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Alveolar rhabdomyosarcoma in a dog confirmed using myogenin immunohistochemistry: a case report

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ABSTRACT: A large mass from the right forelimb of a five-year-old, male, Maltese dog, was resected surgically and examined histopathologically. Grossly, the 4 × 6 cm mass was well-demarcated and firm. Microscopically, the mass was characterised by neoplastic mononuclear cells with large round hyperchromatic nuclei and scant cytoplasm. The neoplastic tumour cells were separated by thick or thin collagen septa and were arranged in an alveolar pattern forming cell nests. Immunohistochemical detections of the tumour revealed positive reactions for vimentin, desmin and myogenin, but the tumour was negative for alpha smooth muscle actin (α-SMA), S-100, CD3, CD79a, CD68, cytokeratin 8 (CK8) and cytokeratin 18 (CK18). These results showed that the tumour cells originated from skeletal muscle; therefore, the tumour was diagnosed as an alveolar rhabdomyosarcoma.

Keywords: alveolar rhabdomyosarcoma; desmin; myogenin; skeletal muscle; vimentin

INTRODUCTION

Rhabdomyosarcoma is a malignant mesenchymal neoplasm composed of cells having an origin of striated skeletal muscle, striated muscle progenitor cells or primitive mesenchymal cells capable of differentiation into striated muscle cells (Cooper and Valentine 2002). In dogs, rhabdomyosarcoma has been reported in the pharynx, larynx, striated muscle, cardiac muscle, gingiva, urinary bladder, greater omentum, urethra, skin and trachea (Brockus and Myers 2004). Rhabdomyosarcoma can be classified into three main types based on their histopathological features; pleomorphic, embryonal and alveolar rhabdomyosarcoma (Kim et al. 1996). Alveolar rhabdomyosarcoma has rarely been reported in humans or animals (Lambert et al. 2004).

Immunohistochemistry is the most suitable method for differentiating rhabdoymyosarcoma from other tumours and for elucidating the origin of the tumour cells. Most rhabdomyosarcoma cases reported previously were confirmed by using an anti-myoglobin antibody (Seibold 1974; Sarnelli et al. 1994; Kim et al. 1996; Bae et al. 2007). However, myogenin is also considered as the most critical marker for human alveolar rhabdomyosarcoma (Dias et al. 2000). So far, only two cases of canine alveolar rhabdomyosarcoma confirmed by immunohistochemistry using a myogenin antibody have been published (Murakami et al. 2010; Otrocka-Domagala et al. 2015). However, those cases were diagnosed as a solid variant of alveolar rhabdomyosarcoma characterised by formation of solid sheets composed of round neoplastic cells packed densely without a typical alveolar pattern. In this article, we describe the gross findings, microscopic findings and immunohistochemical results using an anti-myogenin antibody in a rare case of alveolar rhabdomyosarcoma in the forelimb of a dog.

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Case description

A five-year-old, male, Maltese dog was presented to a local veterinary hospital owing to the rapid growth of a large subcutaneous mass on the right forelimb, which was firm and immovable. Chest radiography revealed no other evidence of metastasis. To respect the large mass, a surgical incision of the right scapular region was performed under general anaesthesia. The mass had a smooth surface, well-demarcated border, spherical appearance and firm consistency (Figure 1.). The mass was 4 × 6 cm in size and the cut surface revealed a heterogeneous whitish-pink colour and a multi-focal reddish component.

