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Case Study

Invasive Ductular Carcinoma in 2 Rhesus Macaques (*Macaca mulatta*)

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In the United States, breast cancer is the most common malignancy among women, with an estimated lifetime incidence of approximately 12% in American women. Invasive ductal carcinoma is the most common form of breast cancer in women, accounting for approximately 60% of all breast carcinomas. Prognostic markers are used to assess aggressiveness, invasiveness, and extent of spread of a neoplasm and thus may be correlated with patient survival. Immunohistochemistry is currently widely used for this purpose, with a variety of prognostication markers available. Classic markers for breast cancer in women include estrogen and progesterone receptor steroid hormone proteins and human epidermal growth factor receptor 2. Many additional markers have been used in diagnosis and prognostication, including p53, p63, and E-cadherin and cell proliferation markers such as Ki67. Despite an estimated lifetime incidence of approximately 6.1%, naturally occurring mammary neoplasms in nonhuman primates are uncommonly reported, with only sporadic references over the past 75 y. The majority of reported tumors occur in rhesus macaques, although this prevalence has been suggested to be a consequence of their high frequency of usage in biomedical research. Here we present 2 cases of mammary carcinoma in adult female intact rhesus macaques, with cytology, histopathology, and extensive immunohistochemical analysis. According to current classifications for human breast tumors, both tumors were classified as invasive ductal carcinoma. The prognostic value of immunohistochemical markers in human breast cancer and in reported cases in nonhuman primates is discussed.

Abbreviations: DCIS, ductal carcinoma in situ; ER, estrogen receptor; HER, human epidermal growth factor receptor; IDC, invasive ductal carcinoma; PR, progesterone receptor.

In the United States, breast cancer is the most common malignancy among women, with an estimated lifetime incidence of approximately 12% in American women.[4,32] In addition to age and family history, multiple major risk factors are recognized for women.[4,24] Postmenopausal hormone therapy has been reported to increase the risk by as much as 53%, particularly with use of combined estrogen–progesterone compounds for 5 y or longer.[4,14,24] Other factors documented to increase the risk of breast cancer in women include nulliparity or late first full-term pregnancy (after the age of 30 y), shorter breast feeding times, older age at menopause, obesity after menopause, young age at menarche, use of oral contraceptives, alcohol consumption, a Westernized diet, and exposure to ionizing radiation.[4,24]

Invasive ductal carcinoma (IDC) is the most common form of breast cancer in women, accounting for approximately 60% of all breast carcinomas and 40% to 75% of invasive breast carcinomas.[10,24,28,31,32,34] IDC can spread via both lymphatics and blood, and by the time of diagnosis, approximately 50% of patients have extension to draining lymph nodes.[28] In the World Health Organization classification system, IDC is also known as "invasive ductal carcinoma, not otherwise specified," because these are a heterogeneous group of tumors that do not exhibit sufficient characteristics for classification into a specific histologic subtype.[12,24] However, despite the considerable variation in histologic appearance, there are some general similarities among tumors that are classified as IDC. The neoplastic cells are arranged in nests, glands, tubules, cords or trabeculae, or may be in a relatively solid pattern.[13,24,27,28] The neoplastic cells themselves may also be highly variable, with abundant eosinophilic cytoplasm, regular or pleomorphic nuclei, and wide variation in mitotic rate.[13,24] Frequently, there will be foci of ductal carcinoma in situ (DCIS) associated with the carcinoma at the time of diagnosis, and the associated DCIS is often of the high-grade comedo type.[24]

Three of the strongest prognostic determinants used in the assessment of malignant human breast cancer are primary tumor size, lymph node stage, and the histologic grade of a malignant tumor.[5,9,17,22-24] The most widely used histologic grading system of human breast cancer is the Nottingham combined histologic grade, also known as the Elston–Ellis modification of the Scarff–Bloom–Richardson grading system (Figure 1).[5,17,24] This system considers 3 microscopic features of the tumor: tubule or gland formation (as an expression of differentiation), degree of nuclear pleomorphism, and mitotic index.[5,17,24] The neoplasm then is assigned a score from 3 to 9, corresponding to a low- (grade I), intermediate- (grade II) or high- (grade III) grade tumor.[5,17,24]

