Pathology

Note

Three neoplasms in a Eurasian otter (*Lutra lutra*): malignant melanoma, trichoblastoma and mammary gland adenoma

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Running head: TRIPLE NEOPLASMS IN AN OTTER
Abstract

An eighteen-year-old female Eurasian otter became emaciated and died. Necropsy examination revealed nose and thoracic cutaneous masses, abdominal subcutaneous mass, and multiple nodules in the liver and lungs. Malignant melanoma was found in the nose cutaneous mass and to have metastasized to the liver, lungs, kidneys, adrenal glands, mammary glands and left mandibular lymph node. The neoplastic cells were labeled for vimentin, melanoma, and S100. The cutaneous mass in the thoracic area consisted of spindle shaped neoplastic epithelial cells and was diagnosed as trichoblastoma. Mammary gland adenoma was observed in the abdominal subcutaneous mass. This is the first report of primary three neoplasms of malignant melanoma, trichoblastoma and mammary gland adenoma in a Eurasian otter.

Key Word: Eurasian otter, malignant melanoma, spindle cell trichoblastoma, triple neoplasms
Otters are classified as sub-family Lutrinae of the family Mustelidae and have been in several countries, especially Eurasian otters (*Lutra lutra*) are a kind of the *Lutra* and have been in Europe, Asia and Africa [10]. The Eurasian otters live in a wide aquatic habitats and life expectancy is around seventeen-year-old [1, 10]. In Lutrinae, several types of tumors have been described, such as leiomyoma and basal cell carcinoma, and so on [11, 17, 19]. In Eurasian otters, malignant melanoma, intestinal lymphoma and hepatocellular adenoma were reported [3, 4, 18]. A previously reported malignant melanoma comprised multiple subcutaneous nodules, were present in the dorsal right flank and left thoracic area, metastasized to the lymph nodes and liver [18]. The multiple primary tumors have been only reported in a sea otter [17]. Here, we report a case of malignant melanoma with concurrent trichoblastoma and mammary gland adenoma in a Eurasian otter.

An 18-year-old female Eurasian otter, had been born at a zoological garden in Japan and been transported and kept at another zoo in Miyazaki, was observed to have an abdominal subcutaneous mass at 16-year-old of age. Two years later, a reddish nose and thoracic cutaneous masses were observed. Two months later, a biopsy was performed at the nose mass (2.1 x 2.6 cm) and left mandibular lymph node (2.2 x 3.0 cm), and a fine needle aspiration cytology was performed at the thoracic cutaneous mass (2.2 x 2.2 cm) and abdominal subcutaneous mass (3.8 x 4.0 cm) of the animal under anesthesia. The animal showed anorexia and hypokinesia three days later after the biopsy and died six days later. The otter was transferred to the Department of Veterinary Pathology, University of Miyazaki for necropsy. The necropsy found a black firm irregular mass (2.3 x 2.8 cm) with hemorrhages and ulcer at the skin of nose that almost covered the surface of the nose and was adherent to the overlying skin (Fig. 1). The cutting surface of the mass was gray and a mottled black color, solid and the borders were unclear between mass and surrounding normal tissues (Fig. 1). No infiltration growth of nose cutaneous mass was observed in the nasal cavity or oral cavity. Cutaneous and subcutaneous masses, 2.5 x 3.5 cm and 4.0 x 4.0 cm in diameter, were observed at the left lateral thoracic and the ventral abdominal areas,
respectively (Supplementary Fig. 1). Severe subcutaneous edema was found at the peripheral area of thoracic mass and cutting surface of mass was black-gray and cystic (Supplementary Fig. 2). The abdominal subcutaneous mass was cystic, contained blood, and its cutting surface was whitish with papillary projections (Supplementary Fig. 3). The borders were clear between each mass and surrounding normal tissue. The left mandibular lymph node was enlarged (3.5 x 4.5 cm) with a solid white-grayish cutting surface. The liver had four nodules, dark-red or dark-green, soft, 7.0 x 9.0, 2.5 x 3.0, 2.0 x 2.0 and 1.5 x 1.0 cm in diameter, in the left lateral, caudate lobe, right median and quadrate lobe, respectively. The capsule of the hepatic nodule in left lateral was rough and the fibrin and blood clot were observed. The cutting surface of all nodules was dark red and solid with hemorrhage at the peripheral area of nodules, and the borders were clear between all nodules and surrounding normal tissue. Multiple white to black nodules, 1 to 5 mm in diameter, were spread throughout the lungs. The abdominal cavity contained hemorrhagic ascites (about 250 ml). The visible mucous membranes were pale.

