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Case Report

A Phyllodes-like Mammary Tumor in a Breeding Galago (*Otolemur garnettii*)

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In humans, phyllodes tumors of the breast are rare fibroepithelial tumors that are further characterized as benign, borderline, or malignant according to their histomorphologic features. Phyllodes tumors are poorly responsive to treatment other than excision. NHP have a much lower frequency of mammary neoplasia than do humans, and none of the lesions reported previously in NHP are consistent with phyllodes tumors. Here we present the case of a mammary tumor in a northern greater galago (*Otolemur garnettii*) that was histologically characteristic of a malignant phyllodes tumor. An 11-y-old, multiparous, pregnant galago presented with a mass in the right middle mammary gland. A fine-needle aspirate yielded neoplastic epithelial cells. Because the animal was pregnant and showed no signs of skin ulceration, pain, or distress, she was allowed to deliver and nurse the infant. At 20 wk after initial presentation, the infant was weaned and the mother was euthanized. At necropsy, the mammary mass measured 3.5 × 2.5 × 1.5 cm, a 13-fold increase in volume since initial presentation. There was no evidence of metastasis in draining lymph nodes, lungs, or any other tissue examined. The tumor was composed of neoplastic stromal, glandular, and adipose tissues and was diagnosed as a malignant phyllodes tumor in light of its high stromal cellularity, high mitotic rate, and marked atypia. This tumor also exhibited liposarcomatous differentiation, which occurs frequently in malignant phyllodes tumors. To our knowledge, this report represents the first described case involving an NHP of a mammary tumor with characteristics consistent with human phyllodes tumors.

Abbreviations: α-SMA, α-smooth muscle actin; FNA, fine-needle aspirate

Phyllodes tumors of the breast in humans are characterized by neoplastic proliferation of both stromal and glandular tissue components and have a propensity for rapid growth.[22,26] Comprising less than 1% of all breast tumors and only 2% to 3% of mammary fibroepithelial neoplasms,[10,31,33] phyllodes tumors are rare biphasic neoplasms of epithelium and expanded stroma and typically are characterized by leaf-like fronds residing in clefts.[6,26,34] Phyllodes tumors are classified as benign, borderline, or malignant according to their histomorphologic features, including stromal cellularity, degree of atypia, mitotic activity, presence or absence of stromal overgrowth, and infiltration of surrounding breast tissue.[22,26,31] Although recurrence of phyllodes tumors is common after local excision, phyllodes tumors metastasize infrequently even when histologically classified as malignant.[8,36] However, malignant phyllodes tumors have a poor prognosis.[36,37] Phyllodes tumors are insensitive to chemotherapy and radiation and behave more like pure sarcomas than like mammary carcinomas.[13,15,31,36] In contrast, whereas human fibroadenomas likewise have epithelial and stromal components, they lack the atypia, stromal cellularity, and propensity for local recurrence seen with phyllodes tumors.[34,39]

From a comparative medicine perspective, NHP have a much lower frequency of naturally occurring mammary neoplasms than do humans.[9,14,44] Mammary neoplasms are more frequently reported in Old World than New World primates, with the majority of reported mammary neoplasms described in rhesus macaques.[9,12,14,17,25,44,45] The only reported case of a mammary tumor in a New World primate involved an adenocarcinoma in a male squirrel monkey.[25,43] To our knowledge, phyllodes tumors have not been reported in either Old or New World primates.[9,12,14,17,25,44,45] Within prosimians, 2 cases of mammary tumors in the brown greater galago (*Otolemur crassicaudatus*) were published more than 40 y ago.[4,5,21,32] These 2 mammary tumor cases were described as a spindle-cell sarcoma and a mixed mammary tumor.[5,21] Here we present a case of a mammary tumor in a northern greater galago (*Otolemur garnettii*) that was histologically characteristic of a malignant phyllodes tumor and contained neoplastic stromal, glandular, and adipose components.

Case Report

History. An 11-y-old, multiparous, pregnant galago (*Otolemur garnettii*) in an established breeding colony presented during midterm gestation with an alopecic, semimovable, firm, multilobulated subcutaneous mass (1 × 1 × 0.5 cm) of the right middle mammary gland. A fine-needle aspirate (FNA) showed sheets of densely packed epithelial cells with anisokaryosis, anisocytosis, and an increased nuclear:cytoplasmic ratio, consistent with a neoplasia of epithelial origin. Individual cells contained abundant cytoplasm, stippled chromatin, and multiple nucleoli. Because the animal was pregnant and had no signs of skin ulceration, pain,

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or distress, she was allowed to deliver and nurse the infant while being closely monitored. Both mother and infant remained in good body condition throughout the monitoring period. Given that galagos have 3 pairs of mammary glands, any decline in milk production from the affected gland appeared to be well compensated. At 20 wk after initial presentation, the infant was weaned and the mother euthanized and presented for necropsy.

