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Spontaneous Peritoneal Malignant Mesothelioma in a Geriatric Japanese Macaque (Macaca fuscata)

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Abstract: *A 28.5-year-old female Japanese macaque (*Macaca fuscata*) was euthanatized because of abdominal distension due to severe ascites. Nodular lesions of varying sizes up to 5 mm in diameter were distributed diffusely on the surface of the omentum, mesentery and parietal peritoneum. No neoplastic masses were detected in any visceral organ. The nodules were composed of proliferation of mono- or multi-layered epithelial-like cells occasionally showing papillary growth and sheets of small round or polygonal cells. Signet ring-like cells and tubular structures were occasionally present. Neoplastic cells were strongly positive to cytokeratin, and occasionally to vimentin. Based on gross and histopathological findings, this tumor was diagnosed as an epithelial type of peritoneal malignant mesothelioma, the first reported case in the non-human primates.*

Key words: *Japanese macaque, peritoneal malignant mesothelioma, signet ring-like cell*

The Japanese macaque (*Macaca fuscata*) is a macaque native to Japan. The animals have been used in studies on biological behavior and physiology (in particular, seasonal breeding cycle) [16]. In these studies, it appears to be important to establish base-line data on the incidences of spontaneous age-related neoplastic or non-neoplastic lesions, to avoid bias or errors due to pathological conditions. Although odontoameloblastoma and basal cell tumor have been recorded [26, 27], there are few papers describing spontaneous tumors in geriatric Japanese macaques. Recently, we encountered spontaneous malignant mesothelioma (MM) in an aged Japanese female macaque. MMs are uncommon tumors derived from mesothelial cells lining the body cavities such as the peritoneal, pleural and pericardial cavities [7, 14], and the tumors have been reported in dogs, cats, goats, horses, rats, hamster and cattle [12, 14]. This paper documents pathological characteristics of a spontaneous MM found in a female Japanese macaque.

The macaque, named Takahama 337, was born in 1976 in the Takahama Group (Primate Research Institute, Kyoto University) housed in an outdoor enclosure. She had been kept in the outdoor group up to 25 years old, and then was transferred to an indoor individual cage to perform some examinations. She was fed a

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regular monkey chow supplemented with fruits and sweet potatoes, and was supplied water *ad libitum*. At the age of 28.5 years, all of a sudden she showed anorexia and abdominal distention apparently due to ascites, and thus, after three days was euthanatized with pentobarbital because of poor prognosis. Euthanasia and sample collections were approved by the Institutional Animal Care and Use Committee of the Primate Research Institute.

At necropsy, a large amount of dark, turbid fluid was observed in the abdominal cavity, and the omentum, mesentery and parietal peritoneum developed numerous nodules of varying sizes, up to 5 mm in diameter. These diffusely disseminated nodules were whitish-gray, and occasionally coalescent (Figs. 1 and 2). No neoplastic masses were detected in any visceral organ such as the liver, spleen, kidneys, lungs, heart, large and small intestines, pancreas, ovaries, uterus and adrenal glands. Major organs as well as some nodular lesions were fixed in 10% formalin, subsequently dehydrated and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin (HE), alcian blue (pH 2.5) with and without hyaluronidase (from bovine testes) digestion, and periodic acid-Schiff (PAS) stains with and without diastase digestion. Sections were also labeled by immunohistochemistry with the avidin-biotin complex method (Dako, Copenhagen, Denmark). The primary antibodies used were monoclonal antibodies against cytokeratin (clone MNF116; Dako, Copenhagen Denmark; predilution) and vimentin (Dako; ×200), and polyclonal antibody against carcinoembryonic antigen (CEA; Biomeda Corp., Cali., USA; ×100). After deparaffinization, microwave antigen retrieval for 10–20 min was performed. Then, sections were treated with 5% skimmed milk for 30 min, and incubated with each primary antibody for 14 h at 4°C, followed by 1-h incubation with biotinylated goat anti-mouse antibody or biotinylated goat anti-rabbit antibody. Final incubation was carried out for 1 h with an avidin-horseradish peroxidase complex, and positive reactions were visualized with 3,3'-diaminobenzidine. Sections were counterstained lightly with hematoxylin. Non-immunized mouse or rabbit serum in place of the primary antibody served as a negative control.

