**Case Report**

**Cutaneous Extraskeletal Myxoid Chondrosarcoma with Recurrence and Multiple Pulmonary Metastasis in a Dog**

再発と多発性肺転移を伴った皮膚の骨外性粘液性軟骨肉腫の犬の一例

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**Abstract:** A small cutaneous mass in the right thigh of an 8-year-old, intact male dog was diagnosed as an extraskeletal myxoid chondrosarcoma. Histologically, the neoplasm had lobularity and was characterized by rich myxoid stroma and hypovascularity. The tumor cells displayed polymorphism, and were often arranged in characteristic cords. Immunohistochemically, the tumor cells were positive for vimentin, S-100a, neuron-specific enolase, and synaptophysin. Six months after the surgery, no recurrence or metastasis were observed; however, a large recurrent tumor in the right thigh extending to the abdomen and multiple pulmonary tumors were found in the dog 15 months after the surgery. The necropsy confirmed that the recurrent and pulmonary tumors were the same as the primary tumor and did not involve any cartilage or bone.

**Key words:** dog, extraskeletal myxoid chondrosarcoma, skin

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**Introduction**

Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue tumor of uncertain differentiation characterized by an abundant myxoid matrix with a multilobular structure and uniform cell cord (9). In humans, EMC accounts for less than 3% of soft tissue sarcoma and usually occurs in adults (7). EMC mostly develops in the deep soft tissues of the proximal...
extremities and limb girdles, and the thigh is the most common site. In animals, there are only three reports of EMC— in a rat, a cow, and a dog. Some cases of extraskeletal mesenchymal chondrosarcoma and regular-type extraskeletal chondrosarcoma have been reported in dogs, but only one canine necropsy case of pulmonary EMC with systemic metastasis has been reported. Therefore, the clinical behavior of canine EMC has not been confirmed.

In this study, we described the gross, histological, immunohistochemical, and ultrastructural features of cutaneous EMC in a dog with recurrence and multiple pulmonary metastasis 15 months after the surgical resection of the primary cutaneous tumor.

Case Report

An 8-year-old, mixed-breed, intact male dog was presented with a solid, round, cutaneous mass (25 × 25 × 15 mm) with partial surface necrosis in the right femoral area. The mass did not tightly adhere to the underlying tissues, and the regional lymph node showed no enlargement. The tumor was surgically excised and submitted to our laboratory for histopathological examination. On cut sections, the mass was shiny white and partially transparent. Grossly, the lesion composing the mass was poorly demarcated.

When the dog was presented to the veterinary clinic six months after surgery, no recurrence or metastasis was detected. However, 15 months after the surgery, a large recurrent mass had developed in the right femoral region extending to the abdomen, and thoracic radiographs showed the presence of small, multiple masses throughout the lungs. Five months later, the dog was euthanized because of the poor prognosis. At necropsy, a large mass (200 × 105 × 65 mm) with surface necrosis was found in the inguinal region of the abdomen (Fig. 1). The penis and scrota were dislocated to the left side of the abdomen because of the mass. The recurrent mass developed in the dermal and subcutaneous regions and did not involve surrounding skeletal muscle, penis, cartilage, or bones. Therefore, the mass was easily separated from the underlined muscles (Fig. 2). The lungs had multiple masses in whole pulmonary lobes (Fig. 3A). The cut surface of the masses were shiny and slimy in appearance, similar to those of the primary and recurrent tumors (Fig. 3B).

No conspicuous abnormalities were found in the lymph nodes or other organs.

