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Case Report

A spontaneous thymic carcinosarcoma in a young Sprague Dawley rat

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Abstract: We encountered a case of spontaneous thymic carcinosarcoma in a young Crl:CD (Sprague Dawley) rat. Grossly, a white multinodular mass replaced the thymus in the thoracic cavity. Histologically, multiple nodules were separated by fibrous stroma, and each nodule included isolated regions that were composed of epithelial or non-epithelial tumor cells. The epithelial tumor cells were relatively large and round to polygonal cells with large nuclei and weakly eosinophilic cytoplasm. These cells were cytokeratin-positive and vimentin-negative. These cells infiltrated the lungs. The non-epithelial tumor cells were poorly differentiated, small, round to spindle-shaped cells with small nuclei and basophilic cytoplasm. These cells were vimentin-positive and mostly cytokeratin-negative. Many islands of cartilage were observed near non-epithelial cells. Based on these findings, the tumor was diagnosed as a primary thymic carcinosarcoma consisting of a malignant thymoma composed of epithelial tumor cells and a mesenchymal chondrosarcoma composed of non-epithelial tumor cells. (DOI: 10.1293/tox.2020-0073; J Toxicol Pathol 2021; 34: 235–239)

Key words: carcinosarcoma, thymus, spontaneous, Sprague Dawley rat

Carcinosarcoma is a rare malignant tumor with an epithelial carcinomatous component and a non-epithelial sarcomatous component[1]. In humans, carcinosarcoma can originate in various organs, including the uterus, ovary, breast, esophagus, lung, urinary system, and thymus[2–8]. In rats, only a few cases of spontaneous carcinosarcoma have been reported in the kidney, uterus, and mammary gland[9–11]. Here, we report a rare case of spontaneous thymic carcinosarcoma, consisting of a malignant thymoma and a mesenchymal chondrosarcoma, in a young Sprague Dawley rat.

A 3-week-old male Crl:CD (SD) rat was purchased from Charles River Laboratories Japan (Kanagawa, Japan). The animal was housed in a group of 4 animals used as sentinels for microbiological surveillance under controlled environmental conditions (temperature, 21–25 °C; humidity, 36–67%) as approved by the Institutional Animal Care and Use Committee of Seikagaku Corporation (Tokyo, Japan). A pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were provided *ad libitum*. The animal was kept without treatment until it was found dead at 21 weeks of age without any abnormal clinical signs.

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At necropsy, a white multinodular mass filled the thoracic cavity. The mass surrounded the heart, and the thymus was invisible (Fig. 1). No clear continuity or adhesion between the mass and the lungs was observed. Approximately 10 mL of bloody pleural effusion was found in the cavity.

The mass and intrathoracic organs were removed together, fixed in 10% neutral buffered formalin, and processed routinely for microscopic examination of paraffin-embedded sections (3 μm) stained with hematoxylin and eosin (HE). For special staining, sections were stained with Movat pentachrome (MP). For immunohistochemistry, sections were incubated overnight at 4 °C with primary antibodies against cytokeratin (mouse monoclonal, clone AE1/AE3, ready to use; Dako, Tokyo, Japan), vimentin (mouse monoclonal, clone V9, 1:100; Dako), ki-67 (mouse monoclonal, clone MIB-5, 1:25; Dako), C-ERC/mesothelin (mesothelin, rabbit polyclonal, 0.05 μg/mL; Immuno-Biological Laboratories Co., Ltd., Gunma, Japan), podoplanin (mouse monoclonal, clone LF3 (B7) D5B3, 5 μg/mL; AngioBio Co., San Diego, CA, USA), smooth muscle actin (SMA; mouse monoclonal, clone 1A4, 1:50; Dako), and S-100 (rabbit polyclonal, ready-to-use, Dako). For retrieval of all antigens, deparaffinized sections were heated in 10 mM citrate buffer (pH 6.0) by autoclaving at 121 °C for 10 min. To detect primary antibodies, universal immunoperoxidase polymers (Histofine Simple Stain MAX PO, anti-rat or anti-rabbit; Nichirei, Tokyo, Japan) were used, and staining was completed by incubation with 3,3′-diaminobenzidine tetrahydrochloride, which resulted in a brown precipitate at the antigen site. Mayer's hematoxylin was used as a counterstain. For negative controls, the primary antibodies were replaced

with non-immune sera. The normal lung was examined as a positive control. The percentage of ki-67-positive tumor cells was calculated by counting ki-67-positive cells per approximately 1,200–1,300 tumor cells.

