Mammary Ductal Carcinoma with Comedo Pattern in a Rhesus Macaque

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A 23-year-old female rhesus macaque presented with a 2.5-cm diameter, firm, moveable, lobulated subcutaneous mass associated with a supranummary teat on the right side of the chest. This animal was a retired breeder, currently in an aging study. No exogenous hormone treatments were noted in the animal's history. Chest radiographs were within normal limits. Blood screens showed no noteworthy variations from normal. Needle aspirate cytology showed clusters of neoplastic cells. Grossly the mass was well circumscribed, firm, and homogeneously tan, with a glandular appearance. Differential diagnoses included sebaceous or mammary adenoma, carcinoma in situ, and lobular or ductular carcinoma. Histopathology was consistent with a mammary ductal carcinoma with comedo pattern. Subsequent needle aspirate cytology from an adjacent right axillary lymph node showed tumor cells with a few lymphoid cells, interpreted as lymphatic spread. Chest radiographs 2 and 6 weeks postbiopsy showed no evidence of pulmonary metastasis. After 1 year, there was no marked change on chest radiographs, but a small cluster of new nodules was palpable in the right axillary region. Histopathology of an excisional biopsy of the new nodules indicated tumor growth subjacent to regional lymph nodes. Further treatment was not performed and the animal remained clinically normal five years after the initial diagnosis. Spontaneous mammary neoplasia is a major concern in human medicine, yet it rarely has been reported to occur in nonhuman primates. This case is important in documenting an additional case of spontaneous mammary tumor development.

Case Report

History. A 23-year-old intact female rhesus macaque (*Macaca mulatta*) weighing 8.4 kg was examined for a mass in the region of a supranummary right teat located adjacent to and below the normal right teat. This subcutaneous mass was 2.5 cm in diameter, firm, lobulated, and moveable on palpation. This monkey was born in 1977 in a domestic colony; was used in a breeding colony from 1985 to 1993; was negative for B virus, produced seven live offspring; and received no reported treatment with exogenous hormones. She was transferred to the University of Maryland School of Medicine in 1996 and placed on an institutional animal care and use committee (IACUC)-approved study of aging in macaques. The presence of a mass first was noted on arrival, when it was 1.5 cm in diameter. It was not considered a noteworthy finding initially. When enlargement was noted during examination for an unrelated problem in 1999, further diagnostic evaluation was undertaken. Differential diagnoses for this mass included mammary or sebaceous gland hyperplasia, and neoplasia (adenoma, carcinoma in situ, lobular carcinoma, ductal carcinoma, lipoma, or granulomatous tissue). This animal had been singly housed, with visual and auditory interaction with other monkeys, per approved protocol justification, in an Association for the Assessment and Accreditation of Laboratory Animal Care, International-accredited facility since 1996, was fed a commercial primate diet, and received environmental enrichment treats including fruit, cereals, and fresh vegetables and other enrichment devices per an IACUC-approved primate enrichment plan.

In November 1999, the macaque was presented for veterinary evaluation of mildly decreased appetite and soft-tissue swelling of the left shoulder joint. An acute soft tissue injury was diagnosed and responded well to analgesic therapy. However, during the physical examination, enlargement of the reported mass was noted, and a decision was made for diagnostic evaluation because of increasing size of the mass.

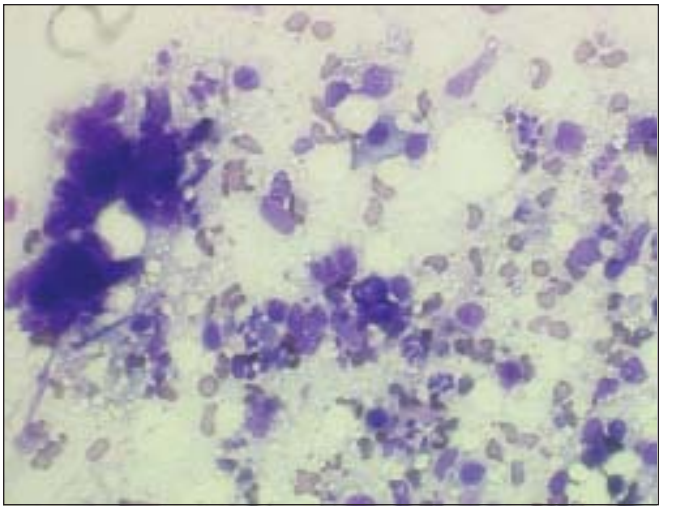

Figure 1. Cytology of fine needle aspirate. Presurgical cytologic preparation showing multiple clusters of large, round, dark blue staining pleomorphic cells with high nuclear to cytoplasmic ratio, surrounded by subacute inflammatory cells including neutrophils, lymphocytes, and monocytes. Clusters interpreted as neoplastic cells. DiffQuick stain.