Excised tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 3-μm thickness, and stained with hematoxylin and eosin (HE). Selected sections of the mass were stained with periodic acid-Schiff (PAS) and Alcian blue (pH 2.5) with or without hyaluronidase (Wako Pure Chemical Industries, Osaka, Japan) predigestion. Immunohistochemical staining was performed using the immunoenzyme polymer method and the primary antibodies shown in Fig. 1.
Table 1. Peroxidase-conjugated anti-mouse (Histofine Simple Stain MAX-PO (M); Nichirei, Tokyo, Japan) or peroxidase-conjugated anti-rabbit (Histofine Simple Stain MAX-PO (R); Nichirei) immunoglobulin (Ig) G was used as a secondary antibody. After immunoreaction, the sections were colorized with 3, 3′-diaminobenzidine (DAB) and counterstained with Mayer’s hematoxylin. A part of the formalin-fixed tissue specimen from the mass was cut into 1-mm³ cubes, re-fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were cut, double-stained with uranyl acetate and lead citrate, and examined using a JEOL 1210 transmission electron microscope (JEOL, Tokyo, Japan) at 80 kV.

Histologically, the primary cutaneous tumor was divided into irregular lobules by fibrous connective tissues extending from the dermis to the subcutis (Fig. 4A). The vascularity of the tumor was characteristically very low. The tumor cells were surrounded by abundant myxoid stroma and arranged in irregular short cords in which the tumor cells singly interconnected with each other in small clusters (Fig. 4B). The tumor cells had eosinophilic cytoplasm and displayed marked polymorphism in shape (round, spindle, stellate, or polygonal) (Fig. 4C). The nuclei were round, ovoid, or spindle-shaped with small nucleoli. Mitotic activity of the tumor cells was moderate, approximately two mitoses per high-power field (400×). The myxoid stroma of the tumor stained positively with Alcian blue but not with PAS. Staining reactivity to Alcian blue was preserved after hyaluronidase digestion. The cellular density of the tumor varied between the lobules. Neutrophilic infiltration was occasionally observed in the tumor. Chondroid and osseous components were not observed in the tumor. The surgical margin of the resected primary tumor was free of tumor cells. The

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Fig. 2. Macroscopic features of the cut surface of the recurrent tumor with shiny white appearance. Necrosis and hemorrhage were observed.

Fig. 3. The metastatic tumors in the lungs. A) Multiple masses throughout the lung. The largest mass (38 × 30 × 28 mm) was present in the right anterior lobe. The left anterior lobe showed hypoplasia. Bar = 4 cm. B) The cut surface of metastatic tumors showed solid and shiny white appearances. Bar = 3 cm.
tumors developed in the right femoral area and in the lungs after surgery shared the same histopathological features as the primary tumor; therefore, we diagnosed these as recurrent and metastatic tumors. The necrosis developed in the center of the recurrent and metastatic tumor masses. There were no tumors in the other organs, including the lymph nodes.

Immunohistochemistry showed that all the tumor cells were positive for vimentin, about half of them for S-100a (Fig. 4D), and more than three quarters of them for NSE and synaptophysin (Fig. 4E). However, the tumor cells were negative for cytokeratin, α-SMA, neurofilament protein, chromogranin A, Iba-1, GFAP, PGP9.5, desmin, and melan A. PCNA was positive in most tumor cells, indicating they had high proliferative activity (Table 2). The myxoid stroma was diffusely positive for type I and type II collagen (Fig. 4F). These immunohistochemical properties of the tumor cells were identical in the primary resected, recurrent, and metastatic pulmonary neoplasms.

Ultrastructural examination showed that the tumor cells had well-developed intermediate filaments, scattered rough endoplasmic reticulum, and a few

| Antibody           | Clone     | Dilution | Antigen retrieval | Antibody source         |
|--------------------|-----------|----------|-------------------|-------------------------|
| Cytokeratin        | AE1/AE3   | 1:100    | trypsin           | Dako                    |
| Cytokeratin        | CAM 5.2   | pre-diluted | proteinase K  | Becton Dickinson         |
| Vimentin           | V9        | 1:25     | MW                | Dako                    |
| α-SMA              | 1A4       | 1:50     | NT                | Dako                    |
| S-100a             | polyclonal | 1:200    | MW                | Dako                    |
| Neuron specific enolase (NSE) | NSE-IgG | 1:100 | MW | Zymed |
| Synaptophysin      | SY38      | 1:20     | NT                | Dako                    |
| Neurofilament protein | 2F11    | 1:100    | MW                | Dako                    |
| Chromogranin A     | polyclonal | pre-diluted | MW | Zymed |
| Iba-1              | polyclonal | 1:100    | MW                | Wako Pure Chemical Industries |
| GFAP               | 1B4       | 1:100    | MW                | Becton Dickinson         |
| PGP9.5             | B14       | 1:100    | MW                | UltraClone Limited       |
| PCNA               | PC10      | 1:100    | MW                | Dako                    |
| Desmin             | MDE II    | 1:30     | MW                | Bio-SCIENCE PRODUCTS AG |
| Melan A            | A103      | 1:50     | MW                | Dako                    |
| Type I collagen    | polyclonal | 1:500    | MW                | COSMO BIO               |
| Type II collagen   | polyclonal | 1:200    | proteinase K      | COSMO BIO               |

