

Lymphoepithelial Thymoma Characterized by Proliferation of Spindle Cells in a Samoyed Dog

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ABSTRACT. Lymphoepithelial thymoma was diagnosed in a 14-year-old Samoyed dog with clinical symptoms of *myasthenia gravis* at 6 months of age. At necropsy, dark red-colored mass with many nodular protuberances was found in the anterior mediastinal area. Histologically, the mass consisted of solid proliferation of neoplastic cells with spindle nuclei and cytoplasm and a few lymphocytes, which is separated by an abundant fibrous and adipose tissue. Immunohistochemically, spindle cells were positive for cytokeratin, and infiltrating lymphocytes were positive for CD3. On the basis of these findings, this tumor was diagnosed as lymphoepithelial thymoma, which is morphologically similar to type A thymoma in humans.

KEY WORDS: canine, spindle cell, thymoma.

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Thymomas are uncommon tumors derived from the epithelial components of the thymus in which there are various proportions of benign lymphocytic infiltration. Thymomas are classified into lymphoid thymoma and lymphoepithelial thymoma on the basis of amount of infiltrating lymphocytes in animals [11]. Dog is known to more frequently develop lymphoid thymoma than lymphoepithelial thymoma [11]. Some of thymoma cases are associated with acquired *myasthenia gravis* (MG) and autoimmune paraneoplastic syndrome [10]. MG, characterized by muscle weakness and megaesophagus, is seen in up to 40 percent of dogs with thymoma [6, 10]. On the other hand, classification of thymoma in human is essentially based on the shape of the neoplastic epithelial cells; whether they adopted an oval or spindle shape (type A), a round or polygonal shape (type B) or a combination of the two (type AB) [2, 7]. To our knowledge, lymphoepithelial thymoma characterized by proliferation of spindle cells has not been reported in animals.

A 14-year-old female Samoyed dog was diagnosed with MG from young age. At 6 months of age the dog showed aspiration pneumonia due to megaesophagus, and muscular atrophy of limbs gradually progressed. Systemic symptoms of MG were clinically exhibited from 3 years of age. The dog died after showing abnormal behavior (circling, prowl etc.), convulsion and coma.

At necropsy, dark red-colored mass of 7 cm in diameter with many nodular protuberances was found in the anterior mediastinal area (Fig. 1). No significant lesions were observed in other organs except for atrophy of wall of the esophagus and skeletal muscles in four limbs.

Tissue samples of systemic organs were fixed in 10% formalin, embedded in paraffin wax, sectioned at 3 μ m, and

stained with hematoxylin and eosin (HE) for light microscopy. Selected sections were stained with Silver stain. For immunohistochemical examination, a standard labeled streptavidin-biotin (LSAB) method (DAKO, Glostrup, Denmark) was employed on paraffin wax-embedded thymoma tissue with rabbit polyclonal antibodies to S-100 protein (DAKO), CD3 (DAKO), CD20 (Thermo, Tokyo, Japan) and mouse monoclonal antibodies to cytokeratin (clone AE1/AE3) (DAKO), smooth muscle actin (clone 1A4) (DAKO), Ki-67 protein (clone MIB-1) (DAKO) and vimentin (clone V9) (YLEM, Roma, Italy). Some formalin-fixed samples of thymoma tissue were rinsed in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in alcohol, and embedded in epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a TEM-100CX electron microscopy (Japan Electron Optics Laboratory, Tokyo).

Histopathologically, the neoplastic mass in the anterior mediastinal area showed lobular structure, which was separated by an abundant fibrous and adipose tissue (Fig. 2). The mass consisted of neoplastic cells with spindle nuclei and cytoplasm and a few lymphocytes (Fig. 3). The neoplastic cells with spindle nuclei and spindle and eosinophilic cytoplasm showed mild to moderate atypism and few mitotic figures. Cornification of the neoplastic cells and Hassall's bodies were not seen. Reticular fibers were not detected around the spindle cells in Silver stain. Immunohistochemically, the neoplastic spindle cells were most positive for cytokeratin (Fig. 4) and negative for vimentin, smooth muscle actin and S-100. A few neoplastic cells showed positive for Ki-67. Infiltrating lymphocytes were positive for CD3, suggesting T cells (Table 1). Ultrastructurally, tonofilament bundles were observed in the cytoplasm of spindle cells (Fig. 5). In the skeletal muscle and esophagus, diffuse atrophy of striated muscles, mild fibrosis and fatty infiltration were found, whereas there were no