Samples were collected, then fixed in 10% neutral buffered formalin, routinely processed for paraffin embedding, sectioned at 3 μm and stained with hematoxylin and eosin (HE). Immunohistochemistry was performed using primary antibodies specific for cytokeratin (clone AE1/AE3; Dako, Glostrup, Denmark; ready to use), vimentin (clone V9; Dako; ready to use), melan A (clone A103; Dako; dilution 1 in 50), melanoma (clone PNL2; Dako; ready to use) and S100 (polyclonal; Dako; ready to use). Antigens were retrieved by incubation with a citrate buffer (pH 6.0) for cytokeratin, vimentin and S100 or a Tris-EDTA buffer (pH 9.0) for melan A and melanoma at 105°C for 10 minutes. The secondary antibody was Histofine Simple Stain MAX-PO™ (Multi) (Nichirei Bioscience, Tokyo, Japan) for all primary antibodies. The chromogen was 0.05% 3, 3’-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, U.S.A.) and 0.03% hydrogen peroxide in a Tris-hydrochloric acid buffer. Hematoxylin was used as the counterstain. Primary antibodies were confirmed
to be cross-reactive with otters by using nose tissue of this case and isotype IgG.

Microscopic examinations of the nose cutaneous mass, liver, lungs, kidneys, adrenal glands, mammary glands and the left mandibular lymph node revealed proliferation of neoplastic melanocytes. Neoplastic cells were found in the dermis, having invaded the surrounding normal tissue without capsule, connected at the dermo-epidermal junction in the nose (Fig. 2A). The neoplastic cells were consisted of two morphologic types, as epithelioid or polygonal and spindle in shape. Epithelioid or polygonal neoplastic cells were arranged in myxoid bundles, cords and nests, contained single to double, ovoid nuclei, a single prominent nucleolus, and scanty cytoplasm (Fig. 2B). Spindle neoplastic cells were arranged in streaming bundles, contained single, elongated spindle nuclei and a single small nucleolus (Fig. 2C). Melanin pigments were contained in a few epithelioid or polygonal neoplastic cells, but not spindle type, in primary and metastatic tissues. Cellular atypia was moderate and nuclear to cytoplasm rations was high. The frequency of mitotic figures was 2 or 3 per high-power field. Foci of the neoplastic cells were found in the liver, lungs, kidneys, adrenal glands, and mammary glands and were also observed in the lymphatic or blood vessels of those tissues (Fig. 3). Normal lymph node structure was replaced by the proliferation of neoplastic cells and necrosis in the left mandibular lymph node. The four hepatic and multiple lung nodules consisted of proliferation of the neoplastic melanocytes and hemorrhage was observed in the hepatic nodules. All tumor cells were positive for vimentin and the epithelioid or polygonal neoplastic cells were positive for melanoma but negative for S100 in the nose cutaneous mass, liver, lungs, kidneys, adrenal glands, mammary glands and left mandibular lymph node (Figs. 3 and 4A). The spindle neoplastic cells in the nose cutaneous mass were positive for S100 but negative for melanoma (Fig. 4B). Neither cell types were labeled with cytokkeratin and melan A. Normal melanocytes in the skin of this case were positive for vimentin, melanoma and melan A and negative for S100.

The thoracic cutaneous mass consisted of spindle to round epithelial neoplastic cells in the dermis
extending to the subcutaneous tissue. The neoplastic cells formed multiple cysts and whorled arrangement without differentiation to three segments of the hair follicle, keratin horn cysts, and trichilemmal keratinization (Fig. 5A and 5B). The cells had little cytoplasm, containing only a few melanin pigments with elongated spindle nucleus. Degenerated inflammatory cells and necrotic debris were found in the center of the cysts, while melanin-laden macrophages were present in the stroma and within the center of the cysts. The neoplastic cells were labeled with the cytokeratin and not with other antibodies (Fig. 5C). Considering the histological and immunohistochemical findings, the mass was diagnosed as a spindle cell trichoblastoma.