A fine-needle aspirate showed multiple irregular clusters of large, basophilic, pleomorphic epithelial cells with no distinct architecture, demonstrating a high nuclear-to-cytoplasmic ratio, enlarged nucleoli, and numerous mitotic figures, thus meeting criteria for neoplasia. The neoplastic cells were surrounded by a background of inflammatory cells including neutrophils, lymphocytes, and macrophages (Fig. 1). Complete blood count with differential and standard serum biochemistry (electrolytes, glucose, blood urea nitrogen, creatinine, calcium, phosphorus, serum protein, albumin, globulin, creatinine phospho-

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kinase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactic dehydrogenase, gamma glutamyltransferase, direct and indirect bilirubin, triglycerides, cholesterol, magnesium, iron, amylase, and lipase) essentially were normal. Chest radiographs showed normal aging changes with no radiographic evidence of pulmonary metastasis.

In light of the cytology and favorable results of preliminary hematologic, serologic, and radiologic screening, wide surgical excisional biopsy was performed according to standard veterinary surgical procedures. These included use of aseptic surgical technique, isoflurane anesthesia after induction with ketamine, continuous evaluation of vital signs and anesthetic depth by a trained veterinary technician using visual and mechanical monitoring techniques, intravenous fluid support, analgesics, and antibiotics. The tumor was well circumscribed, with no visible extensions beyond the local borders. It was firm with a homogeneous consistency, tan on cut surface, and surrounded by fat and overlying skin. The tumor was placed in 10% neutral buffered formalin and submitted for histopathology. Recovery of the animal was routine. Postoperative treatment included buprenorphine at 0.03 mg/kg twice daily for 2 days and enrofloxacin at 5 mg/kg once daily for 5 days.

One week after surgery, the animal opened a portion of the incision and was sedated for examination and repeat closure of the incision. At the time there was mild swelling in the right axillary region with a small lump presumed to be an axillary lymph node. Needle aspirate from this lump showed cells similar to the presurgery cytology in a background of adipose tissue. There were macrophages, neutrophils, and lymphocytes consistent with chronic, but active, mild inflammation. Further treatments such as radical mastectomy, ovariohysterectomy, chemotherapy, and hormone therapy were considered. Because of a lack of historical treatment protocols for mammary neoplasia in macaques and the desire not to add confounding variables that might exclude the animal from the research protocol for the study of aging, the attending veterinarian and investigator decided to monitor the animal closely without further therapy. There were no clinical signs related to the tumor that indicated current pain or distress necessitating euthanasia. It was agreed that if decline in the animal's well-being became an issue, euthanasia would be performed.

Quarterly follow-up examinations of the animal included physical examination, chest radiography, hematology, and serum chemistry panels. At 1 year postsurgery, a small cluster of 2- to 5-mm focal nodules were palpable in the right axillary region. These were removed surgically, and histopathology showed tumor growth associated with lymph nodes and lymphatics, with appearance very similar to the cytology shown in Fig. 1. Chest and abdominal radiographs did not show visible metastases, and blood work remained essentially normal. After five years of follow up the monkey continued to do well, with small localized, nodules but no clinical or radiographic signs of metastatic disease.

Histology procedures. Paraffin-embedded samples of mammary gland were cut into 5-μm sections and stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), PAS–diastase, and Alcian blue (pH 2.5 and 1.0). For mucicarmine staining, a commercial kit (Bio Optica, Milano, Italy) was used.

