

## Extraskelletal chondroblastic osteosarcoma of the subcutaneous tissue in a Maltese dog

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**Abstract:** A 10-year-old spayed female Maltese dog presented to a local animal hospital with a subcutaneous mass (4 × 3 × 3 cm) in the right shoulder region. The mass was well circumscribed, with soft tissue opacity and variable levels of mineralization, but with no bony involvement in radiography. The mass was surgically removed. Upon histological examination, the mass consisted of malignant mesenchymal cells that had produced a chondroid matrix and osteoid. Round to polygonal neoplastic cells with large nuclei showed moderate anisokaryosis and variable numbers of mitotic figures. The tumor cells were positive for vimentin and osteocalcin and negative for pancytokeratin, S100, and C-kit. On the basis of histopathologic and immunohistochemical features, the tumor was diagnosed as an extraskelletal chondroblastic osteosarcoma.

**Key words:** Dogs, extraskelletal osteosarcoma, soft tissue, chondroblastic osteosarcoma, immunohistochemistry

### 1. Introduction

Extraskelletal osteosarcoma is defined as an osteoid-producing mesenchymal tumor with no skeletal involvement (1). Extraskelletal osteosarcomas are highly malignant and extremely rare, comprising only 1% of all osteosarcomas excluding those of mammary gland origin (1–3). Of the few canine cases reported in the subcutaneous tissues and extremities, the majority of cases have been reported in larger breeds of dog, with no reports specifically involving small-breed dogs.

Since the number of cases reported is extremely low, here we describe a case of an extraskelletal osteosarcoma in the skin of a Maltese dog, a small breed, which is of considerable interest.

### 2. Case history

A 10-year-old spayed female Maltese dog weighing 4.7 kg presented to a local animal hospital with a soft tissue mass in the right shoulder area. Radiographically (Figure 1), the mass was well circumscribed, with soft tissue opacity and variable levels of mineralization, but with no bony involvement. There was no enlargement of the axillary lymph node or thorax lymph node. Upon review of the medical records, the dog had no history of injections in that area before the mass was found. The mass was surgically

removed under general anesthesia. An encapsulated mass was located beneath the dermis and was completely removed with enough margin.

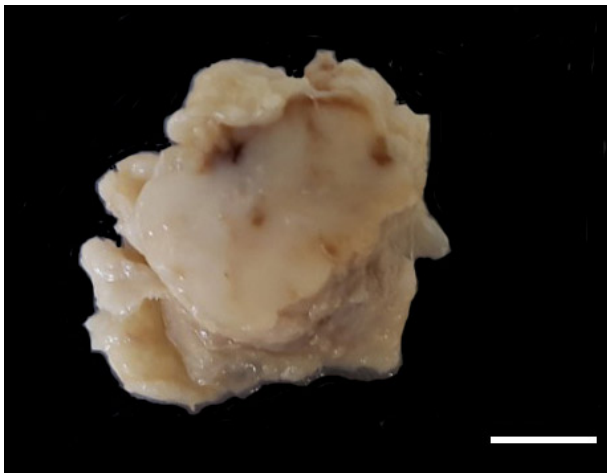
For definitive diagnosis, the excised mass was fixed in a 10% buffered formalin solution before being routinely processed and embedded in paraffin. The mass was sent to the Department of Veterinary Pathology, College of Veterinary Medicine, Konkuk University. Macroscopically, the pale white mass was well circumscribed and firm (approximately 4 × 4 × 3 cm), but could readily be cut by a blade without prior decalcification (Figure 2). Sections were cut and stained with hematoxylin and eosin (H&E) for evaluation of histological features, while others were stained with Alcian blue for detection of the chondroid matrix. For immunohistochemical examination, primary antibodies to pancytokeratin (M3515; Agilent Technologies, Santa Clara, CA, USA), vimentin (M0725; Agilent Technologies), osteocalcin (NB600-1394; Novus Biologicals, Littleton, CO, USA), S100 (Z0311; Agilent Technologies), and C-kit (A4502; Agilent Technologies) were used. Deparaffinized slides were rehydrated with PBS and incubated in 3% hydrogen peroxide for 20 min. Heat-induced antigen retrieval was performed in Tris-EDTA (pH 9.0) for the detection of vimentin, C-Kit, and osteocalcin and in citric acid (pH 6.0) for detection of pancytokeratin using a

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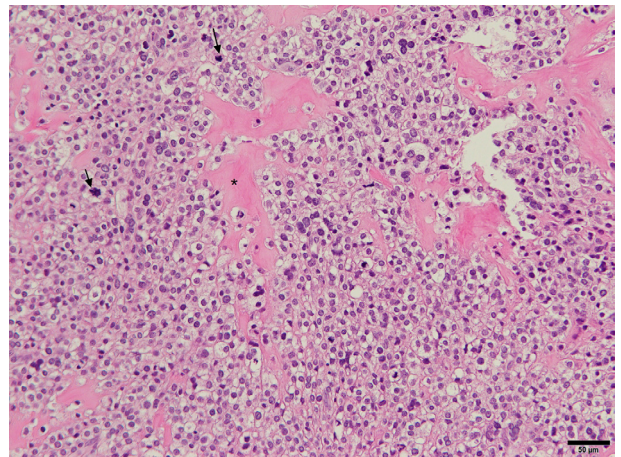
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**Figure 1.** Radiograph of subcutaneous mass excised from a Maltese dog. A well-circumscribed mass was detected, with soft tissue opacity and variable levels of mineralization with no bony involvement.