The abdominal subcutaneous mass showed papillary proliferation of the mammary gland epithelium in the subcutaneous tissue. The mass was encapsulated and well-demarcated without invading the surrounding normal tissue. Neoplastic cells proliferated in papillary or tubular formation with squamous metaplasia (Fig. 6A). Cellular atypia was minimal and mitotic figures were few. The neoplastic cells were positive for cytokeratin and negative for vimentin (Fig. 6B). The normal mammary glands were found around a neoplastic tissue. Therefore, the abdominal mass was diagnosed as a mammary gland simple adenoma with squamous metaplasia.

Although other types of tumors have been reported in Eurasian otters, neither trichoblastoma nor mammary gland adenoma has been reported [3, 4, 18]. Moreover, multiple tumors in otters are very rare, although cholangiocellular adenocarcinoma, leiomyoma and pheochromocytoma in a sea otter has been reported [17]. In the present case, three neoplasms were observed. Since this is the first presentation of a trichoblastoma or mammary gland adenoma in a Eurasian otter, reporting the occurrence of such a case is valuable.

Melanomas, which originate from melanocytes, are one of the tumors of haired skin, mouth, eyes and feet. They have been reported as common in several domestic animals, such as dogs, cats, horses and pigs, and in wildlife species, such as wildebeests (Connochaetes taurinus), white tigers (Panthera
tigris) and rhesus macaques (Macaca mulatta) [2, 5, 7, 13]. In dogs, melanomas have cellular variations such as balloon and spindle types. Several criteria for the malignancy of melanomas in dogs are well known, for example, mitotic figures of more than 3 per 10 high per fields indicate malignancy [7, 8]. In a previous case of malignant melanoma in an 11-year-old Eurasian otter, the neoplastic cells were presented with right flank and left thoracic subcutaneous nodules and metastasized to the lymph nodes and liver [18]. Malignant melanoma can metastasize to the regional lymph nodes and organs via the lymph vessels in animals [7]. In the present case, primary tumor cells were observed in the nose mass and to have metastasized to the liver, lungs, kidneys, adrenal glands, mammary glands and the left mandibular lymph node via the lymph vessels or blood vessels. The neoplastic melanocytes might initially have metastasized to the left mandibular lymph node because the lymph node structure was almost completely replaced by the neoplastic cells. Since this animal contained hemorrhagic ascites and had multiple friable hepatic nodules, the cause of death might be anemia due to hemorrhage from hepatic nodules in addition to emaciation due to cachexia by malignant tumors.

Melanocytic neoplasms in animals usually react with vimentin, melan A, PNL2, S100, TRP-1 and TRP-2, among others. In humans, HMB-45 and tyrosinase marker are also useful [7, 14-16]. In the present case, neoplastic melanocytes were immunolabeled for vimentin, melanoma, and S100, but were not immunolabeled for melan A. On the other hand, normal melanocytes were positive with melanoma and melan A. Based on these results, it was suspected that the expression of melan A in neoplastic melanocytes of otter could be reduced or lost. In human and canine, melan A is poorly reactive with several type of melanoma than other melanocytic markers [9, 12]. It might be important to diagnose malignant melanoma in Eurasian otter by using several antibodies, such as vimentin, melan A, melanoma, and S100.

The spindle cell trichoblastoma, previously classified as a basal cell tumor in cats, is most frequently seen in cats [7, 8]. In the present case, the morphology of the neoplastic cells of the thoracic
cutaneous mass included long spindle nuclei, scanty cytoplasm, and only a few melanin pigments. Moreover, the neoplasms showed multifocal cystic degeneration and a whorled appearance. Those findings are similar to spindle cell type trichoblastomas in cats. Since this case had been lacking differentiation to three segments of the hair follicle, keratin horn cysts, and trichilemmal keratinization, we ruled out the possibility of trichoepithelioma.