On gross exam (Figure 1), the mammary mass measured 3.5 × 2.5 × 1.5 cm, a 13-fold increase in volume since initial presentation. On cut surface, the otherwise solid-appearing mass contained a cavity (1 × 0.7 cm) filled with milky fluid. Histologically, the cavity was consistent with the site of the prior FNA and was characterized by mild histiocytic inflammation, hemosiderin, and serous fluid.

Material and Methods

All animals involved were managed according to a breeding protocol approved by the Vanderbilt University IACUC and were housed in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals*,[18] the Public Health Service Policy on Humane Care and Use of Laboratory Animals,[29] and the Animal Welfare Act.[3] Galagos were maintained on a 12:12-h reversed light cycle using red-light filters during the day, received a commercial feline laboratory diet (LabDiet 5003, PMI Nutrition International, Brentwood MO) supplemented with sweet potatoes and a foraging mix (nuts, grain mixes), and had unrestricted access to water. The galago enrichment plan included social, structural, and sensory items. Social housing was provided for compatible breeding pairs and juveniles. Elevated perches (swinging or stationary), nest boxes, or hide tubes comprised structural enrichment. Sensory enrichment routinely included auditory (music, nature sounds) and olfactory (spices, scents) elements.

Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Additional sections were labeled with cytokeratin (dilution, 1:1000) and vimentin (1:500; dual stain, catalog no. Z0622, Cytokeratin Wide-Spectrum Screening and Vimentin, Dako, Carpinteria, CA), cytokeratin only (dilution, 1:4000; Dako), p63 (dilution, 1:500; catalog no. P3737, Sigma–Aldrich, St Louis, MO), and α-smooth muscle actin (α-SMA; dilution, 1:500; reference no. 0909, Dako) on the Bond-Max Immunostainer (Leica Biosystems, Wetzlar, Germany).

Results

The adjacent normal mammary tissue was compressed by an expansile, nonencapsulated, well-demarcated, nodular mass that extended subcutaneously to the underlying pectoral musculature. The mass consisted of malignant stromal, glandular, and adipose components, with complex morphology and multiple cysts (Figure 2 A). The stromal component was composed of densely cellular streams and whorls of markedly atypical spindle cells with a high mitotic rate of 24 per 10 high-power fields (Figure 2 B). In addition, the tumor contained an adenocarcinomatous component, composed of malignant glandular structures, moderately pleomorphic nuclei with multiple nucleoli, and an intermediate mitotic rate (7 per 10 high-power fields; Figure 2 C). The tumor also demonstrated heterologous differentiation, as indicated by the presence of a well-differentiated liposarcomal component with numerous lipoblasts (Figure 2 D). There was no gross or microscopic evidence of metastasis in draining lymph nodes, lungs, or other tissues. Unrelated to the mammary tumor, the capsular surface of both kidneys was irregular and pitted. Histologically, mild lymphohistiocytic interstitial nephritis with fibrosis was present. Renal tubules were dilated and often contained protein. Rare mineralization of tubular epithelial cells was noted, as well as occasional glomerulosclerosis (that is, increased glomerular mesangial matrix).

Figure 1. Photograph of the mass in the right middle mammary gland of the galago, with a normal gland on the left (arrowhead).

Cytokeratin Wide-Spectrum Screening. The glandular epithelium demonstrated cytoplasmic cytokeratin staining, whereas the stroma was negative. Glandular epithelium consisted of both benign (Figure 3 A) and malignant components, with loss of myoepithelial cells around malignant, infiltrative-appearing glands (Figure 3 B).

Vimentin. Intense cytoplasmic vimentin labeling was present in more than 80% of the stromal component of the tumor (Figure 3 A).

p63. Intensely p63-positive myoepithelial cells had nuclear staining and formed a continuous layer surrounding the ducts and acini. Stromal tumor cells were negative for p63 (Figure 3 C).

α-SMA. Intensely α-SMA-positive, basally located myoepithelial cells formed a continuous layer surrounding the normal ducts and acini. The malignant epithelial and stromal components were negative for α-SMA (Figure 3 D).

