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Comparative Medicine Section

Spontaneous Benign Thymoma in a 10-Week-Old Female ICR (CD-1) Mouse

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Thymomas are tumors originating from thymic epithelial cells and are uncommon in animals, including pets and domestic and laboratory animals. A 10-wk-old female ICR (CD-1) mouse in the control group of a toxicity study presented a thymic mass consisting of epithelial and lymphocytic components. Histopathological evaluation showed epithelioid neoplastic cells intermixed with small lymphocytes and areas of hemorrhage. Immunohistochemistry revealed positivity for proliferating cell nuclear antigen and pan-cytokeratin (AE1/AE3), and negativity for S100 and vimentin. Based on these features, the mass was diagnosed as a benign thymoma. To the best of our knowledge, this is the first report of a thymoma in a laboratory mouse at such a young age.

Abbreviations and Acronyms: IHC, immunohistochemistry; INHAND, International Harmonization of Nomenclature and Diagnostic Criteria for Lesions; PCNA, proliferating cell nuclear antigen

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Introduction

Thymic epithelial tumors, including thymomas, are of considerable biologic interest due to their association with paraneoplastic syndromes, particularly myasthenia gravis in humans and animals.[1] Thymomas originate from thymic epithelial cells and are generally slow-growing, well-encapsulated tumors infiltrated by lymphocytes.[2–4] According to the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND), thymomas in rats and mice are characterized by solid growth of epithelial tubules and cords centrally located within thymic lobules.[5] INHAND criteria also classify benign thymomas into 2 main subtypes, that is, epithelial and spindeloid types, based on the predominant cell morphology in rats and mice. Metastasis is extremely rare, and the incidence of thymomas in humans and animals tends to increase with age.[6,7] The World Health Organization classifies human thymomas into 6 subtypes: type A (medullary or spindle cell thymoma), type AB (mixed thymoma), type B1 (predominantly cortical thymoma), type B2 (cortical thymoma), type B3 (well-differentiated thymic carcinoma), and type C (thymic carcinoma).[6,8] In contrast, animal thymomas are classified based on predominant cell type into lymphoid, epithelial, or mixed types.[3] Meanwhile, there have been several studies and case reports of thymomas in animals. Thymomas are commonly associated with older animals, with the average age of onset in dogs being ~9 to 10 y,[7,9,10] and there have been reports of thymomas in cats, dogs, sheep, goats, rats, rabbits, monkeys, and the Java sparrow.[2,4,11–20] In rodents, thymoma is common in Wistar Han rats. Especially in female Wistar Han rats, lymphoid subtype thymoma occurred with a high frequency of 13.5%. In addition, thymomas have also been reported in other rat strains, including Fischer 344 (F344/N), Sprague–Dawley (Ico:OFA-SD), and Wistar (RccHan:WIST) rats, although with a relatively lower frequency compared with Wistar Han (Han/Wistar) rats.[21–24] In rats, thymomas occur in various strains at varying frequencies, whereas in mice, there are reports of thymoma in NZB × CFW mice, which is a thymoma-prone strain.[25] In European hamsters (*Cricetus cricetus*), another rodent species, thymomas have been reported in 22 out of 40 individuals, showing a frequency of 55%. Among these 22 thymomas, 18 were B1 type and 2 each were B1/AB and AB/B1, based on the World Health Organization classification.[26] However, reports of spontaneous thymomas in young laboratory mice are exceedingly rare. Differential diagnoses of thymomas include lymphoma, thymic carcinoma, schwannoma, fibrosarcoma, and leiomyosarcoma.[27]

Herein, we report a case of a spontaneous benign thymoma in a 10-wk-old ICR (CD-1) mouse. The rarity of this occurrence in a young laboratory mouse highlights its significance, raising considerations about genetic predisposition, early-onset tumorigenesis, and implications for toxicologic pathology studies.

