High-Grade Myofibroblastic Sarcoma of Inguinal Region in a Dog

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ABSTRACT. A subcutaneous tumor in the left inguinal region was present in an 11-year-old female bloodhound. Histopathologically, the tumor showed invasive growth and extensive necroses, and it was composed of spindle-shaped, elongated, and stellate neoplastic cells accompanied by occasional giant cells arranged in fascicular, herringbone, or irregular storiform patterns with abundant production of collagen fibers. The cytoplasm of most tumor cells was positive for vimentin, alpha-smooth muscle actin, and calponin, but was negative for desmin, smoothelin, and S-100. Furthermore, most of the tumor cells were negative for Iba1 while some tumor cells were weakly positive. Thus, this tumor was diagnosed as a high-grade myofibroblastic sarcoma according to the diagnostic criteria for human myofibroblastic sarcomas.

Key words: canine, immunohistochemistry, neoplasm, pathology.

Canine cutaneous fibrosarcomas are mainly composed of fibroblastic proliferation and variable amounts of collagen fibers, and are characterized by a high rate of local recurrence and a relatively low rate of metastasis [3, 18]. We sometimes encounter cases of these tumors showing focal myofibroblastic differentiation, but we usually diagnose them as fibrosarcomas based on the fact that the predominant tumor cells are fibroblasts. We encountered a dog with a subcutaneous tumor composed of exclusive myofibroblast proliferation with abundant collagen production. In humans, the term “myofibroblastic sarcoma” has been proposed for sarcomas composed predominantly of myofibroblasts [4, 20], which are subdivided into four categories: infantile myofibrosarcoma occurring in infants less than 4 years old and having consistent genetic abnormalities, inflammatory myofibroblastic tumor having a characteristic morphological features such as fasciitis-like lesions and sclerosing areas with a prominent chronic inflammatory infiltrate, low-grade myofibroblastic sarcoma, and high-grade myofibroblastic sarcoma [20]. Of these subtypes, high-grade myofibroblastic sarcoma is characterized by proliferation of elongated spindle-shaped tumor cells with fascicular or herringbone growth patterns that show numerous mitoses and abundant collagen production [22]. In domestic and companion animals, there are several reports of inflammatory myofibroblastic tumors in dogs [2, 13, 17] and domestic animals [19], cardiac myofibroblastic tumor in a dog [14] and low-grade myofibroblastic sarcomas in cats [1]. In addition, we have recently reported a case of low-grade myofibroblastic sarcoma in a dog [28]. In contrast, to the best of our knowledge, there is no report investigating tumors with morphological features of high-grade myofibroblastic sarcomas in dogs. In this article, we report a canine case of high-grade myofibroblastic sarcoma.

An 11-year-old female bloodhound had a mass in the left inguinal region. The mass was surgically removed, but the dog died for unknown reasons two months after the surgery. Necropsy of this dog could not be performed, because the owner rejected the necropsy. Therefore, additional information such as regional lymphadenopathy, internal spread of the tumor and metastasis to the other organs could not be obtained from this dog. The removed mass was fixed in 10% neutral buffered formalin and subjected to routine histological processing.

The mass was composed of two elastic nodules, 6.0 × 8.0 × 1.0 cm and 4.0 × 3.0 × 1.0 cm, and its color was grayish-white or locally light brown. The tumor was located in the subcutis, and the skin covering it had an ulcer 4.0 cm diameter.

The tissues fixed in 10% buffered formalin were embedded in paraffin. Histological sections of 4 µm were made and stained with hematoxylin and eosin (HE) and Masson’s trichrome. For immunohistochemistry, sections were deparaffinized, rehydrated, and incubated with 0.3% hydrogen peroxidase in methanol for 30 min at room temperature to block endogenous peroxidase activity. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) in a microwave at 90°C for 10 min (vimentin, desmin, and alpha-smooth muscle actin) or in an autoclave at 121°C for 10 min (calponin, smoothelin, and Iba1). The primary antibodies used in the present study were mouse monoclonal antibodies against vimentin (Mo725, clone V9, 1:100; Dako, Glostrup, Denmark), desmin (MA-18547, 1:100; Thermo Fisher Scientific Inc., Rockford, IL, U.S.A.), alpha-smooth muscle (clone 1A4, 2801, 1:100; Discove...
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M0851, 1:100; Dako), calponin (C2687, 1:30000; Chemicon International, Temecula, CA, U.S.A.), smoothelin (MAB3242, 1:200; Chemicon International, Temecula, CA, U.S.A.), and a polyclonal antibody against S-100 (Z3011, 1:200; Dako), and Iba1 (1:250; Dako). Sections were incubated with these primary antibodies at 4°C overnight. All immunodetections were carried out by the avidin-biotin complex method (VECTASTAIN® Elite ABC Kit; Vector Laboratories Inc., Burlingame, CA, U.S.A.) with 3,3′-diaminobenzidine as a chromogen, followed by light counterstaining with hematoxylin. Negative controls consisted of omitting the primary antibody. Formalin fixed sections of

Fig. 1. (A) The tumor shows a fascicular proliferative pattern of spindle-shaped tumor cells and is accompanied by proliferation of collagen fibers. There are scattered extensive necroses in the tumor. HE stain. × 400. (B) The collagen fibers are stained in blue with Masson’s trichrome stain. × 100. (C) The tumor is mainly composed of spindle-shaped cells with numerous mitotic figures (arrows) arranged in fascicular or herringbone patterns. HE stain. × 400. (D) Tumor cells are composed of atypical pleomorphic cells. There are occasional tumor cells with giant nuclei (arrows) within the nests composed of stellate cells arranged in fascicular patterns. HE stain. × 400. (E) Tumor cells of the mass are positive for alpha-smooth muscle actin. Avidin-biotin complex method counterstained with hematoxylin. × 200. (F) Tumor cells show weak immunoreactivity for calponin. Avidin-biotin complex method counterstained with hematoxylin. × 400.
canine skin, brain, and lung were used as positive controls and to check for the normal distribution of the antigens.