Discussion

Histologically, the mammary tumor in this galago had diagnostic features of a malignant human phyllodes tumor, given the presence of both epithelial and stromal components in the lesion and the characteristics of the stromal component, specifically the high stromal cellularity, high mitotic rate, marked cytologic atypia, stromal overgrowth, and heterologous liposarcomatous differentiation.[22,26,31] Phyllodes tumors of the breast in humans are defined as mixed epithelial and stromal or mesenchymal neoplasms and are further characterized as benign, borderline or malignant according to their histomorphologic features, including stromal cellularity, degree of atypia, mitotic activity, presence or absence of stromal overgrowth (low-power microscopic fields without epithelium), and infiltration of surrounding breast tissue.[6,26,34] These tumors often display a 'leaf-like' architecture, but some are solid and vaguely lobulated, as in the current case.[33,38] Differential diagnoses for a phyllodes tumor include

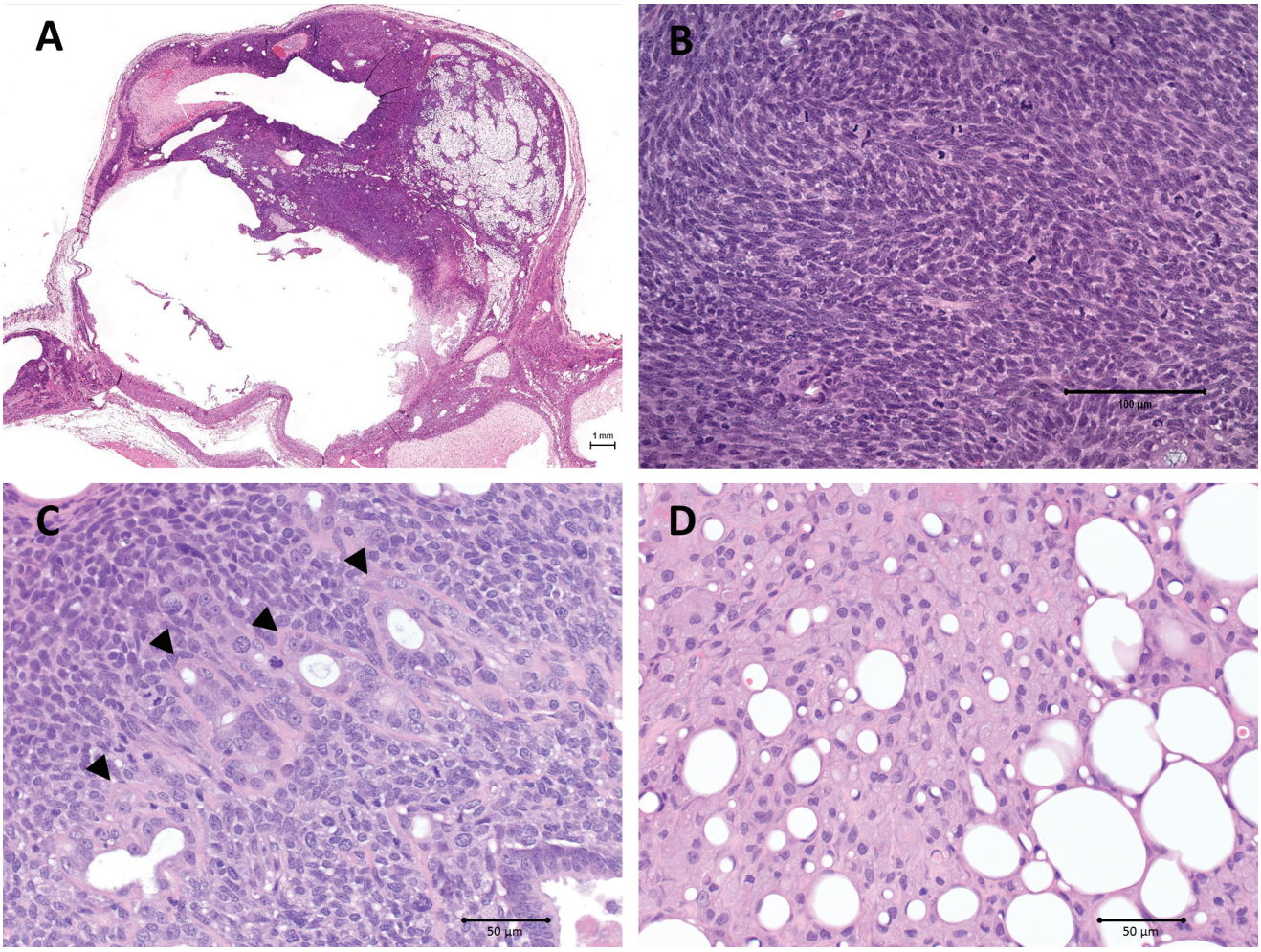

Figure 2. Photomicrographs. (A) Whole-mount cross-section of the mammary tumor. Note seromatous cavity due to prebious FNA (lower left). Bar, 1 mm. (B) Densely cellular neoplastic stroma composed of streams and whorls of mitotically spindle cells with moderate to marked pleomorphism. Magnification, 20×; bar, 100 µm. (C) Malignant epithelial component (arrowheads) comprised of moderately pleomorphic infiltrative-appearing glands. Magnification, 40×; bar, 50 µm. (D) Liposarcomatous element of the neoplasm. Magnification, 40×; bar, 50 µm. Hematoxylin and eosin stain.

fibroadenoma, metaplastic carcinoma, and pure sarcoma. In humans, fibroadenomas are a much more common fibroepithelial mammary lesion than are phyllodes tumors, and fibroadenomas lack atypia, stromal overgrowth, significant mitotic activity, and increased stromal cellularity.[39] In contrast, the tumor here had a highly cellular, sarcomatous stroma consistent with a malignant phyllodes tumor. Also unusual to our case is the presence of both benign and malignant epithelial components, a rare but described finding.