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- 단 보 -

Mixed Osteosarcoma with Metastatic Alveolar Carcinomatous Appearance in Canine Mammary Gland Tumor

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We describe here a case of malignant mixed osteogenic tumor of the mammary gland with alveolar carcinomatous appreance. A firm, 2 to 2.5cm (in diameter) mass under the 5th nipple, showing the structure of extraosseous osteogenic sarcoma, was removed from the left 5th mammary gland of 12-year-old female dog. When investigated under the microscope, the osteoid material undergoing mineralization was surrounded by numerous scattered osteoblasts and a few osteoclastic cells throughout the osteoid tumorous stroma. The osteoid lesions were continuous with hypercellular myoepithelial cells of a very immature character with several mitotic figures. In addition, there were also carcinomatous tubules and alveoli, with invading cells into peripheral stroma, surrounded by myoepithelial cells in the mammary gland. In these lesions, emanating cords of tumor cells appear to be continuous with the myoepithelial cell layer of a duct. The presence of all these cell types suggests the existence of a common malignant origin, the stem cell being differentiated into epithelial carcinomatous and mesenchymal sarcomatous chondral and osteogenic tissues.

Key words : Malignant mammary mixed tumor, carcinoma, osteosarcoma, myoepithelial cell, canine

Introduction

It has been reported that malignant mixed mammary tumor in canine occurs only 1%~4% according to the different reporters [9-10], although malignant tumors including carcinoma, sarcoma and malignant mixed tumor account for 50% of mammary gland tumor [1]. Ectopic cartilaginous or bony metaplasia accompanied by abundant proliferation of myoepithelial cells is one of the most common patterns of growth in canine mammary mixed tumors [7,11,12]. Recent studies suggest that the formation of various mesenchymal tissues, including cartilage and bone in canine mammary mixed tumor, is related to these types of cells by demonstrating the expression of cytoskeletal proteins in hypercellular myoepithelial cells [13,14]. The osseous material as well as myxoid or chondroid matrix has been considered to develop from metaplasia of myoepithelial cells in mixed tumors [14]. In case of malignant mixed tumor, there may be malignant changes in any of the tissues of mixed tumor as carcinoma, malignant myoepithelioma, chondrosarcoma and osteosarcoma, or rarely a mixture of carcinoma and any of the other malignant types [7]. However, this malignantly changed mixed tumor in canine mammary gland has been reported few until now [7,9,10]. Mounlton *et al.* reported that metastasis in malignant mixed tumor usually appears as carcinoma, is less often found as chondrosarcoma or osteosarcoma, and rarely occurs as myoepithelioma [9]. In this study, we report here rare malignant mixed tumor concomitantly with osteosarcoma and metastastic carcinomatous changes in Yorkshire Terrier.

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Materials and Methods

A 12-year-old female Yorkshire Terrier was presented to a local animal hospital with a mass under the left 5th nipple. In the biochemical analysis, Complete Blood Chemistry (CBC) and Biochemistry tests were normal. For histological assessment, the tissues were immediately fixed in 10% neutral buffered formalin and presented to Kyungpook National University. Then, they were routinely processed and embedded in paraffin. Sections were cut at 4 um thickness and they were stained with hematoxylin

and eosin (H&E) for light microscopy. For Immunohistochemistry, sections of the tissue were deparaffinized in xylene, rehydrated through graded ethanol solutions and washed in distilled water. Endogenous peroxidase activity was inhibited using 3% hydrogen peroxide for 40 min and executed a microwave antigen process in 10 mmol/L citrate buffer. The mouse monoclonal antibodies used for immunostaining were CK8 (diluted in 1:100, Novocastra Laboratories Ltd, UK) and HER-2/neu (diluted in 1:100, Biogenex, CA, USA). Immunoreactive materials were visualized with avidin-biotin-peroxidase complex solution using an ABC kit (Vector Laboratories, Burlingame, CA, USA) with 3,3-diaminobenzidine (Zymed Laboratories Inc., San Francisco, CA, USA).

