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NOTE

Basosquamous carcinoma with systemic metastasis in a miniature Pinscher

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Abstract

Basosquamous carcinoma (BSCC) is a rare malignancy, primarily composed of basal cells with foci of squamous differentiation. It is considered to be histologically an intermediate type between basal cell carcinoma and squamous cell carcinoma, and is known to have aggressive behaviors. BSCC occurred in a 17-year-old female minipin with a history of surgical excision for a mammary tumor. The right upper hindlimb was severely enlarged to 8 × 5 cm. Cross-section showed a homogenous white to yellow-white mass compressing the surrounding muscular tissues. The tumor metastasized also to the lungs, heart, abdominal cavity, liver and salivary gland. Microscopically, basaloid cells were crowded into solid nests or lobules separated by well-developed fibrous tissues with occasional keratinizations. Since there was no skin lesions, the tumor is assumed to be originated from the formerly present tumor in mammary gland. To our literature review, this case is the first BSCC with systemic metastasis in a dog.

Key words: basosquamous carcinoma, canine, cytokeratin 34βE12, pancytokeratin AE1/AE3

Basosquamous carcinoma (BSCC) is a rare skin tumor, with features of both basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Therefore, it has been characterized histologically as an intermediate type between BCC and SCC. However, the existence and diagnosis of BSCC is still controversial in both human and veterinary pathology. Some authors described it as a distinct entity[8], whereas others believe it as a variant of SCC[18] or BCC[5]. It is now generally considered to be a new entity of skin cancer. Unlike BCC, it has more aggressive and metastatic behavior, and poor prognosis. BSCC has also been referred to as basaloid squamous carcinoma, basal-squamous cell carcinoma, metatypical carcinoma and metatypical basal cell carcinoma[9,12,17].

Although the incidence, predilection and histological features of BSCC are well characterized in humans, it has been poorly documented in the veterinary literature. In humans, BSCC has a predilection for the base of

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the tongue, hypopharynx and supraglottic larynx in the upper aerodigestive track[6,14,19]. In dogs, it accounts for 0.35% of all skin tumors with the occurrences most often in the head, neck and hindlimb[7,8].

Several morphological features can be diagnostic criteria of BSCC, including carcinoma with a basaloid pattern in association with SCC, carcinoma *in situ* or focal squamous differentiation[18]. Therefore, BSCC has biphasic histological appearances with a prominent basaloid component and a conventional SCC component. The growth of the basaloid component is characterized by large nests, contoured lobules, or trabecular cord-like arrangements of small, crowded or even single cells with scant cytoplasm and hyperchromatic nuclei. The lobules of malignant basaloid cells often display a high mitotic index and comedo-type central necrosis. Cystic spaces filled with mucinous material can be present in a minority of the tumors[2,3,15,18].

Cytologically, malignant basaloid cells have sparse cytoplasm and round to oval hyperchromatic nuclei that have a high nuclear/cytoplasmic ratio. The nuclei of the cells often display peripheral palisading cells around the lobules[3,10,15]. The conventional SCC component is invasive and is found in the differentiated region, which may show a range of squamous cell differentiation or basaloid component[18]. Although BSCC is usually distinguishable in routine hematoxylin and eosin (H&E)-stained sections, immunohistochemical labeling is helpful for differential diagnosis of BSCC in humans. Most BSCCs are positive for pancytokeratin AE1/AE3 and high molecular weight cytokeratin 34βE12[2], and are commonly negative for chromogranin, synaptophysin, muscle-specific actin, and neuron specific enolase (NSE)[2,3,13]. To distinguish BSCC, BCC and SCC, epithelial membrane antigen (EMA) and Ber EP4 could be used as markers. EMA was positive only in SCCs[4], and Ber EP4 was positive in almost all BCCs and more than half of all BSCCs[4,11].

In this study, we report a case of BSCC with massive femoral osteolysis and systemic metastasis in a dog.