**Figure 2.** Cut surface of a formalin-fixed mass excised from a Maltese dog. The pale white mass was well circumscribed and firm (approximately 4 × 4 × 3 cm). Bar = 1 cm.



**Figure 3.** Subcutaneous mass excised from a Maltese dog. Polygonal neoplastic cells were observed around the osteoid matrix (asterisks). Osteoblasts with prominent nucleoli and mitotic figures (arrowhead) are visible. H&E staining. Bar = 50 µm.

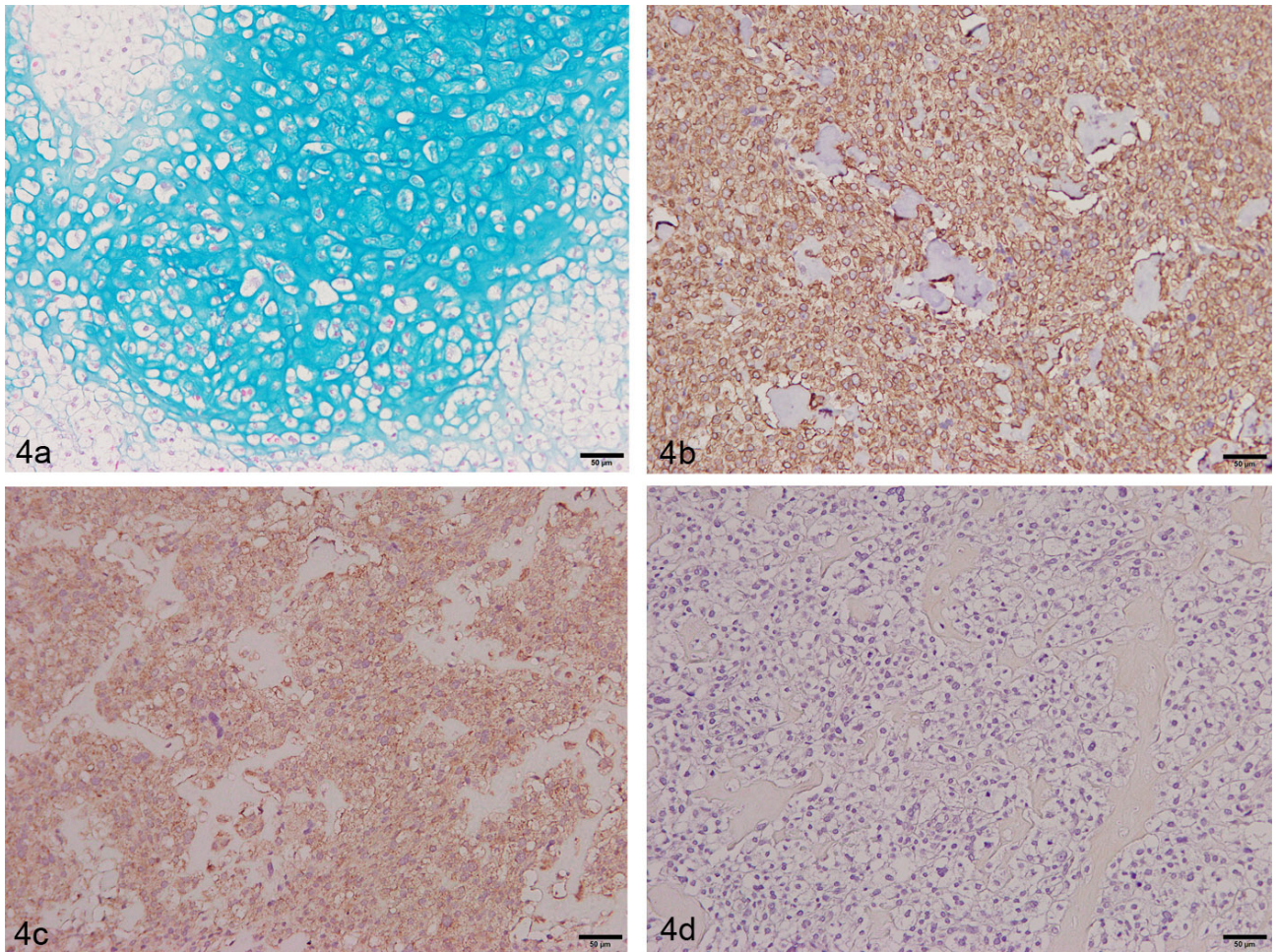
microwave oven at high power. Antigen retrieval was not performed for the S100 antibody. After blocking with 5% normal goat serum for 30 min at room temperature, the slides were individually incubated with each of the primary antibodies: the slides were washed three times in PBS and incubated with horseradish peroxidase secondary antibodies (K5007; Agilent Technologies) for 40 min. All slides were counterstained with Gill's hematoxylin. Positive control tissue sections were included for each antibody, and isotype-matched immunoglobulins were used as negative controls.