The excised mass was referred to the laboratory of veterinary pathology, Kyungpook National University for histopathological evaluation. The six representative tissue pieces of excised mass were fixed in 10% formalin, processed routinely and embedded in paraffin wax. The paraffin sections were prepared with a thickness of 4 μm and stained with haematoxylin and eosin for light microscopic examination. To identify the origin of the neoplastic cells, sections were stained immunohistochemically using the avidin-biotin-peroxidase complex method (Vectastatin ABC kit; Vector, USA). The primary antibodies used for the neoplastic tumour mass sections were, anti-desmin (mouse monoclonal, Dako, Denmark), anti-α-smooth muscle actin (α-SMA) (mouse monoclonal, Sigma, USA), anti-vimentin (mouse monoclonal, DakoCytomation, Denmark), anti-CD68 (mouse monoclonal, Dako, Denmark), anti-CD3 (mouse monoclonal, Santa Cruz Biotechnology, Inc, USA), anti-CD79a (mouse monoclonal, Santa Cruz Biotechnology, Inc, USA), anti-cytokeratin8 (CK8) (mouse monoclonal, Novocastra Laboratories Ltd, UK), anti-cytokeratin18 (CK18) (mouse monoclonal, Novocastra Laboratories Ltd, UK), anti-S-100 (mouse monoclonal, Santa Cruz Biotechnology, Inc, USA) and anti-myogenin (mouse monoclonal, Dako, Denmark). For detection of nuclear myogenin expression, antigen retrieval was performed. Slides were incubated in 0.01M citric acid buffer (pH 6.0) at 100 °C for 10 min. Negative controls included sections incubated with normal mouse serum and were processed as above. Skeletal muscle sections, smooth muscle sections, histiocytoma sections, T-cell lymphoma sections, B-cell lymphoma sections, skin sections and spinal cord sections were considered as positive controls. In addition, phosphotungstic acid haematoxylin (PTAH) staining was performed to detect cross striation of tumour cells which originated from skeletal muscle.

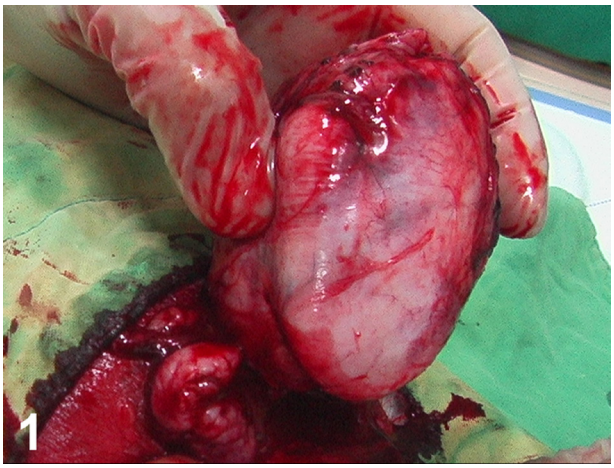

Figure 1. Mass from the right scapular region. The excised large mass is characterised by a smooth surface, and by a well-demarcated, round, spherical and reddish appearance

Microscopically, the mass was composed of numerous basophilic small and large neoplastic mononuclear cells with prominent round-to-ovoid nuclei and scant cytoplasm. The neoplastic cells indicated moderate anisokaryosis and poorly differentiated stages. Neoplastic tumour cells grouped in nests were separated by thick or thin collagenous septa and arranged in an alveolar pattern. These neoplastic cells lined or covered the collagenous septa and were arranged in a discohesive manner in the centre of both large and small alveolar spaces (Figure 2). The poorly differentiated neoplastic tumour cells also frequently displayed numerous mitotic figures. There were four to five mitotic figures per high power field (× 400) as well as infiltration of a few inflammatory cells including eosinophils and macrophages among the neoplastic tumour cells. Cross striated neoplastic cells were not detected in PTAH stain.

Immunohistochemical evaluation confirmed the rhabdomyosarcomatous nature of the neoplastic cells, which were positive for anti-myogenin (Figure 3) in their nuclei, and positive for anti-desmin (Figure 4) and anti-vimentin (Figure 5) in their cytoplasm. The tumour also showed negative reactions for anti-CD68, anti-CD3, anti-CD79a, anti-CK8, anti-CK18, anti-SMA and anti-S-100. Positive control slides were positive for each antibody and all negative control slides revealed negative reactions. In the present case, the tumour cells showed a posi-

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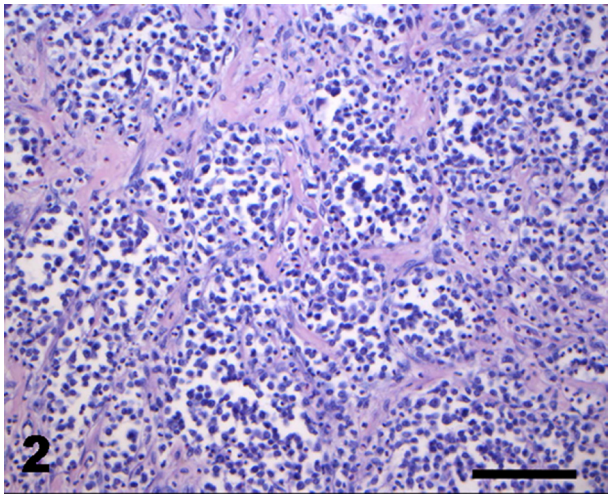