Immunohistochemical assays were done according to standard methods by using a commercial streptavidin–biotin peroxidase labeling kit (DAKO, Glostrup, Denmark) and diaminobenzidine as chromogen (0.04% for 7 min). Endogenous peroxidase was blocked by treatment with 3% hydrogen peroxide for 30 min. The sections then were immersed in citrate buffer (2.1 g citric acid monohydrate per liter of distilled water; pH 6.0), heated for four 5-min. periods in a microwave oven at 750 W, and cooled at room temperature for 20 min. The primary antibodies (DAKO) consisted of monoclonal antibodies against cytokeratin 19 (clone BA 17, diluted 1:40), smooth muscle alpha-actin (clone 1A4, diluted 1:100), vimentin (clone V9, diluted 1:40), and Mac 387 (monoclonal mouse anti-human myeloid/histiocyte antigen, clone Mac 387; 1:100). All antibodies were incubated overnight at 4°C. The reaction was developed by using peroxidase (Labeled Streptavidin biotin System-LSAB, DAKO). The chromogen diaminobenzidine (0.04%, 7 min at room temperature) was used, and then the sections were counterstained with Papanicolau hematoxylin, rinsed in tap water, dehydrated, and mounted with DPX Mountant (distrene, plasticizer, xylene, Fluka Chemie AG, Buchs, Switzerland). Pathology procedures were performed at the University of Bologna (Italy).

Pathology. Grossly the tumor appeared as a firm, tan, lobulated, subcutaneous mass, without a distinct capsule. There was no gross evidence of invasion into the subjacent musculature. At low magnification, the tumor showed multilayered to solid carcinomatous areas intermingled with an intense desmoplastic stroma. The carcinoma appeared as distended ductal structures lined by a multilayered epithelium, often lacking lumina, and with surrounding necrotic debris in a comedo pattern. At higher magnification, the neoplastic cells showed indistinct cell borders, scant slightly eosinophilic cytoplasm, a prominent nucleus with clumped chromatin, and a single nucleolus. Mitotic figures were frequent. Among the neoplastic cells there were a number of globoid cells, with distinct cell boundaries, cytoplasm from uniformly eosinophilic to finely vacuolated, often with an eccentric nucleus ("signet ring" cells). These larger cells were frequently intermingled with the carcinomatous counterpart and sometimes formed small clusters (Fig. 2A and B). Both cell types stained positively for cytokeratin 19 and negatively for vimentin and Mac 387. (Fig. 3 and 4). Histochemical stains did not reveal any mucicarmine, PAS, Alcian blue pH 2.5-and 1.0-positive content in either cell type (Fig. 5). The desmoplastic stroma surrounding the carcinoma was infiltrated with inflammatory cells, mostly lymphocytes and eosinophils.

Pathology discussion. The presence of large multilayered to solid areas suggests a ductal origin of the tumor. The cells forming the neoplastic mass have the features of a comedo carcinoma of ductal origin, with accumulation of cell detritus and necrosis in the lumina. The epithelial origin of the cells is demonstrated by the immunohistochemical positive reaction to anti-cytokeratin 19 and the negative reaction to vimentin. The only cells that stained positive for alpha-actin, which were those of the smooth muscle admixed with the connective tissue forming the adjacent stroma and the vascular walls.

The numerous globoid cells observed among the typical epithelial neoplastic cells (which resembled morphologically those of a mucinous carcinoma were confirmed to be of epithelial origin (cytokeratin 19 positive) but do not produce mucinous substance. In fact, although their morphology resembles signet ring cells in mucinous carcinoma their content stains negative for mucicarmine, PAS, and Alcian blue (pH 2.5 and 1.0), which are all methods to reveal mucin. The PAS negativity also indicates low glycogen content, allowing for the exclusion of the so-called glycogen-rich carcinoma of human breast cancer. The vacuolated appearance of most cells is suggestive of a lipid content (not demonstrable in our formalin-fixed and paraffin embedded material) as in the so-called lipid-rich carcinoma known in human breast as well as animal mammary gland tumors.

The possible inflammatory nature of these globoid cells as foamy macrophages is excluded by the immunohistochemical negative stain with the antibody Mac 387. As alpha-actin did not stain these globoid cells, their nature as myoepithelial cells can also be excluded.

