pISSN 1598-298X
J Vet Clin 30(6) : 478-481 (2013)

Journal of Veterinary Clinics
www.ksvc.or.kr

Peripheral Giant Cell Granuloma in a Dog

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(Accepted: December 11, 2013)

Abstract : A mass was detected in the oral cavity from a 18-year-old female miniature poodle dog. Grossly, the mass was soft to hard, red to purple, and 1.5 × 1.5 × 1 cm in size. Histopathologically, the mass was composed of hyperplastic gingival epithelium, well-vascularized stroma, closely packed pleomorphic cells, and numerous giant cells with multiple nuclei and abundant eosinophilic cytoplasm. Immunohistochemically, tumor cells were positive for alkaline phosphatase and cytokeratin 7, but not positive for CD68 unlike in human. The mass was diagnosed to peripheral giant cell granuloma in oral cavity through typical clinical and histopathological features.

Key words **:** peripheral giant cell granuloma, canine, alkaline phosphatase, cytokeratin 7, CD68.

Introduction

Variety of neoplastic lesions, odontogenic or non-odontogenic in origin, occur in the oral cavity. They can be confused with non-neoplastic lesions such as swelling, gingival hyperplasia, infective conditions etc. Conversely, oral neoplasms may present as nonhealing ulcerative lesions rather than as masses (5).

The term epulis is a clinically descriptive term referring to a localized swelling on the gingiva (5). Epulis is common in the dog and they accounted for approximately 59% of benign canine oral neoplasm (19). They constitute a variety of pathologic entities and can be classified into four types on the basis of histologic appearance: fibromatous, ossifying, acanthomatous and giant cell (7).

The giant cell epulis (GCE) is regarded as hyperplastic or granulomatous lesion and has occurred at the site of tooth extraction (2,7). Three of the four GCE were found on the gingiva around the maxillary premolars (22). GCE is an infrequent reactive, exophytic lesion of the oral cavity, also known as peripheral giant cell granuloma (PGCG), osteoclastoma, giant cell reparative granuloma, or giant cell hyperplasia (3,18). In recent studies, giant cell epulis is called a peripheral giant cell granuloma mainly. PGCG is a rare tumor in dog, also accounts for approximately 1% of all oral pathologic lesions in man (4). PGCG originates from the connective tissue of the periosteum or from the periodontal membrane, in response to local irritation or chronic trauma (3). It generally shows the histopathological features, such as thin loose fibrovascular connective tissue, hemorrhage, hemosiderosis and multinucleated giant cells interspersed with lymphocytes, plasma cells, osteoclast and macrophages in the submucosal stroma (20,22).

Detailed analysis of phenotypic markers of tumors cells may help to clarify tumor histogenesis and to improve their diagnosis in surgical veterinary pathology. Amelogenins, keratins, collagens type III and IV, vimentin, fibronectin, osteonectin, alkaline phosphatase, osteopontin, bone sialoprotein and osteocalcin are used to comparative investigations of odontogenic cells (12,16). In human, PGCG was demonstrated that the tumor tissues were derived from osteoclast which is positive for CD68. CD68 is a specific marker showing that PGCG is derived from osteoclasts (3). And PGCG was also positive for alkaline phosphatase (AP) (11). In addition, osteoclast-like giant cells were weakly positive for cytokeratin 7 (CK7) (15). This report illustrates a case of canine PGCG by clinical and histopathological features.

Case

An 18-year-old female miniature poodle dog with the clinical signs of dysmasesis and anorexia was presented to local animal hospital. The swelling with turgidity in the premolar to molar maxillary region was detected in physical examination, because it was suspected to interalveolar abscess, the patient was treated with antibiotics and anti-inflammatory drugs. However, the treatment was ineffective and surgical excision of the mass was performed.