Histopathologically, a mass surrounded the atrophied thymus, which had retained the lobule structures, mainly consisting of lymphocytes, although no clear structure of the thymic cortex and medulla was found. Continuity between the mass and atrophied thymus was observed (Fig. 2a). The mass extended to the adjacent adipose tissue (Fig. 2a) and multiple sites of the lung (Fig. 2b), but not to the heart or esophagus. All mass extensions into the lungs were located only in the lobular periphery. The mass was composed of multiple nodules separated by fibrous stroma (Fig. 2c), and islands of cartilage formation (Fig. 2a, 2c, 2d) and necrosis (Fig. 2b) were found in the nodules.

In almost all nodules, two types of tumor cell were found, and each type was located in isolated regions (Fig. 2d, 2e, 2f). The first type was characterized by solid, glandular, or sheet-like growth. These cells were relatively large and round to polygonal in shape, with large nuclei and weakly eosinophilic cytoplasm, and showed cellular atypia, such as pleomorphism and a clear nucleolus (Fig. 2e). Mitotic figures were sporadically found in this region (Fig. 2e). Small necrotic foci of the first type of tumor cell were occasionally observed. The mass extensions into the lungs consisted primarily of the first type of tumor cell (Fig. 2b′). The second type of tumor cell was characterized by solid growth of poorly differentiated small round to spindle-shaped cells with small nuclei and basophilic cytoplasm (Fig. 2f). Mitotic figures were sporadically observed in this region. In addition, lace-like eosinophilic matrices, as well as many islands of cartilage consisting of well-differentiated chondrocytes and cartilage matrix, were found near the second type of tumor cell at a high frequency (Fig. 2d). One or several aggregated atypical chondrocytes with large nuclei were found around some of these islands of cartilage. No inflammatory cells, including neutrophils, macrophages, or lymphocytes, were found.

MP stained the matrices of the islands of cartilage strongly blue, and the lace-like eosinophilic matrices weakly or strongly blue. Collagen fibers that separated the nodules were stained red (Fig. 2c).

Immunohistological staining showed that the first type of tumor cell was positive for cytokeratin and negative for vimentin, while the second type of tumor cell was mostly positive for vimentin and negative for cytokeratin (Fig. 3a, 3b, 3c). Both types of tumor cells were negative for S-100, but the chondrocytes in the islands of cartilage were positive for S-100 (Fig. 3d). Both types of tumor cells were partly positive for ki-67 and negative for mesothelin, podoplanin, and SMA. The percentage of ki-67-positive tumor cells was 24.1% for the first type of tumor cell, and 23.1% for the second type of tumor cell, although the values of the second type of tumor cell varied (9.2–32.8%) among the 10 different fields observed.

In the present case, approximately 10 mL of bloody pleural effusion was found in the cavity. Hemorrhage from the mass or rhexis of the thoracic aorta due to extension of the mass was considered as possible causes of the effusion.

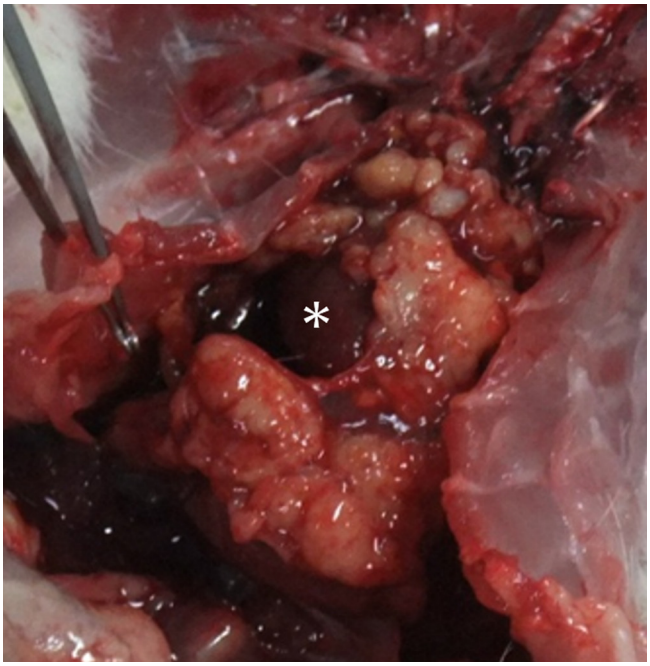

Fig. 1. Macroscopic view of the thoracic cavity. A white multinodular mass (approximately 4 × 3 cm) is surrounding the heart (asterisk). The thymus is invisible.

