Adenocarcinoma of the Lung in a Steller Sea Lion (*Eumetopias jubatus*)

Shinji SATO, Hiroki KITAMURA, Masaaki MORI, Masahiro FUKAZAWA¹⁾, Masanori TAKEDA²⁾ and Koichi KADOTA³⁾

Nansei Livestock Hygiene Service Center, 83-2 Hayamase, Matsusaka, Mie 515-0217, ¹⁾Toba City Office, 1-6 Daimyohigashi, Toba, Mie 517-0022, ²⁾Futami Sea Paradise, 580 Futami, Watarai, Mie 519-0602, and ³⁾Hokkaido Research Station, National Institute of Animal Health, Toyohira, Sapporo 062-0045, Japan

(Received 31 March 1998/Accepted 3 August 1998)

ABSTRACT. The histological and ultrastructural characteristics of an adenocarcinoma of the lung are described in an about 16-year-old female Steller sea lion with a 1.5 month history of cough and anorexia. The animal had multiple neoplastic nodules in the lungs and diaphragmatic pleura. The bronchial and mediastinal lymph nodes were replaced by neoplastic tissue, and there were several metastatic lesions in the liver and spleen. The lung tumor was characterized by accumulations of encapsulated lesions with central necrosis, and the neoplastic cells showing a papillary growth pattern produced small amounts of mucin. Ultrastructurally, some cells contained basal bodies, and cilia were rarely seen. This neoplasm was considered to be of ciliated bronchial or bronchiolar epithelium origin.

— **KEY WORDS:** adenocarcinoma, lung, pinnipedia.

J. Vet. Med. Sci. 60(12): 1349–1351, 1998

Reported neoplasms in pinnipeds have been few. The majority of the neoplasms have been in California sea lions (*Zalophus californianus*), and this fact is probably attributable to their popularity as zoo and aquarium display animals [4]. Undifferentiated carcinomas of the lung were recorded without histology in two California sea lions [4]. This report describes a case of adenocarcinoma of the lung in a Steller sea lion which was characterized by accumulations of encapsulated lesions with central necrosis.

An about 16-year-old female Steller sea lion showed slight loss of appetite, and began to cough conspicuously 4 days later. Its condition deteriorated, and the animal had no appetite 36 days after the onset of anorexia. Clonic convulsions occurred 5 days later, and the animal died 3 days later.

At necropsy, there were multiple well-demarcated subpleural nodules, up to 2 mm in diameter, throughout the lungs. They were white and relatively soft, and frequently accumulated to form tumor masses of varying sizes. On cut section, similar nodules were densely or sparsely distributed. Mediastinal tumor masses were composed of enlarged bronchial and mediastinal lymph nodes and tumor nodules, whose cut surfaces were firm and fibrous, and yellowish white in color or white with yellowish white areas. There were many subpleural nodules, 2 mm to 4 cm in diameter, in the diaphragm. There were several subcapsular nodules, 1 to 2 cm in diameter, in the liver and spleen.

Tissues were fixed in 10% phosphate-buffered formalin, embedded in paraffin wax, and routinely processed for histological examination. Sections were stained with hematoxylin and eosin (HE) and Alcian blue (pH 2.5). Immunohistochemistry was applied to paraffin wax sections. The samples were incubated with antibodies to keratin (BioGenex Laboratories, U.S.A.) and vimentin (Dako Corporation, U.S.A.), and an immunoperoxidase staining system (BioGenex Laboratories) was used in the subsequent processes. Before staining, selected slides stained with the keratin reagent and the vimentin reagent were, respectively,

pretreated with 0.05% solutin of pepsin and microwave heating. For electron microscopy, small blocks taken from formalin-fixed tissues were post-fixed in 1% osmium tetroxide, embedded in epoxy resin, stained with uranyl acetate and lead citrate, and examined with an electron microscope.

Histologically, each of the nodules in the lungs was encapsulated by dense fibrous tissue, and had a necrotic center (Fig. 1). In such lesions, there were papillary growths of neoplastic cells, and remaining alveolar walls were detected, but were largely thickened and fibrosed (Fig. 2). There was diffuse interstitial fibrosis elsewhere in the lungs, and larger blood vessels showed thickening of the walls. In places, neoplastic cells, most of which formed 'inverted acini', were present within lymphatic vessels (Fig. 3).

The neoplastic cells were cuboidal or columnar in shape, and had eosinophilic cytoplasm and ovoid or round vesicular nuclei with medium sized or small nucleoli. Small amounts of Alcian blue-positive mucin was detected in the surface layers of the neoplastic cells. Mitotic figures were sometimes seen.

The enlarged lymph nodes were almost completely displaced by neoplastic tissue with severe stromal fibrosis and widespread areas of necrosis. Nearly all of the other metastatic lesions also had very abundant fibrous stroma and necrotic areas. As in the lung lesion, the neoplastic cells in the metastases showed a papillary growth pattern, but tended to be non-cohesive.