a) trypsin, 0.1%, 37°C, 30 min; MW = microwave/citrate buffer (pH 6.0), 90°C, 10 min; NT = no treatment; MW = microwave/citrate buffer (pH 6.0), 95°C, 20 min; MW = microwave/citrate buffer (pH 9.0), 90°C, 10 min; proteinase K, 0.4mg/ml, 37°C, 30 min. b) Dako, Copenhagen, Denmark; Zymed, California, U.S.A.; Wako Pure Chemical Industries, Osaka, Japan; Becton Dickinson, Heidelberg, Germany; UltraClone Limited, Isle of Wight, England; Bio-SCIENCE PRODUCTS AG, Emmenbrucke, Switzerland; COSMO BIO, Tokyo, Japan.

| Antibody            | Tumor cell |
|---------------------|------------|
| Cytokeratin AE1/AE3 | −*         |
| Cytokeratin CAM 5.2 | −          |
| Vimentin            | +++        |
| α-SMA               | −          |
| S-100a              | +          |
| NSE                 | ++         |
| Synaptophysin       | ++         |
| Neurofilament protein | −        |
| Chromogranin A      | −          |
| Iba-1               | −          |
| GFAP                | −          |
| PGP9.5              | −          |
| PCNA                | +++        |
| Desmin              | −          |
| Melan A             | −          |
| Type I collagen**   | ++         |
| Type II collagen**  | ++         |

*+++ = positive (>75%); ++ = positive (50~75%); + = positive (<50%); − = negative; ** = staining reactivity of myxoid stroma.
**Fig. 4.** Histological and immunohistochemical characteristics of the primary tumor. A) The tumor was located in the dermis and showed irregular multiple lobulation separated by fibrous septa. Hematoxylin and eosin (HE) stain. Bar = 200 μm. B) Tumor cells arranged irregularly in short cords, in small clusters, and singly in the myxoid stroma. HE stain. Bar = 100 μm. C) Tumor cells with round, ovoid to spindle-shaped nuclei with eosinophilic cytoplasm. Mitotic figure (arrow) and neutrophilic infiltration were observed. HE stain. Bar = 40 μm. D) Approximately half of the tumor cells were positive for S-100a. Immunohistochemical staining with Mayer’s hematoxylin counterstain. Bar = 40 μm. E) More than three quarters of the tumor cells were positive for synaptophysin. Immunohistochemical staining with Mayer’s hematoxylin counterstain. Bar = 40 μm. F) The myxoid stroma was diffusely positive for type II collagen. Immunohistochemical staining with Mayer’s hematoxylin counterstain. Bar = 100 μm.
**Fig. 5.** Ultrastructural appearance of tumor cells. A) Tumor cells showed well-developed intermediate filaments (*), scattered rough endoplasmic reticulum, a few microvillus processes (arrows), and a cell junction (arrowhead). Collagen fibrils were scattered in the stroma. Bar = 5 μm. B) Higher magnification of microvillus processes (arrow in A). Bar = 500 nm. C) Higher magnification of intermediate filaments (* in A). Bar = 100 nm. D) Higher magnification of a desmosome-like structures without tonofilaments (arrowhead in A). Bar = 100 nm.

microvillus processes but no neuroendocrine granules (Fig. 5A–C). The cells were sometimes attached to each other by desmosome-like structures without tonofilaments (Fig. 4D). Some tumor cells contained lipid droplets. No basal lamina-like structure was observed around the tumor cells, and collagen fibrils were scattered in the stroma.