Results and Discussion

Canine mammary gland tumors frequently have different amounts of epithelial, myoepithelial, and connective tissue components [3]. In general, the tumors accompanied by mesenchymal tissue, such as cartilage and bone, together with the proliferation of myoepithelial cells are called mixed tumors [3]. Most mixed tumor has benign appearance, which generally composed of benign luminal epithelium and numerous myoepithelium mixed with mesenchymal cells [1]. From the data offered from the literature about 65% of mammary gland tumors in canine are benign mixed tumors, and 25% are carcionomas; the rest are hyperplasias, adenomas, malignant mixed tumors, and myoepitheliomas [7]. But these figures are variable according to the methods of classifying the tumors, especially the separation of mixed tumor from carcinoma [1,7,9]. Recently, Meuten *et al.* in 2002 reclassified mammary carcinoma in canine noninfiltrating carcinoma, complex carcinoma (two cell types), simple carcinoma (one cell type), simple carcinoma (tubulopapillary type), simple carcinoma (solid type), and simple anaplastic carcinoma [6].

In this study we found the obvious features to attribute to diagnose as a malignant mixed tumor arised in benign mixed tumor. The mass, a firm and 2 to 2.5 cm (in diameter), was surgically excised and submitted to us for histopathological examination. Removed mass was well-circumscribed and multilobulated and hard to cut due to bonny substances in the mass (Fig. 1). Histologically this tumor was composed of osteoid or osseous tissues which are continuous with increased proliferation, especially of myoepithelial cells, which are polygonal with clear cytoplasm, or spindle shaped (Fig. 2 and 3). In addition, another area

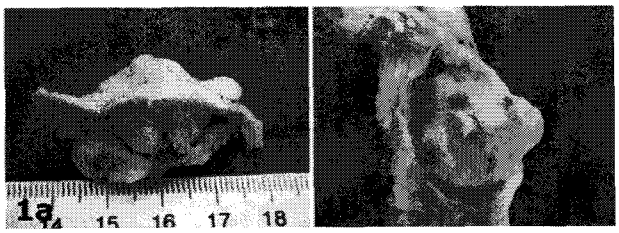

Fig. 1. (a) Mass of the mammary gland; dog. well-encapsulated and multilobulated mass was located under the nipple. (b) Mass of the mammary gland; dog. The cut surface contained yellowish soft areas in the center surrounded by whitish solid areas with mulifocal hemorrhagic foci in the peripheral region.

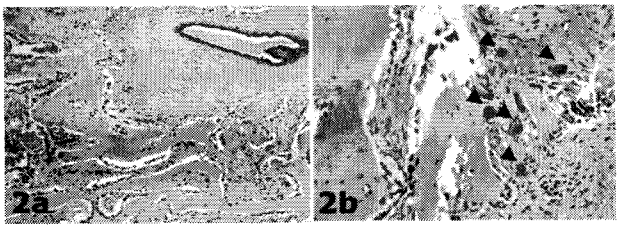

Fig. 2. (a) Osteosarcoma; dog. Bony material with highly densed area are admixed with proliferated myoepithelial cells arised in basement membrane of the gland. Stromal hemorrhage and deposition of hemosiderin are observed in osteogenic region in this tumor. H&E. Original magnification × 100. (b) Osteosarcoma; dog. Osteoid (light pink) and bony trabeculae are surrounded by osteoclastic multinucleated giant cells (*arrowhead*). Osteoblast-like cells participate in forming the bone, which appears as osteoid tissue. In hypercellular area, pleomorphism and occasional mitotic figures are observed in the mucinous stroma. H&E. Original magnification × 400.

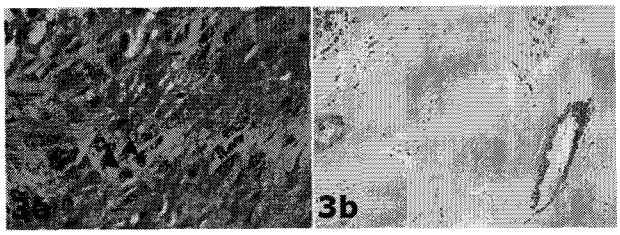

Fig. 3. (a) Osteosarcoma; dog. Metaplastic myoepithelial cells with mitotic figures (*arrowhead*) occupy the spaces between the trabecular networks of osseous material. Most of these cells have more than two nucleoli. H&E. Original magnification × 400. (b) Osteosarcoma; dog. The osteoid lesions including early formation of cartilage are continuous with hypercellular myoepithelial cells of a very immature character with several mitotic figures. H&E. Original magnification × 200.