Figure 2. Neoplastic tumour cells characterised by small and large mononuclear cells with large round-to-ovoid hyperchromatic nuclei and scant cytoplasm were separated by thick or thin collagenous septa and arranged in an alveolar pattern forming cell nests. H&E stain; bar = 100 μm

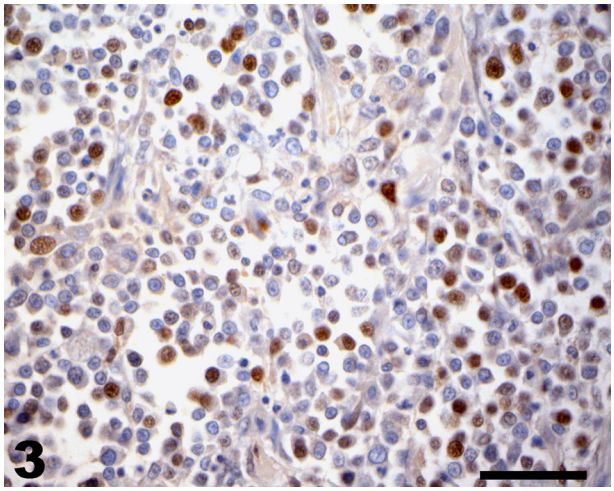

Figure 3. Strong immunopositive reaction for myogenin in the nuclei of neoplastic cells. Avidin-biotin-peroxidase method, Mayer's haematoxylin counterstain; bar = 100 μm

tive reaction for vimentin, desmin and myogenin, which confirmed the poor differentiation, as well as the mesenchymal and skeletal muscle cell origin of tumour cells. The intensity of vimentin staining was stronger in small immature cells than in large neoplastic cells. However, desmin and myogenin were expressed more intensely in the large cells than the small cells. In particular, the expression of myogenin was only detected in the nucleus of relatively mature large cells (Figure 3). Small cells showing weak or no expression of myogenin exhibited a more intensely positive reaction for vimentin. These results suggest that this tumour was poorly differentiated and originated from skeletal muscle.

DISCUSSION AND CONCLUSIONS

The immunohistochemical markers of skeletal muscle such as vimentin, desmin, myoglobin and myogenin play a critical role in differential diagnosis of rhabdomyosarcoma (Dias et al. 2000; Suzuki et al. 2006). To the authors' knowledge, in dogs, four cases of alveolar rhabdomyosarcoma (Seibold

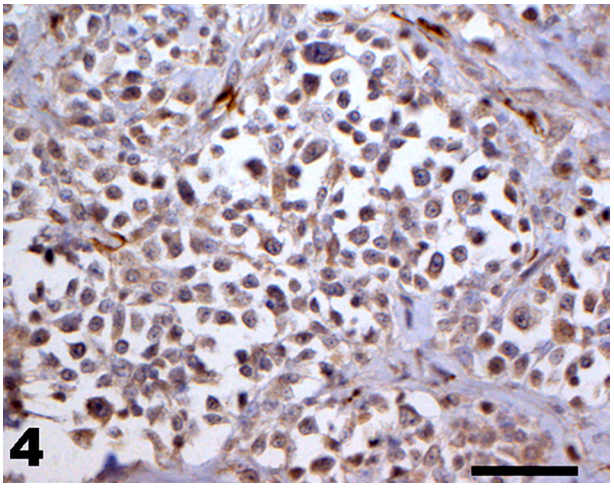

Figure 4. Neoplastic cells show an immunopositive reaction for desmin in their cytoplasm. Avidin-biotin-peroxidase method, Mayer's haematoxylin counterstain; bar = 50 μm