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Feature	Description	Points
Tubule and gland formation		
	Majority of tumor (>75%)	1
	Moderate degree (10%–75%)	2
	Little or none (<10%)	3
Nuclear pleomorphism		
	Small, regular, uniform cells	1
	Moderate increased in size, variability	2
	Marked variation	3
Mitotic index		
	0–5	1
	6–10	2
	>10	3
Total	Low grade (Grade I), well differentiated	3–5
	Intermediate grade (Grade II), moderately differentiated	6–7
	High grade (Grade III), poorly differentiated	8–9

Figure 1. Modified Scarff–Bloom–Richardson grading system for breast carcinoma histopathology. The mitotic index refers to the number of mitotic figures in 10 consecutive high-power (magnification, 400×) fields. Adapted from Tavassoli and Devilee[24] and Bansal and colleagues.[2]

Prognostic markers are used to assess aggressiveness, invasiveness, and extent of spread of a neoplasm and thus may be correlated with patient survival.[23] Many diverse immunohistochemical markers are currently available for use in estimating prognosis.[23,29] Classic markers include estrogen receptor (ERα and ERβ) and progesterone receptor (PR) steroid hormone proteins and human epidermal growth factor receptor 2 (HER2).[15,23,29,34] ER and PR normally function together to direct mammary epithelial growth, differentiation and survival by acting via nuclear receptors to modulate transcription of target genes.[23] Loss of expression of these receptors is an important negative prognostic indicator for human breast carcinomas, whereas those tumors that retain positive staining are associated with slow tumor growth, lower histology grade and a better overall prognosis.[23,29,34] HER2 (also known as HER-2/Neu/c-ErbB2) was first used in the assessment of human breast cancer in 1987.[15,29] Amplification or overexpression is associated with a poor 5-y prognosis in women with breast cancer and is found in approximately 30% of human breast carcinomas.[15,23,29,34] Additional markers that have been used in diagnosis and prognostication include p53, p63, E-cadherin, vimentin, and various cell proliferation markers including PCNA, Ki67, and AgNOR.[15,32,34]

Despite an estimated lifetime incidence of approximately 6.1% (as compared with 12.4% in humans), naturally occurring mammary neoplasms in nonhuman primates are reported infrequently, with only sporadic references over the past 75 y.[1,5,8,10,11,15,20,25,27,28,32,34] The majority of reported tumors have been found in rhesus macaques, although this prevalence may be a consequence of their high frequency of usage in biomedical research, as compared with that of other nonhuman primate species.[5,8,15,20,32,34] Here we present 2 cases of mammary carcinoma in adult female intact rhesus macaques, with cytology, histopathology, and immunohistochemical analysis. According to current classifications for human breast tumors, both macaque tumors were classified IDC.

Case Report

All experimentation (unrelated to their mammary tumors) was performed under independent protocols approved by the Yale University IACUC. The macaques were pair-housed indoors in an AAALAC-accredited facility and fed Harlan Teklad 8714: 15% protein primate chow (Madison, WI) with a variety of fruits and vegetables provided daily. They were seronegative for simian T-cell leukemia virus types 1 and 2, simian retrovirus type D, SIV, *Macacine herpesvirus* 1 (B virus), and measles virus. In addition, they tested negative for tuberculosis by semiannual intradermal tuberculin testing and annual in vitro tuberculin immunoassay. All examinations were performed under sedation by using intramuscular ketamine hydrochloride (10 mg/kg). Surgical removal of the masses was performed after sedation using intramuscular ketamine hydrochloride (10 mg/kg) followed by intubation and isoflurane (1.5% to 3%) inhalation anesthesia. Masses were removed by using standard sterile surgical procedures. Analgesic support included local anesthetic infiltration prior to incision (bupivacaine 0.5%, 2 mL locally), and postoperative administration of buprenorphine (0.1 mg/kg SC every 12 h for 1 to 2 d) and meloxicam (0.2 mg/kg PO once daily for 5 d).