**Discussion**

Despite its name, EMC is now categorized by the WHO as a soft tissue tumor of uncertain differentiation because there is no convincing evidence of cartilaginous differentiation\(^9, 18\). In humans, EMC is histologically characterized by a multilobular structure divided by fibrous connective tissue, abundant myxoid stroma, and uniform cells arranged in cords or clusters which are often interconnected forming cell networks\(^9\). Hypovascularity of the tumor stroma is also conspicuous. In the present study, the canine tumor demonstrated all these histological features of EMC and did not involve any joints or bones, indicating it had an extraskeletal origin.

The myxoid stroma seen in the present case was composed of acid mucopolysaccharides without hyaluronic acid, similar to that of human EMC\(^1, 3, 4, 8, 10\). Some investigators regard hyaluronidase resistance as one of the key points for the diagnosis of EMC\(^3\), but this is now regarded as controversial\(^10\). Hyaluronidase resistance was demonstrated in the present case, but not in the previous cases of a rat\(^13\) and a cow\(^19\). Thus, more detailed investigations about myxoid stroma may be necessary in animal cases with EMC. In the present case, type II collagen, known to be specific to cartilaginous tissues, was detected by immunostaining. This indicates that the present tumor had the potential to produce cartilaginous matrix, as observed in human EMC\(^1, 8\). In addition, the ultrastructural findings of the tumor cells and their positive immunoreactivity to S-100a and vimentin suggest that the present tumor possessed some characteristics normally associated with chondroblasts\(^6, 10\). These immunohistochemical and ultrastructural findings of the tumor and the myxoid stroma suggest chondrocyte differentiation, which was also previously observed in rat EMC\(^11\). Some studies have reported that neuroendocrine markers were expressed in EMC\(^2, 7, 8, 14\). In the present case, the tumor cells were positive for NSE and synaptophysin, indicating neuroendocrine differentiation\(^2, 4, 14, 15\). However, no neuroendocrine granules were detected by electron microscopy.

Based on the macroscopic, histological, immunohistochemical, and ultrastructural findings, we conclude the present tumor was EMC. This is the first report of primary cutaneous EMC in dogs. The differential diagnosis of this tumor includes chondrosarcoma, myxoid liposarcoma, malignant melanoma, mixed tumor/myoepithelioma of the sweat gland, and nervous system tumors\(^5, 10, 18\). We excluded these tumors mainly by macroscopic, histological, immunohistochemical (negative reactions to epithelial, muscular, and melanocytic markers), and ultrastructural features. Among these tumors, chondrosarcoma was the main differential diagnosis of the tumor in the present case, because myxoid change is a common feature of chondrosarcoma\(^12\). Chondrosarcoma usually arises in the bones or chondroid tissues, and is characterized by the formation of distinct cartilaginous tissue\(^12\). However, the tumors in the present case did not contain cartilaginous tissue and did not involve any bones or cartilage tissues.

In humans, EMC occurs at a median age of approximately 50 years and has high rates of recurrence.
and tumor-associated death. Local recurrence ranges from 37% to 48%, while metastasis occurs in about half of the cases. Metastasis usually develops in the lung, as seen in the present case. In the present case, recurrence and metastasis to the lungs were found 15 months after the primary tumor had been resected, suggesting that canine EMCs could be similar to human EMCs. In contrast, a recently reported canine case of EMC showed systemic metastasis from a primary pulmonary neoplasm at the age of 5 months. The features of tumor cells in both cases were histologically comparable. Therefore, the difference in the metastatic behavior of the two cases may be related to the tumor size and the primary site. In conclusion, canine EMC is a rare tumor, but it is malignant and carries the potential of systemic metastasis. Therefore, practitioners should excise the tumor with a wide margin even in cases of small tumor, and should carefully perform follow-up examinations after surgery.

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