Squamous Cell Carcinoma with Systemic Metastases

Squamous Cell Carcinoma with Systemic Metastases in a Spotted Seal (Phoca largha)

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ゴマフアザラシ (Phoca largha) の全身転移を伴う扁平上皮癌の一例

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ABSTRACT. A 31-year-old male spotted seal (Phoca largha) was examined at necropsy. Multiple masses were observed in systemic organs, including the lung, stomach, pancreas, greater omentum, and pulmonary lymph nodes. Histologically, pulmonary masses contained numerous solid nests composed of polygonal tumor cells and often central keratinization, whereas those were less keratinization in the stomach, which tumor emboli were observed in the vessels. On immunohistochemistry, tumor cells were positive for keratin 5, pancytokeratin AE1/AE3, and p63 but negative for vimentin, cytokeratin CAM5.2, and calretinin. Based on macroscopical, histological, and immunohistochimical features, this case was diagnosed as squamous cell carcinoma with systemic metastases. The primary site in this case was probably the lung, with vascular metastases to distant organs including the stomach, omentum, and pulmonary lymph nodes. To the authors’ knowledge, this is the first description of squamous cell carcinoma in a spotted seal.

Key words: spotted seal; squamous cell carcinoma; systemic metastases


Reports of tumors in captive marine mammals have increased recently, although they are extremely low compared with domestic animals [10]. Tumors reported include lymphoma, papilloma, fibroma, and squamous cell carcinoma, and the types and distribution of these tumors in marine mammals are assumed to be similar to those in domestic animals [9, 10]. Squamous cell carcinoma has been reported in marine mammals, including beluga whales, California sea lions, dolphins, and gray seals [2-4, 7, 10, 14, 15]. However, there have been no reports of squamous cell carcinoma in spotted seal (Phoca largha). Here we describe the histological and immunohistochimical features of squamous cell carcinoma with systemic metastases in a spotted seal. This is the first report of squamous cell carcinoma in a spotted seal.

A 31-year-old male spotted seal that had been kept at Enoshima Aquarium in Fujisawa City, Kanagawa Prefecture, Japan died with clinical symptoms including continuous loss of appetite. The animal was subjected to necropsy within 10 h of death. Body length and weight at necropsy were 164.5 cm and 95.0 kg, respectively. Grossly, each of the six tumor masses, measuring 2.0-4.0 cm in diameter, was located randomly in the left and right lobes of the lungs. All masses were firm and uniform, and the cut surface of the masses was grayish white. Pulmonary lymph nodes were slightly enlarged with hemorrhage. In addition, numerous nodules measuring 0.1-0.5 cm and grossly reddish brown in color were found in the greater omentum and mesenterium. Other findings included enlarged pancreatic lymph nodes, dense adhesions to the greater curvature of the stomach and pancreas, and renal calculi. No mass was observed in the mucosa of oral, esophagus and stomach, and skin. Microbiological examination of the lung, pulmonary lymph nodes, greater omentum, spleen, and anal tissues, and blood and nasal swabs revealed low to moderate numbers of Escherichia coli and Vibrio alginolyticus.

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Fig. A ~ E
A) Low-power view of the tumor showing various solid nests. Large nests with central keratinization were observed. Hematoxylin and eosin (HE).
B) High-power view of the mass consisting of tumor cells characterized by round to oval nuclei and abundant eosinophilic cytoplasm. HE.
C) Distinct intercellular bridges were observed. HE.
D) Strong positive immunoreaction for keratin 5 in the cytoplasm of tumor cells. Keratin 5 immunohistochemistry with hematoxylin counterstain.
E) Positive immunoreaction for p63 in the nucleus. p63 immunohistochemistry with hematoxylin counterstain.
Several tissue samples from the trachea, liver, spleen, intestine, pancreas, lymph nodes, adrenal gland, kidney, esophagus, lung, heart, stomach, and greater omentum were fixed in 10% neutral buffered formalin. Following fixation, the tissues were embedded in paraffin wax, sectioned at 4 μm, and stained with hematoxylin and eosin. Immunohistochemical staining of serial sections was also performed using the streptavidin-biotin complex method. The primary antibodies used were as follows: pancytokeratin (clone AE1/AE3; 1:50, Dako Denmark A/S, Glostrup, Denmark), vimentin (clone V9; 1:200, Dako), keratin 5 (clone XM26; 1:25, NeoMarkers Inc., Fremont, CA), low-molecular-weight cytokeratin (clone CAM5.2; prediluted, BD, Franklin Lakes, NJ), p63 (clone 4A4; 1:150, NeoMarkers), calretinin (clone DAK-Calret1: 1:40, Dako), chromogranin A (rabbit polyclonal; 1:150, Dako), and papilloma virus (rabbit polyclonal; prediluted, Dako). After reaction with the specific primary antibodies, the sections were incubated with biotinylated goat anti-mouse immunoglobulin (Ig) G or anti-rabbit IgG antibodies (Dako), followed by peroxidase-conjugated streptavidin (Dako). Finally, the reaction with each antigen was visualized following the addition of diaminobenzidine tetrahydrochloride and counterstaining with hematoxylin. The antibodies used were validated by a positive reaction with their corresponding normal tissues and by a negative reaction on replacement with normal mouse or rabbit serum.