Case 1 involved an 18-y-old intact female rhesus macaque (*Macaca mulatta*) that was noted on routine physical examination to have a firm subcutaneous mass (4 cm × 5 cm × 1 cm) in the region of the left mammary gland. Fine-needle aspiration of the mass (Figure 2) revealed frequent clusters of polygonal epithelial cells with distinct borders and small to moderate amounts of basophilic vacuolated cytoplasm. Nuclei were round to oval to irregular, with finely stippled chromatin and 1 or 2 variably prominent dark magenta nucleoli. Anisocytosis and anisokaryosis were moderate, and there were rare binucleated cells. There were also moderate numbers of macrophages with abundant cytoplasm containing well-demarcated, clear vacuoles (secretory product). Because cytologic findings were suggestive of malignancy, the mass was removed 10 d later and submitted for histopathology. The mass was fixed in 10% buffered formalin, processed routinely for sectioning, and then stained with hematoxylin and eosin.

On histopathologic examination, the dermis and subcutis contained an unencapsulated, poorly demarcated, infiltrative neoplasm that extended into a large lactiferous duct within the nipple. The neoplasm was composed of neoplastic cells arranged in lobules, nests, and acini separated by a large amount of dense fibrovascular stroma (Figure 3 A). Neoplastic cells were polygonal, with indistinct cell borders and small to moderate amounts of vacuolated eosinophilic cytoplasm. Nuclei were round and vesicular

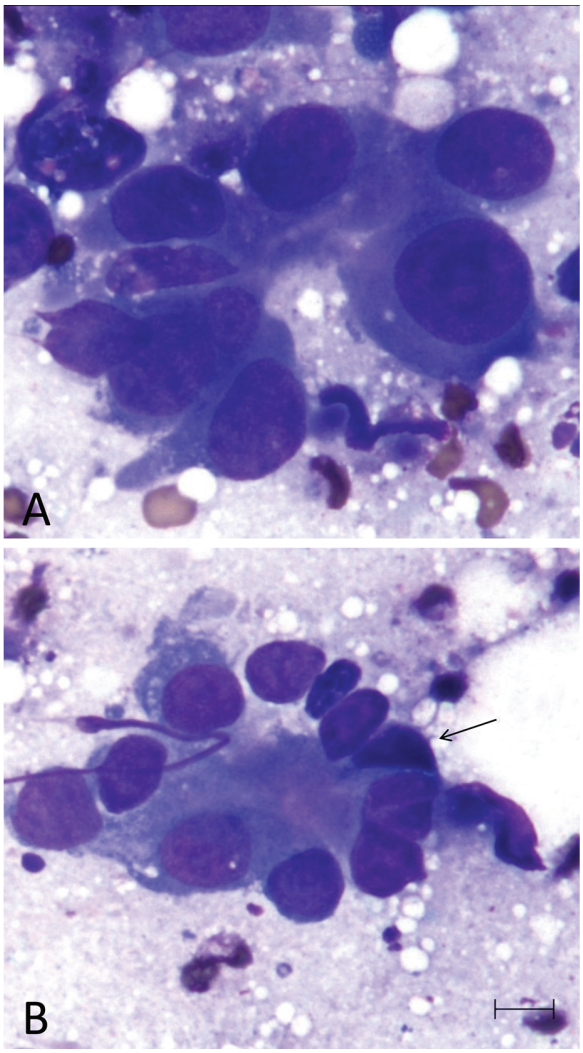

Figure 2. Fine-needle aspirate of the mammary tumor of case 1. (A, B) Clusters of polygonal epithelial cells arranged in crude acini are present with (arrow, B) occasional angular basal epithelial cells. Cells have distinct borders and variable amounts of basophilic vacuolated cytoplasm. Nuclei are round to oval to irregular, with finely stippled chromatin and 1 or 2 variably prominent dark-magenta nucleoli. Anisocytosis and anisokaryosis are moderate. Modified Giemsa stain; scale bar, 10 µm.

with 1 to 3 prominent basophilic nucleoli. Anisocytosis and anisokaryosis were mild to moderate, and mitoses were 6 to 9 per 10 high-power fields. Lobules of neoplastic cells often contained small central accumulations of necrotic cell debris or homogenous eosinophilic secretory material. The stroma contained large numbers of lymphocytes with lesser plasma cells and neutrophils.