contained invasive alveolar and ductular carcinomatous appearance. This tumor shows small focus of invasive carcinoma cells surrounded by whorls and sheets of proliferative myoepithelial cells that envelop ductal-lobular epithelium, situated on the epithelial basal lamina. Epithelium lining the alveoli showed early stage of malignancy characterized by cellular pleomorphism, frequent mitotic figures, and piling up into lumen, one of the first changes indicative of carcinomatous change [9] (Fig. 4).

Histological features observed as carcinomatous appearance in this present tumor were supported by immunohistochemical study as shown in Fig. 5. To observe obscure malignancy including invasiveness and metastasis we conducted immunohistochemical stain using the avidin-biotin-peroxidase complex method. Immunohistochemistry was conducted with antibody for CK8, marker for mammary glandular epithelium. Positive immunostained single or clustered tumor cells were observed to invade to adjacent stromal tissue and metastize into lymphatic vessel. Moreover, the presence of faintly stained or negative reacted epithelial cells, indicating poor differentiation, may mean that this tumor progresses to malignant pattern. These poorly differentiated cells are seen more frequently in the center rather than in peripheral area of this present tumor. Additionally, we observed HER-2/neu expression by immunohistochemical method in this tumor. Oncoprotein HER-2 is known as a member of the tyrosine kinase receptor family which is growth factor receptor with 50% homology to the epidermal growth factor receptor, and has surface membrane, transmembrane, and cytoplasmic domains. The gene product has been reported by the association of HER-2 amplification with worse clinical outcome in human [2]. Anti-human HER-2 protein antibody has cross-reactivity with canine mammary tissues. Therefore, HER-2 overexpression may be also useful features indicative of poor prognosis in a canine mammary gland tumor [5]. In this present tumor, a few individual positive cells for HER-2/neu appeared in the alveoli but immunoreactive epithelial cells are not plentiful as that in pure carcinoma. This may indicate that the tumor, arised in benign mammary mixed tumor, progress into early malignant features.

Complex carcinoma also has both epithelial and myoepithelial components, occasionally arranged in reticulated pattern. In this tumor, intercellular mucoid material and cartilage often found must be diffentiated from osteoid or woven bone, true bone formation [6]. The tumor in the present case is characterized by extensive bone formation

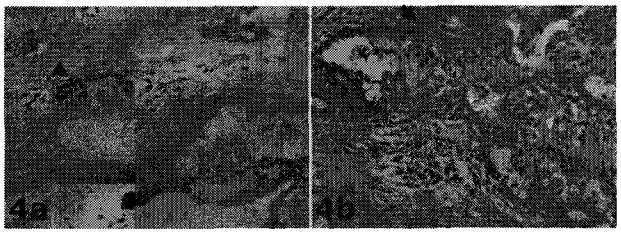

Fig. 4. (a) Carcinomatous change; dog. Focus of metastasis (*arrowhead*) to regional lymphatic channel in adjacent tissues of ductal carcinoma was identified. H&E. Original magnification × 400. (b) Carcinomatous change; dog. Focal disruption (*arrow*) of the thick basement membrane is evident. It contained necrotic area in an enlarged acinus. H&E. Original magnification × 400.

