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Case report

A RARE CASE OF OSSEOUS METAPLASIA IN CANINE LEIOMYOMA AND PLASTICITY OF SMOOTH MUSCLE CELL

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Metaplasia is a transformation of one mature cell type to other kinds of mature cells. Metaplasia is hardly detected in benign tumors, whereas it is frequently observed in malignant tumors. In this study, we report the first case of osseous metaplasia in canine leiomyoma. The region of osseous metaplasia was highly eosinophilic and had various sizes of distinct lacunae. The osseoid material was confirmed by Von Kossa staining. Except for the osseous metaplasia, the mass presented typical features of leiomyoma without any histopathological features of malignancy. The characteristics of malignant tumors, including pleomorphism, mitotic figures, and lymphatic metastasis were not identified and the mass was proven to be a benign tumor. The osseous metaplasia in leiomyoma without dysplastic changes might be due to plasticity which is a unique feature of smooth muscle cells, whether the tumor is malignant or not. This case suggests the possible occurrence of osseous metaplasia in leiomyoma, which has been overlooked due to the lack of reports. Also, it is recommended to pay attention to making a diagnosis of smooth muscle tumor with metaplasia so as not to be confused with leiomyosarcoma and leiomyoma variants.

Keywords: Osseous metaplasia, smooth muscle, benign, leiomyoma, dog

INTRODUCTION

Metaplasia refers to the replacement of one type of differentiated somatic cells with other types of differentiated cells that do not normally exist in the tissue. It occurs in response to external stimuli to adapt to a new environment [1,2]. As the stimuli are prolonged and accumulated, more cells transform their identity with accompanying low-grade dysplasia [1]. Importantly, metaplasia with high-grade dysplasia tends to culminate in malignant tumors [1]. Thus, metaplasia is commonly used as one of the criteria of malignancy in tumor diagnosis, especially when accompanies a high-grade dysplasia [1,3].

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Leiomyoma is a slow-growing benign tumor which is originated from smooth muscle and commonly occurs in domestic and companion animals [4-6]. It is observed in various organs which are composed of smooth muscle such as the gastrointestinal tract [5], reproductive tract [7,8], urinary bladder [4], laryngopharynx [9], eyelid [10], and skin [11]. According to the histopathological findings, leiomyoma is composed of homogeneous spindle cells with elongated and blunt-ended nuclei. The neoplastic cells showed an interlacing arrangement. It is hard to observe dysplasia and pleomorphism in leiomyoma.

Thus, osseous metaplasia is rarely reported in leiomyoma. Osseous metaplasia in leiomyoma has been reported in only two cases in humans [12,13], one case in feline [14], and no case in dogs. Due to the rarity of osseous metaplasia in leiomyoma, the study on the relationship of metaplasia and malignancy in smooth muscle cell tumors has not been conducted so far. As the first case report of osseous metaplasia in canine leiomyoma, this report will help to better understand the pathogenesis of osseous metaplasia in leiomyoma and its association with malignancy.

CASE PRESENTATION

A 10-year-old female Miniature Pinscher's perineum was swollen (Figure 1A). All vital signs, including body temperature, appetite, defecation, and urination, were normal. Serum chemistry and complete blood count were performed, and the results were normal, except for rather high platelets counts ($5.26x10^{11}$/L). On X-ray imaging, a