According to the modified Scarff–Bloom–Richardson method for histologic grading of invasive carcinomas, this tumor was characterized as IDC of low to intermediate grade. The neoplasm was classified as an invasive carcinoma due to its infiltrative nature into the surrounding dermis and subcutis, as well as the histopathologic features of the cells themselves (multiple nucleoli and mild to moderate pleomorphism). This grade was given because a moderate degree of tubule and glandular formation was present (2 points), the nuclear pleomorphism was mild to moderate (1 to 2 points), and the mitotic index was 6 to 9 (2 points), thus putting the total score right at the line between low and intermediate grade (5 to 6 points).

Case 2 involved an 18-y-old intact female rhesus macaque (*Macaca mulatta*), who developed a firm, mobile subcutaneous mass (1.2 cm × 0.8 cm × 0.5 cm) approximately 1 cm lateral to the left nipple. Fine-needle aspiration revealed occasional small to moderately sized clusters of polygonal epithelial cells, with generally distinct borders and a moderate amount of basophilic vacuolated cytoplasm. Nuclei were round to oval to irregular, with finely stippled chromatin and 1 or 2 variably prominent dark-magenta nucleoli. Anisocytosis and anisokaryosis were moderate, and no mitotic figures were seen. Again, because the cytologic findings were suggestive of malignant neoplasia, the mass was removed 10 d later and submitted for histopathology.

On histopathologic examination, the dermis and subcutis contained an unencapsulated, poorly demarcated, infiltrative neoplasm composed of neoplastic cells arranged in lobules, nests, and acini separated by a large amount of fibrovascular stroma (Figure 3 C). Histopathologic features were very similar to those of the first case, and in case 2, anisocytosis and anisokaryosis were moderate and mitoses were 12 to 14 per 10 high-power fields. Lobules of neoplastic cells often contained large central accumulations of necrotic cell debris, which was mineralized occasionally. According to the modified Scarff–Bloom–Richardson grading system, this tumor was characterized as IDC, intermediate grade. This grade was given because moderate tubule and glandular formation was present (2 points), the nuclear pleomorphism was moderate (2 points), and the mitotic index was greater than 10 (3 points), giving a total score of 7 points.

The stroma of both neoplasms was strongly positive after staining with periodic acid–Schiff (data not shown), implying a basement membrane component to the stroma elaborated by the neoplastic cells. The periodic acid–Schiff stain also highlighted the basement membranes of the ducts and lobules of the mammary glands, allowing for visualization of the regions in which neoplastic cells breached the membrane and indicating the invasive nature of the tumors. Immunohistochemical staining for ERβ1 (dilution, 1:50; MA1-81281, Pierce Biotechnology, Rockford, IL), c-ErbB2 (1:200; CME342, Biocare Medical, Concord, CA), AE1/AE3 (1:100; MS343, Neo Markers, Fremont, CA), smooth-muscle actin (MS113; Neo Markers), p63 (1:300; M7247, Dako, Carpenteria, CA), E-cadherin (1:80; M3612, Dako), p53 (1:100; MS187, Thermo Scientific, Waltham, MA) and Ki67 (CRM325, 1:100; Biocare Medical) was performed on both tumors, and the summarized results are presented in Table 1. In addition, 3 different immunostains (CRM302, Biocare Medical; M3569, Dako; and no. 3157, Cell Signaling Technology, Danvers, MA) for PR were attempted but all failed to stain both normal and neoplastic tissue in both cases. All immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues, and positive and negative controls were used for each antibody. Antigen-retrieval methods were used for all antibodies except smooth-muscle actin (citrate buffer pH 6.0 for ERβ1, c-ErbB2, p53, E-cadherin and Ki67; proteinase K for AE1/AE3; TRIS–EDTA, pH 9.0 for p63), and primary antibodies were incubated for 30 min each. The detection method was either Envision Mouse (K4001; Dako; ERβ1, AE1/AE3, SMA, p63, p53, E-cadherin) or Envision Rabbit (K4007; Dako; c-erbB2, Ki67), according to the species source of the antibody. The chromogen was DAB (K3468; Dako) and the counterstain was hematoxylin for all antibodies. All antibodies were monoclonal, and the ERβ1, AE1/