As described previously, the tumor grossly filled the thoracic cavity, and the thymus was invisible. Histologically, continuity between the tumor and atrophied thymus was found. Therefore, the tumor was considered to originate from the thymus and grew as though it replaced the thymus. The first type of tumor cell was considered to be epithelial, while the second type was non-epithelial according to the immunohistochemical results for cytokeratin and vimentin.

The epithelial cells were considered to be components of the malignant thymoma because the tumor site, morphology, and growth pattern of the first type of tumor cell, that is, round to polygonal with sheet-like, glandular, or solid architecture, were consistent with the features of a thymoma. The thymoma component was considered to be malignant because of cellular atypia, sporadic mitotic figure, high percentage of ki-67-positive cells, infiltration of the surrounding tissues, and presence of necrotic foci.

Thymoma is a cytokeratin-positive thymic epithelial cell tumor characterized by a variable admixed population of lymphocytes[12, 13]. The arrangement and appearance of neoplastic epithelial cells in thymomas vary considerably[12]. Tumor cells of thymoma are known to exhibit a variety of growth patterns, including sheet-like, cord-like, nodular, tubular, or intracystic papillary growth[12]. In a malignant thymoma, there is infiltration to adjacent tissues, but metastasis is rare[12]. In the present case, the location and infiltration sites of the tumor, as well as the cell morphology and growth