A 17-year-old female minipin was referred to the Veterinary Medical Center (VMC) of Chungbuk National University with lameness, severe swelling and pain in the right leg. On physical examination, body temperature, pulse rate and respiration were normal. The right upper hindlimb was severely swollen with moderately firm consistency. The palpation gave the dog severe pain. In survey radiographs, there was an aggressive osteolytic lesion with periosteal reaction in proximal diaphysis, great trochanter, and neck of right femur. There were two mass lesions in caudal lung field, consistent with pulmonary metastasis (Fig. 1A & B). The

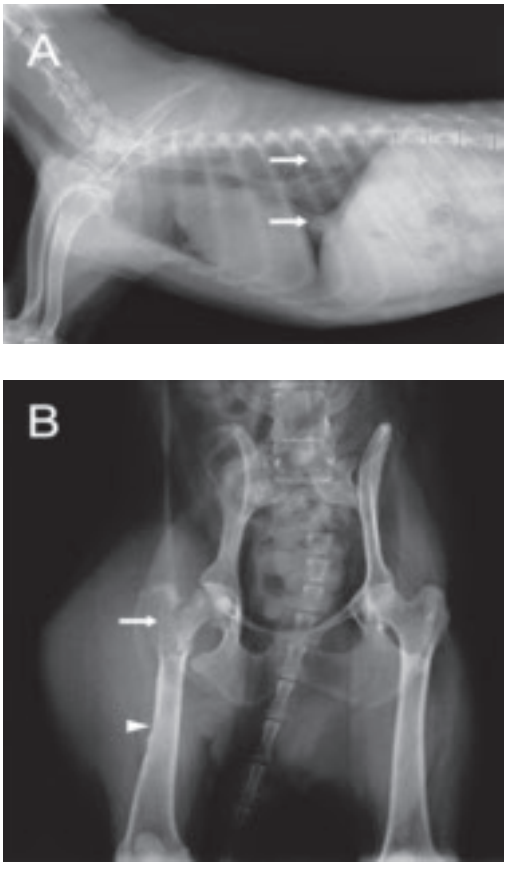

Fig. 1. Radiographs. (A) Two mass lesions in caudal lung field, consistent with pulmonary metastasis (arrows). (B) An aggressive osteolytic lesion with periosteal reaction in proximal diaphysis (arrowhead), great trochanter, and neck of right femur (arrow).

patient had a history of surgical excision of a mammary tumor in a local clinic 4 months before referral to the VMC. Histological diagnosis of the mammary tumor was unknown. At the time of the excision, right upper hindlimb had already been swollen to 4 cm in diameter. After intensive medication for 3 months at the VMC, the dog was euthanized by the request of the owner.

At necropsy, the right upper hindlimb was severely enlarged to 8 × 5 cm. The skin was easily separated from the mass occupying almost the entire upper hindlimb (Fig. 2A). In cross-section, the mass presented a homogeneous white to yellow-white appearance compressing the surrounding muscular tissues without any bone-like consistency (Fig. 2B). Inguinal lymph nodes enlarged to 3–4 cm in diameter. In the abdominal cavity, a 3 × 1 cm-sized peanut-shaped mass was found attached to the abdominal wall just beneath the lumbar vertebrae. Well-delineated nodules ranging from a few millimeters to 2.5 cm were evident in the lungs. In the heart, yellowish white irregular plaques were found on the ventricular walls (Fig. 2C).

After autopsy, tissue samples were fixed in 10% neutral buffered formalin and processed for histopathological examinations. Four micrometer-thick tissue sections were stained using H&E stain, and immunohistochemistry (IHC) was performed. For IHC, monoclonal anti-human cytokeratin 34βE12 (1 : 10, DAKO, Glostrup, Denmark), pancytokeratin AE1/AE3 (1 : 2, Abcam, Cambridge, UK), EMA (1 : 50, DAKO), Ki-67 (1 : 5, BioGenex, San Roman, CA, USA), chromogranin A (CGA) (1 : 500, Enzo, Ambler, PA, USA), synaptophysin (1 : 20, Abcam), smooth