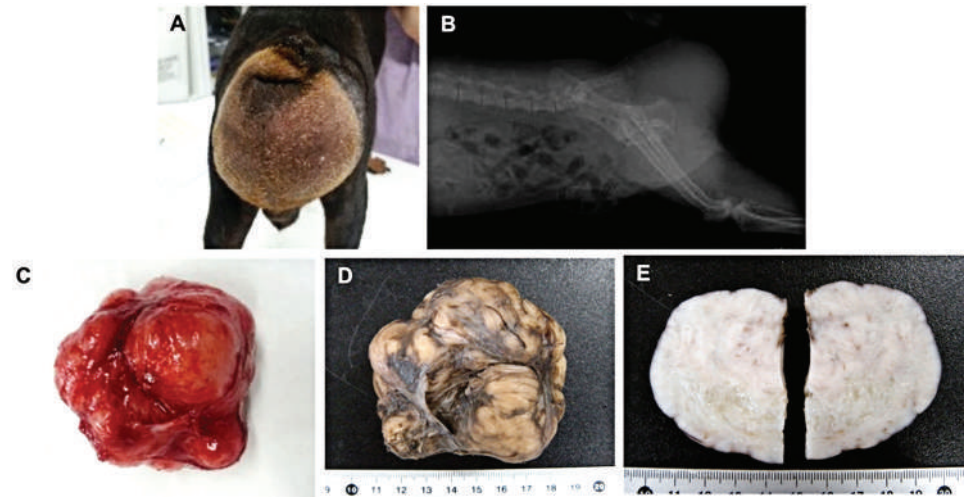

Figure 1. (A) Peritoneal lesion of the tumor. The peritoneum was distended due to the tumor growth. **(B)** X-ray image of the tumor lesion. The tumor was found in the peritoneum and showed a globular shape. **(C)** Gross findings prior to fixation. The mass was well-circumscribed. **(D)** Gross findings after fixation. The mass was covered with a collagen capsule. The mass was composed of several globular-shaped masses. (E) Cut surface of the mass. The cut surface was white to pale pink. In some parts, a yellowish semitransparent lobule was found.

large well-delineated mass was found in the perineum (Figure 1B). The mass was removed surgically (Figure 1C). No recurrence and metastasis to other organs were observed after resection of the mass during the follow-up period.

Macroscopically, the mass was firm and round; the size of the mass was 8×8×5 cm^3. The mass was well-circumscribed and encapsulated, and it was covered and lobulated with a thin collagen capsule (Figure 1D). On the cut surface, the mass was white to pale pink (Figure 1E). The tissue sample was submitted to the veterinary pathology laboratory of the Kyungpook National University from a local animal hospital. The tissue sample was fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin wax. The embedded tissue was cut into 6 μm thickness. The slides were depariffinized and stained with hematoxylin and eosin (H&E). Histopathologically, the neoplastic cells formed broad interlacing fascicles that mimicked normal smooth muscle. The mass was composed of proliferating spindle cells with blunt-ended nuclei called cigar-shaped nuclei (Figure 2A). The cytoplasms of the neoplastic cells were strongly eosinophilic with vacuolation (Figure 2B). There was no striation in the cytoplasm. The pleomorphism of neoplastic cells and nucleus

