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Case Report

Spontaneous histiocytic sarcoma originating from the epididymis in a CD-1 mouse

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Abstract: We report a histiocytic sarcoma originating from the epididymis observed in a 110-week-old male CD-1 mouse in a carcinogenicity study. At necropsy, no lesions were observed in the epididymis. Histologically, a neoplastic lesion was observed in the cauda of the epididymis that was well demarcated from the surrounding tissues. The lesion mainly consisted of spindle-shaped tumor cells with oval to elongated nuclei and abundant eosinophilic or foamy cytoplasm. The tumor cells were arranged in a fascicular pattern, interlacing bundles, or a whorl pattern. The nuclei showed mild atypia with irregular shapes and varied sizes, whereas few mitotic figures and no typical multinucleated cells were observed. The epididymal ducts remained within the neoplastic lesion, and the tumor cells invaded between the epithelium and the smooth muscle layer of the epididymal duct. Immunohistochemically, the tumor cells were positive for vimentin and macrophage markers (Iba1, CD204, F4/80, and Mac-2) but negative for cytokeratin and other mesenchymal cell (α-smooth muscle actin, desmin, CD31, and platelet-derived growth factor receptor-β), neural cell (S-100 and nestin), or Leydig cell markers (calretinin). Proliferating cell nuclear antigen-positive tumor cells were sporadically observed in the lesion. Based on these results, the tumor was diagnosed as a histiocytic sarcoma originating from the epididymis. This report provides additional histopathological evidence of spontaneous histiocytic sarcomas originating from the epididymis of aged mice. (DOI: 10.1293/tox.2024-0022; J Toxicol Pathol 2024; 37: 133–137)

Key words: male reproductive organ, macrophage, histiocyte, neoplasm, carcinogenicity study

Histiocytic sarcomas are common neoplasms arising from the hematolymphoid system in aged mice[1], and invasion by these tumors can occur in lymphoid organs as well as non-lymphoid organs, such as the liver, uterus, vagina, and skin[2, 3]. However, histiocytic sarcomas originating from the epididymis in mice are rare[4–8], and in case of experimental animals, these have only been observed in mice[4]. Previous reports on histiocytic sarcomas of the epididymis have described the morphological and ultrastructural characteristics of the tumor and the differences between epididymal histiocytic sarcoma and uterine or systemic histiocytic sarcoma[6, 7]. Although immunoreactivity for proliferating cell nuclear antigen (PCNA) and S-100 has been reported in these tumors, detailed immunohistochemical evaluations of other antibodies have not been reported. Herein, we describe our experience with a histiocytic sarcoma originating from the epididymis in an aged mouse. This report describes the histological and immunohistochemical characteristics of the tumor.

The animal was a 110-week-old male Crl:CD-1(ICR) mouse (Charles River Laboratories, Raleigh, North Carolina) in the low-dose group of a carcinogenicity study. No treatment-related neoplastic lesions, including histiocytic neoplasms, were observed in any treated animals. The animal was individually housed in a suspended stainless-steel cage, under barrier conditions of 22 ± 4°C with 50 ± 20% relative humidity, 10 times or greater air changes per hour, and a 12-hour light-dark cycle. The animal had free access to a standard laboratory diet (PMI Certified Rodent Diet® #5002) and tap water provided via an automatic water supply system. The animal was observed twice daily (morning and afternoon) for clinical signs, mortality, and moribundity. All animal handling procedures were performed in accordance with the animal welfare bylaws of a contract research organization that is now accredited by AAALAC International.

The animal showed no clinical signs until terminal necropsy. Necropsy revealed bilateral atrophy of the testes, however, there were no gross findings in the epididymis.