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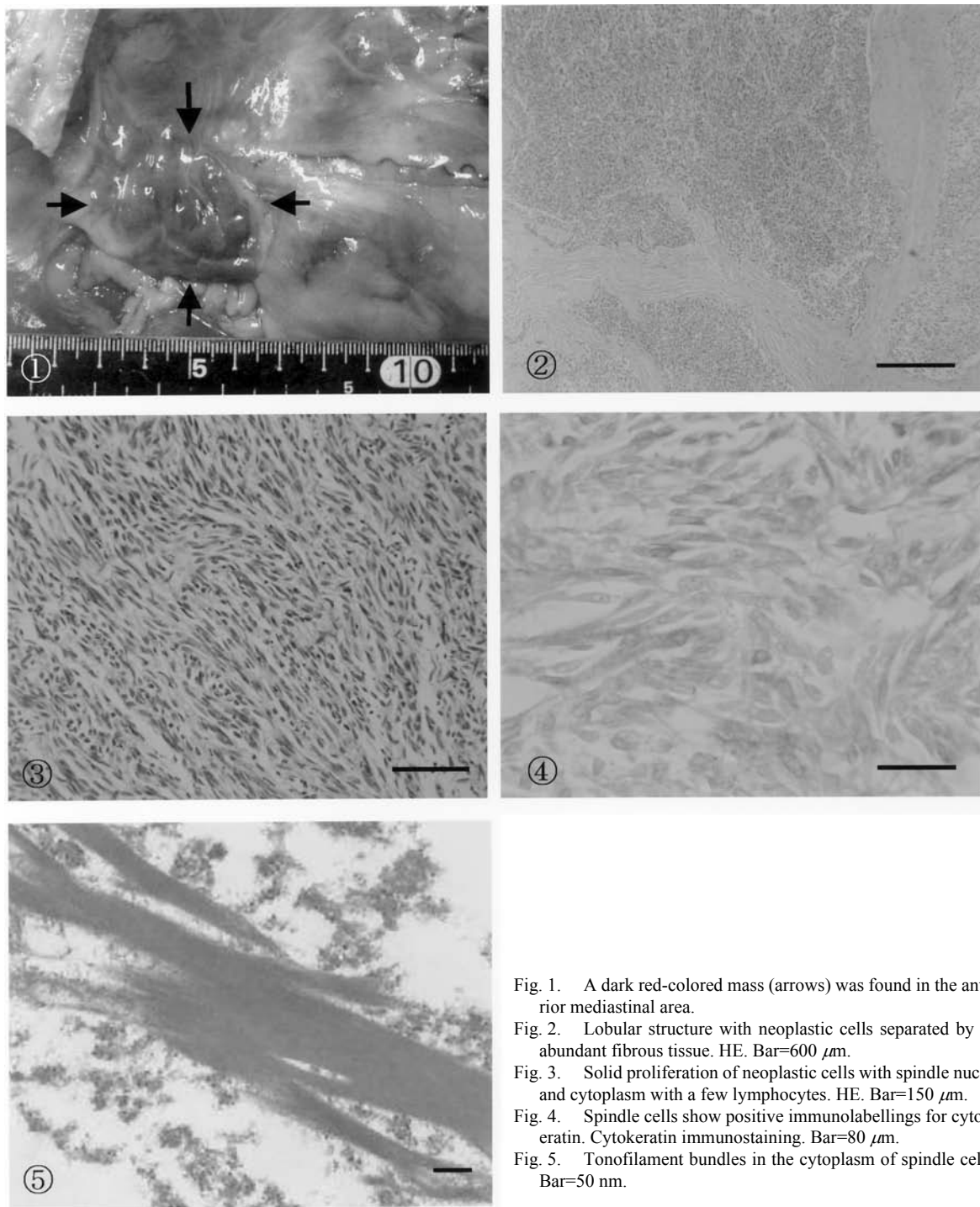


Fig. 1. A dark red-colored mass (arrows) was found in the anterior mediastinal area.