Histopathologically, the nodular lesions in the omentum, mesentery and parietal peritoneum were composed of proliferation of mono- or multi-layered cuboidal or round epithelial-like cells on the surface, occasionally showing papillary growth (Figs. 3, 4, and 7). Small round or polygonal cells arranged in solid sheets were also seen, forming clusters and nests (Figs. 4 and 5). These neoplastic proliferation was accompanied by desmoplastic development of collagen fibers (Figs. 4 and 5), resulting in thickening of the omentum and mesentery as seen in the gross finding (Figs. 1 and 2). In the clusters and nests of neoplastic cells, signet-ring like cells were occasionally present (Fig. 5). The cells had mucin-containing cytoplasm positive with alcian blue (Fig. 6) and PAS stains; the PAS reaction was resistant to diastase digestion. The interstitial matrix reacted with alcian blue stain, and the reactivity was reduced by hyaluronidase treatment. Tubular structures with space or ambiguous space were sometimes observed within the collagenous (Fig. 8) and adipose tissues. Mitotic figures were infrequent. Mild lymphocyte infiltrates (Fig. 7) and hemorrhages were seen in tumor tissues. The majority of neoplastic cells showed positive immunoreactivity to cytokeratin (Fig. 7), and a few neoplastic cells were labeled with an antibody to vimentin (Fig. 8). Cells reacting to CEA were not detected. Proliferation of neoplastic cells was seen on the capsule of the liver, and the serosa of the intestines and uterus. The proliferation was simply superficial, and invasive growth deep into the visceral organs was not seen.

Differential diagnosis should be made from adenocarcinomas that may generate from the stomach, ovaries, pancreas and intestines. Grossly, no neoplastic masses were detected in these organs. Uniform growth of nodules of varying sizes on the surface of the omentum, mesentery and parietal peritoneum is a characteristic gross finding in human and rat peritoneal MMs [7, 21]. While adenocarcinomas are histologically characterized by tubular, acinar or cystic growths in which the spaces are lined by cuboidal to columnar epithelial cells [2, 7, 17, 19], such growth patterns were not evident in the present case. According to the histological growth patterns, human and rat MMs are classified as epithelial, sarcomatoid (fibrous) or biphasic (mixed) type [7, 14, 21]. Besides the three major types, several subtypes have been described in human, rat and canine cases, such as lymphohistiocytoid, desmoplastic (sclerosing), microcystic, clear cell, small cell, and deciduoid [7, 12,