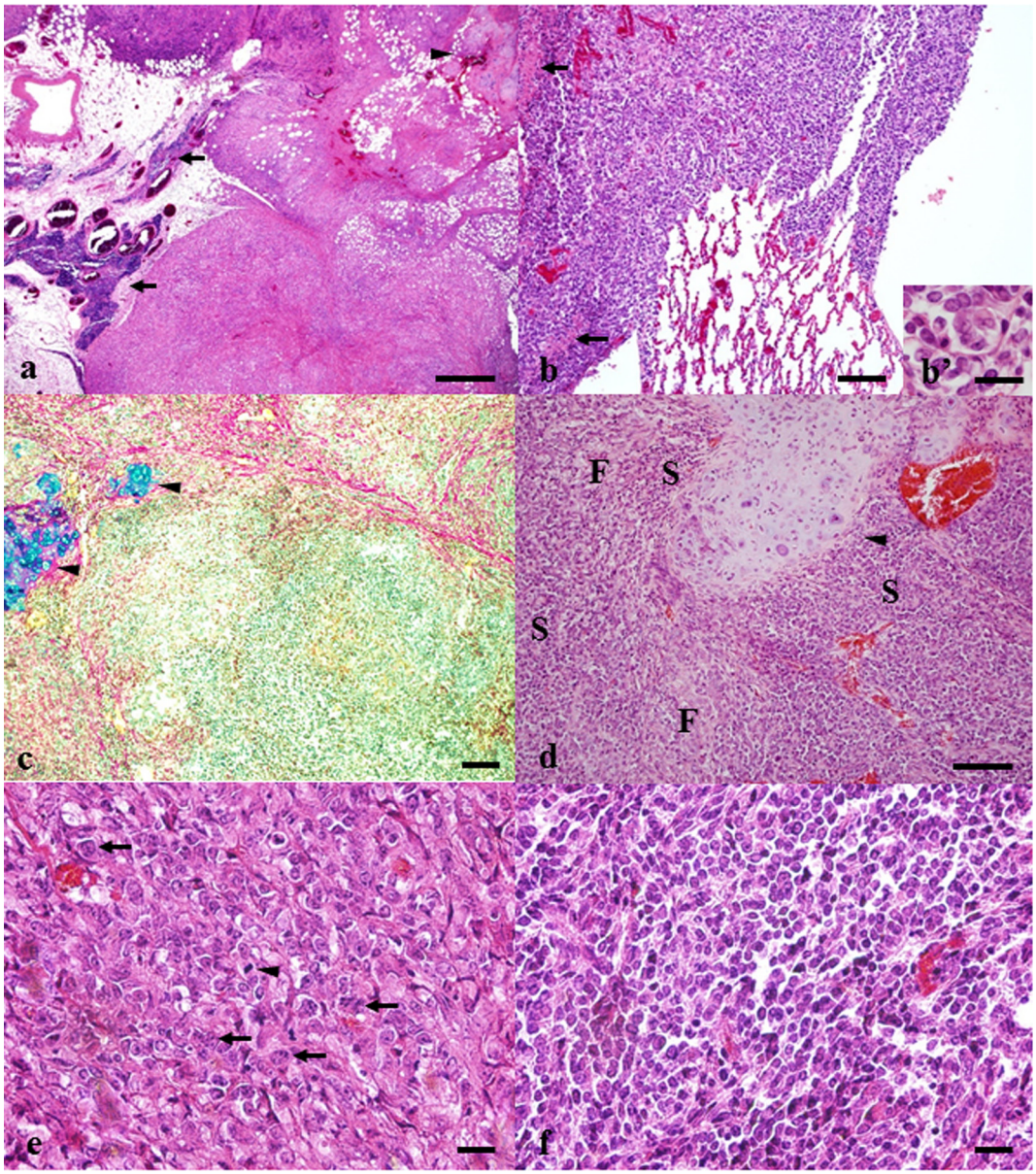

Fig. 2. Photomicrograph of the tumor. (a) Mass is continuous with the severely atrophied thymus (arrows) and extends to the adipose tissue. The mass contains island of cartilage (arrowhead). HE stain. (b) Mass extends to a lobular periphery of the lung. Necrotic foci (arrows) are found in the mass. HE stain. (b') Large and round cells with large nuclei and weakly eosinophilic cytoplasm from Fig. 2b. HE stain. (c) Mass is composed of multiple nodules separated by fibrous stroma (collagen fibers are stained red). The mass contains many islands of cartilage (arrowheads). Movat pentachrome stain. (d) A nodule contains island of cartilage (arrowhead) and 2 distinct regions (F and S). Regions F show weakly eosinophilic and regions S show basophilic. HE stain. (e) Large and round to polygonal cells with large nuclei and weakly eosinophilic cytoplasm compose region F. A part of the cells has clear nucleoluses (arrows). Mitotic figures (arrowhead) are sporadically found. HE stain. (f) Small and round cells with small nuclei and basophilic poor cytoplasm compose region S. HE stain. Bar=500 µm (a), 1 mm (b), 100 µm (c, d), 20 µm (b', e, f).

pattern of the epithelial tumor cells, were consistent with the features of a malignant thymoma. Based on the histopathological features, the epithelial component was considered a malignant thymoma with epithelial predominance.

A bronchiolo-alveolar carcinoma was considered as a differential diagnosis in view of tumor extension into the lungs. However, the present case was not identified as a bronchiolo-alveolar carcinoma, because tumor cell infiltration was localized only in the lobular periphery of the lungs.

The non-epithelial component was identified as a mesenchymal chondrosarcoma, as defined by the World Health Organization (WHO) for humans[14], because the morphology and growth pattern of the second type of tumor cell, that is, poorly differentiated, small, round to spindle-shaped cells, and many islands of cartilage containing tumor cells surrounded by well-differentiated S100-positive chondro-

