Clinicopathological and immunological features of pleomorphic rhabdomyosarcoma in the subcutis of a 9-year-old Lolo dog

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Received: 24 August 2021 / Accepted: 13 January 2022 / Published online: 25 January 2022
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Abstract

A 9-year-old female Lolo dog presented with a subcutaneous, large mass protruding from the right-side of her flank region with intact covering skin. Surgical excision of the mass revealed a well demarcated, large firm mass without invading the underlying muscle of the flank. The gross examination revealed a fish-flesh colored, large firm mass, histologically neoplastic cells exhibiting pleomorphism, multinucleated giant cells, and bizarre mitosis. Using immunohistochemical staining, the tumor cell was expressed with vimentin, desmin, and myogenin. Based on histological and immunohistochemical staining, it was confirmed as PRMS. The dog was followed up to 2 years after surgical removal. There was no recurrence, and the dog had normal health. The present case indicated that PRMS had characteristically histological and immunohistochemical features that could be used in differential diagnosis from highly confusing alveolar and embryonal rhabdomyosarcoma in dogs, and it must be included in differential diagnosis of anaplastic sarcoma in adult dogs.

Keywords Pleomorphic rhabdomyosarcoma · Myogenin · Subcutis · Dog

Introduction

Rhabdomyosarcoma (RMS) is a malignant mesenchymal neoplasm that exhibits variable degrees of differentiation towards the skeletal myocytes. Diagnosis and classification of RMS neoplasms are difficult due to complexity and variations of the gross morphology, histological architecture, and

cellular phenotyping with subsequent relatively rare reports (Caserto 2013). RMS has four variants — embryonal, alveolar, botryoid, and pleomorphic — depending on histological, immunohistochemical expressions, and age of onset (Parham 2001).

Pleomorphic rhabdomyosarcoma (PRMS) is the least common among other subclasses in human and animals. PRMS is a high complex neoplasm and is considered a pitfall for pathologists. This is first due to all subclasses of RMS showing cellular pleomorphism. A number of reports in veterinary journals describe “pleomorphic” tumors that should be classified as embryonal. Second, many highly pleomorphic sarcomas have been diagnosed as RMS without IHC confirmation (Cooper and Valentine 2017). To the best of our knowledge, this is the first report describing clinicopathological, immunohistochemical, and the outcome of a rare case of PRMS in the subcutis of an adult dog. PRMS must be included in a differential diagnosis with undifferentiated pleomorphic sarcoma in the subcutis of the dog, and immunohistochemistry should be pursued.

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Case report

A 9-year-old female Lolo dog was presented to the teaching hospital of veterinary medicine at Mansoura University in Egypt. The owners mentioned the rapidly growing mass, a non-painful, protrusion from right flanks since she was 2 months old. The dog had a history of no mating. The clinical examination revealed a subcutaneous firm mass protruding from the right flank with intact covering skin (Fig. 1). Surgically removing the mass under general anesthesia and dissecting the mass from the surrounding tissue, there was no invasion of the underlying muscle, muscle of flank or the overlying skin, and it was covered by subcutaneous fascia. The gross examination of the mass was of fish-flesh in color, firm in consistency, and about 22 cm × 19 cm × 31 cm. It had an irregular surface, and it was non-encapsulated. The mass on the cut section was firm. Histologically, the mass revealed plumped spindle and epithelioid-shaped neoplastic cells. The nucleus was vacuolated and with prominent nucleoli. Some cells had two nucleoli. There were variable amounts of eosinophilic cytoplasm. Widely distributed giant cells throughout the mass were seen (Fig. 2), and focal coagulative necrosis were noticed (Fig. 2). Strap cells had multiple nuclei, and racket-shaped cells and bizarre mitosis were seen (Fig. 3). With anisocytosis and anisokaryosis, some cells displayed karyomegaly and minimal fibrocollagenous stroma.

The immunophenotype of the tumor cells was analyzed using immunohistochemistry. Vimentin, (Lab Vision™, Int'l: RUO, USA, RM9120S1), desmin, and myogenin (BioGenex, Milmont Drive, Fremont, California, USA) were used. Sections (4-µm thick) were cut and mounted on silane-coated glass slides. The sections were deparaffinized in xylol and dehydrated through a graded ethanol series.

Fig. 1 Lolo dog displays large subcutaneous mass at the right flank. Bar, 20 mm

Fig. 2 Plump of spindle and epithelioid neoplastic cells and bizarre multinucleated giant cells. HE. Bar, 100 µm. Inset, focal coagulative necrosis among the pleomorphic neoplastic cells. HE. Bar, 200 µm

Antigen retrieval was performed by 0.01-M citric acid buffer (pH 6.0) at 100 °C for 10 min. Endogenous peroxidase activity was blocked with 3% H₂O₂ for 10 min. Tissue sections were then incubated with mouse monoclonal primary antibodies against myogenin (ready to use) and desmin (1:100), overnight incubation, in addition to the rabbit monoclonal vimentin (1:400), at room temperature for 1 h. The tissue slides were incubated, anti-mouse and anti-rabbit secondary antibodies at the room temperature for 30 min and visualized by incubation with the 3,3'-diaminobenzidine tetrahydrochloride liquid system (Dako, autostainer-link-48) at room temperature for 5 min. The sections were subsequently

Fig. 3 Strap shape cell with multiple nucleus, racket-shape cell (arrow), and bizarre mitosis (arrow heads). HE, bar, 200 µm. Inset, the tumor cells displayed diffuse cytoplasmic, brown immunostaining against vimentin. IHC, DAB, immunostaining, hematoxylin as counterstain, bar, 200 µm

counterstained with hematoxylin and observed by light microscopy (Olympus Corporation, Tokyo, Japan). A negative control was carried out using antibodies of the same species but of irrelevant specificity.