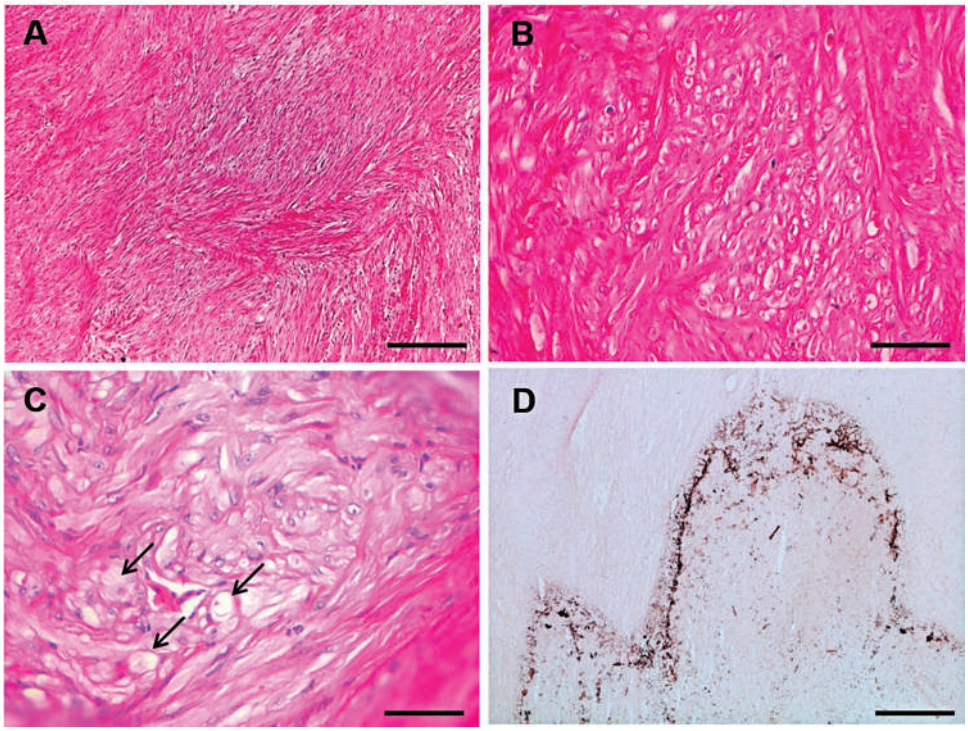

Figure 2. Histopathological findings. **(A)** The mass was composed of spindle shape neoplastic cells. The neoplastic cells had blunt-ended nuclei (cigar-shaped). Scale bar = 200 μm. **(B)** Osseous metaplasia was observed in some parts of the mass. Early stage of immature osseous metaplasia was observed. Scale bar = 100 μm. **(C)** Prominent lacunae (arrows) were observed in the osseous metaplasia lesion. Scale bar = 50 μm. H&E staining. **(D)** Special staining for calcium deposition which is presented as dark brown or black color. Scale bar = 200 μm. Von kossa staining.

was hard to observe. Lymphatic metastasis and mitotic figures were not observed. Interestingly, the early stage of osseous metaplasia was observed among the well-differentiated neoplastic cells. Variable sizes of lacunae were observed in the lesions of immature osseous metaplasia. The lacunae had eosinophilic, round, and condensed material in the center (Figure 2C). To confirm osseous metaplasia, Von Kossa staining was performed, which is a special staining for detecting calcium deposition. It was stained as distinct dark brown to black by Von Kossa staining and determined as osseous metaplasia (Figure 2D).

Table 1. Information on primary antibodies used in this study

	Concentration	Manufacturer	Catalog no.
α-smooth muscle actin	1:200	SantaCruz	SC-53142
Vimentin	1:200	SantaCruz	SC-6260
Desmin	1:100	SantaCruz	SC-23879
MyoD	1:200	SantaCruz	SC-32758
S100	1:100	SantaCruz	SC-53438