Histologically, the pulmonary masses consisted of differently sized nests of polygonal cells, occasionally showing central keratinization (Fig. A). Tumor cells had round to oval nuclei with 1-2 prominent nucleoli and abundant eosinophilic cytoplasm (Fig. B). The frequency of mitotic figures was 0-1 per high-power field. Similar tumor cells were observed in the pulmonary lymph nodes. In the stomach, tumor cells were also observed in various nests but with less keratinization. Tumor emboli were frequently observed in the blood vessels. Their cell borders were well defined and showed well-formed intercellular bridges (Fig. C). The greater omental and mesenteric nodes and pancreatic lymph nodes showed the same histological features as those observed in the stomach. Tumor emboli were also observed in renal blood vessels. Other microscopic lesions included pulmonary congestion, chronic interstitial nephritis, and calcification in the renal medullary tubular epithelial cells.

Immunohistochemically, the tumor cells were strongly positive for cytokeratin AE1/AE3, keratin 5 (Fig. D), and p63 (Fig. E) and negative for vimentin, cytokeratin CAM5.2, chromogranin A, calretinin, and papilloma virus.

Based on macroscopical, histological, and immunohistochemical features, this case was diagnosed as squamous cell carcinoma with systemic metastases. Based on the degree of differentiation such as the presence of intercellular bridges and/or keratinization, squamous cell carcinoma is divided into three types: well, moderately, and poorly differentiated [6]. In the present case, the morphological features of tumors in the lung and pulmonary lymph nodes were consistent with those of the well-differentiated-type tumors. In contrast, the morphological features of tumors in the stomach, pancreatic lymph nodes, and greater omental and mesenteric nodes were consistent with those of the poorly differentiated-type tumors compared with those in the lung.

Squamous cell carcinomas in marine mammals are reported to occur primarily in the skin, tongue, esophagus, stomach, and lung [2-4, 7, 10, 14, 15]. In Atlantic bottlenose dolphin, pulmonary squamous cell carcinoma often metastasizes to regional lymph nodes and the kidney [7]. In humans, pulmonary squamous cell carcinoma often metastasizes to the gastrointestinal organs, such as the esophagus and stomach [1]. In contrast, primary squamous cell carcinoma, which rarely occurs in the stomach, metastasizes to many organs, including the lung [15]. In the present case, the primary site was probably the lung followed by vascular metastasizes to distant organs, although an apparent transition of the bronchial epithelium to neoplastic squamous cells and obviously larger masses in the lung were not observed.

Immunohistochemical evaluation by several markers, such as keratin 5, p63, thyroid transcription factor (TTF-1), and calretinin, in addition to cytokeratins and vimentin, is often used in the differential diagnosis of adenocarcinoma, squamous cell carcinoma, and mesothelioma of the lung [7, 15]. Ordóñez reported that all 30 cases of squamous cell carcinoma arising in the lung were positive for both p63 and keratin 5 [12], suggesting that these are useful markers in the diagnosis of this neoplasm. In marine mammals, immunoreactivity for keratin 5 was demonstrated in squamous cell carcinoma in a harbor porpoise [15]. However, the usefulness of p63 as a marker in squamous cell carcinoma of marine mammals remains unknown. Although p63 activity has not been reported in regard to squamous cell carcinoma in dogs and cats, one example was observed in epithelial cells with squamous differentiation in mammary tumors and in myoepithelial/basal cells in normal and neoplastic mammary tissues in dogs [8, 13]. In the present case, immunoreactivity for p63 and keratin 5 was consistent with that observed in case of squamous cell carcinoma in humans. Furthermore, p63 is a useful marker in the diagnosis of squamous cell carcinoma in marine mammals. On the other hand, squamous cell carcinoma is negative for TTF-1, which is a specific and sensitive marker of pulmonary adenocarcinomas in humans [5, 12]. Pulmonary squamous cell carcinoma in an Atlantic bottlenose dolphin was negative for TTF-1 [7]. Unfortunately, TTF-1 could not be evaluated in that case. Mesothelioma is positive for calretinin, cytokeratin, and vimentin [11] and could be eliminated from the differential diagnosis in the present case by the negative immunostaining for vimentin and calretinin.

Some cases of primary squamous cell carcinoma of the lung in humans are closely related to viral infection such as infection by papilloma virus [16]. It has been reported that this virus can induce cutaneous papillomas in marine mammals [3]. No
include inclusion bodies or viral antigens were observed in the present case. However, the relationship between papilloma virus infection and squamous cell carcinoma in marine mammals remains unknown and merits further study.

要約
創育ゴマファザラン成雄（31歳）の全身性腫瘤を確認した。各腫瘤は有棘細胞由来の腫瘤細胞が充実核様に増殖し、脈管内腔を認められた。免疫組織学的に腫瘤細胞はサイトカタラチンAE1/AE3、クレアチン5、p63に陽性を示し、ピメチン、サイトカタラチンCAM5.2、カルセチンに陽性であった。本症例は扁平上皮癌と診断し、肺原発と推察した。本例はゴマファザランにおける全身転移を伴う扁平上皮癌の初報告である。

キーワード：ゴマファザラン、全身転移、扁平上皮癌

REFERENCES