### 3. Results and discussion

Microscopically, the mass consisted of malignant mesenchymal cells that had produced a chondroid matrix and osteoid, with chondroblastic and osteoblastic

components in nearly equal volumes. Chondroid tissue formed by abnormal chondroblastic cells showed a moderate degree of anisokaryosis with vacuolated cytoplasm and large nucleoli. Polygonal cells with prominent nucleoli showed a moderate degree of anisokaryosis, and these had formed the osteoid components (Figure 3). There were minimal numbers of inflammatory cells. There were three to four mitotic figures per 40× high-power field. The chondroid material stained positive with Alcian blue (Figure 4a). Immunohistochemical analysis showed that most neoplastic cells were positive for vimentin (Figure 4b) and osteocalcin (Figure 4c) and negative for pancytokeratin, S100 (Figure 4d), and C-kit. On the basis of these findings, the tumor was diagnosed as an extraskeletal chondroblastic osteosarcoma.





**Figure 4.** Subcutaneous tissue mass excised from a Maltese dog. The chondroid material show positive staining reaction with Alcian blue (a). Immunohistochemical analysis showed that most neoplastic cells were positive for vimentin (b) and osteocalcin (c) and negative for S100 (d). Bar = 50 µm.

Osteosarcomas are malignant mesenchymal tumors that produce osteoid (2,4). Osteosarcoma accounts for 85% of malignant primary bone tumors in dogs (5,6), commonly occurring in middle-aged to older dogs and in large breeds (4). In one study, giant breeds of dog (>36 kg in weight) and large breeds (18–36 kg) were demonstrated to have a much higher probability of developing osteosarcoma than small breeds (<9 kg) (7).

Extraskeletal osteosarcoma is a malignant mesenchymal tumor that produces either osteoid or cartilage in the viscera or soft tissues, with no bone involvement (2). It is a rare tumor in dog, excluding tumors originating from the mammary gland (1,2). In contrast to human cases, most canine extraskeletal osteosarcomas arise from the viscera rather than from the subcutaneous tissues (2). Of the few canine cases reported in the subcutaneous tissues, one report described cases involving four dogs (1) and another occurred in a 7-year-old spayed female Labrador retriever

(3), while the most recently reported cases were in seven dogs (8), in an 11-year-old spayed female Labrador retriever (associated with a retained surgical sponge) (9), and in a 6-year-old spayed female Labrador retriever (associated with injection) (10). The majority of cases have thus been reported in larger breeds of dog, with no reports specifically involving smaller breeds.

Injection-site sarcomas commonly occur in cats but are rare in dogs (11). In this case, there is no evidence of chronic inflammation due to injection with no history of injection according to the medical records. In addition, histologic findings that can be commonly found in injection-site sarcomas such as central necrosis rimmed by inflammation and macrophages containing adjuvant materials were not observed. Based on these results, it was determined that the tumor was not associated with injection.

To the authors' knowledge, this is the first report of primary subcutaneous extraskeletal chondroblastic osteosarcoma in a Maltese dog. Among reports published in the 21st century, only a few cases of subcutaneous extraskeletal osteosarcoma were documented, all of which occurred in large or unspecified breeds of dog or unspecified locations (8,10). Since the location of the tumor is very unusual, the differential diagnosis of extraskeletal osteosarcoma in subcutaneous tissue may be missed if no calcification is observed radiographically. Furthermore, as extraskeletal osteosarcoma is a malignant tumor, accurate diagnosis is very important for prolonged survival of the dog. If calcification is observed radiographically, the possibility of extraskeletal osteosarcoma should be considered and the excised mass should be evaluated histologically and immunohistochemically to obtain an accurate diagnosis. In this case, 4 months after the surgical

removal, the tumor recurred at the same site and was confirmed as a result of biopsy.

In our report, we describe an extraskeletal osteosarcoma arising in a small breed of dog (Maltese), with radiographically evident calcification, for which we performed histopathological and immunohistochemical diagnosis. This finding suggests that, although subcutaneous extraskeletal osteosarcoma in a small breed of dog is extremely rare, it is important to be aware of possible differential diagnoses. In addition, immunohistochemical staining as performed in this study will help to confirm the diagnosis of extraskeletal osteosarcoma.

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