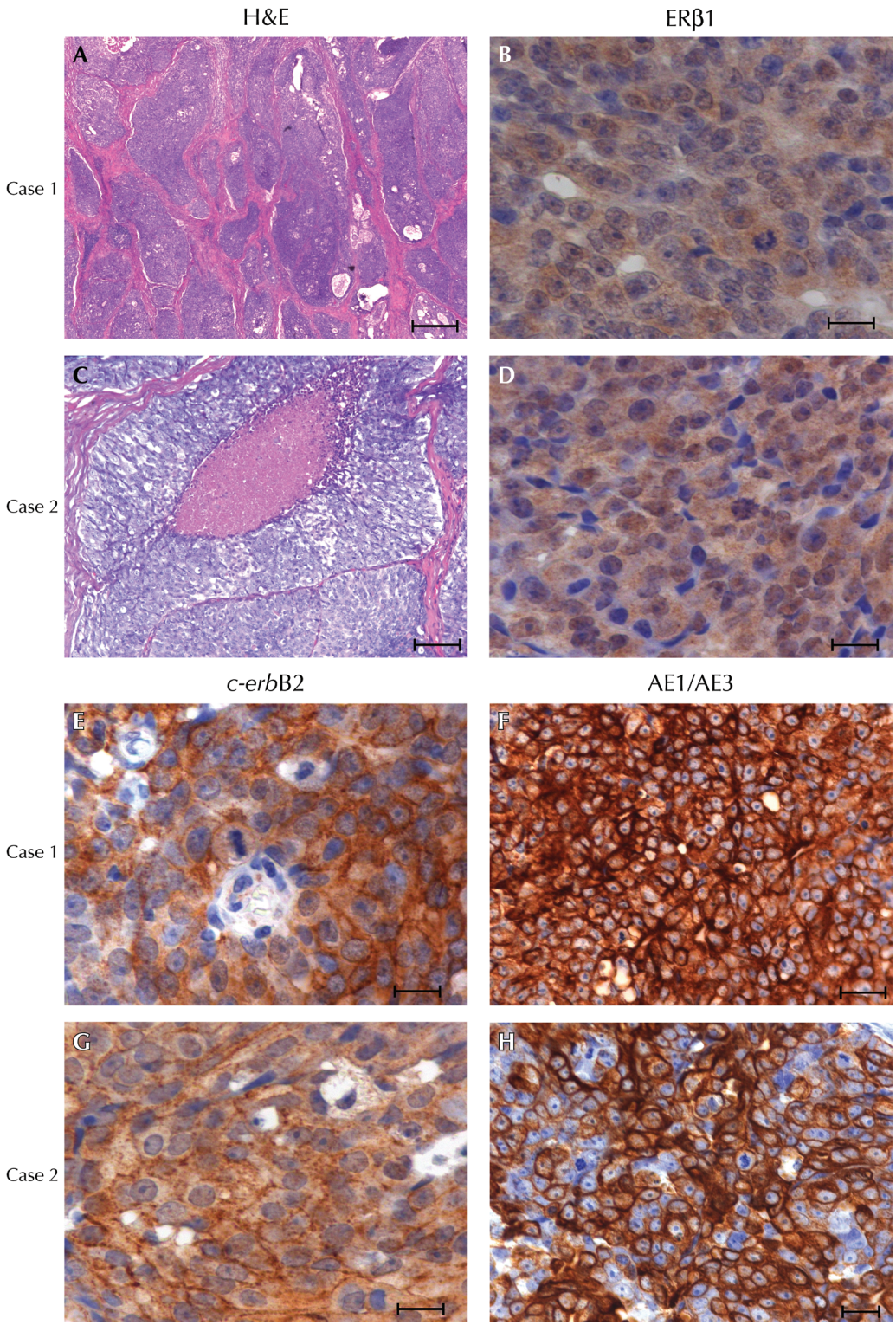

Figure 3. Histopathology and immunohistochemical features of the mammary tumors from cases 1 and 2. In both cases, the dermis and subcutis contained an unencapsulated, poorly demarcated, infiltrative neoplasm composed of neoplastic cells arranged in lobules with a (A) solid to (C) cribriform pattern, separated by a large amount of dense fibrovascular stroma. Hematoxylin and eosin stain; scale bar: 200 µm (A, case 1); 100 µm (case 2). (B and D) Estrogen receptor β1 immunohistochemistry. Positive nuclear and cytoplasmic staining of neoplastic cells for estrogen receptor β1 was present in both cases. Hematoxylin counterstain; scale bar, 20 µm. (E and G) c-ErbB2 immunohistochemistry. Strong positive membranous and occasional cytoplasmic staining of neoplastic cells for c-ErbB2 was present in both cases. Hematoxylin counterstain; scale bar, 20 µm. (F and H) AE1/AE3 immunohistochemistry. Strongly positive staining of cytoplasm of neoplastic cells for AE1/AE3 (pancytokeratin) was present in both cases. Hematoxylin counterstain; scale bar, 20 µm.

Table 1. Immunohistochemical staining characteristics of neoplastic cells of the mammary carcinomas in cases 1 and 2

Primary antibody	Case 1	Case 2
ERβ1	+	+
c-ErbB2	+	+
AE1/AE3	+	+
Smooth-muscle actin	+ (weak)	+ (weak)
p63	—	—
p53	+	+
E-cadherin	+	+
Ki67	10% to 15%	20% to 25%