Case Discussion

Spontaneous mammary neoplasia in nonhuman primates has been described infrequently, and there is little to no information on

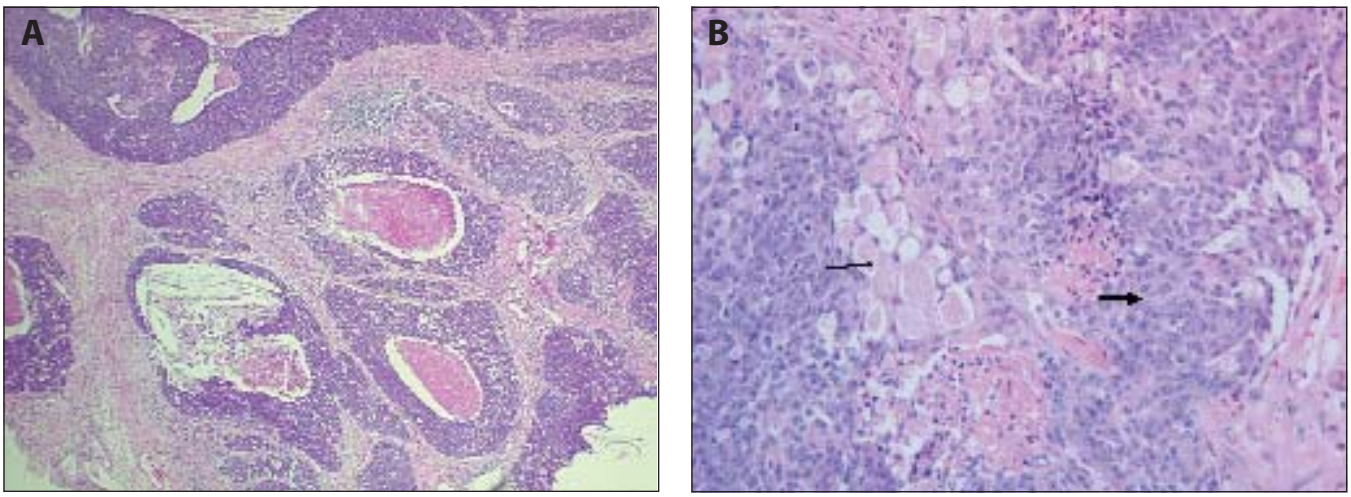

Figure 2. Surgical biopsy of ductal carcinoma with comedo pattern. (A) Multilayered to solid carcinomatous areas intermingled with intense desmoplastic stroma. Note distended ductal structures lined by a multilayered epithelium, either surrounding necrotic debris in a comedo pattern or with absent lumina. H&E; magnification, ×4. (B) The ductal carcinoma appears formed by two cell types, one characterized by large globoid cells filled with abundant granular faintly eosinophilic cytoplasm, with an eccentric nucleus (signet ring cell, thin arrow) the other showing indistinct cell borders, a prominent nucleus and a single nucleolus (large arrow). H&E; magnification, ×20.

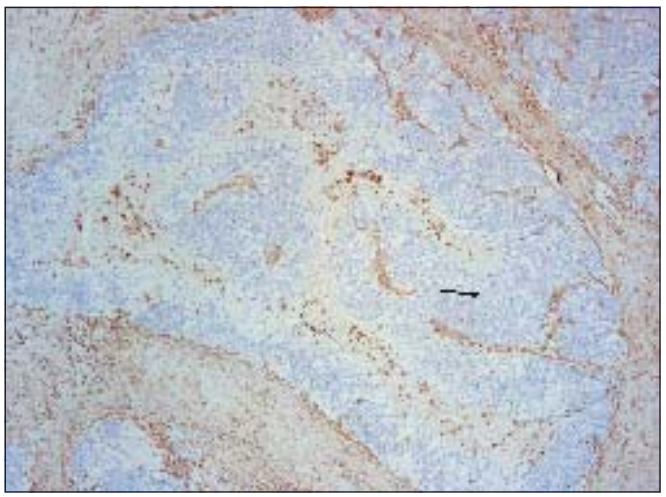

Figure 3. Immunohistochemical stain by anti-vimentin antibody showing negative carcinomatous fields (arrow) and positive stromal septa. Magnification, ×5.

treatment beyond surgical excision. Few cases of primary mammary neoplasia or neoplasia in supranummary mammary glands have been described in the rhesus macaque, or other species of nonhuman primates. The majority of reports involving rhesus monkeys addressed carcinomas, while other masses were described as nodular hyperplasia (1-3, 5-8, 10, 11, 15-17). Four cases of spontaneous neoplasia in rhesus macaques including ductal neoplasia similar to this case have been reported (7, 8, 10, 17).