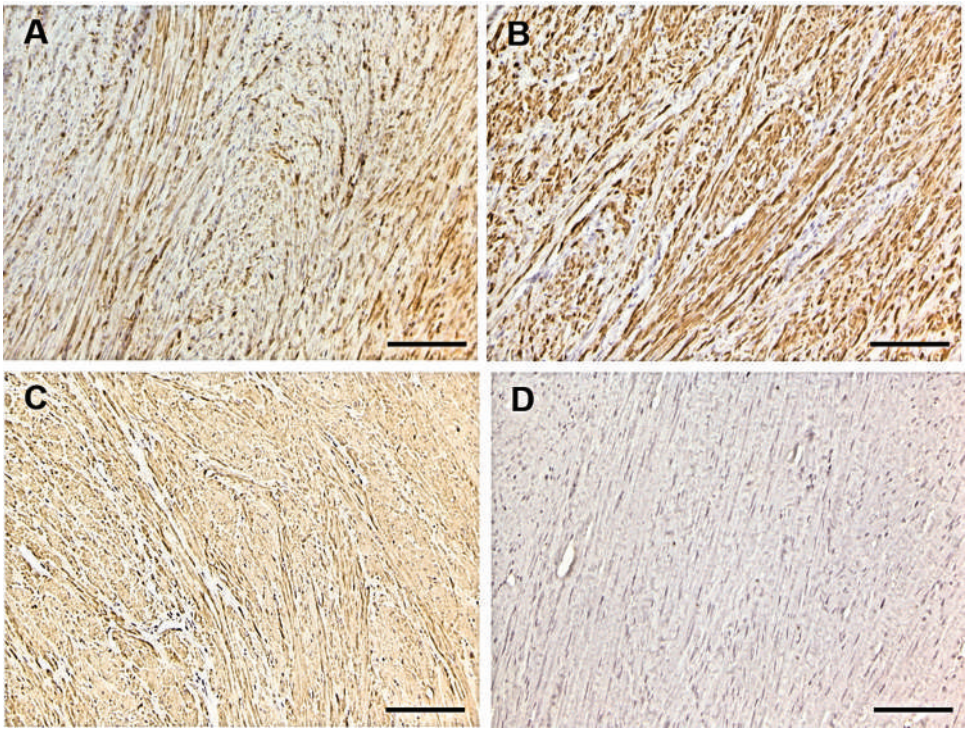

Figure 3. Immunohistochemical staining for **(A)** vimentin **(B)** desmin **(C)** alpha-smooth muscle actin, and **(D)** MyoD. Scale bar = 200 μm. Avidin-biotin Complex (ABC) detection method was used for IHC staining.

The mass was considered to be one of the spindle cell tumors, such as leiomyoma, fibroma, Schwannoma, malignant nerve sheath tumor, rhabdomyoma, and hemangiopericytoma. For differential diagnosis, IHC staining was performed for alpha-smooth muscle actin, vimentin, desmin, MyoD, and S100. For immunohistochemistry staining, the deparafinized slides were incubated with 0.01M citrate buffer to retrieve antigen. After incubation in blocking solution, primary antibodies were applied and incubated for 1 hour (Table 1). The antigen-antibody complex was detected by an avidin-biotin complex (ABC) kit (VECTA Stain Elite ABC kit) and visualized by using DAB substrate kit. The neoplastic cells were strongly positive for alpha-smooth muscle actin, vimentin, and desmin, moderately positive for myoD, but negative for S100, indicating that the cells were originated from smooth muscle (Figure 3). On the basis of the results of macroscopic and microscopic observation, and IHC staining, the mass was diagnosed as a benign leiomyoma.

DISCUSSION

Metaplasia in leiomyoma is extremely rare. There was no article on osseous metaplasia in canine leiomyoma when searched by the key words "canine", "leiomyoma", and "metaplasia" on MEDLINE/Pubmed for the period between January 1974 and January 2022. Only a few cases of osseous metaplasia in leiomyoma have been reported in humans and cats [12-14]. Of note, metaplasia is typically observed in malignant tumors and is one of the features of malignancy [3]. Malignant tumors are composed of undifferentiated immature cells, which have flexibility and multipotency. These features of malignant neoplastic cells are suggested to contribute to the frequent occurrence of metaplasia in malignant tumors [15]. Also, the relationship between metaplasia and malignancy could be explained by the fact that metaplasia with prolonged external stimuli is occasionally accompanied by dysplasia, which leads to pleomorphism and aggressiveness in malignant tumors. In other words, the reason for the frequent occurrence of metaplasia in malignant tumors could be explained by the flexibility in cell fate and dysplasia of malignant tumors.

In this case, however, we report osseous metaplasia in leiomyoma which is benign tumor originating from smooth muscle. It presents typical features of benign tumors including abscence or low grade of nuclear pleomorphism, mitotic index, and dysplasia. After resection of the mass, there was no recurrence of tumor and the prognosis was good. Although leiomyoma presents typical features of benign tumor basd on morphological criteria, it can also show atypical behaviors which are generally regarded as malignant features.