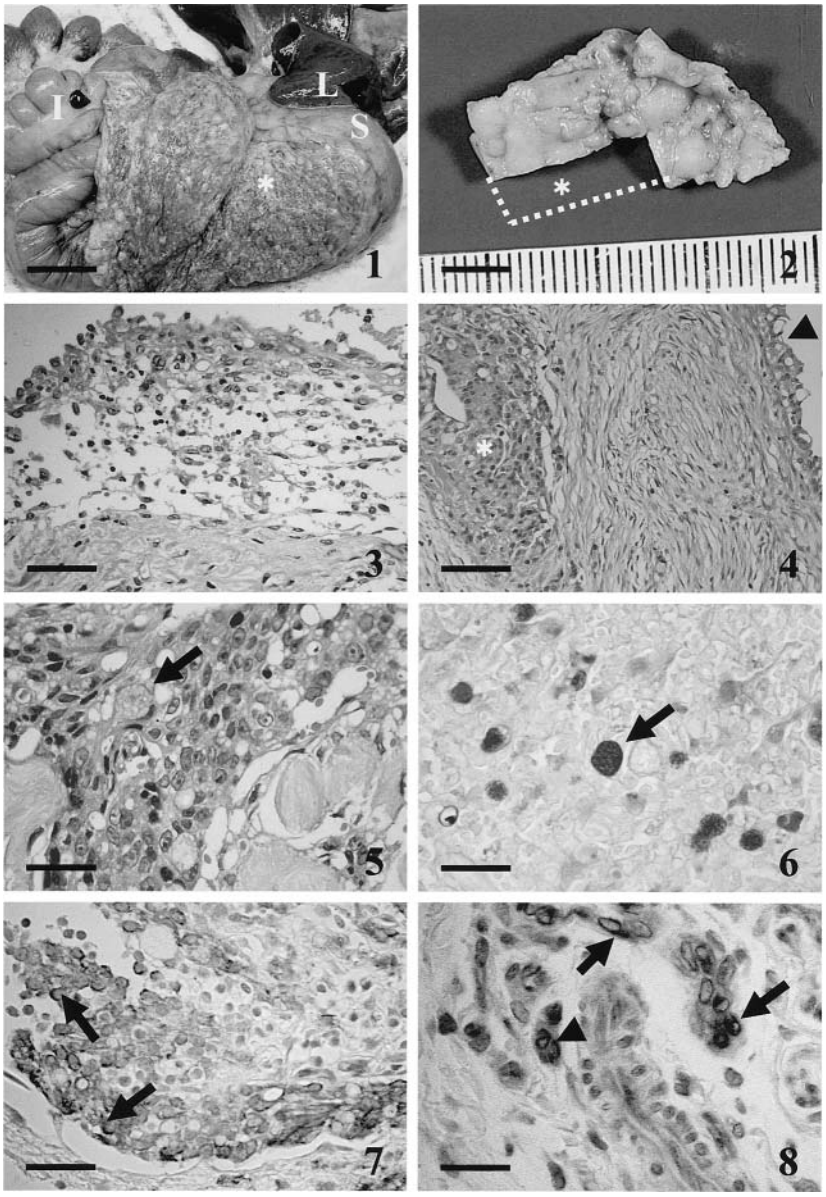

Fig. 1. Gross finding of a Japanese macaque with peritoneal malignant mesothelioma (MM). In the omentum, numerous nodules of varying sizes are seen (*). L, liver; S, stomach; I, intestines. Bar = 5 cm.

Fig. 2. Gross finding of the MM. A portion of formalin-fixed omentum developing multifocal-to-coalescent nodules. A piece (*) was cut for the histopathological examination. Bar = 5 mm.

Fig. 3. Nodular lesion of the MM in the mesentery showing mono-layered growth of cuboidal epithelial-like cells on the surface. Beneath the surface, edema and hemorrhage are seen. HE stain. Bar = 30 μm.

Fig. 4. Nodular lesion of the MM in the omentum. There is multi-layered proliferation of epithelial-like cells on the surface (arrow) and sheets consisting of small round or polygonal neoplastic cells (*), accompanied by abundant collagen fibers. HE stain. Bar = 40 μm.

Fig. 5. Nodular lesion of the MM in the omentum. Sheet-like proliferation consists of small round or polygonal neoplastic cells, and a signet ring-like cell is present (arrow). HE stain. Bar = 25 μm.

Fig. 6. Nodular lesion of the MM in the omentum. Signet ring-like cells present in the cluster of neoplastic cells are positive with alcian blue stain (arrow). Alcian blue (pH 2.5) stain. Bar = 25 μm.

Fig. 7. Nodular lesion of the MM in the mesentery showing papillary growth of epithelial-like cells. The majority of neoplastic cells give a positive reaction to cytokeratin (arrows). Lymphocyte infiltrates are seen among neoplastic cells. Immunohistochemistry, counterstained with hematoxylin. Bar = 30 μm.