Fig. 2. Lobular structure with neoplastic cells separated by an abundant fibrous tissue. HE. Bar=600 μ m.

Fig. 3. Solid proliferation of neoplastic cells with spindle nuclei and cytoplasm with a few lymphocytes. HE. Bar=150 μ m.

Fig. 4. Spindle cells show positive immunolabellings for cytokeratin. Cytokeratin immunostaining. Bar=80 μ m.

Fig. 5. Tonofilament bundles in the cytoplasm of spindle cells. Bar=50 nm.

findings suggesting myositis or myopathy.

Pathological findings of the present case can be summarized as follows: 1) the dark red-colored tumor tissue was found in the anterior mediastinal area, 2) the neoplastic lesion consisted of proliferation of spindle cells and a few lymphocytes, 3) the spindle cells were immunohistochemi-

cally positive for cytokeratin and negative for vimentin, which suggests that the neoplastic cells were derived from epithelial cell line, and 4) tonofilament bundles were identified in spindle cells. Tonofilament bundles have been reported to be a distinctive feature of epithelial thymoma [4, 8]. Based on these findings, this tumor was diagnosed as

Table 1. Immunohistochemical labeling in spindle cells and infiltrating lymphocytes

Antibody	Spindle cells	Infiltrating lymphocytes
Cytokeratin	+	–
Vimentin	–	–
S-100	–	–
Smooth muscle actin	–	–
CD3	–	+
CD20	–	–
Ki-67	±	–

+, Positive; ±, Weakly positive; –, Negative.

lymphoepithelial thymoma. The characteristic finding that neoplastic cells have spindle-shaped cytoplasm is similar to that in type A thymoma in humans. World health organization classification of thymoma in humans is essentially based on the shape of the neoplastic epithelial cells; whether they adopted an oval or spindle shape (type A) [2, 7], a round or polygonal shape (type B) or a combination of the two (type AB). A fourth category, thymoma type C (thymic carcinoma), was also introduced and defined as a tumor showing overt cytological features of malignancy, independent of the shape of the cells [2, 9]. Type A thymoma is also admixed in variable proportions with small (usually few) lymphocytes. In addition, patients who has type A thymoma develop complication of MG less than compared with patients who had type B thymoma [7]. This is, to our knowledge, the first report in animals of lymphoepithelial thymoma characterized by spindle shape cells, as has been described in human type A thymoma.

Because the proliferation of spindle cells was observed, as the differential diagnosis for the present case, mesenchymal tumors with feature of spindle shape are adopted. The first differential diagnosis is malignant schwannoma. Immunohistochemically, the neoplastic cells are positive for S-100 protein, and ultrastructurally, external basal lamina around neoplastic cells are confirmed [3]. The second differential diagnosis is fibrosarcoma, which has neoplastic spindle cells. Immunohistochemically, these neoplastic cells are positive for vimentin [12]. The third differential diagnosis is leiomyosarcoma, which also has neoplastic spindle cells. Immunohistochemically, the neoplastic cells are positive for smooth muscle actin [1]. The morphology in this case is similar to that in these tumors, however, immunohistochemical and ultrastructural findings enabled us to exclude a diagnosis of these nonepithelial tumors. Type C carcinoma is defined as a tumor exhibiting clear-cut cytological atypia [9]. The present case would be distinguished from thymic carcinoma because of its small atypia.

Acquired MG has been considered to occur as a result of disruption or dysfunction of motor end plate acetylcholine receptors (AChRs) due to deposition of circulating autoantibody at the neuromuscular junction [10]. The normal thymus contains myoid cells that express muscle proteins and

AChRs. It is thought that induction of self-recognition of these proteins occurs due to thymoma. The present case showed clinical symptoms of MG at 6 months of age. This indicates that the present tumor may exist before then, additionally, it is possible that the tumor may be congenitally formed. In humans, some cases of ectopic hamartomatous thymoma (EHT) have been reported [5]. EHT is considered to be a benign tumor which is believed to be derived from remnants of the cervical sinus of His. EHT usually occurs subcutaneously in the lower neck, and has characteristic features of triphasic with spindle cell elements, adipocytes and epithelial elements. Thus, developmental region and histological features of the present case were different from those of EHT.

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