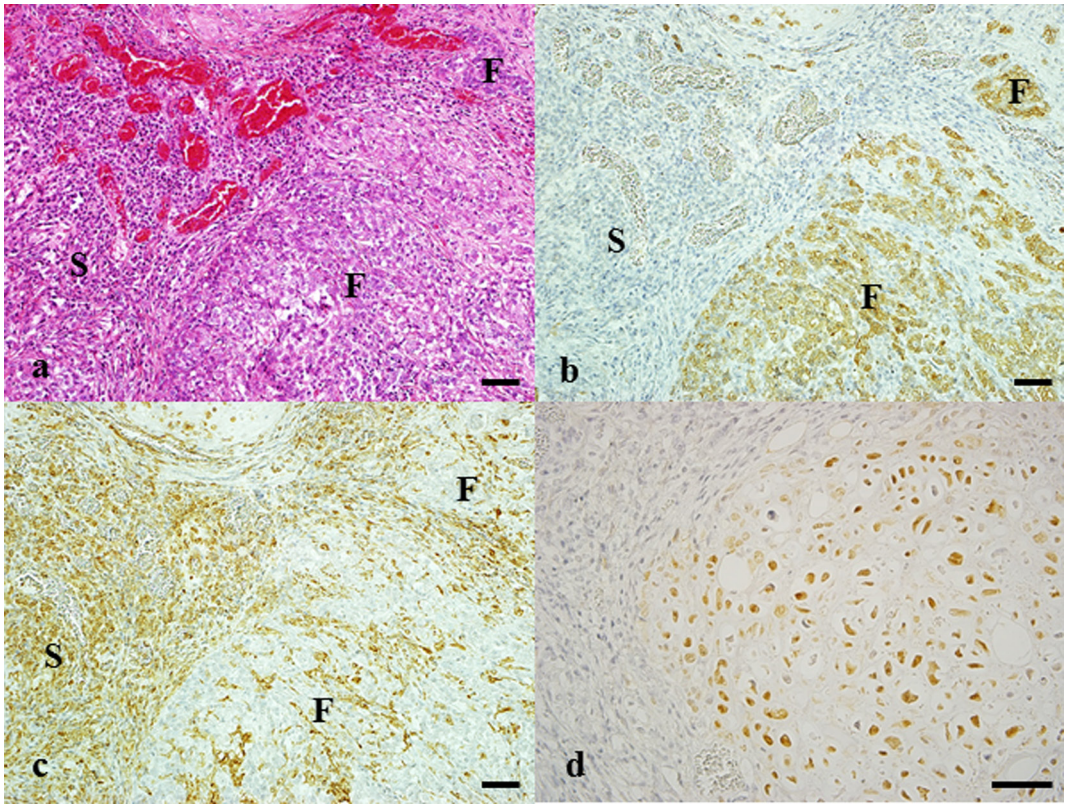

Fig. 3. Immunohistochemistry of the tumor cells. (a, b, c) Serial sections. The area of first type of tumor cell (F) and second type of tumor cell (S) are positive for cytokeratin and vimentin, respectively, and the staining property of 2 types of antibodies does not overlap. (d) The chondrocytes in the island of cartilage are S100-positive, but the tumor cells around the island of cartilage are S100-negative. (a) HE stain. (b) Cytokeratin immunostaining. (c) Vimentin immunostaining. (d) S-100 immunostaining. Bar=50 µm (a, b, c, d).

cytes, were consistent with the features of a mesenchymal chondrosarcoma.

Mesenchymal chondrosarcoma is a rare, high-grade malignant tumor of the bone or soft tissue[15]. Although the tumor cells in typical chondrosarcoma are well-differentiated large cells[16], the tumor cells with diffuse growth in mesenchymal chondrosarcoma are poorly differentiated, small, nearly round to spindle-shaped, and vimentin-positive[15]. In nests of these cells, differentiated islands of cartilage are sporadically present[15]. In the islands of cartilage, the chondrocytes are S100-positive, and the small tumor cells are S100-negative[15, 17]. In the present case, islands of cartilage consisting of well-differentiated chondrocytes and cartilage matrix are considered a feature described in the literature on mesenchymal chondrosarcoma. Lace-like eosinophilic matrices were considered to be cartilage matrices because they were positive for Alcian blue on MP staining. In addition, the morphology of cartilage, as well as growth, cell morphology, and immunohistochemical features of non-epithelial tumor cells, were consistent with the features of mesenchymal chondrosarcoma. Moreover, mesenchymal chondrosarcoma develops not only in bone tissues, but also in various soft tissues[15]. This was consistent with the location of the tumor. Based on the histopathological features, the non-epithelial component was considered a mesenchymal chondrosarcoma, as defined by the WHO for humans[14].