[27,30] Although fibroadenomas have been reported in macaques,[14,44] to our knowledge the current report represents the first described case of a mammary tumor in an NHP that is consistent with human phyllodes tumors. The results of the immunohistochemistry we used here—cytokeratin wide-spectrum screening, vimentin, p63, and α-SMA—were consistent with a phyllodes tumor. Metaplastic carcinoma, a potential differential diagnosis due to the incidence of reported carcinomatous mammary lesions in macaques,[14,25,44] was ruled out by the negative staining of the stromal component for p63 and wide-spectrum cytokeratin.[1] In addition to having epithelial and fibrosarcomatous components consistent with those of human phyllodes tumors,[22,26] this tumor exhibited liposarcomatous differentiation, which is a common heterologous element in malignant phyllodes tumors.[16,19,24,28,35,41,42]

The clinical presentation, disease progression, and age at presentation in this case were consistent with a human phyllodes tumor. Phyllodes tumors have been reported in pregnant women but are rare and frequently malignant,[2,7,11,23] as was the tumor we report here. The affected galago was at midterm gestation when the tumor was first detected as a less than 1-cm^3 mass; within 20 wk, the mass grew 13-fold to encompass considerably more volume than a normal galago mammary gland. Likewise, in humans, phyllodes tumors during pregnancy present most frequently during the third trimester as large, rapidly growing masses.[11] Finally, although still producing offspring, the affected galago had reached an age typical for retirement from the breeding colony (10 to 15 y), whereas most humans at presentation for phyllodes tumors are premenopausal.[11,40] Female galagos have estrus cycles and therefore do not undergo menopause, but the age at presentation is comparable between our galago and humans with phyllodes tumors.

It is unsurprising that FNA failed to support the diagnosis of a phyllodes tumor in our galago. The only cells recognized on FNA suggested neoplastic epithelial cells consistent with a carcinoma.