Immunohistochemically, almost all neoplastic cells were positive for keratin, and occasional cells were positive for vimentin.

Although the formalin-fixed neoplastic tissues were inadequately preserved for electron microscopy, a few electron-lucent mucous granules could be observed in the cytoplasm. The neoplastic cells had poorly developed luminal microvilli, rarely with cilia, and basal bodies were seen in some cells.

Primary pulmonary tumors are rare in domestic animals,

Fig. 1. The individual neoplastic lesions are encapsulated, and their centers are necrotic. Lung. HE stain. $\times 12.5$.

but metastatic lesions are relatively common. To make a diagnosis of primary lung tumor, we must exclude possible primary sites elsewhere in the body [1]. Because neoplastic lesions were located mainly in the thorax, the present tumor was thought to be a primary lung carcinoma. In contrast to this neoplasm with subpleural lesions, mesotheliomas had multiple papilliform lesions on the surfaces of body cavities [6].

Coexpression of keratin and vimentin was detected in canine mesotheliomas, which were readily distinguished from lung adenocarcinomas showing keratin positivity alone [7]. Although vimentin is an important marker to distinguish malignant mesothelioma from lung adenocarcinoma in human beings [3], sometimes there is coexpression of keratin and vimentin in lung carcinomas [10]. Immunohistochemistry for intermediate filaments was not useful in differentiating between our tumor and mesothelioma, but ultrastructural findings such as electron-lucent mucous granules and poorly developed microvilli showed that the tumor was different from a mesothelioma [10].

The presence of ciliated cells in the primary tumor or in metastatic sites was an aid in localizing the origin of a lung carcinoma as the surface epithelium of the bronchial passages rather than the bronchial submucosal glands [8]. Ciliated neoplastic cells, which resembled goblet cells, were observed in an equine adenocarcinoma of the lung [8]. In a study of 218 lung carcinomas in dogs and cats, some neoplastic cells had cilia in a few adenocarcinomas [9]. Although not prominent, cilia, basal bodies and mucin production were seen in the present tumor. This tumor was considered to be related to both ciliated and goblet cells and to have arisen from ciliated bronchial or bronchiolar epithelium.

The present neoplasm was characterized by accumulations of lesions with central necrosis. In women, similar lesions have been observed in intraductal carcinoma (comedo type) of the breast, which is characterized by a neoplastic growth within the mammary ducts and may show extension into the acini [2]. Although sea lions seem often to dive with at least partially inflated lungs, considerable part of the breath is exhaled before they dive [5]. When sea lions dive deeply, their lungs are compressed by the water pressure but are not crushed. Such functions of the lungs may be related to metastasis via the airways within the lungs and may have helped to produce a unique histological appearance in our case. Further cases are needed to elucidate histological features of lung carcinomas in marine mammals.

Fig. 2. The alveolar walls showing fibrous thickening (arrow) are lined by neoplastic cells, which form papillary growths into the lumen. Mitoses are present (arrowheads). Lung. HE stain. $\times 400$.

Fig. 3. A large space, presumably a lymphatic vessel, is filled with neoplastic cells forming free-floating 'inverted acini'. The acini are closely associated with papilliform growths of tumor. Lung. HE stain. $\times 200$.

REFERENCES

1. Dungworth, D. L. 1993. pp. 539–699. *In: Pathology of Domestic Animals*, 4th ed. (Jubb, K. V. F. *et al.* eds.), Academic Press, San Diego.
2. Gompel, C., Faverly, D. and Silverberg, S. G. 1990. pp. 311–374. *In: Principles and Practice of Surgical Pathology*, 2nd ed. (Silverberg, S. G. ed.), Churchill Livingstone, New York.
3. Jasani, B. and Schmid, K. W. 1993. pp. 65–75. *In: Immunohistochemistry in Diagnostic Histopathology*, Churchill Livingstone, Edinburgh.
4. Landy, R. B. 1980. pp. 579–584. *In: The Comparative Pathology of Zoo Animals* (Montali, R. J. and Migaki, G. eds.), Smithsonian Institution Press, Washington, D. C.
5. Macdonald, D. W. 1984. pp. 238–291. *In: The Encyclopedia of Mammals*, Facts on File, New York.
6. Madewell, B. R. and Theilen G. H. 1987. pp. 535–565. *In: Veterinary Cancer Medicine*, 2nd ed. (Theilen, G. H. and Madewell, B. R. eds.), Lea & Febiger, Philadelphia.
7. McDonough, S. P., MacLachlan, N. J. and Tobias, A. H. 1992. *Vet. Pathol.* 29: 256–260.
8. Moulton, J. E. 1978. pp. 205–239. *In: Tumors in Domestic Animals*, 2nd ed. (Moulton, J. E. ed.), University of California Press, Berkeley.
9. Moulton, J. E., von Tscharner, C. and Schneider, R. 1981. *Vet. Pathol.* 18: 513–528.
10. Rosai, J. 1996. pp. 289–434. *In: Ackerman's Surgical Pathology*, 8th ed., Mosby, St. Louis.