Case Description

The animal described here was a female ICR (CD-1) mouse (Orient Bio, Seongnam, Republic of Korea), allocated to the control group in a 4-wk main study followed by a 2-wk recovery phase of an oral toxicity study. All mice were fed a pellet diet (Envigo RMS, Indianapolis, IN) and provided with tap water ad libitum. They were housed in metal cages within a barrier facility maintained at 23 ± 3 °C and 50% ± 5% relative humidity on a 12-h light/12-h dark cycle. Mice were housed in solid-bottom metal cages lined with hardwood bedding material. The study protocol was approved by the IACUC of Biotoxtech (approval no. 180789). Regardless of test group assignment, animals received 0.5% CMC-Na with Tween 80 as a vehicle at a dose of 10 mL/kg. Necropsy, clinical pathology, and histopathologic evaluation were conducted following euthanasia. For histopathologic examination, organs and tissues were fixed in 10% neutral-buffered formalin, routinely processed,

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embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Immunohistochemistry (IHC) was performed using the following primary antibodies: rabbit monoclonal anti-S100 alpha antibody (1:1000, catalog no. ab183979, Abcam, Cambridge, MA), anti-vimentin antibody (1:200, catalog no. ab92547, Abcam), anti-pan-cytokeratin (AE1/AE3) antibody (1:1000, catalog no. ab308262, Abcam), and anti–proliferating cell nuclear antigen (PCNA) antibody (1:100, catalog no. ab92552, Abcam). Antigen retrieval was conducted using citrate buffer (pH 6.0) for 10 min. Sections were incubated with primary antibodies overnight at 4 °C, followed by a secondary antibody (EnVision HRP-conjugated anti-rabbit; Dako, Agilent Technologies, Santa Clara, CA) for 1 h at room temperature. DAB was used as the chromogen, and hematoxylin was applied for counterstaining. AE1/AE3 was used to confirm epithelial origin, vimentin to assess mesenchymal differentiation, S100 to rule out neural crest–derived tumors, and PCNA to evaluate proliferative activity relative to adjacent normal thymic tissue. Adjacent normal thymic tissue was used as an internal control for AE1/AE3 antibody. Appropriate positive (normal epithelial tissue) and negative (primary antibody omitted) controls were included for all IHC procedures.

The thymic mass, measuring 1.3 mm in diameter, was not grossly visible at necropsy but was discovered incidentally during histopathologic examination. Due to its small size, it likely did not cause any clinical symptoms. Histologically, the tumor appeared as a distinct, partially encapsulated mass within the thymus, clearly demarcated from the adjacent normal thymic tissue. Although generally encapsulated, focal areas showed partial disruption and compression of adjacent thymic parenchyma. The neoplastic lesion was predominantly composed of epithelioid cells arranged primarily in solid cords and trabecular structures, consistent with thymic epithelial proliferation according to INHAND criteria. These epithelial tumor cells exhibited round to oval nuclei with finely stippled chromatin, and moderate amounts of lightly eosinophilic cytoplasm, with minimal cellular atypia or pleomorphism. The arrangement in cords and trabeculae was interspersed with areas of lymphocytic infiltration containing numerous small mature lymphocytes. Hemorrhagic foci and small cystic spaces containing erythrocytes and occasional hemosiderin deposits were present within lymphocyte-rich regions. In certain regions, the epithelial cords appeared to infiltrate these lymphocyte-dominated areas, forming distinct epithelial islands surrounded by lymphocytes (Figure 1). IHC results demonstrated that the neoplastic cells were negative for S100 and vimentin, but showed strong cytoplasmic reactivity for AE1/AE3 and moderate nuclear positivity for PCNA (Figure 2). AE1/AE3 confirmed the epithelial nature of the tumor. PCNA expression was observed in ~80% of neoplastic cells, compared with <5% in adjacent normal thymic epithelial cells. AE1/AE3 is a marker that indicates that a thymoma originates from epithelial cells.[28,29] No other significant findings were observed in clinical pathology or histopathology of other organs. Taken together, the histopathologic and immunohistochemical features confirmed the diagnosis of benign thymoma.