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After gross postmortem examinations, all organs routinely assessed in carcinogenicity studies were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E) for histopathological examination. Serial sections of the epididymal lesion were stained with periodic acid-Schiff (PAS) reaction with diastase digestion, Masson's trichrome staining, and immunohistochemically with antibodies for vimentin (clone: D21H3, dilution 1:200, Cell Signaling Technology, Danvers, MA, USA), Iba1 (rabbit polyclonal, dilution 1:500, Fujifilm Wako, Osaka, Japan), macrophage scavenger receptor A (MSR-A: CD204, clone: SRA-E5, dilution 1:200, Transgenic Inc., Kobe, Japan), F4/80 (rabbit polyclonal, dilution 1:500, Proteintech, Rosemont, IL, USA), Mac-2 (clone: M3/38, dilution 1:500, Cedarlane, Burlington, NC, USA), α-smooth muscle actin (α-SMA, clone: 1A4, dilution 1:100, Dako, Glostrup, Denmark), desmin (clone: D33, dilution 1:100, Dako), CD31 (rabbit polyclonal, dilution 1:50, Abcam, Cambridge, UK), platelet-derived growth factor receptor-β (PDGFR-β: vascular pericyte marker, clone: C82A3, dilution 1:200, Cell Signaling Technology), S-100 (rabbit polyclonal, dilution 1:1,000, Dako), nestin (Schwann cell marker, clone: Rat-401, dilution 1:200, Abcam), calretinin (Leydig cell marker, clone: DAK Calret 1, dilution 1:100, Dako), wide spectrum cytokeratin (rabbit polyclonal, dilution 1:100, Abcam), and PCNA (clone: PC10, dilution 1:500, Dako) using Histofine® Simple Stain mouse MAX-PO (mouse stain kit, Nichirei, Tokyo, Japan). Antigen retrieval for immunohistochemical staining was performed using a microwave oven at 98°C with a HistVT One solution (pH 7.0; Nacalai Tesque, Kyoto, Japan) for 10 min. Tissue sections of the normal liver, kidney (renal artery), sciatic nerve, small intestine, and testes from aged mice were concurrently stained immunohistochemically as positive controls of macrophages (Kupffer cells), blood vessels, Schwann cells, proliferating cells, and Leydig cells, respectively.

Histologically, a neoplastic lesion was observed in the cauda of the epididymis, which was well demarcated from the surrounding tissues, replacing the normal epididymal tissue (Fig. 1A). The lesion mainly consisted of spindle-shaped tumor cells with abundant eosinophilic or foamy cytoplasm, indistinct cell boundaries, and oval to elongated nuclei. The tumor cells, resembling fibroblasts, were arranged in a fascicular pattern, interlacing bundles, or a whorl pattern (Fig. 1B). The nuclei of the tumor cells had delicate chromatin and distinct nucleoli, and showed mild atypia with irregular shapes and variations in size (Fig. 1C), whereas few mitotic figures or erythrophagocytosis (Fig. 1C, inset) in focal hemorrhages, and no typical multinucleated giant cells were observed. The epididymal ducts remained within the neoplastic lesion, and the tumor cells invaded between the epithelium and the smooth muscle layer of the epididymal duct (Fig. 1D). Mild neutrophil or lymphocytic infiltration was sporadically observed. Histopathological examination of the other organs showed no proliferative lesions, except in the epididymis.

The tumor cells had diastase-resistant PAS-positive granules in their cytoplasm (Fig. 2A). Masson's trichrome staining revealed no obvious proliferation of collagenous fibers. Immunohistochemically, the tumor cells were positive for vimentin (Fig. 2B) and the macrophage markers Iba1 (Fig. 2C), CD204, F4/80, and Mac-2 (Fig. 2D) but were negative for cytokeratin (Fig. 2E) and other cell markers, such as α-SMA (Fig. 1E), desmin, S-100, nestin (Schwann cell marker) (Fig. 2F), CD31, PDGFR-β (vascular pericyte marker) (Fig. 2G), or calretinin (Leydig cell marker). PCNA-positive tumor cells were sporadically observed in the lesion (PCNA labeling index, 17.2%; 5 high-power fields) (Fig. 2H).

Based on the results of the histopathological examinations described above, the proliferative lesion of the epididymis in the present case was a histiocytic neoplasm. The tumor cells had malignant properties because they invaded beyond the smooth muscle layer into the epithelial side of the normal epididymal ducts and showed mild nuclear atypia. Furthermore, the proliferative activity of the tumor cells was confirmed by PCNA staining. Therefore, the tumor was diagnosed as a histiocytic sarcoma originating from the epididymis. The histological findings of the present case were mostly consistent with the diagnostic features of epididymal histiocytic sarcomas in mice in the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND)[4]: (i) pleomorphic cells ranging from round to fusiform in shape comprising the tumor; (ii) multinucleated giant cells may be present; (iii) eosinophilic foamy cytoplasm with PAS-positive granules; (iv) erythrophagocytosis with the presence of pigments such as hemosiderin; (v) pleomorphic nuclei displaying sharp cleavage and frequent mitotic figures; and (vi) invasion of epididymal tissues and systemic growth, primarily in the liver. However, multinucleated giant cells and systemic growth were absent, and mitotic figures were rarely observed in the present case. Itagaki *et al.* reported that multinucleated giant cells were not observed in epididymal histiogenic tumors, and that there was no systemic growth in organs other than the epididymis[6]. The incidence of histiocytic sarcomas originating from the epididymis in mice is generally very low, and only a few cases have been reported in B6C3F1 mice (eight animals out of 1,970 animals; 0.40%) and CD-1 mice (one animal out of 1,189 animals; 0.08%)[9]. In addition, histiocytic sarcomas in mice are difficult to diagnose because of their variable cellular morphologies, growth patterns, and organ distributions[10]. Therefore, epididymal histiocytic neoplasms can be misdiagnosed as malignant Schwannoma, undifferentiated sarcoma, or Leydig cell tumor[3, 8].