The mass was fixed in 10% neutral buffered formalin. After paraffin embedding, 4 μm sections were stained with hematoxylin and eosin (H&E) and applied for histopathological analysis. Also, the tumor tissue was examined immunohistochemically by the avidin-biotin peroxidases complex method (Vector Laboratories, CA, USA), using primary antibodies (Table 1). Briefly, 4 μm sections were deparaffinized

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Table 1. Primary antibodies used for immunohistochemistry

Antibody	Clone	Dilution	Positive Control tissue
Alkaline phosphatase	Rabbit polyclonal	1 : 150	Canine mammary tumor
CD68	Mouse monoclonal	1 : 150	Mouse bone marrow
Cytokeratin 7	Mouse monoclonal	1 : 150	Mouse tonsil

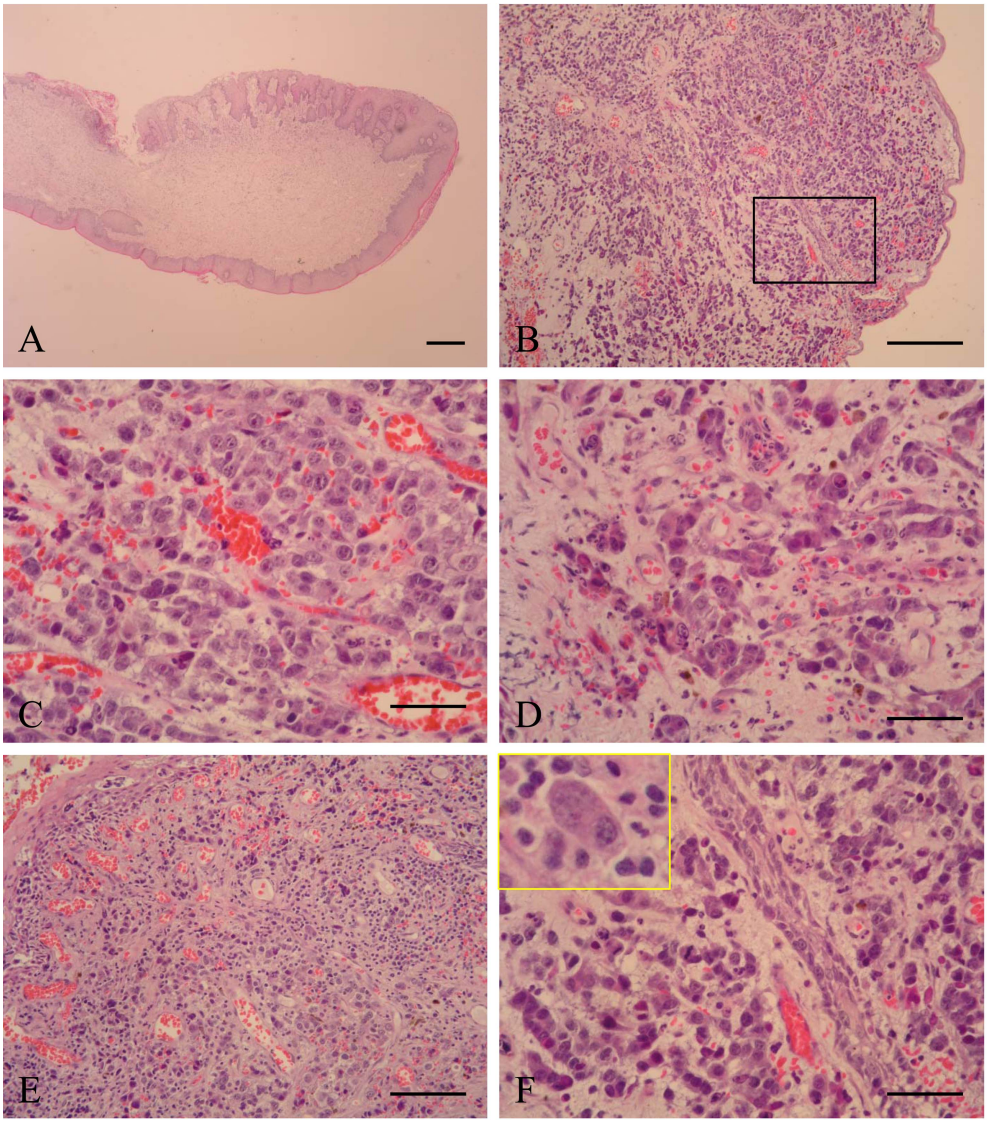

Fig 1. Tumor is covered with thin or hyperplastic gingival epithelium (A; Bar = 500 μm). Tumor is composed of nodules with fibrovascular connective tissue and anastomosing cords of hyperplastic gingival epithelium (B; Bar = 200 μm). Densely packed irregular shaped neoplastic cells are formed variably sized tumor clusters and cords (C, D; Bar = 50 μm). Small areas of hemorrhage are dispersed throughout the tissue with dilated veins and capillaries (E; Bar = 100 μm). Irregularly shaped multinucleated giant cells, often containing many visible nuclei with abundant eosinophilic cytoplasm (box: high magnification) are noted (F; Bar = 50 μm, Higher magnification of B). H&E.