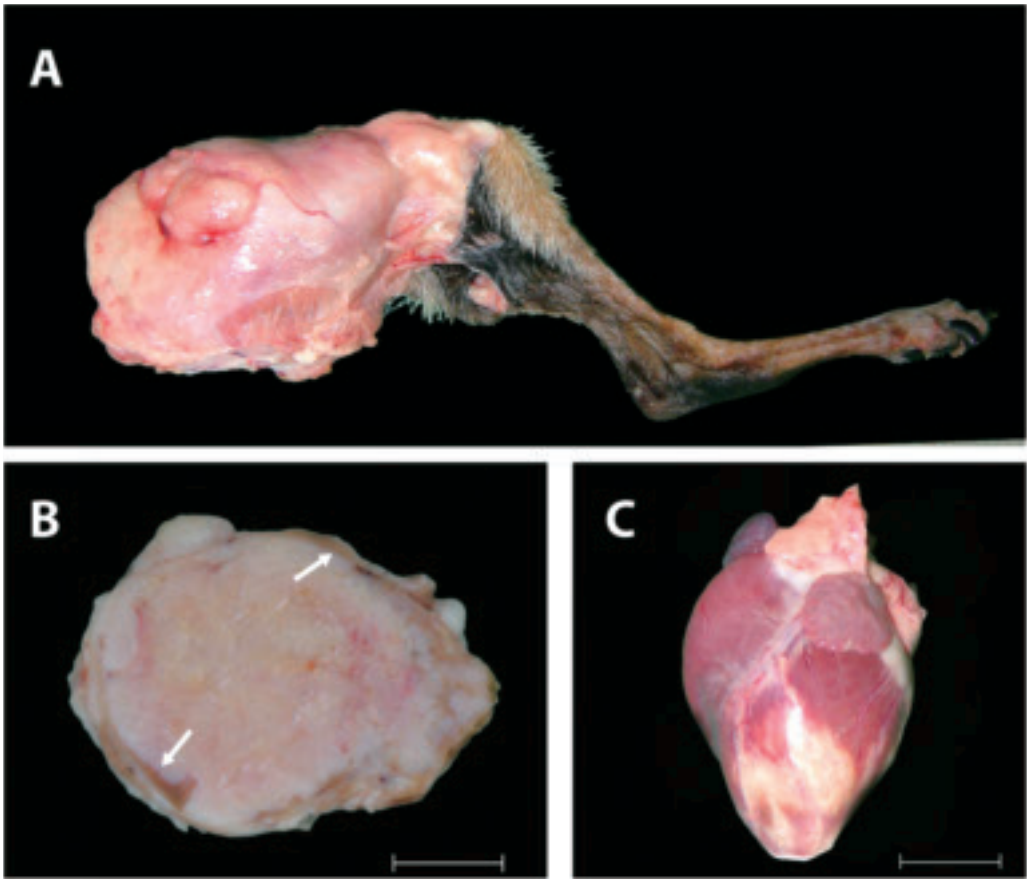

Fig. 2. Gross findings. (A) The primary tumor mass in right upper hindlimb enlarged to 8 × 5 cm. (B) Cross-section of the mass. Note yellowish white homogeneous tissues compressing the surrounding muscular tissues (arrows) (bar = 2 cm). (C) Metastasis to heart (bar = 2 cm).