Histiocytic sarcomas originating from the epididymis should be differentiated from these tumors. In the present case, the tumor was composed of spindle-shaped cells resembling those of tumors derived from Schwann cells. However, the tumor cell morphology and cellular arrangement in the present case differed from those of malignant Schwannomas, which typically form nuclear palisades and Verocay bodies with adjacent palisades and intervening cytoplasm. In addition, the tumor cells showed negative immunoreac-

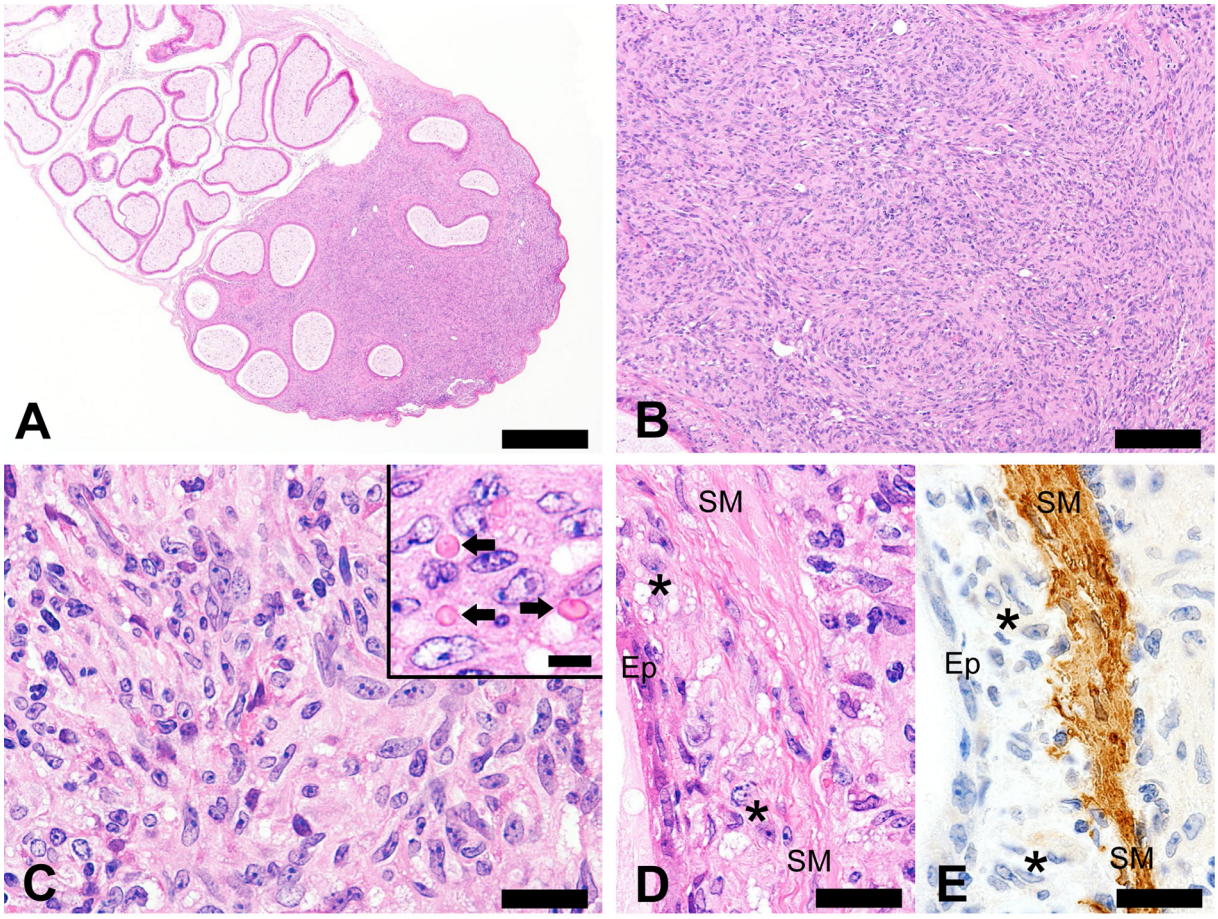

Fig. 1. Histopathological findings of the tumor in the cauda of the epididymis. (A) Low-power magnification of the tumor in the cauda of the epididymis. The tumor is well demarcated from the surrounding tissues and replaced normal epididymal tissue. H&E. Bar=500 µm. (B) Middle-power magnification of the tumor. The tumor mainly consists of fibroblast-like spindle-shaped cells with abundant eosinophilic or foamy cytoplasm. These tumor cells are arranged in a fascicular pattern, interlacing bundles, or a whorl pattern. H&E. Bar=100 µm. (C) High-power magnification of the tumor. The nuclei of the tumor cells have delicate chromatin and distinct nucleoli and show mild atypia with irregular shapes and varied sizes. H&E. Bar=20 µm. Inset: erythrophagocytosis (arrows) is rarely observed. Bar=5 µm. (D) High-power magnification of the tumor. The tumor cells invade (asterisks) between the epithelium (Ep) and the smooth muscle layer (SM) of the epididymal duct. Bar=20 µm. (E) Immunohistochemical staining for α-smooth muscle actin (α-SMA). Note the invasive growth of tumor cells (asterisks) between the epithelium (Ep) and the smooth muscle layer (SM; α-SMA-positive) of the epididymal duct. The tumor cells are negative for α-SMA. Bar=20 µm.