Fig. 8. Nodular lesion of the MM in the omentum. Neoplastic cells present solitarily (arrowhead) or forming ducts (arrows) show a positive reaction to vimentin. Immunohistochemistry, counterstained with hematoxylin. Bar = 25 μm.

14, 21, 22]. The present case was characterized by mono- and multi-layered proliferation of mesothelial cells showing papillary growth and by sheet-like proliferation of small round or polygonal cells; tubular structures were occasionally seen. Diffusely distributed lesions in the abdominal cavity and heterogeneity in histological and cellular features indicated the malignant potency of this tumor. These characteristics are in agreement with those of the epithelial type of human MMs, although the tubulopapillary and tubuloglandular patterns are more prominent in human cases [7]. Immunohistochemically, neoplastic cells in our case were strongly positive for cytokeratin, and occasionally for vimentin. Neoplastic cells in the epithelial type of MMs showed sole immunoreaction to cytokeratin or dual immunoreactions to both cytokeratin and vimentin [3, 7, 21, 22]. In contrast to adenocarcinomas, some of which are believed to be reactive for CEA, the negative reaction to CEA may support the mesothelial origin of this tumor [7, 19]. It is interesting to note that mucin-containing signet ring-like cells were seen in the present tumor. Although the cells appear characteristically in the signet ring cell carcinoma (a subtype of adenocarcinoma) [2], the presence of neoplastic cells similar to signet ring cells has been reported in the epithelial type of human MMs [6]. Presumably, the signet ring cells might be due to metaplasia of neoplastic mesothelial cells toward mucin-containing epithelial cells.

To our knowledge, this is the first report of spontaneous peritoneal MM in non-human primates, although a pericardial MM, that was characterized by branching fronds and glandular structures lined by epithelial-like cells, was reported in an 18-year-old female rhesus monkey [4]. In humans, the development of pleural MMs after exposure to asbestos is well documented [7], and such processes have recently become a topical issue in Japan. Chrysotile asbestos fibers have been found in the lung tissues of pet dogs with MMs in the U.S.A., in association with exposure of their owners to asbestos [11]. However, the relationship between asbestos fibers and MMs has not yet been established in animals. Non-human primates, such as rhesus and cynomologus monkeys, have been used in toxicity studies [1, 9], and these animals are regarded as valuable experimental models for the evaluation of human carcinogenic risks [20]. Toxicologic pathologists who perform evaluations in these studies require detailed knowledge concerning the characteristics of spontaneously occurring neoplastic lesions. In rhesus monkeys, intestinal adenocarcinoma [17, 19], mammary gland ductal carcinoma [5], ampullary carcinoma [24] and ovarian choriocarcinoma [8] have been reported. In cynomologus monkeys, hepatocellular neoplasm [18], choriocarcinoma [23], ovarian teratoma [23], oral squamous cell carcinoma [15], pulmonary tumor [13], cervical and vaginal epithelial neoplasm [25], and ovarian epithelioid trophoblastic tumor [10] have been documented. In addition to these monkey species, Japanese monkeys are also a valuable experimental animal [16]. Accumulation of data on spontaneously occurring neoplasms is needed to establish the biological characteristics of the Japanese macaque.