Immunohistochemical staining was done against mouse monoclonal for CD68 (1:1000), Melan-A (ready-to-use), and chromogranin (1:100) (BioGenex, Milpitas Drive Fremont, California, USA), in addition to rabbit polyclonal antibody S100 (1:300) (Lab Vision™, Int'l: RUO, USA), using the same technique as vimentin, desmin, and myogenin. The tumor cells expressed against vimentin (Fig. 3), desmin (Fig. 4), and myogenin (Fig. 4) and they were negative for CD68, S100, Melan-A, and chromogranin. Based on the pathological and immunohistochemical staining, the diagnosis was RMS. A careful histological examination was done to determine the absence of any area that had alveolar or an embryonal pattern of growth, and the final diagnosis was confirmed to be PRMS. The dog was followed for up to 2 years after the surgery. There was no recurrence, and she maintained normal health.

A PRMS diagnosis is treacherous for pathologists as it displays a marked degree of pleomorphism and anaplasia so it can be misdiagnosed as anaplastic sarcoma. Also, alveolar, especially solid subtype, and embryonal RMS, the most aggressive of RMS subclasses, displays pleomorphism and the presence of strap cells and multinucleated giant cells (Cooper and Valentine 2017). The present case provides a novel insight into subcutaneous PRMS describing the clinicopathological features, immunohistochemical properties, and clinical outcomes of an adult dog.

Fig. 4 The tumor cells displayed frequently moderate, mainly in strap cells, nuclear brown immunostaining against myogenin. IHC, DAB, immunostaining, hematoxylin as counterstain, bar, 200 μ m. Inset, the tumor cells displayed sparse and patchy pattern, cytoplasmic immunostaining against desmin. IHC, DAB, immunostaining, hematoxylin as counterstain, bar, 200 μ m

Cutaneous skeletal muscle neoplasm of an adult dog is rare, with only two cases of rhabdomyoma — one occurring on the skin and subcutis of a leg and the other on the skin of a foot in 11-year-old and 14-year-old dogs (Caserto 2013). Nevertheless, the cutaneous PRMS is often misdiagnosed with undifferentiated or pleomorphic sarcoma. PRMS, especially, is not incorporated in the category of soft tissue sarcomas by some investigators (Dennis et al. 2011).

The histological criteria can be misdiagnosed as alveolar or embryonal RMS, but the age of onset should be considered as embryonal and alveolar RMS usually at less than 2 years old (Cooper and Valentine 2017). The histological architecture of PRMS must be not displaying any alveolar or embryonal pattern (Cooper and Valentine 2017).

Immunohistochemical, using different markers for rhabdomyosarcoma, such as desmin and myogenin, is fundamental as specific diagnostic markers. Also, they give an indication about the degree of differentiation and, used in humans to differentiate between alveolar and embryonal RMS (Caserto 2013). Desmin is a low specific marker for RMS and must be accompanied by myogenin in order to confirm RMS (Caserto 2013). There are no previous clear reports about the expression pattern of the different markers, but the correlation to alveolar and embryonal RMS in dogs. myogenin is strongly expressed in tumor cells in alveolar RMS. Meanwhile, it is patchy and sparse at embryonal RMS and vice versa for desmin (Caserto 2013). In comparison with the PRMS in dogs of that case, myogenin was lower in the expression in relation to alveolar RMS. Also, desmin was lower in the expression in relation to embryonal RMS. It is estimated that RMS that is composed of relatively undifferentiated cells should be expected to express less desmin and more myogenin (Caserto 2013). Other myogenic regulating factors lead to variation in their expressions (Costa et al. 2002). That may explain the variation in the expressions of desmin and myogenin in the different RMS.

The differential diagnosis of subcutaneous PRMS from anaplastic sarcoma is inevitable, and this can be pursued by immunohistochemical markers. Vimentin, desmin, myogenin, which are diagnostic markers of rhabdomyosarcoma, were positive (Caserto 2013). Other markers were used to exclude the confusion neoplasm exhibiting undifferentiated pleomorphic sarcoma — CD68 for histiocytic sarcoma (Ide et al. 2011), S100 for peripheral nerve sheath tumor (Nonaka et al. 2008), melan-A for melanoma (El-Shafaey and Hamed 2020), and chromogranin exclude neuroendocrine differentiation (Ogawa et al. 2011).

The surgical removal of the subcutis PRMS in the dog was competent, and the mass was well demarcated without any evidence of invasion of the surrounding tissue. It was of favorable prognosis and long survival, up to 2 years without any complications. The previous report about PRMS about the dog at Cornell University, without follow-up or detailed

information, indicates that PRMS shows a clear invasion of the adjacent muscle (Cooper and Valentine 2017). In humans, 13 reported cases of subcutaneous PRMS in different areas of the body, surgical enucleations were the standard method for the treatment. In some cases that showed invasion, the prognosis rate was 33.3% (Watanabe et al. 2017).

In conclusion, PRMS in the subcutis of adult dogs must be included with undifferentiated pleomorphic sarcoma and immunohistochemistry should be pursued. Subcutaneous PRMS in dogs is of different expressions to other RMS subclasses in dog and of favorable prognosis, if not invading the surrounding tissue.

Declarations

Ethical approval None of this article.

Consent for publication All the authors gave their consent for publication.

Conflict of interest The authors declare no competing interests.

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