First, for expample, there is a growing number of reports that benign leiomyomas metastasize to remote organs, which is named benign metastasizing leiomyoma (BML) [16-18]. BMLs are often observed in lungs with forming multiple nodules and bone [19,20]. Owing to the metastasis, BML can be misdiagnosed as leiomyosarcoma, which is a malignant counterpart of leiomyoma. In general, however, the primary tumor and

the metastasized tumors of BML do not show aggressive progress which is related to a poor prognosis. The osseous metaplasia observed in this case also could be one of the atypical features of leiomyoma like metastasis of leiomyoma. The atypical features of leiomyoma could be explained by the unique characteristics of smooth muscle. Unlike other mature somatic cells, smooth muscle cell has remarkable plasticity which can change their phenotypical status between contractile and synthetic in response to environmental cues [21]. The plasticity of smooth muscle cells is regulated by epigenetic changes, especially histone modification and DNA methylation, which lead to transcriptional changes depending on environmental stimuli [21].

Second, another example of the plasticity of the smooth muscle cells is that osseous metaplasia is reported in normal endometrium and cervix even without any history or presence of neoplasms [22,23]. The cell fate of smooth muscle is not strictly confined to contractile phenotype, suggesting the potential of smooth muscle to transform other cell types such as osteoblasts which secrete osteoid and form osseous metaplasia.

Besides leiomyosarcoma as a malignant counterpart of leiomyoma, there are four types of leiomyoma variants, whose malignancy is between leiomyoma and leiomyosarcoma. The four leiomyoma variants are mitotically active leiomyoma, cellular leiomyoma, atypical leiomyoma, and smooth muscle tumors of uncertain malignant potential (STUMP) leiomyoma [24]. Metaplasia of leiomyoma may be classified as a new "variant" of leiomyoma as more cases are reported like this study.

It is recommended not to apply "metaplasia" as one of the criteria to determine malignancy in smooth muscle-originated tumors due to its plasticity, although it is commonly regarded as a malignant feature in many tumors [3]. Also, the exact mechanisms involved in osseous metaplasia in neoplastic cells are not revealed yet. Thus, studying this case could be helpful to reveal the mechanisms of osseous metaplasia in neoplastic cells. Unless neoplastic cells of leiomyoma present moderate to severe dysplasia, it is suggested not to consider leiomyosarcoma, although metaplasia is observed. Thus, it is suggested to pay attention to makimg a diagnosis of smooth muscle tumor with metaplasia.

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Authors' contributions

EJL drafted and edited the manuscript. KSJ edited the manuscript. Both authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

the owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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REDAK SLUČAJ KOŠTANE METAPLAZIJE KOD LEJOMIOMA PSA I PLASTIČNOST GLATKIH MIŠIĆNIH ĆELIJA

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Metaplazija je transformacija jednog tipa zrelih ćelija u druge tipove zrelih ćelija. Metaplazija se retko uočava kod benignih tumora, dok je kod malignih tumora često prisutna. U ovoj studiji izveštava se o prvom slučaju koštane metaplazije kod leiomioma pasa. Oblast koštane metaplazije bila je visoko eozinofilna i imala je lakune različitih veličina. Oseoidni materijal je potvrđen Von Kossa bojenjem. Osim koštane metaplazije, masa je imala tipične karakteristike lejomioma bez histopatoloških karakteristika maligniteta. Karakteristike malignih tumora, uključujući pleomorfizam, atipične mitotske figure i limfne metastaze, nisu identifikovane i dokazano je da je masa benigni tumor. Kostna metaplazija kod lejomioma bez displastičnih promena može biti posledica plastičnosti koja je jedinstvena karakteristika ćelija glatkih mišića, bez obzira da li je tumor maligni ili ne. Ovaj slučaj ukazuje na moguću pojavu koštane metaplazije kod lejomioma, što je zanemareno zbog nedostatka dostupnih izveštaja. Takođe, preporučljivo je obratiti pažnju na postavljanje dijagnoze tumora glatkih mišića sa metaplazijom kako se ne bi mešali sa varijantama leiomiosarkoma i lejomioma.