A malignant mesothelioma was considered the differential diagnosis in view of the location of the tumor, but was ruled out because few cells were positive for both cytokeratin and vimentin, the tumor cells were negative for mesothelin and podoplanin, and no spontaneous malignant mesothelioma with cartilage formation has been reported in any species.

Based on gross morphological and histopathological findings, the tumor was diagnosed as a primary thymic carcinosarcoma consisting of a malignant thymoma and mesenchymal chondrosarcoma. Malignant thymoma comprises the first type of tumor cell, while mesenchymal chondrosarcoma comprises the second type of tumor cell.

The histogenesis of primary thymic carcinosarcoma is explained by the following two hypotheses[18]:

1) Non-epithelial malignant tumor develops due to metaplasia of the epithelial component.
2) Epithelial and non-epithelial malignant tumors develop from multipotential stem cells.

Since the malignant epithelial and non-epithelial components coexisted in multiple nodules in a complex manner in the present case, the second hypothesis may be applicable, although DNA clonality was not analyzed for the non-epithelial or epithelial component. In addition, the possibility

of a collision tumor was considered. A collision tumor is characterized as a mixture of 2 histogenetically distinct malignant cell populations that have arisen in separated primary sites[19]. In the present case, the possibility of a collision tumor was ruled out because 2 types of tumor cells coexisted in almost all nodules and the separated primary sites of each tumor could not be determined.

Spontaneous primary thymic carcinosarcoma has not been reported in rats and has only rarely been reported in humans[8, 20, 21]. Thymic carcinosarcoma is synonymous with sarcomatoid carcinoma and is classified as thymic carcinoma, according to the WHO classification for humans[22]. The incidence of sarcomatoid carcinoma is as low as 7% of thymic carcinoma[21]. In addition, this tumor often infiltrates surrounding organs[23]. In conclusion, based on the histological findings, the tumor was diagnosed as a primary thymic carcinosarcoma comprising a malignant thymoma and a mesenchymal chondrosarcoma. This is the first report of a spontaneous primary thymic carcinosarcoma in a young Sprague Dawley rat.

Disclosure of Potential Conflicts of Interests: The authors declare that there are no conflicts of interest.

