A Case Report of Carcinoma of Mammary Gland with Systemic Metastases in a Captive California Sea Lion (*Zalophus californianus*)

Yuko TAJIMA¹⁾, Masayuki NAKAMURA²⁾ and Tadasu K. YAMADA¹⁾

1) Department of Zoology, National Museum of Nature and Science, 3-23-1, Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan.

2) Marine World - Uminonakamichi, 18-28, Saitozaki, Higashi-ku, Fukuoka-shi, Fukuoka 811-0321, Japan.

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カリフォルニアアシカ (*Zalophus californianus*) の全身転移を伴う乳腺癌の一例

田島木綿子¹⁾, 中村雅之²⁾, 山田 格¹⁾

1) 国立科学博物館 動物研究部 〒169-0073 新宿区百人町 3-23-1

2) マリンワールド海の中道 〒811-0321 福岡市東区西戸崎 18-28

ABSTRACT. Invasive carcinoma of the mammary gland with systemic metastases was found in a captive nulliparous female California sea lion, over 17 years old. Numerous yellowish white masses of various sizes were discovered in the mammary region and many organs. Microscopically, all the masses consisted of epithelial tumor cells that were irregular in size and shape. Epithelial tumor cells had often invaded adjacent tissues and vessels, especially lymphatics. The tumor cells were arranged mainly in tubular to papillary formations with some solid sheets in the mammary region, whereas principally solid formations were found in the metastatic regions. We diagnosed this as a case of simple tubulopapillary to solid carcinoma of mammary gland with systemic metastases.

Key words: California sea lion, adenocarcinoma, mammary gland

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The California sea lion (*Zalophus californianus*) is a species classified in Family Otariidae, Order Carnivora [1]. Mammary gland tumor is rather well known in dogs and cats that are classified also in Order Carnivora, and various studies and treatments have been reported [2, 3]. In pinnipeds, however, although various neoplasia were reported [4-12], the reports of mammary gland tumor are limited [13, 14]. This study introduces systemic metastasis of invasive carcinoma of the mammary gland in a captive California sea lion. The affected sea lion had been kept for about 17 years before death at Marine World - Uminonakamichi in Fukuoka City, Fukuoka Prefecture, Japan. The animal was a female without an experience of pregnancy. The clinical signs including loss of appetite and activity had developed gradually, although the animal was treated with antibiotics for several weeks. A subcutaneous mass had been palpable in her right axilla until death. She died on March 18, 2005, and the entire course was about a month from the time of noticed loss of appetite until death.

The animal was transported by air to the National Museum of Nature and Science and necropsied within 24 hours after death. Body length and weight measured prior to the necropsy

were 165 cm and 66.7 kg, respectively. The animal was extremely emaciated, otherwise the overall pelage appeared healthy (Fig. 1). Numerous masses of various sizes, the largest of which was 17 cm in diameter and situated near the right anterior nipple, were extensively discovered subcutaneously in the mammary region. These masses possessed dilated cysts containing some brownish-yellow viscous fluid, however, no cutaneous ulcer and injury were observed. Gross appearances of these masses varied widely from soft to firm in texture, and yellow to pale brown in color. Subcutaneous tissues were extensively and severely edematous and congestive. Many circumscribed round masses up to 2 cm in diameter and yellowish-white in color, were also found extensively in other areas including the heart, lungs, kidneys, stomach, small and large intestines, adrenal glands, thyroid gland, pancreas, genital organs, many lymph nodes, peritoneum and even skeletal muscles (Fig. 2). However, there were few masses in the liver and the spleen. Severe pulmonary congestive edema, and swollen, fragile and discolored liver were observed.

Collected tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE) for histopathology. Selected

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Fig. 1 External feature. Ventral view.
Severely emaciated otherwise the pelage all over appeared healthy.