Discussion

Although thymomas have been reported in pets and domestic animals, they rarely occur spontaneously in laboratory animals.[3,30] However, juvenile thymomas have been documented in humans and other species, suggesting that early-onset thymomas can arise across species, possibly due to intrinsic genetic or developmental factors.[31] In juvenile animals, differential diagnoses for mediastinal tumors include lymphoma, germ cell tumors, neurogenic tumors, and metastatic lesions. In the present case, these differential diagnoses were ruled out based on morphologic and immunohistochemical findings. Recent studies have established genetically engineered mouse models of thymic epithelial tumors involving PI3K and TP53 pathway dysregulation, providing new insights into thymoma pathogenesis and supporting the translational value of such tumors in toxicologic pathology studies.[32] Despite limitations in scope, a previous study evaluating animals that sporadically died before week 50 found no spontaneous thymomas in 101 ICR (CD-1) mice and 48 Sprague–Dawley rats, indicating the rarity of thymomas in young laboratory rodents.[33] Based on INHAND classification, thymomas are categorized by epithelial cell morphology, and in rodents, thymomas are commonly divided into epithelial and spindeloid subtypes. The epithelial type comprises >80% epithelial cells, whereas the spindeloid type consists of fusiform epithelial cells, and may also exhibit features of medullary differentiation.[5] In the present case, epithelioid-shaped neoplastic cells were observed with small lymphocytes. The differential diagnoses included lymphoma, schwannoma, and fibrosarcoma. Nonneoplastic differential diagnoses such as thymic hyperplasia, thymic cysts, and thymic involution were ruled out based on the absence of cystic structures and the lack of epithelial lining indicative of cysts. The cavitary areas observed within the mass were interpreted as secondary to tumor-associated hemorrhagic changes and degenerative alterations, rather than true cyst formation or necrosis. Although focal disruption into the adjacent thymic parenchyma was present, the lesion remained largely well-circumscribed without evidence of vascular invasion or significant nuclear atypia. These histopathologic features are

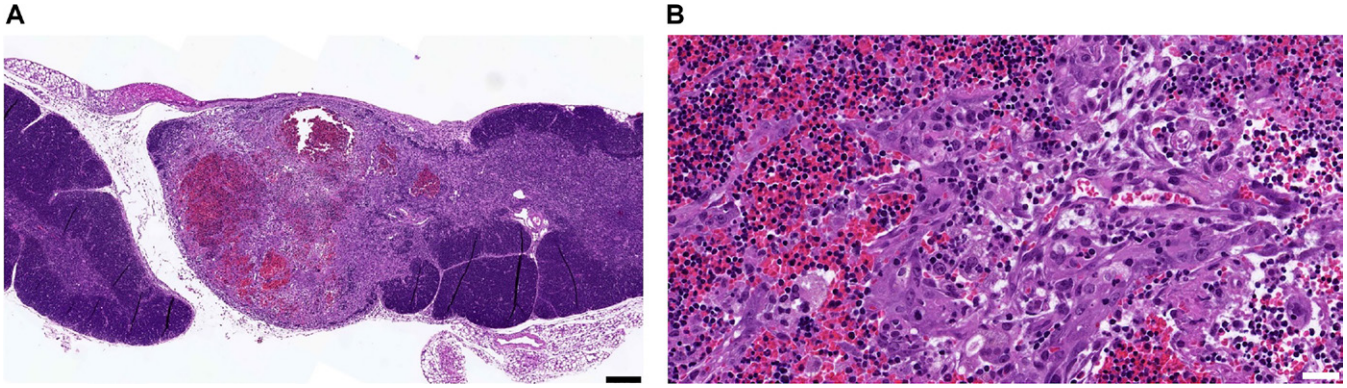

Figure 1. The mass of the thymus composed of epithelial area and lymphocytes area. (A) Low-power field. (B) High-power field. Scale bars, 200 µm (black) and 100 µm (white).