tivity for S-100 and nestin. In undifferentiated sarcomas, the tumor morphology shows highly pleomorphic patterns with bizarre spindle-shaped cells and giant cells. However, the present case showed mild nuclear atypia, few mitotic figures, and no multinucleated giant cells, therefore, the present case was different from an undifferentiated sarcoma. In Leydig cell adenoma and carcinoma in mice, the tumor cells are round or polygonal in shape with eosinophilic or vacuolated cytoplasm and poorly differentiated basophilic cells, respectively[4, 11]. However, the tumor in the present case was mainly composed of spindle-shaped cells with abundant eosinophilic cytoplasm, showing no invasion into the capsule or adjacent tissues, and the tumor cells showed negative immunoreactivity for calretinin. Other differential diagnoses included granulomatous inflammation (sperm granuloma), leiomyosarcoma, and hemangiopericytoma (perivascular wall tumor). The chronic response to the escape of sperm into epididymal interstitial tissues can mimic neoplasia, but organization with a central area of sperm surrounded by a granulomatous capsule composed of epithelioid giant cells and mixed inflammatory cell infiltration differentiates granulomatous inflammation from the present case[4, 12]. The present case resembled tumors derived from smooth muscle cells as well as Schwann cells. However, the shape of the nuclei in the tumor cells in the present case differed from that of leiomyosarcomas, which typically exhibit blunt-ended or cigar-shaped nuclei. In addition, the tumor cells showed negative immunoreactivity for α-SMA and desmin. In perivascular wall tumors, particularly in dogs, spindle-shaped tumor cells are characterized by a perivascular concentric arrangement[13, 14]. However, the present case did not show a perivascular concentric arrangement, although the tumor