Fig. 2 Numerous circumscribed round and white masses were found extensively in other sites, even skeletal muscles.

paraffin sections were examined immunohistochemically using a monoclonal mouse anti-human smooth muscle actin (clone 1A4, Dako, Carpinteria, CA) at 1:100 dilution. Microscopically, in the masses of the mammary region, the epithelial tumor cells were arranged mainly in tubular and/or papillary formations (Fig. 3a), occasionally with some solid sheets. Since the epithelial tumor cells were demonstrated principally in solid formations, masses of other organs were recognized as metastatic foci of the carcinoma of mammary gland (Fig. 4a). The epithelial tumor cells were mainly cuboidal to columnar with intense to moderate basophilic cytoplasm and an ovoid nucleus in the mammary areas (Fig. 3b), while round to ovoid cells had slightly basophilic cytoplasm and a round nucleus in the metastatic areas (Fig. 4b). Nests consisting entirely of myoepithelium-like cells were present mainly in the mammary gland area. The myoepithelium-like cells were mainly short spindle-shaped and occasionally arranged in a stellate or reticulate pattern. They had vacuolated cytoplasm and an ovoid or round nucleus. These cells showed a positive

reaction to smooth muscle actin. Epithelial tumor cells had several mitotic and hyperchromatic figures under high power and often had some degree of infiltrative growth into adjacent tissues with many inflammatory cells including lymphocytes and macrophages, and invasion of vessels with high ratio of metastases along lymphatics. The mammary ducts were extremely dilated, occasionally with some proteinous fluid and *cholesterin* crystals in the lumen.

We diagnosed this case as a simple tubulopapillary to solid carcinoma of the mammary gland with extensive systemic metastases [9]. As a result of the invasive proliferation of epithelial tumor cells, multiple organs would have fallen into functional failure, and possibly caused death of the animal. The tumor cells were found to infiltrate many *lymphatic* vessels, and occasionally into blood vessels in both the mammary gland and metastatic areas. Besides, as there were only few white masses in the liver and spleen, the metastatic route of tumor cells was assumed to be lymphogenous rather than hematogenous in this case, similar to those usually seen in dogs and cats [9].

Carcinoma of Mammary Gland in a California Sea Lion

Fig. 3a Mammary gland.

The epithelial tumor cells were arranged mainly in tubular and/or papillary formations.

Hematoxylin and eosin (H-E) stain, Bar: 50 μ m

Fig. 3b Mammary gland.

Epithelial tumor cells were mainly cuboid to columnar with moderate basophilic cytoplasm and ovoid nucleus.

H-E stain, Bar: 20 μ m

Fig. 4a Lung.

In the metastatic areas, epithelial tumor cells formed mainly solid formations.

H-E stain, Bar: 50 μ m

Fig. 4b Lung.

Epithelial tumor cells were relatively round having slightly basophilic cytoplasm and a round nucleus in the metastatic regions.

H-E stain, Bar: 20 μ m

Matsuda et al. [14] found distinct proliferation of myoepithelial cells in the carcinoma of mammary gland of a captive California sea lion. In general, although significantly more mitotic figures are observed in malignant than in benign tumors in dogs, this was not true of the case under study, which was similar to those of cat [9]. Although the urogenital carcinomas in California sea lions were recently reported to associate with the presence of Otarine Herpesvirus-1 (OtHV-1) [15], there were no specific findings related with the viral infection in this case.

Although myoepithelial cell proliferation often occurs in canine mammary tumors [16], in the present case, there were only few nests of myoepithelial cell proliferation, even in mammary gland areas.

要 約

飼育カリフォルニアアシカ未経産メス（17歳以上）の乳腺領域および多数の臓器に大小不同の黄白色腫瘍を確認した。各腫瘍は異型性の高い乳腺上皮細胞由来の腫瘍細胞が、乳腺領域

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では管状乳頭型, 転移巣領域では充実型増殖で構成されていた。腫瘍細胞の周囲組織への浸潤は著しく, リンパ管内にしばしば観察された。本症例は全身転移を示す乳腺癌(管状乳頭型から充実型増殖)と診断した。

キーワード: カリフォルニアアシカ, 腺癌, 乳腺

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