References

1. Wick MR, and Swanson PE. Carcinosarcomas: current perspectives and an historical review of nosological concepts. Semin Diagn Pathol. **10**: 118–127. 1993. [Medline]
2. Cantrell LA, Blank SV, and Duska LR. Uterine carcinosarcoma: a review of the literature. Gynecol Oncol. **137**: 581–588. 2015. [Medline] [CrossRef]
3. Harris MA, Delap LM, Sengupta PS, Wilkinson PM, Welch RS, Swindell R, Shanks JH, Wilson G, Slade RJ, Reynolds K, and Jayson GC. Carcinosarcoma of the ovary. Br J Cancer. **88**: 654–657. 2003. [Medline] [CrossRef]
4. Esses KM, Hagmaier RM, Blanchard SA, Lazarchick JJ, and Riker AI. Carcinosarcoma of the breast: two case reports and review of the literature. Cases J. **2**: 15. 2009. [Medline] [CrossRef]
5. Ishida H, Fujishima F, Onodera Y, Konno-Kumagai T, Maruyama S, Okamoto H, Sato C, Heishi T, Sakurai T, Taniyama Y, Kamei T, and Sasano H. Esophageal carcinosarcoma with basaloid squamous cell carcinoma: a case report and review of the literature. Tohoku J Exp Med. **249**: 255–263. 2019. [Medline] [CrossRef]
6. Braham E, Ben Rejeb H, Aouadi S, Kilani T, and El Mezni F. Pulmonary carcinosarcoma with heterologous component: report of two cases with literature review. Ann Transl Med. **2**: 41. 2014. [Medline]
7. Baschinsky DY, Chen JH, Vadmal MS, Lucas JG, Bahnson RR, and Niemann TH. Carcinosarcoma of the urinary bladder--an aggressive tumor with diverse histogenesis. A clinicopathologic study of 4 cases and review of the literature. Arch Pathol Lab Med. **124**: 1172–1178. 2000. [Medline] [CrossRef]
8. Suarez Vilela D, Salas Valien JS, Gonzalez Moran MA, Izquierdo Garcia F, and Riera Velasco JR. Thymic carcinosarcoma associated with a spindle cell thymoma: an immunohistochemical study. Histopathology. **21**: 263–268. 1992. [Medline] [CrossRef]
9. Inomata A, Hayakawa K, Aoki T, and Hosokawa S. Spontaneous carcinosarcoma originating from the renal pelvis in a rat. J Toxicol Pathol. **29**: 185–189. 2016. [Medline] [CrossRef]
10. Kaspareit-Rittinghausen J, and Deerberg F. Spontaneous malignant mixed müllerian tumors and rhabdomyosarcoma of the uterus in rats. Toxicol Pathol. **18**: 417–422. 1990. [Medline] [CrossRef]
11. Komitowski D, Sass B, and Laub W. Rat mammary tumor classification: notes on comparative aspects. J Natl Cancer Inst. **68**: 147–156. 1982. [Medline]
12. Pearse G. Histopathology of the thymus. Toxicol Pathol. **34**: 515–547. 2006. [Medline] [CrossRef]
13. Willard-Mack CL, Elmore SA, Hall WC, Harleman J, Kuper CF, Losco P, Rehg JE, Rühl-Fehlert C, Ward JM, Weinstock D, Bradley A, Hosokawa S, Pearse G, Mahler BW, Herbert RA, and Keenan CM. Nonproliferative and proliferative lesions of the rat and mouse hematolymphoid system. Toxicol Pathol. **47**: 665–783. 2019. [Medline] [CrossRef]
14. Nakashima Y, Pinieux G, and Ladanyi M. Mesenchymal chondrosarcoma. In: WHO Classification of Tumours of Soft Tissue and Bone, 4th ed. CDM Fletcher, JA Bridge, PCW Hogendoorn, and F Mertens (eds). IARC, Lyon. 271–272. 2013.
15. Shakked RJ, Geller DS, Gorlick R, and Dorfman HD. Mesenchymal chondrosarcoma: clinicopathologic study of 20 cases. Arch Pathol Lab Med. **136**: 61–75. 2012. [Medline] [CrossRef]
16. Fossey S, Vahle J, Long P, Schelling S, Ernst H, Boyce RW, Jolette J, Bolon B, Bendele A, Rinke M, Healy L, High W, Roth DR, Boyle M, and Leininger J. Nonproliferative and proliferative lesions of the rat and mouse skeletal tissues (bones, joints, and teeth). J Toxicol Pathol. **29**(Suppl): 49S–103S. 2016. [Medline] [CrossRef]
17. Nakamura Y, Becker LE, and Marks A. S-100 protein in tumors of cartilage and bone. An immunohistochemical study. Cancer. **52**: 1820–1824. 1983. [Medline] [CrossRef]
18. Okudela K, Nakamura N, Sano J, Ito T, and Kitamura H. Thymic carcinosarcoma consisting of squamous cell carcinomatous and embryonal rhabdomyosarcomatous components. Report of a case and review of the literature. Pathol Res Pract. **197**: 205–210. 2001. [Medline] [CrossRef]
19. Ishiwata I, Ishiwata C, Nagayama T, and Ishikawa H. Histogenesis and culture of human uterine carcinosarcoma. Cancer Res. **41**: 1978–1983. 1981. [Medline]
20. Chalabreysse L, Etienne-Mastroianni B, Adeleine P, Cordier JF, Greenland T, and Thivolet-Bejui F. Thymic carcinoma: a clinicopathological and immunohistological study of 19 cases. Histopathology. **44**: 367–374. 2004. [Medline] [CrossRef]
21. Suster S, and Rosai J. Thymic carcinoma. A clinicopathologic study of 60 cases. Cancer. **67**: 1025–1032. 1991. [Medline] [CrossRef]
22. Marx A, Chan JK, Coindre JM, Detterbeck F, Girard N, Harris NL, Jaffe ES, Kurrer MO, Marom EM, Moreira AL, Mukai K, Orazi A, and Ströbel P. The 2015 World Health Organization classification of tumors of the thymus: continuity and changes. J Thorac Oncol. **10**: 1383–1395. 2015. [Medline] [CrossRef]
23. Moran CA, and Suster S. Thymic carcinoma: current concepts and histologic features. Hematol Oncol Clin North Am. **22**: 393–407. 2008. [Medline] [CrossRef]