The percentage of neoplastic cells staining positive for Ki67 was evaluated according to published recommendations,[6] which suggest the assessment of at least 3 randomly selected high-power fields, providing that staining is homogenous across the section.

AE3, smooth-muscle actin, and p53 antibodies react to nonhuman primate tissue, as reported by the respective manufacturers.

Both tumors and their surrounding normal mammary lobular and ductal tissue exhibited similar staining patterns for all antibodies evaluated. There was positive staining of neoplastic cells (primarily nuclear, with lesser cytoplasmic) for ERβ1 in both tumors (Figure 3 B and D), with occasional positive nuclear staining of acinar and ductal epithelial cells in normal internal control tissue adjacent to lesions.[12,16] Diffusely, there was strong positive membranous staining of neoplastic cells in both cases for c-ErbB2 (Figure 3 E and G), with occasional cytoplasmic staining as well.[12,21] Multifocally, positive membranous and cytoplasmic staining was found in normal acinar and ductal epithelial cells adjacent to lesions. There was diffuse strongly positive cytoplasmic staining of neoplastic cells in both cases for AE1/AE3 (Figure 3 F and H), a pancytokeratin marker, consistent with an epithelial origin of both neoplasms.[18] As expected, there was strongly positive cytoplasmic staining of ductal and acinar epithelial cells for AE1/AE3 in adjacent normal mammary tissue.