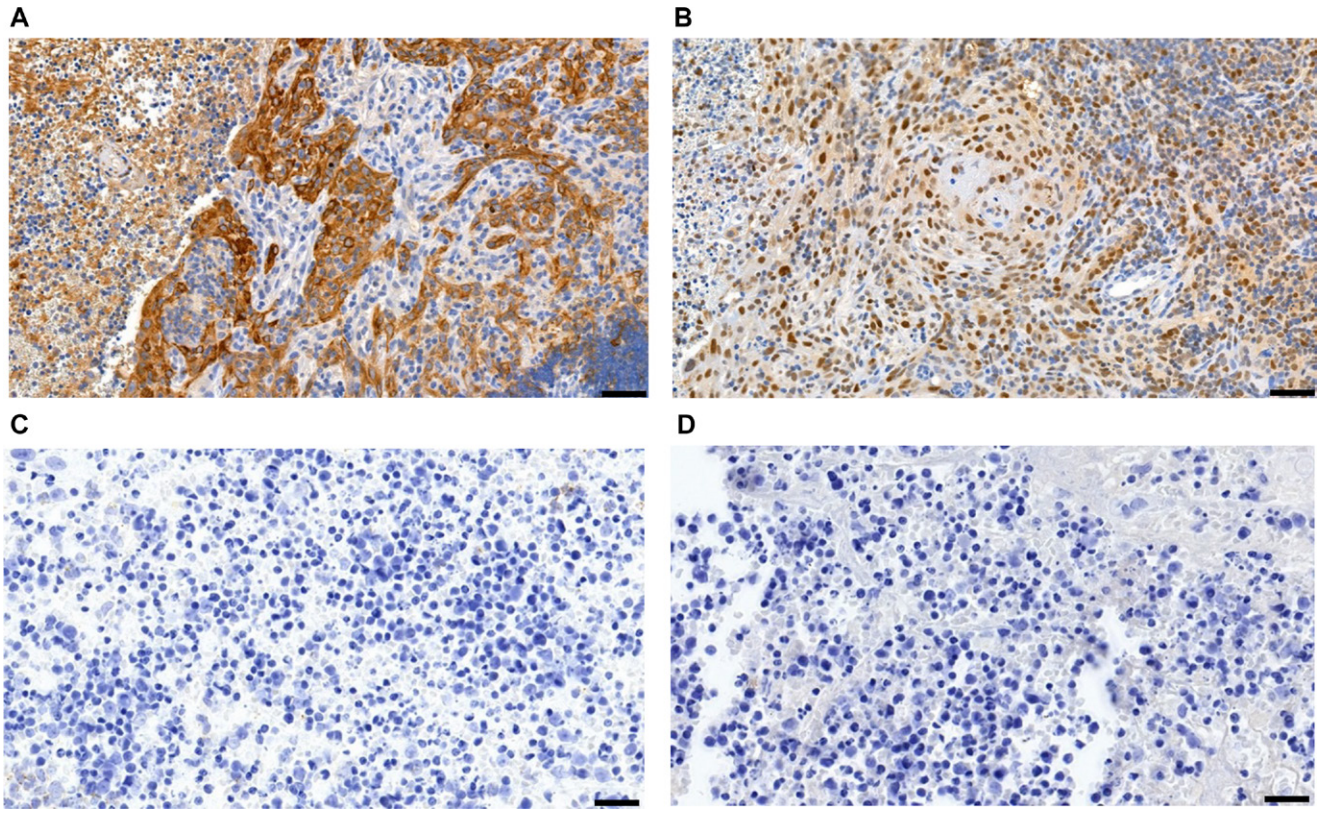

Figure 2. In IHC, the neoplastic cells were positive for pan-cytokeratin (AE1/AE3) (A) and PCNA (B), but negative for vimentin (C) and S100 (D). Scale bar, 50 µm.

consistent with a diagnosis of benign thymoma according to the INHAND criteria for rodent thymic tumors. Differential diagnosis between thymic hyperplasia and thymoma is essential in laboratory rodents. While thymic hyperplasia presents as a diffuse expansion of thymic tissue with polyclonal lymphocytic proliferation, thymomas are characterized by discrete, encapsulated masses composed predominantly of neoplastic epithelial cells. In this case, the presence of a well-circumscribed epithelial proliferation with AE1/AE3 reactivity and the absence of diffuse thymic involvement ruled out hyperplasia. Immunoreactivity of the neoplastic cells revealed that S100 and vimentin were negative whereas PCNA and AE1/AE3 were strongly positive in nuclear and cytoplasm, respectively. The tumor exhibited a well-differentiated epithelial component with minimal nuclear pleomorphism and no evidence of necrosis or vascular invasion, which are hallmark features of thymic carcinoma. In addition, the absence of metastasis further supports the classification as a benign tumor. Based on these results, a benign thymoma was diagnosed. In laboratory rodents, spontaneous thymomas are uncommon and primarily observed at low frequencies (0.18% to 1.9%) in aged populations.[34–36] Previous studies specifically reported a very low incidence of spontaneous thymomas in CD-1 mice, with only 3 confirmed cases out of 325 control mice, all in females.[37] Our observation of a spontaneous thymoma in a 10-wk-old CD-1 mouse thus represents an exceptionally rare finding and may suggest an underlying genetic or developmental susceptibility. Furthermore, the importance of distinguishing reactive from neoplastic thymic lesions in rodents has been reported, as has the incidence and morphology of spontaneous thymic proliferative lesions in aging CD-1 mice.[34] The absence of thymomas in young CD-1 mice across these studies highlights the rarity and scientific value of the current case. Our case is unique in that a thymoma spontaneously developed in a 10-wk-old ICR (CD-1) mouse.

Conflict of Interest

The authors have no conflicts of interest to declare.

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