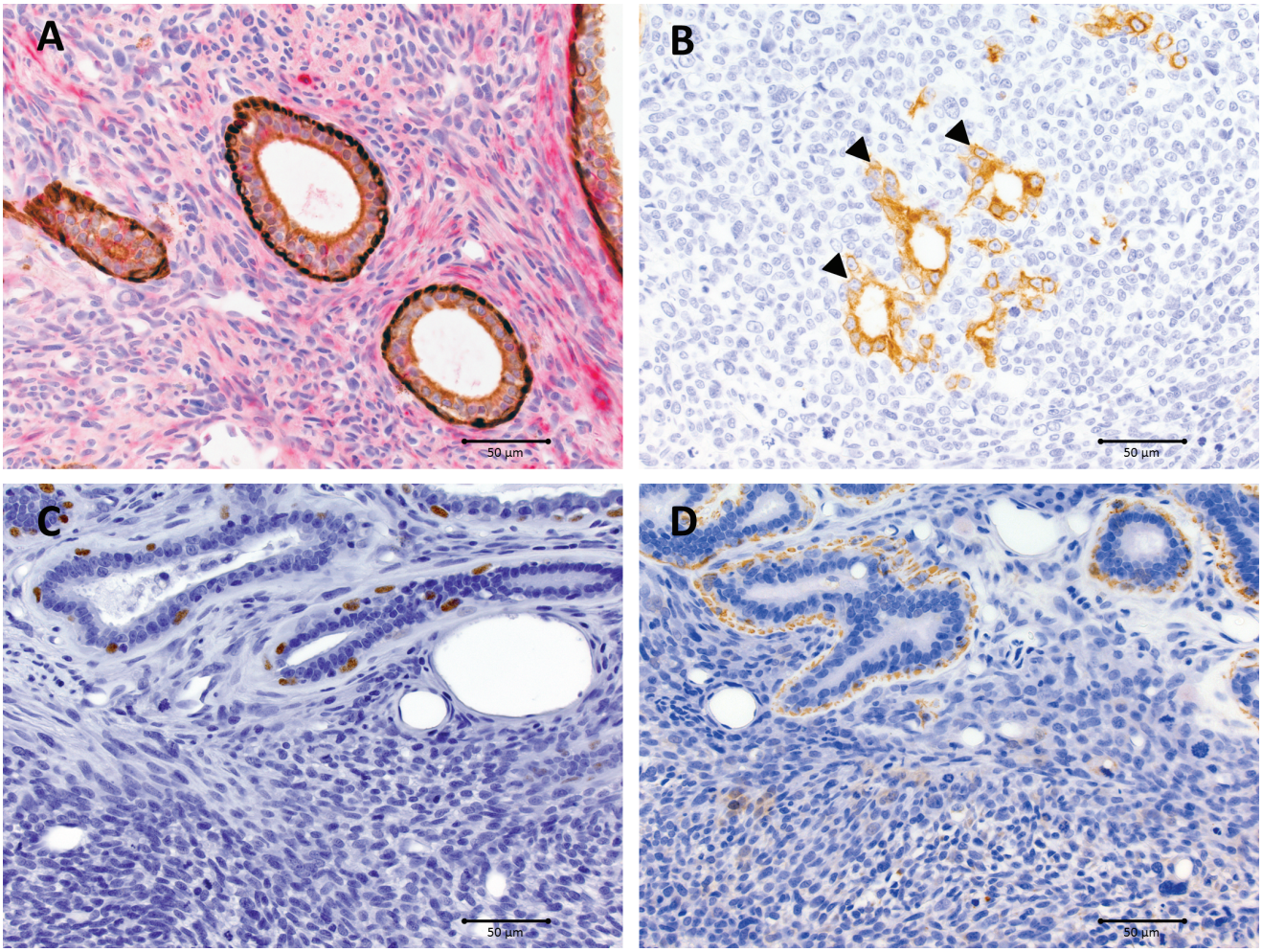

Figure 3. Photomicrographs of immunohistochemistry. (A) Wide-spectrum cytokeratin and vimentin dual stain. The benign epithelial component is highlighted by cytokeratin staining (brown), with intense nuclear staining of myoepithelial cells and moderate cytoplasmic staining of glandular epithelial cells. The malignant stromal component was vimentin-positive (red). (B) Cytokeratin only. Malignant epithelial component (arrowheads). Note the loss of myoepithelial staining. (C) p63. Nuclear labeling of myoepithelial cells in the benign epithelial component. Neoplastic stromal cells were negative. (D) α-SMA. Cytoplasmic labeling of myoepithelial cells in the benign epithelial component. Neoplastic stromal cells were negative. Magnification, 40×; bar, 50 µm.

Diagnosing phyllodes tumors on the basis of results of FNA is known to be challenging,[15,20] because these lesions are heterogeneous and the tumor architecture may not be appreciated on FNA. In addition, phyllodes tumors are very rare in humans and have not previously been reported in NHP. In the absence of prior experience in diagnosing phyllodes tumors and of known tissue architecture, our FNA was interpreted to indicate a neoplasm of epithelial origin only. Retrospectively, we noted that the FNA also contained cells consistent with the neoplastic stromal component. Given the advanced age of the affected galago, case management would likely have been the same even had a phyllodes tumor been diagnosed according to the FNA results.

As previously mentioned, 2 cases of mammary tumors in the brown greater galago (*Otolemur crassicaudatus*) were reported more than 40 y ago.[5] The histologic appearance of phyllodes tumors can vary widely, so although it is unclear whether these cases have more than superficial similarities to phyllodes tumors, the cell types described previously (spindle-cell sarcoma and benign mixed mammary tumor)[5] might represent a histopathogenesis similar to that we reported here.

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