Teleki and Ford reported a spontaneous intraductal mammary carcinoma in an adult female rhesus macaque with presentation very similar to the present case. This animal was also treated by surgical excision of the initial and subsequently developing nodules without other therapy and remained clinically healthy for at least 2 years from the initial surgery (17). Eydelloth and Swindle reported a case of concurrent intraductal mammary carcinoma and benign ovarian teratoma in an adult female rhesus macaque, but this animal had received exogenous hormones as part of a reproductive study and died due to trauma from a harem cagemate (8). Cohen, Saidla, and Schlafer recently reported a spontaneous mammary gland ductal carcinoma in situ in a 6- to 8-year-old multiparous female rhesus macaque, which also was treated surgically (7). Spontaneous mammary carcinoma with widespread metastases in a 22-year-old rhesus was reported by Hubbard and colleagues as similar in behavior to infiltrating duct carcinoma, the most common breast cancer in women. This animal survived for 5 years after detection of the initial tumor, having been observed with no treatment reported (10). Two control rhesus in a contraceptive study developed multiple small mammary nodules, which were diagnosed as mammary nodular hyperplasia (15). These excised nodules differed from the current tumor, having fewer layers of cells and more distinct acinar borders. Several possible etiologic explanations for macaque mammary neoplasia have been postulated, including the Mason–Pfizer monkey virus described by Chopra and others to occur in nonhuman primate mammary tumors (5, 6). This virus has been found in mammary tissue of animals which do not develop neoplasia as well and is not considered a primary etiologic factor (7). Cohen and colleagues postulate that androgens, growth hormones, irradiation, and aging may all have tumorigenic influence on the mammary gland (7).

For the purpose of the present report, we are not including those cases in which the animals had received exogenous hormone treatment as part of a research protocol. However, one such report noted the rarity of breast cancer in monkeys not receiving hormone therapy as support for their case having a hormonal cause (11). Another noted that the occurrence of spontaneous tumors in control animals needed to be considered in the evaluation of mammary tissue in toxicologic studies of contraceptives and other drugs (15).

From a review of the literature, spontaneous mammary neoplasia appears to be rare, or rarely reported, in nonhuman primates. Multiple reports on the same tumor cases and disagreement among pathologists, both veterinary and human, on the naming of mammary tumors make quantification difficult, but the number of spontaneous mammary neoplastic lesions reported to have occurred in rhesus macaques is approximately 12. The infrequent occurrence of spontaneous mammary neoplasms in macaques may be due to the limited numbers of middle-aged to geriatric nonhuman primates available for study, to lack of reporting, or to true low incidence. In Beniashvili's 1988 overview article, he suggested that the relative youth of monkeys usually kept in primate facilities might be a reason for the infrequent reports of spontaneous neoplasia, and he also noted a general increase in neoplasia in monkeys older than 10 years when compared with younger animals (2). Again in 2001, Cohen and coworkers commented on the rare reporting of spontaneous mammary gland tumors in rhesus monkeys, postulating that this paucity might reflect species resistance or a lack of thorough routine necropsy or examination (7).