Mammary neoplasms are common in female domestic animals but are rare in otters [6]. In this case, the neoplasm presented on the abdominal subcutaneous area. The epithelial neoplastic cells that were observed between the normal mammary glands did not show any malignant features. Goldschmidt et al reported a new classification of mammary gland tumors in canines [6]. Considering those findings and the criteria, this tumor was diagnosed as a mammary gland adenoma.

To our knowledge, only one case of multiple neoplasms in a sea otter has been reported in subfamily Lutrinae. Therefore, this is the first report of multiple neoplasms consisting of a malignant melanoma, mammary gland adenoma, and trichoblastoma in a Eurasian otter (*Lutra lutra*).

**Conflict of Interest**

The authors declare no potential conflicts of interest with respect to the research, authorship, and the publication of this article.

**References**

1. Acharjyo, L. N. and Mishra, C. G. 1983. A note on the longevity of two species of Indian otters in captivity. *J. Bombay Nat. Hist. Soc.* **80**: 636.

2. Adetunji, S. A., Krecek, R. C., O’Dell, N., Prozesky, L., Steyl, J. and Arenas-Gamboa, A. M. 2018. Melanoma in golden and king wildebeests (*Connochaetes taurinus*). *J. Zoo Wildl. Med.* **49**: 134-142.

3. Bae, I. H., Pakhrin, B., Jee, H., Shin, N. S. and Kim, D. Y. 2007. Hepatocellular adenoma in a Eurasian otter (*Lutra lutra*). *J. Vet. Sci.* **8**: 103-105.
4. Bartlett, S. L., Imai, D. M., Trnpkiewicz, J. G., Garner, M. M., Ogasawara, S., Stokol, T., Kiupel, M., Abou-Madi, N. and Kollias, G. V. 2010. Intestinal lymphoma of granular lymphocytes in a fisher (Martes pennanti) and a Eurasian otter (Lutra lutra). J. Zoo Wildl. Med. 41: 309-315.

5. Frazier, K. S., Herron, A. J., Hines II, M. E. and Altman, N. 1983. Immunohistochemical and morphologic features of an intradermal nevocellular nevus (benign intradermal junctional melanocytoma) in a Rhesus Monkey (Macaca mulatta). Vet. Pathol. 30: 306-308.

6. Goldschmidt, M., Pena, L., Rasotto, R. and Zappulli, V. 2011. Classification and grading of canine mammary tumors. Vet. Pathol. 48: 117-131.

7. Goldschmidt, M. H. and Goldschmidt, K. H. 2017. Epithelial and Melanocytic Tumors of the Skin. pp. 88-141. In: Tumors in Domestic Animals, 5th ed. (Meuten, D. J. ed.), John Wiley & Sons, Inc, Ames, Iowa.

8. Goldschmidt, M. H., Dunstan, R. W., Stannard, A. A., Tscharner, C. V., Walder, E. J. and Yager, J. A. 1998. Histological Classification of Epithelial and Melanocytic Tumors of the Skin of Domestic Animals. pp. 18-101. In: World Health Organization International Histologic Classification of Tumors of Domestic Animals, 2nd ed, Vol 3. (Schulman, F. Y. ed.), Armed Forces Institute of Pathology, Washington, D.C..

9. Jungbluth, A. A. 2008. Serological reagents for the immunohistochemical analysis of melanoma metastases in sentinel lymph nodes. Semin. Diagn. Pathol. 25: 120-125.

10. Mason, C. F. and Macdonald, S. M. 1986. LUTRA LUTRA. pp. 7-45. In: Otters: Ecology and Conservation. (Mason, C. F. ed.), Cambridge University Press, Cambridge.

11. Nakamura, K., Tanimura, H., Katsuragi, K., Shibahara, T. and Kadota, K. 2002. Differentiated basal cell carcinoma in a Cape clawless otter (Aonyx capensis). J. Comp. Patho. 127: 223-227.

12. Ramos-Vara, J. A. and Miller, M. A. 2011. Immunohistochemical identification of canine melanocytic neoplasms with antibodies to melanocytic antigen PNL2 and tyrosinase: comparison
with Melan A. Vet. Pathol. 48: 443-450.

13. Rao, A. T., Achaejyo, L. N. and Mohanty, A. K. 1991. Malignant melanoma in a white tiger. Indian J. Vet. Pathol. 15: 113-114.