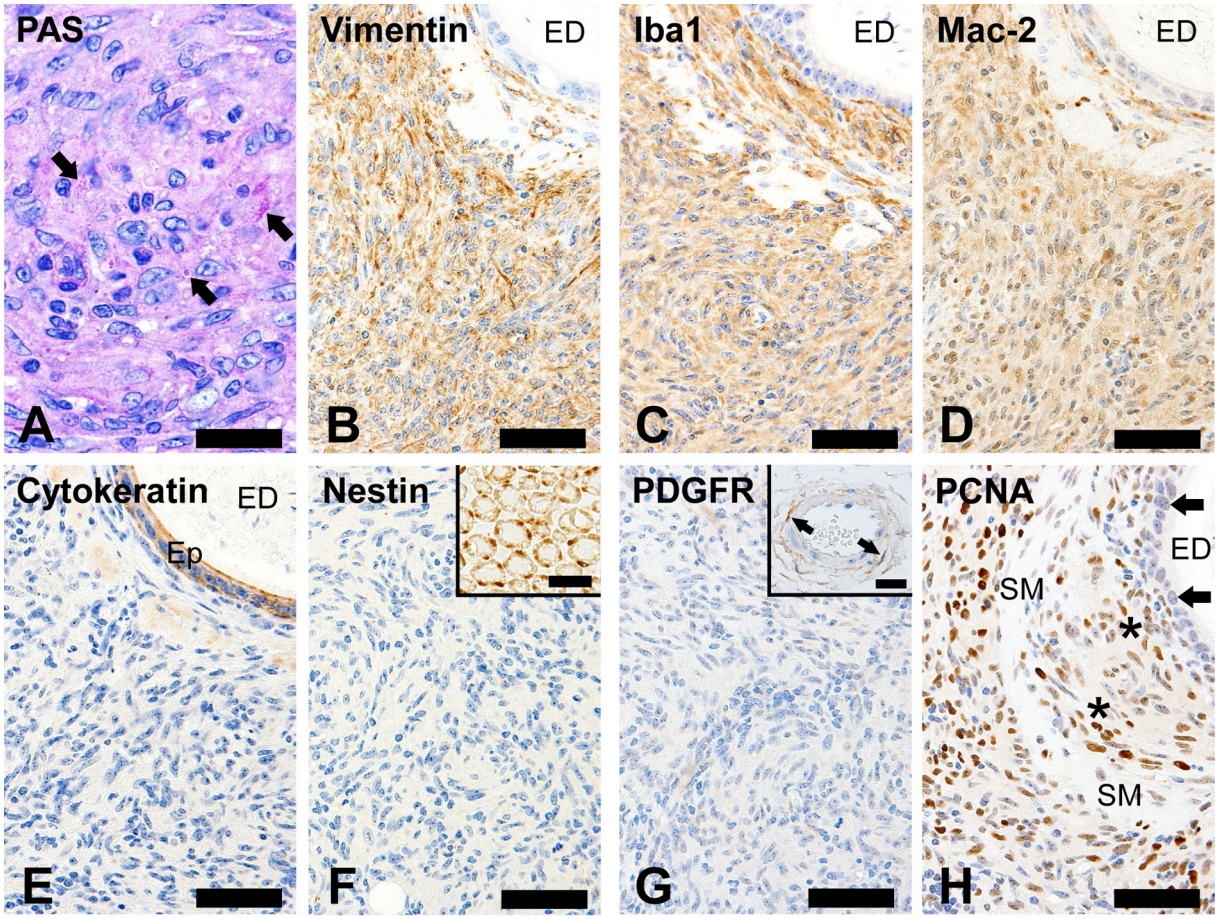

Fig. 2. Special staining and immunohistochemical staining of the tumor. (A) Periodic acid-Schiff (PAS) reaction with diastase digestion. The tumor cells have PAS-positive granules (arrows) in the cytoplasm, which are diastase-resistant. Bar=20 µm. (B) Immunohistochemical staining for vimentin. The tumor cells are diffusely positive for vimentin. ED: epididymal duct, Bar=50 µm. (C) Immunohistochemical staining for Iba1. The tumor cells are diffusely positive for Iba1. ED: epididymal duct, Bar=50 µm. (D) Immunohistochemical staining for Mac-2. The tumor cells are diffusely positive for Mac-2. ED: epididymal duct, Bar=50 µm. (E) Immunohistochemical staining for cytokeratin. The tumor cells are negative for cytokeratin. The epithelium (Ep) of the epididymal duct (ED) shows immunopositivity for cytokeratin. Bar=50 µm. (F) Immunohistochemical staining for nestin. The tumor cells are negative for nestin. Bar=50 µm. Inset: Schwann cells of the sciatic nerve showing immunopositivity for nestin. Bar=10 µm. (G) Immunohistochemical staining for platelet-derived growth factor receptor-β (PDGFR-β). The tumor cells are negative for PDGFR-β. Bar=50 µm. Inset: vascular pericytes (arrows) of the renal artery showing immunopositivity for PDGFR-β. Bar=20 µm. (H) Immunohistochemical staining for proliferating cell nuclear antigen (PCNA). PCNA-positive tumor cells are sporadically observed in the tumor including in the invasive growth area (asterisks). In contrast, the epithelium (arrows) and the smooth muscle layer (SM) of the epididymal duct (ED) are negative for PCNA. Bar=50 µm.

cells were arranged in a whorl pattern. Hemangiopericytomas are extremely rare in rodents, and there have been no reports of primary epididymal tumors. In addition, the tumor cells in the present case showed negative immunoreactivity for PDGFR-β[15]. Considering the differential diagnoses, the present case was a histiocytic sarcoma.

To our knowledge, there are no case reports of histiocytic sarcoma of the epididymis at a single site in aged mice with detailed immunohistochemical staining results. In the present case, it was difficult to diagnose histiocytic sarcoma of the epididymis using only H&E staining, therefore, four histiocyte markers, F4/80 and Mac-2, which are recommended by INHAND, and Iba1 and CD204, which are common histiocyte markers for immunohistochemical staining, were used for definitive diagnosis of the histiocytic sarcoma. The present report provides useful information and additional histopathological evidence for spontaneous histiocytic sarcomas originating from the epididymis of aged mice.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest to disclose in connection with this report.

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