Histologically, the subcutaneous tumor was not encapsulated and showed invasive growth to the surrounding dermis, skeletal muscles, and adipose tissues. The tumor was composed of spindle-shaped, elongated, and stellate cells, and tumor cells were arranged in fascicular, herringbone, or irregular storiform patterns (Fig. 1A). There were scattered extensive necroses in the center of the mass (Fig. 1A) and scattered infiltration of inflammatory cells consisting of macrophages, lymphoid and plasma cells around the necrotic areas. The tumor frequently had various degrees of cellularity accompanied by abundant fibrous collagen that was stained in blue with Masson’s trichrome (Fig. 1B) or myxoid matrix. The tumor cells were characterized by eosinophilic cytoplasm with indistinct cell boundaries, and their nuclei were varied in shape: round, oval, or tapering, showing moderate atypia and numerous mitotic figures (16 to 20 per 10 fields examined with the ×40 microscope objective) (Fig. 1C and 1D). There were occasional tumor cells with giant nuclei or binuclei within the nests composed of stellate cells arranged in fascicular patterns (Fig. 1D).

Immunohistochemically, strong cytoplasmic immunoreactivity for vimentin was diffusely observed in most of the tumor cells, and the cytoplasm of the most spindle-shaped cells was positive for alpha-smooth muscle actin (Fig. 1E) and calponin (Fig. 1F). However, neoplastic cells were negative for desmin, smoothelin that is localized in mature smooth muscle cells [29], and S-100. In addition, Iba1 that is a marker of macrophages in dogs [16] was strongly positive for infiltrating macrophages that were located around the necrotic areas of the tumor. On the contrary, most of the tumor cells were negative for Iba1, although some of the tumor cells were weakly positive.

Myofibroblasts are mesenchymal spindle cells that share morphological characteristics of both fibroblasts and smooth muscle cells. They play an important role in the regulation of smooth muscle contraction [10, 27]. They have variable immunoreactivity for some muscle actin markers, including alpha-smooth muscle actin, calponin, and desmin [25]. Myofibroblasts are present in the stroma of normal organs [6], and in reactive processes such as granulation [12]. In addition, a variety of benign soft tissue tumors show myofibroblastic differentiation, and malignant mesenchymal neoplasms showing dominant myofibroblastic differentiation are described as myofibroblastic sarcomas and myofibrosarcomas [5]. These myofibroblastic sarcomas in humans occur mainly in subcutaneous tissues of the head, neck, and extremities [7, 9, 20, 21] and are occasionally observed in the bone and viscera [24].

High-grade myofibroblastic sarcomas in humans have been described as pleomorphic sarcomas which are composed of atypical spindle, polygonal, and giant cells showing myofibroblastic differentiation [7]. In addition, numerous mitotic figures (more than 10 per 10 high power fields) and tumor necrosis usually accompany these tumors [4, 7, 20]. Furthermore, areas with a fascicular or herringbone pattern composed of elongated spindle-shaped tumor cells that show numerous mitoses and abundant collagen production are also observed in these high-grade myofibroblastic sarcomas [20]. These morphological features in human high-grade myofibroblastic sarcomas are quite similar to those in the present case.

The immunohistochemical findings in the present case (positive for vimentin, alpha-smooth muscle actin, and calponin, but not for desmin, smoothelin, and S100) are almost consistent with those of human myofibroblastic sarcomas. Furthermore, most of the tumor cells were negative for Iba1 although some tumor cells were weakly positive. In addition, it has been reported that 56% of malignant fibrous histiocytomas (MFHs) had detectable myofibroblasts with a mean of 3% (range 0–22%) of myofibroblasts per case [11], but the ratio of myofibroblast differentiation is extremely lower than that in the high-grade myofibroblastic sarcomas. Therefore, the possibility that the present case is the variant of MFHs is extremely low, and this case therefore should be diagnosed as high-grade myofibroblastic sarcoma.

There are no adequate diagnostic criteria for myofibroblastic sarcomas in the international tumor classification of domestic animals [15]. However, we must keep in mind that in the past, many myofibroblastic sarcomas among subcutaneous tumors were likely diagnosed as fibrosarcomas based on microscopic examinations on HE-stained histological sections [23, 26, 30, 31]. Most of these tumors were probably diagnosed as fibrosarcomas because immunohistochemical examinations are seldom performed for spindle-shaped cell tumors accompanied by collagen fiber production. On the contrary, myofibroblastic sarcoma is classified as a distinct tumor entity in humans [8]. In this respect, more detailed studies on myofibroblastic sarcomas in dogs are necessary to consider them a specific tumor entity.

REFERENCES