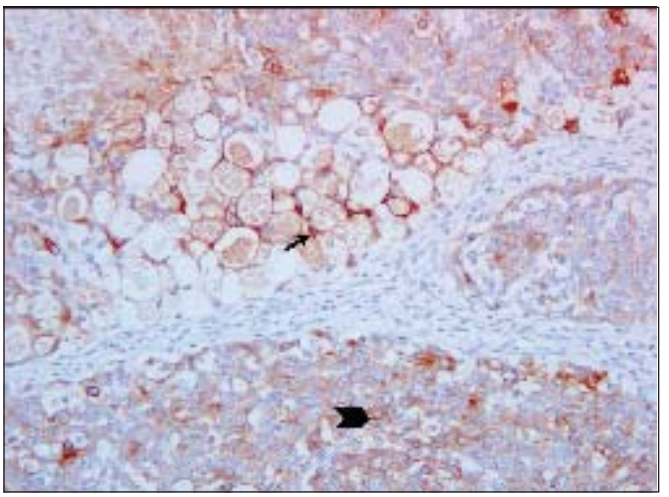

Figure 4. Immunohistochemical stain by anti-CK19 antibody, showing strong positivity in the peripheral condensed rim of the cytoplasm in the globoid cells (arrow) and faint focal positivity in the cytoplasm of ductal carcinomatous cells (arrowhead). Magnification, ×20.

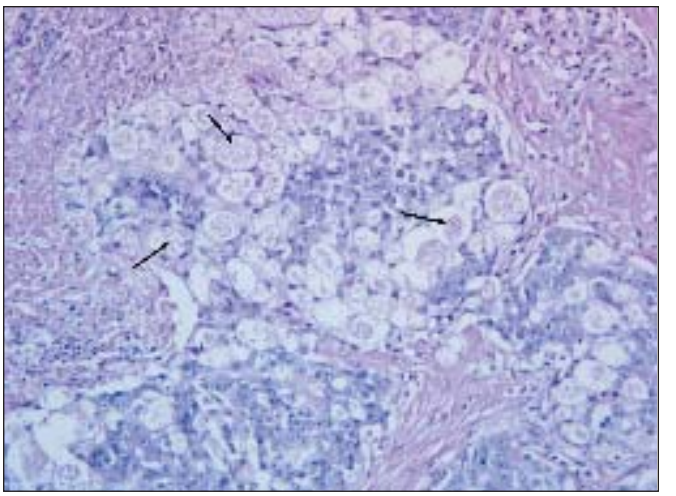

Figure 5. PAS negativity of the globoid cells (arrows) exclude a mucinous content. Magnification, ×20.

In the presented case, the attending veterinarian and investigator elected to use surgical excision of the mass alone, with diligent monitoring postsurgery. This decision was made due to the age of the animal, lack of well-defined protocols for medical management of breast associated tumors for this (or other veterinary) species, and a desire not to add confounding variables to the research protocol. We did not identify definitive factors that would indicate the need for additional therapy based on discussions of human (primate) mammary neoplasia. It was agreed that if metastatic spread to lungs or other tissues occurred and compromised the health of the animal, euthanasia would be performed. As of October 2004, at 28 years of age, the monkey remained clinically unaffected. This case demonstrates a spontaneous, locally invasive, mammary ductal carcinoma in a rhesus macaque, with minimal (if any) clinical effects on the well being of the animal.

Surgical excision with wide margins is the primary treatment for mammary neoplasia in the veterinary literature. Although ovariohysterectomy in dogs at an early age (before the fourth estrus) has been shown to decrease incidence of mammary neoplasia, it has not been shown to alter prognosis after neoplasia develops (4). One study reported the use of tamoxifen (0.42 mg/kg orally twice daily) as effective for treating dogs with mammary adenocarcinoma (12). Effective was defined as objective tumor response in five of seven dogs with inoperable or metastatic tumor. However, mean survival time was reported to be 4 months. Tamoxifen frequently causes estrogen-like side effects such as vulvar swelling, vaginal discharge, urinary tract infections, and incontinence (4, 13, 17). There have also been reports in the veterinary literature of the use of doxorubricin and cyclophosphamide (9), but no consensus has been reached on the efficacy of this approach. In cats, side effects of this treatment include severe anorexia and mild myelosuppressive effects (4). The veterinary literature does not provide clear or convincing consensus on the use of adjunctive chemotherapy or hormonal therapy in the treatment of mammary neoplasia. The likelihood of noteworthy side effects that would reduce quality of life and the uncertain benefit of this tumor also guided a decision not to pursue these options in this case. The decision seems to have been borne out by the significant interval free of clinical disease for this animal.