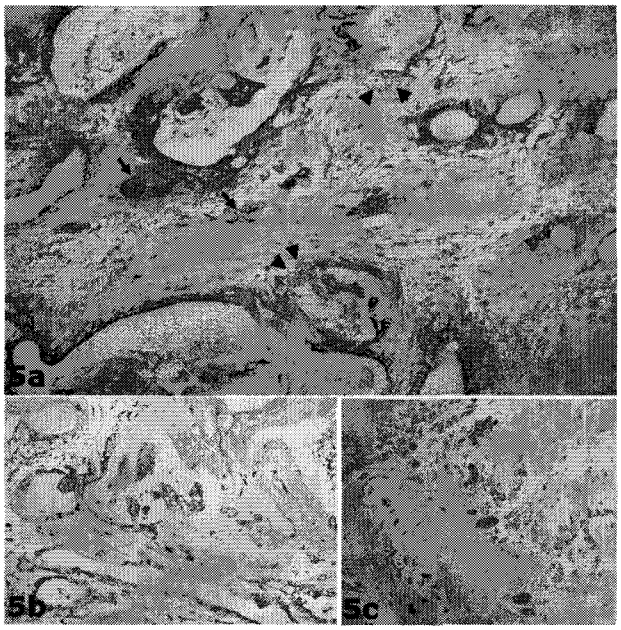

Fig. 5. (a) Immunohistochemical stain with antibody for CK8; dog. Positive reactive glandular epithelial cells (*arrow*) have invasive growth pattern to adjacent tissue. Note negatively stained glandular epithelial cells (*arrowhead*), indicating a poorly differentiated feature. Original magnification × 200. (b) Immunohistochemical stain with antibody for HER-2/neu; dog. Several carcinoma cells in lumen show positive immunoreactivity in carcinomatous changed lesions, indicating more aggressive clinicopathologic features. Original magnification × 200. (c) Immunohistochemical stain with antibody for HER-2/neu; dog. A few sarcoma cells including osteoblst-like cells and osteoclast-like giant cells around hyaline cartilage show positive immunoreactivity in osteosarcoma lesions. Original magnification × 200.

and focal cartilaginous component and these metaplastic changes are similar to pure osteosarcoma. Osteosarcoma may arise in benign mixed tumor and replaced it [6]. Consequently, it was diagnosed as malignant mixed tumor that tumor presented metaplastic sarcoma, composed of bone and cartilage, developed from the myoepithelial elements and carcinoma developed from the epithelial component.

Important information in this report is that the malignancy concomitantly developed both in epithelial and mesenchymal component in a mixed mammary tumor, although the present tumor mass are well-demarcated and small sized approximately as a range of 2~2.5 cm. In fact, malignant mixed tumor is generally indistinguishable from the benign form of mixed tumor but usually grows rapidly and sometimes sizes more than 10 cm in diameter [9]. This tumor appeared as benign in gross finding but in internal structure of this tumor metastasis of tumor cells to regional lymph node was often observed. To our knowledge, this case may be valuable to be reported since in this kind of tumor benign growth pattern and morphologically benign appearance in gross findings could hide to veterinarian a possibility of microscopically malignant changes as an uncommon case of canine mammary gland tumor.

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초록 : 개의 유선에서 발생한 혼합성 골육종 및 샘포 상피세포의 전이성 암종

구문정 · 홍일화 · 박진규 · 양해걸 · 위엔동웨이 · 기미란 · 이혜림 · 홍경숙 · 한정연 · 황옥경 · 김태환 · 도선희[1] · 정규식*
(경북대학교 수의과대학 병리학교실, [1]건국대학교 수의과대학 임상병리학교실)

본 증례는 개의 유선에서 발생한 골형성성의 악성 혼합 유선 종양으로 샘포의 암종성 변화를 동반하고 있다. 종양은 12년령 암캐의 좌측 5번째 유선에서 절제되었으며 직경 2~2.5cm의 단단한 mass로 절단 시 경도가 높은 골성의 구조를 가지고 있었다. 현미경학적 관찰 시, 골유사 물질이 미네랄 침착되고 있었으며 다수의 골형성 세포와 일부 파골세포가 골생성 종양 기질 전반에 걸쳐서 관찰되었다. 이러한 골유사 병변은 높은 밀집도를 보이는 근상피 세포와 연접하고 있었으며 이러한 세포들은 수 개의 유사분열상을 나타내고 있었다. 이와 더불어, 유선 세관과 샘포의 암종성 변화를 보이는 세포들이 인접 기질로 침습하고 있는 모습이 관찰되었고 이것 역시 증가된 근상피 세포들로 둘러싸여 있었다. 본 증례에서 볼 수 있는 이러한 세포들의 출현은 동시 발생된 악성 종양의 형태를 제시할 수 있으며 종양의 기원은 상피 유래의 암종성 조직과 중간엽 유래의 육종성 연골 및 골 조직으로 구별할 수 있겠다.