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References

1. Araya, M., Kelleher, S.L., Arredondo, M.A., Sierralta, W., Vial, M.T., Uauy, R., and Lonnerdal, B. 2005. *Am. J. Clin. Nutr.* 81: 1065–1071.
2. Boutis, A.L., Andreadis, C., Patakiouta, F., and Mouratidou, D. 2006. *World J. Gastroenterol.* 14: 2958–2961.
3. Brower, A., Herold, L.V., and Kirby, B.M. 2006. *Vet. Pathol.* 43: 384–387.
4. Chandra, M. and Mansfield, K.G. 1999. *J. Med. Primatol.* 28: 142–144.
5. Cohen, M., Saidla, J.E., and Schlafer, D.H. 2001. *J. Med. Primatol.* 30: 121–126.
6. Cook, D.S., Attanoos, R.L., Jalloh, S.S., and Gibbs, A.R. 2000. *Histopathol.* 37: 33–36.
7. Enzinger, F.M. and Weiss, S.W. 1995. pp. 787–819. Soft Tissue Tumors, 3rd ed. (Enzinger, F.M. and Weiss, S.W. eds.), Mosby Press, St. Louis.
8. Farman, C.A., Benirschke, K., Horner, M., and Lappin, P. 2005. *Vet. Pathol.* 42: 226–229.
9. Foster, J.R. 2005. *Toxicol. Pathol.* 33: 63–74.
10. Giusti, A.M., Terron, A., Belluco, S., Scanziani, E., and Carcangiu, M.L. 2005. *Vet. Pathol.* 42: 223–226.
11. Glickman, L.T., Domanski, L.M., Maguire, T.G., Dubielzig, R.R., and Churg, A. 1983. *Environ. Res.* 32: 305–313.
12. Head, K.W., Else, R.W., and Dubielzig, R.R. 2002. pp. 477–478. Tumors in Domestic Animals, 4th ed. (Meuten, D.J. ed.), Iowa State Press, Iowa.
13. Kaspareit, J., Friderichs-Gromoll, S., Buse, E., Korte, R., and Vogel, F. 2001. *Exp. Toxicol. Pathol.* 53: 267–269.

14. Morini, M., Bettini, G., Morandi, F., Burdisso, R., and Marcato, P.S. 2006. *Vet. Pathol.* 43: 198–201.
15. Nakamura, S., Sakakibara, I., Ono, F., Shibata, S., Michishita, M., Ishii, Y., Kobayashi, R., Takahashi, K., and Yoshikawa, Y. 2000. *Exp. Anim.* 49: 225–228.
16. Nozaki, M., Shimizu, K., Mitsunaga, F., Watanabe, G., and Taya, K. 2002. *Endocrine.* 19: 131–137.
17. O'Sullivan, M.G. and Carson, C.S. 2001. *J. Comp.Pathol.* 124: 212–215.
18. Reindel, J.F., Walsh, K.M., Toy, K.A., and Bobrowski, W.F. 2000. *Vet. Pathol.* 37: 656–662.
19. Rodriguez, N.A., Garcia, K.D., Fortman, J.D., Hewett, T.A., Bunte, R.M., and Bennett, B.T. 2002. *J. Med. Primatol.* 31: 74–83.
20. Schoeffner, D.J. and Thorgeirsson, U.P. 2000. *In vivo* 14: 149–156.
21. Shibuya, K., Tajima, M., and Yamate, J. 1990. *Jpn. J. Vet. Sci.* 52: 1313–1317.
22. Shibuya, K., Tajima, M., and Yamate, J. 1993. *Toxicol. Pathol.* 21: 87–90.
23. Toyosawa, K., Okimoto, K., Koujitani, T., and Kikawa, E. 2000. *Vet. Pathol.* 37: 186–188.
24. Usborne, A.L. and Bolton, I.D. 2004. *Comp. Med.* 54: 438–442.
25. Wood, C.E., Borgerink, H., Register, T.C., Scott, L., and Cline, J.M. 2004. *Vet. Pathol.* 41: 108–111.
26. Yanai, T., Masegi, T., Tomita, A., Kudo, T., Yamazoe, K., Iwasaki, T., Kimura, N., Katou, A., Kotera, S., and Ueda, K. 1995. *Vet. Pathol.* 32: 57–59.
27. Yanai, T., Wakabayashi, S., Masegi, T., Iwasaki, T., Yamazoe, K., Ishikawa, K., and Ueda, K. 1995. *Vet. Pathol.* 32: 318–320.