and placed in citrate buffer at pH 6.0 for antigen retrieval. After washing, sections were incubated in 3% H_2O_2/methanol to quench endogenous peroxidase activity. Nonspecific protein binding was attenuated by incubation in 10% goat normal serum with PBS. Then, specimens were incubated overnight with primary antibodies and reacted with the second biotinylated antibodies and avidin-biotin-horseradish peroxidase complex and then detected by diaminobenzidine (Vector Laboratories, CA, USA). After developing, the sections were counterstained with hematoxylin and coversliped.

Histopathologically, tumor was covered with thin or hyperplastic gingival epithelium, and composed of multiple densely

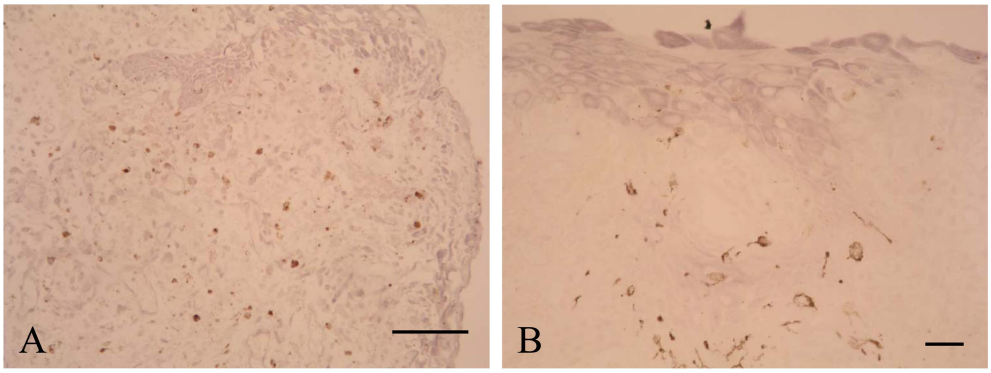

Fig 2. Positive reactions for alkaline phosphatase (A) and cytokeratin 7 (B) are noted in the gingival tissue. Bar = 100 μm, IHC.

cellular and well vascular nodules separated by anastomosing cords of hyperplastic gingival epithelium with a thin band of loose fibrovascular connective tissues. Small areas of hemorrhage were dispersed throughout the tissue with dilated veins and capillaries. Tumor cells was spindled with a background of collagenic fibers, or rounded and pleomorphic with a less fibrotic background. There was occasional inflammatory cells admixed with the tumor cells or within surrounding fibrovascular tissues. The striking histologic feature was the presence of large numbers of irregularly shaped multinucleated giant cells, often containing many visible nuclei with abundant eosinophilic cytoplasm (Fig 1). It is characterized by rich vasculature, particularly in the peripheral areas, consisting mainly of thin walled, small sized vessels.

Immunohistochemically, it was presented that the tumor cells were positive for alkaline phosphatase and CK7 (Fig 2). However, the tumor cells were negative for CD68 unlike in human (Data not shown).