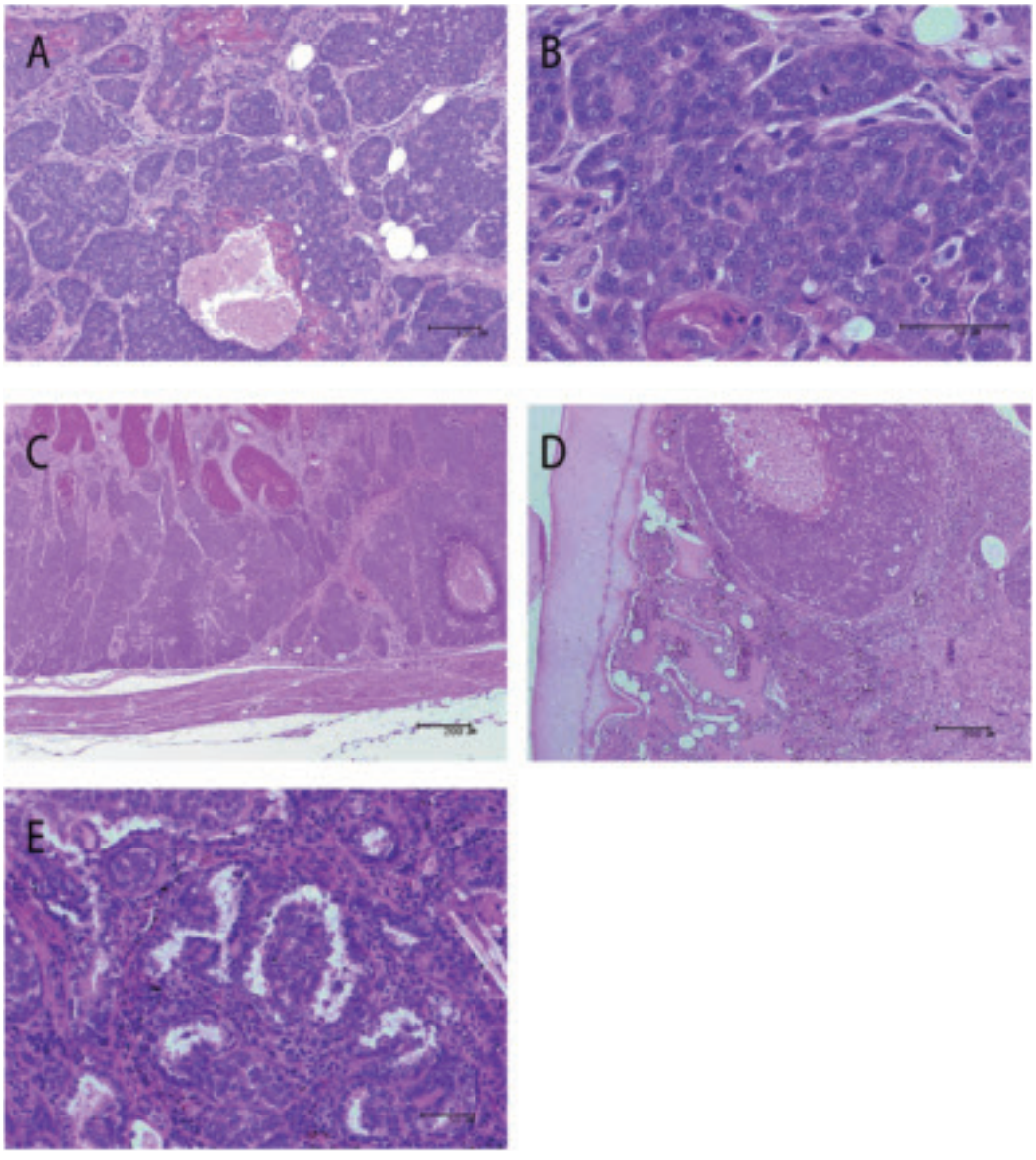

Fig. 3. Microscopic findings. (A) Lobular configurations of basaloid tumor cells with squamous keratinizing differentiation and central cometo-type necrosis. (B) The basaloid cells have hyperchromatic nuclei and scant to moderate cytoplasm with frequent mitoses. (C) Expansive tumor margin. Note the atrophied surrounding muscle layer. (D) Osteolysis in femoral head. Note the thin articular cartilage, trabecular bones and tumor lobules. (E) Ductal differentiation in lungs.

muscle actin (SMA) (1 : 50, Abcam) and NSE (1 : 200, DAKO) were used as primary antibodies. Binding of the primary antibodies were detected using avidin-biotin-peroxidase complex method (ABC kit; Vector Laboratories, Burlingame, CA, USA). Cell proliferation index was measured by counting the number of Ki-67 positive cells in 1,000 tumor cells.

Microscopically, basaloid cells were crowded into solid nests or lobules separated by well-developed fibrous tissues in most tumor masses including the heart and lungs.

In the center of some lobules, abrupt or gradual differentiation into keratinizing squamous cells with keratin pearls and/or comedo-type necrosis were remarkable (Fig. 3A). The basaloid cells possessed hyperchromatic nuclei and scant to moderate cytoplasm with

frequent mitoses (Fig. 3B). In some areas, the basaloid tumor cells producing mucinous materials looked like signet ring cells. At the margins of the limb mass, expansively growing tumor tissues were compressing the surrounding muscular tissues (Fig. 3C). Despite thorough examinations, osseous tissues were not discovered in sections from the longest diameter of the limb mass. However, in the bone marrow of femoral head, osteolytic trabecular bones and articular cartilage with invasion of cancer cells were found (Fig. 3D). The invasive basaloid cell nests forming keratin pearls were also found in lung and myocardium. Ductal differentiation and/or peripheral palisading were occasionally seen in small lobular structures, especially in the lungs (Fig. 3E). Grossly unidentified metastatic cell foci were found in the liver and salivary gland. Histologically, a diagnosis of BSCC was made.

Pancytokeratin AE1/AE3 was expressed in both basaloid cells and keratinizing squamous cells (Fig. 4A), whereas cytokeratin 34βE12 was expressed only in keratinizing squamous cells (Fig. 4B). Moreover, pancytokeratin AE1/AE3 was strongly expressed in the cytoplasms of ductal differentiation (Fig. 4C). However, EMA, CGA, synaptophysin, SMA, NSE were negative. Cell proliferation index by Ki-67 immunolabeling was 19.4%.

