheads) are also observed. Inset: Enlarged image showing cytoplasmic cross-striations. (e) Desmin immunostaining. Parts of the cytoplasm in undifferentiated tumor cells stain positively for desmin. A large pleomorphic rhabdomyosarcomatous tumor cell (arrowhead) can be seen.

tion of myoid cells. In the normal thymus, myoid cells are scattered in the vicinity of Hassall's capsules in the thymic medulla, especially at the corticomedullary junction[9]. Myoid cells are frequently observed in birds[10] and reptiles[11] but are rarely observed in rodents, humans[12], or other mammals (guinea pigs, rabbits, and cattle)[13]. Microscopically, the cells are relatively large with clear oval nuclei and eosinophilic cytoplasm[9]. Electron microscopically, they contain myofibrils and triad-like membranous structures in the cytoplasm. Myoid cells express desmin[14], sarcomeric actin[15], and S-100 protein[16], but not SMA[17] or vimentin[18]. The rhabdomyosar-

comatous tumor cells observed in our case were characterized by large amounts of eosinophilic cytoplasm with cross-striations and immunoreactivity for desmin, sarcomeric actin, and S-100 protein. These features are similar to those of myoid cells. Further, the mass was well encapsulated with fibrous connective tissue, and there was no adhesion to intrathoracic organs, such as striated muscle (esophagus and the thoracic wall). Therefore, the rhabdomyosarcomatous tumor cells found in our case were most likely to be derived from myoid cells in the thymus. In addition, since no independent carcinoma or sarcoma was found in this animal, we

were able to exclude the possibility of a collision tumor involving epithelial thymoma and rhabdomyosarcoma derived from adjacent skeletal muscles.

The presence of undifferentiated tumor cells was another interesting feature in this case. Although the population of this tumor cell type was small in comparison with the populations of the other cell types, undifferentiated tumor cells showed the highest proliferative activity. Further, these cells reacted positively for desmin but not for other antibodies examined. Zółtowska reported that myoid and epithelial cells arise from a common primitive desmin-positive mesenchymal cell existing in the thymus[16]. Therefore, it was speculated that the undifferentiated tumor cells were derived from immature thymic mesenchymal cells before differentiation into myoid or epithelial cells. Although the pathogenesis of the tumor in the present case was not identified, the tumor was mainly characterized by a proliferation of both epithelial and rhabdomyosarcomatous tumor cells and had features similar to those mentioned in previous reports on myoid thymoma[2–4].

Although the malignant potential of thymoma generally depends on whether it invades adjacent tissues and/or metastasizes, it has been reported that benign and malignant thymoma cannot be differentiated completely based on these criteria[4]. Although no invasion or metastasis was observed in our case, we diagnosed this tumor as a malignant tumor based on its histopathologic features, including high proliferative activity, cytologic atypia, and necrotic behavior.

In conclusion, we diagnosed this tumor as a malignant myoid thymoma. A retrospective study of 244 thymomas in F344 rats identified in the National Toxicology Program database reported that only four cases had a myoid component. Further, in one case, the thymoma, which had a myoid component, was diagnosed as a myoid thymoma, which was categorized as a subtype of rodent thymoma[4]. However, its histological and immunohistochemical characteristics were not described in detail. To our knowledge, this is the first case report of a spontaneous malignant myoid thymoma in a rodent.

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