Discussion

To diagnose PGCG in oral cavity, differential diagnoses with malignant tumors such as malignant melanoma, squamous cell carcinoma, fibrosarcoma should be considered (9). In case of malignant melanoma, there are copious intracellular pigment and visibly black, frequent metastasis to regional lymph nodes and then the lungs. In case of squamous cell carcinoma (SCC), there are often quite aggressive locally, invading subjacent tissues. Some SCCs contain more differentiated cell, keratin, often in whorls (keratin pearl) and visible intercellular bridges, whereas others are less well differentiated, with significant mitotic activity. In case of fibrosarcoma, there is collagen producing cell (fibroblasts) of the oral cavity (9).

And other types of epulis such as fibromatous, ossifying, acanthomatous type in oral cavity should be considered (7, 13,20). The fibromatous epulis is composed of an expansile mass of stellate fibroblasts surrounded by various amount of densely packed and fibrillar collagen. The ossifying epulis has all of features of a fibromatous epulis but in addition contains either irregular islands of osteoid of mineralized bone or acellular eosinophilic cementum or dentin-like material. The acanthomatous epulis also has features of a fibromatous epulis but contains broad sheets and cords of stratified squamous epithelium with prominent intercellular bridges typical of stellate reticulum. In this case, the striking histologic feature was the presence of large numbers of irregularly shaped multinucleated giant cells, and well vascular nodules separated by anastomosing cords of hyperplastic gingival epithelium and a thin band of loose fibrovascular connective tissues. In addition, there was no metastasis to other tissues and organ.

Immunohistochemically, tumor cells were positive to AP that indicates osteoclast-origin, because AP is a diagnostic marker of osteoclasts (11). Though it is not a specific marker of PGCG, because it is positive to other tumors such as canine mixed mammary tumor (8), human testicular germ cell tumors (6) etc. Also AP was detected in endothelial cell of mature capillaries, fibroblast and fibrocyte (17). CKs are structural proteins and immunophenotypic markers of epithelial cells and their neoplasms (10). We examined expression of CK7, because osteoclast-like giant cells in the undifferentiated carcinoma of the liver were positive for CK7 (15). In this case, osteoclast-like giant cells were weakly positive for CK7. Although the expression of AP and CK7 may be helpful to identify tumor cell origin, their specificity remains in question. Therefore, further study is needed about the AP and CK7 as a specific marker of canine PGCG. Meanwhile, tumor cells were negative for CD68 unlike in human. CD68 was commonly positive in human PGCG (3). Common synonyms of CD68 is macrosialin, and CD68 is one of the defining markers for monocytes, macrophages, dendritic cells, granulocytes, activated T cells, subset of B cells etc. (1). Although IHC can be a valuable tool, most tumor markers have limitations and some complicating factors exist. Negative reactions could be related to loss of antigenicity during fixation and processing (14). In addition, technical difficulties and poor tumor cell differentiation may produce a negative stain (21). For this reason, more studies are needed regarding to relationship between canine PGCG and CD68.

In conclusions, this case was diagnosed to PGCG through clinical and histopathological features. And it was thought that PGCG consisted of osteoclast-derived cells as human and AP and CK7 could be used as a marker for differential

diagnosis of PGCG in canine.

Acknowledgment

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (2010-0011450).

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개에서 거대세포 치은종의 증례

조은상 · 전성주 · 홍다해 · 류시윤 · 정주영 · 박배근 · 손화영[1]

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요 약 : 18살의 암컷 푸들의 구강에서 종괴가 발견되었다. 육안적으로, 종괴는 단단하고, 적자색을 띠었으며, 크기는 1.5 × 1.5 × 1 cm 였다. 조직병리학적으로 종괴는 과증식된 잇몸 상피와 혈관이 잘 발달된 기질로 구성되어 있었으며, 다형 세포 및 풍부한 호산성 세포질과 다수의 핵을 가진 많은 수의 거대 세포가 관찰되었다. 면역조직화학적으로, 종양 세포는 alkaline phosphatase와 cytokeratin 7에 양성 반응이었지만, CD68에 음성 반응이었다. 종괴는 전형적인 임상적, 조직병리학적인 특징에 의해 구강내 거대세포 치은종으로 진단되었다.

주요어 : 거대세포 치은종, 개, alkaline phosphatase, cytokeratin 7, CD68.