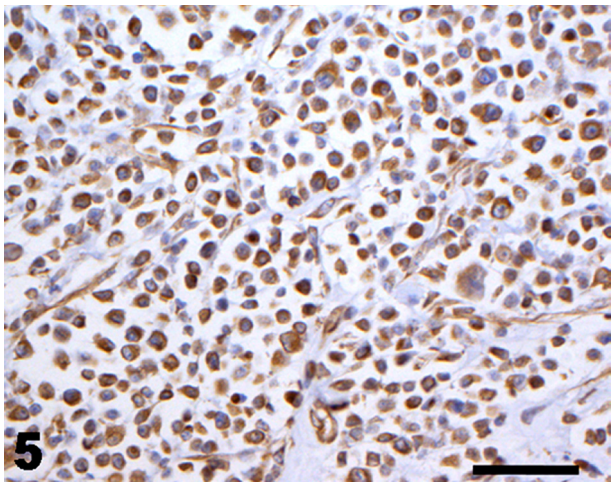

Figure 5. Strong immunopositive reaction for vimentin. Avidin-biotin-peroxidase method, Mayer's haematoxylin counterstain; bar = 50 μm

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1974; Sarnelli et al. 1994; Kim et al. 1996; Bae et al. 2007) have been reported, which occurred in the gingiva, urinary bladder, genital tract, retroperitoneal cavity, left maxillary area and greater omentum, and these cases were confirmed via myoglobin immunohistochemistry. Myogenin is considered as the most critical marker for human rhabdomyosarcoma (Dias et al. 2000). In the veterinary literature, Kobayashi et al. (2004) reported the expression of myogenin and MyoD in two canine botryoid rhabdomyosarcomas, and myogenin was found to be a useful marker for canine rhabdomyosarcoma. However, there has been no reported case of alveolar rhabdomyosarcoma confirmed using an anti-myogenin antibody in the veterinary literature.

It is well known that immunohistochemical expression levels vary depending on the degree of cell differentiation (Chaponnier and Gabbiani 2004). Vimentin is a marker for cells of mesenchymal origin but not differentiated myocytes (Duquette et al. 2005). In the developmental process of striated muscle, vimentin is expressed in the early stage of cell differentiation while it begins to disappear as muscle fibres develop (Yener 2001). Desmin is a major intermediate filament protein in adult striated, cardiac and smooth muscle; therefore, it can be used as a specific muscle marker (Fletcher 2000; Radi 2006). Its expression starts in the early phase of differentiation and persists into later stages (Radi 2006). Myogenin induces differentiation of myoblasts into the multinucleated myotube, which has abundant cytoplasm containing myofibrils. Myogenin is one of the myogenic transcriptional factors expressed in the early stage of muscle development and its expression differs depending on the stage of myogenesis (Kobayashi et al. 2004). Myogenin is intensely expressed during the late stages of myoblast differentiation and during myotube formation. After myotube maturation, myogenin expression begins to decrease to a low level of expression (Kostrominova et al. 2000). Therefore, myogenin may be a useful marker for considering the myogenic origin and differentiation of canine rhabdomyosarcoma (Kobayashi et al. 2004).

Alveolar rhabdomyosarcoma tumour cells line the collagenous septa and tend to be arranged in a discohesive manner in the centre of the alveolar space; therefore, they appear in an alveolar pattern (Fletcher 2000). Cross striations can be difficult to demonstrate in poorly differentiated alveolar rhabdomyosarcomas (Sarnelli et al. 1994). In this case, we tried to detect cross-striations using PTAH staining; however, no cross-striations were observed in the neoplastic cells, which were considered as poorly differentiated cells. Despite this, the typical alveolar pattern strongly suggested a diagnosis of alveolar rhabdomyosarcoma.

Based on the gross findings, microscopic findings, immunohistochemical identification and the knowledge that a lack of cross-striation is frequently observed in a poorly differentiated rhabdomyosarcomas, this case can be diagnosed as an alveolar rhabdomyosarcoma even though the tumour in this case exhibited a well-encapsulated and well-demarcated gross appearance. In addition, our study suggests the possibility of using myogenin as a diagnostic marker in alveolar rhabdomysoarcoma in canines. To the authors' knowledge, this case is the first report that describes the detailed gross, microscopic, immunohistochemical findings of alveolar rhabdomyosarcoma using an anti-myogenin antibody, in the forelimb of a dog.

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