Differential diagnosis should include apocrine ductular carcinoma (ADC) and epitheliomatous sebaceous carcinoma (ESC) in dogs. ADC is multilobular tumor showing apocrine ductular epithelial differentiation. Tumor margins are infiltrative. And some tumors may develop multiple foci of squamous epithelium with keratinization via a granular layer. The important diagnostic feature is that the neoplastic tubules of ADC are generally lined by a double layer of cuboidal epithelial cells. Papillary hyperplasia may also be present within tubular lumens[9]. Meanwhile, the ESC is composed predominantly of basaloid reserve cells in islands, cords, and trabeculae. ESC also has infiltrative margins. In some lesions, small duct-like structures lined by mature squamous epithelium and containing keratin and sebocytic debris are seen. However, ESC usually has occasional sebocytic tumor cells with lipidized cytoplasm and does not exhibit peripheral palisading of the basaloid reserve cells[9]. These histological features are distinct from BSCC.

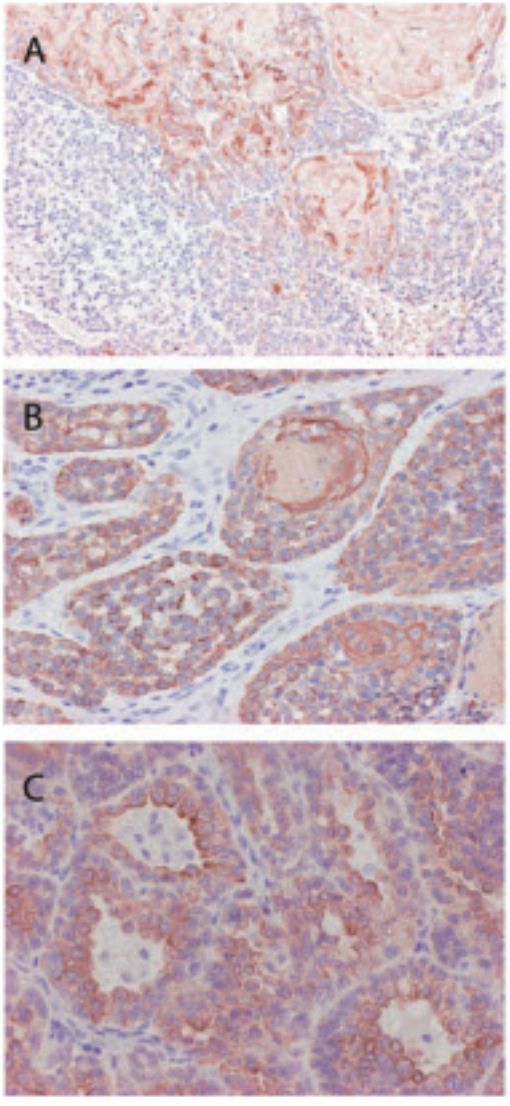

Fig. 4. Immunohistochemistry. (A) Cytokeratin 34βE12 is expressed in keratinizing squamous cells (X40). (B & C) The expression of pancytokeratin AE1/AE3 (X200). Note the expression in both keratinizing cells and basaloid cells (B), and in epithelium of ductal differentiation (C).

Histological features of this case could be summarized as expansive growth of basaloid tumor cell nests or lobules with severe

fibroplasia, frequent squamoid differentiation with keratin pearls, comedo-type necrosis, massive femoral osteolysis and systemic metastases. These lesions are well recognized in human BSCCs, and indicate the severe aggressive entity of the tumor. The Ki-67 immunolabeling index is 19.3%-44.2% in BSCCs[1,16], of which is consistent with that of the current case. The massive femoral osteolysis should be noted in this case. Although the osteolytic lesions were localized and less severe at the time of first referral to the VMC as seen in Fig. 1B, they progressed almost to the entire femur in 3 months.

Considering that BSCC is a skin cancer and the epidermal tissues are not involved in the tumorigenesis, the right upper hindlimb could not be primary site of the tumor. We assume that the systemic spread of the tumor was originated from the formerly present mammary tumor, although the definite diagnosis of original tumor was unknown. To our knowledge, this is the first canine BSCC case that metastasized systemically.

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