14. Smedley, R. C., Spangler, W. L., Esplin, D. G., Kitchell, B. E., Bergman, P. J., Ho, H. -Y., Bergin, I. L. and Kiupel, M. 2011. Prognostic markers for canine melanocytic neoplasms: a comparative review of the literature and goals for future investigation. Vet. Pathol. 48: 54-72.

15. Smith, S. H., Goldschmidt, M. H. and Mcmanus, P. M. 2002. A comparative review of melanocytic neoplasms. Vet. Pathol. 39: 651-678.

16. Spangler, W. L. and Kass, P. H. 2006. The histologic and epidemiologic bases for prognostic considerations in canine melanocytic neoplasia. Vet. Pathol. 43: 136-149.

17. Stetzer, E., Williams, T. D. and Nightingale, J. W. 1981. Cholangiocellular adenocarcinoma, leiomyoma, and pheochromocytoma in a sea otter. J. Am. Vet. Med. Assoc. 179: 1283-1284.

18. Weber, H. and Mecklenburg, L. 2000. Malignant melanoma in a Eurasian otter (Lutra lutra). J. Zoo Wildl. Med. 31: 87-90.

19. Williams, T.D. and Pulley, L. T. 1981. Leiomyomas in two sea otters, Enhydra lutris. J. Wildl. Dis. 17: 401-404.
Figure Legends

**Fig. 1.** Gross findings of the nose mass. A black irregular mass with hemorrhages at the nose. Insert: The cutting surface of the mass is gray and a mottled black color and solid.

**Fig. 2.** Histopathological findings of malignant melanoma in the nose mass. (A) Neoplastic melanocytes are found in the dermis and connect at the dermo-epidermal junction in the nose. Hematoxylin and Eosin (HE). Bar=100 μm. (B) Neoplastic cells are epithelioid or polygonal with large, single to double nuclei and contain a single prominent nucleolus. HE. Bar=30 μm. (C) Spindle neoplastic cells proliferate in a storiform arrangement. HE. Bar=60 μm.

**Fig. 3.** Histopathological and immunohistochemical findings of malignant melanoma in the mammary glands. The neoplastic melanocytes are observed in the lymphatic vessels in the mammary glands. HE. Bar= 60 μm. Inset: Neoplastic cells react with melanoma (PNL2). Immunohistochemistry (IHC) for melanoma (PNL2).

**Fig. 4.** Immunohistochemical findings of malignant melanoma in the nose mass. (A) Almost all epithelioid or polygonal neoplastic cells are moderate immunolabeled for melanoma (PNL2). IHC for melanoma (PNL2). Bar=30 μm. (B) Spindle neoplastic cells are immunolabeled for S100 (B). IHC for S100. Bar=30 μm.

**Fig. 5.** Histopathological and immunohistochemical findings of trichoblastoma in the thoracic cutaneous mass. (A) Neoplastic cells of trichoblastoma form multiple lobular to cystic and a whorled appearance. HE. Bar=300 μm. (B) The cells are long and spindle with spindle or round nuclei. HE. Bar=60 μm. (C) Neoplastic cells react with cytokeratin (AE1/AE3). IHC for cytokeratin (AE1/AE3). Bar=60 μm.
Fig. 6. Histopathological and immunohistochemical findings of mammary gland adenoma in the abdominal subcutaneous mass. (A) Neoplastic mammary gland epithelium proliferate in a papillary pattern. HE. Bar=100 μm. Inset: The squamous metaplasia of tumor cells. HE. (B) Neoplastic cells are immunolabeled for cytokeratin (AE1/AE3). IHC for cytokeratin (AE1/AE3). Bar= 60 μm.
Supplemental figures

Supplemental Fig. 1. Gross findings of the cutaneous and subcutaneous masses. The masses are observed at the thoracic (arrowheads: *) and abdominal (arrow) area.

Supplemental Fig. 2. Gross findings of the thoracic cutaneous mass. The mass shows subcutaneous edema. Inset: The cutting surface of the mass is black-gray and cystic.

Supplemental Fig. 3. Gross findings of the abdominal subcutaneous mass. The mass is grayish and contains blood. Inset: The cutting surface of the mass is whitish with papillary projections.