The current case report is submitted in hopes that increased reporting of spontaneous tumors in these animals may lead to a better understanding of the differences and similarities among primate species and contribute to our understanding of comparative mammary disease in animals and humans. Laboratory animal veterinarians, toxicologists, and research scientists need to have information on the incidence of spontaneous lesions in the animals they study in order to interpret the significance of lesions seen in clinical study situations. This was succinctly stated by Lowenstine ("There is no such thing as a generic monkey!") in her discussion of lesions and nonlesions in primate pathology (14). Although our case does not present an unreported tumor type, it adds additional data to the epidemiologic record and may serve to stimulate further clarification of the true incidence of such tumors in rhesus monkeys, macaques in general, and other species of nonhuman primates.

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References

1. **Appleby, E. C., I. F. Keymer, and J. M. Hime.** 1974. Three cases of suspected mammary neoplasia in non-human primates. J. Comp. Pathol. **84:**351-364.
2. **Beniashvili, D.** 1989. An overview of the world literature on spontaneous tumors in nonhuman primates. J. Med. Primatol. **18:**423-437.
3. **Benirschke, K., F. M. Garner, and T. C. Jones.** 1978. Pathology of laboratory animals, vol. II, p. 1204-1206. Springer-Verlag, New York.
4. **Birchard, S. J. and R. G. Sherding.** 2000. Saunders manual of small animal practice, 2nd ed., p. 222-225. Saunders, Philadelphia.
5. **Chopra, H. C.** 1973. Oncorna type virus particles in a tumor of a rhesus monkey. *In* R.M. Dutcher and L. Chieco-Bianchi (ed.), Unifying concepts of leukemia. Bibl. Haematol. **39:**228-235.
6. **Chopra, H. C. and H. D. Oie.** 1972. Possible etiological role of virus particles detected in rat and monkey mammary tumors. J. Natl. Cancer Inst. **48(4):**1059-1065.
7. **Cohen, M., J. E. Saidla, and D. H. Schlafer.** 2001. A spontaneously occurring mammary gland ductal carcinoma *in situ* in a rhesus macaque (*Macaca mulatta*) and a review of spontaneous mammary gland tumors in rhesus monkeys. J. Med. Primatol. **30:**121-126.
8. **Eydelloth, R. S. and M. M. Swindle.** 1983. Intraductal mammary carcinoma and benign ovarian teratoma in a rhesus monkey. J. Med. Primatol. **12:**101-105.
9. **Hahn, K. A., R. C. Richardson, and D. W. Knapp.** 1992. Canine malignant neoplasia: biological behavior, diagnosis, and treatment alternatives. J. Am. Anim. Hosp. Assoc. **28:**251-256.
10. **Hubbard, G. B., D. H. Wood, and W. I. Butcher**. 1984. Mammary carcinoma with metastasis in a rhesus monkey (*Macaca mulatta*). Vet. Pathol. **21:**531-533.
11. **Kirschstein, R. L., A. S. Rabson, and G. W. Rusten.** 1972. Infiltrat-

ing duct carcinoma of the mammary gland of a rhesus monkey after administration of an oral contraceptive: a preliminary report. J. Natl. Cancer Inst. **48(2):**551-556.
12. **Kitchell, B. E.** 1994. Mammary carcinoma in dogs: an update on biology and therapy, p. 884-886. Proceedings of the 12th ACVIM Forum, June 6-8, 1994, San Francisco, Calif. Omnipress, Madison, Wis.
13. **Kitchell, B. E. and J. L. Fidel.** 1992. Tamoxifen as a potential therapy for canine mammary carcinoma, p. 91. Proceedings of the Veterinary Cancer Society. Eastern States Veterinary Assoc., Gainsville, Fla.
14. **Lowenstine, L. J.** 2003. A primer of primate pathology: lesions and nonlesions. Toxicol. Pathol. **31(Suppl.):** 92-102.
15. **Nelson, L. W. and L. D. Shott.** 1973. Mammary nodular hyperplasia in intact rhesus monkeys. Vet. Pathol. **10:**130-134.
16. **Seibold, H. R. and R. H. Wolf.** 1973. Neoplasms and proliferative lesions in 1065 nonhuman primate necropsies. Lab. Anim. Sci. **23:**533-539.
17. **Teleke, S. and T. M. Ford.** 1980. Spontaneous intraductal mammary carcinoma in a rhesus monkey. Vet. Pathol. **17(4):**502-504.