Multifocally, there was strong positive cytoplasmic staining for smooth-muscle actin in cells surrounding neoplastic lobules, consistent with normal stromal myofibroblasts and myoepithelial cells, as well as within scattered supporting stromal cells (data not shown).[7] Diffusely, there was weakly positive cytoplasmic staining of neoplastic cells for smooth-muscle actin. The adjacent normal mammary tissue demonstrated positive cytoplasmic staining of myofibroblasts and myoepithelial cells surrounding ducts and occasional positive cytoplasmic staining of acinar cells. Immunostaining for p63, a myoepithelial marker, resulted in strong but discontinuous positive nuclear staining of myoepithelial cells constituting the basal layer of neoplastic lobules (Figure 4 A and C).[3,7,30] Rarely, p63 immunopositive cells were noted in superficial aspects of the tumor (Case 2, Figure 4 C, black arrow). There was strongly positive nuclear staining of myoepithelial cells within adjacent normal mammary tissue, and these positively stained cells formed discontinuous rings at the outer margins of acini and ducts. Neoplastic cells in both cases exhibited strong positive membranous, and less prominent cytoplasmic, staining for E-cadherin, consistent with a ductular origin of the tumors (Figure 4 B and D).[7] Within adjacent normal mammary tissues, ductal epithelial cells multifocally exhibited positive membranous staining. Acinar epithelial cells were negative. Immunostaining for the human prognostic factor p53 revealed scattered neoplastic cells in both cases, with strongly positive nuclear expression (Figure 4 E and G).[7,19,26] Adjacent normal mammary tissue was immunonegative for p53.The proliferation marker Ki67 gives a score that is defined as the percentage of positively stained cells among the total numbers of neoplastic cells scored.[6] In the current report, Ki67 was evaluated according to the recommendations of Dowsett and colleagues, in which at least 3 randomly selected high power (40× objective) fields are examined, given that staining is homogenous across the section.[6] In case 1, approximately 10% to 15% of neoplastic cells exhibited strongly positive nuclear staining, compared with approximately 20% to 25% in case 2 (Figure 4 F and H). Positive nuclear staining was present sporadically, but consistently, within acinar and ductal epithelial cells in adjacent normal mammary tissue in both cases.

Discussion

Mammary glands of macaques are similar to those of women in many aspects of anatomy and physiology.[4,34] Like humans, macaques have 2 pectoral mammary glands, with the adult gland consists of a branching ductal system and terminal ductal lobular units.[4] Humans and macaques also share similar patterns of development and regression, sex steroid receptor expression, and mechanisms of glandular secretion, making them a useful model of mammary disease in humans.[34] However, as mentioned earlier, there are only sporadic reports in the literature that describe naturally occurring mammary neoplasia in nonhuman primates. Multiple factors are thought to contribute to the paucity of reports. Most experimental animals are not kept for their entire lifespan, specifically postmenopausal years, when the majority of breast cancer cases occur in women.[28,32,34] Many laboratory nonhuman primates are multiparous and ovariectomized, on a controlled diet, and generally not as overweight as higher risk humans, all of which factors are cited to reduce the risk of breast cancer in women.[28,32,34]

Histopathologically, both of the tumors reported here were classified as IDC. The histopathology of human IDC is extremely diverse, with neoplastic cells arranged in cords, tubules, acini, or solid sheets surrounded by various amounts of desmoplastic stroma.[13,24,27,28] Morphologic features of IDC in nonhuman primates have been described as similar to those in humans.[25,28,34] In addition, comedo-type change is frequent within macaque carcinomas, at approximately twice the incidence reported in human breast carcinomas.[34] In humans, comedo-type necrosis is an indicator of high-grade lesions in women, but the association between necrosis extent and histologic grade in macaque carcinomas remains unclear.[34] Both tumors presented here had comedo-type change, most prominently noted within case 2. In addition, higher grade carcinomas showed extensive stromal invasion and marked loss of tubular architecture,[32,34] features that were present in both of the current cases.

Although IDC is the most common mammary tumor reported in nonhuman primates, a range of spontaneous hyperplastic and neoplastic mammary gland lesions have been described in cynomolgus and rhesus macaques.[10,32,34] Therefore, we felt it important to distinguish the tumors in these 2 cases from other neoplastic lesions, particularly DCIS and invasive lobular carcinoma. The adult mammary gland in primates consists of a branching ductal system and terminal ductal lobular units consisting of a terminal intralobular duct and surrounding alveoli invested with myoepithelium.[4] DCIS is characterized by a proliferation of ductal cells

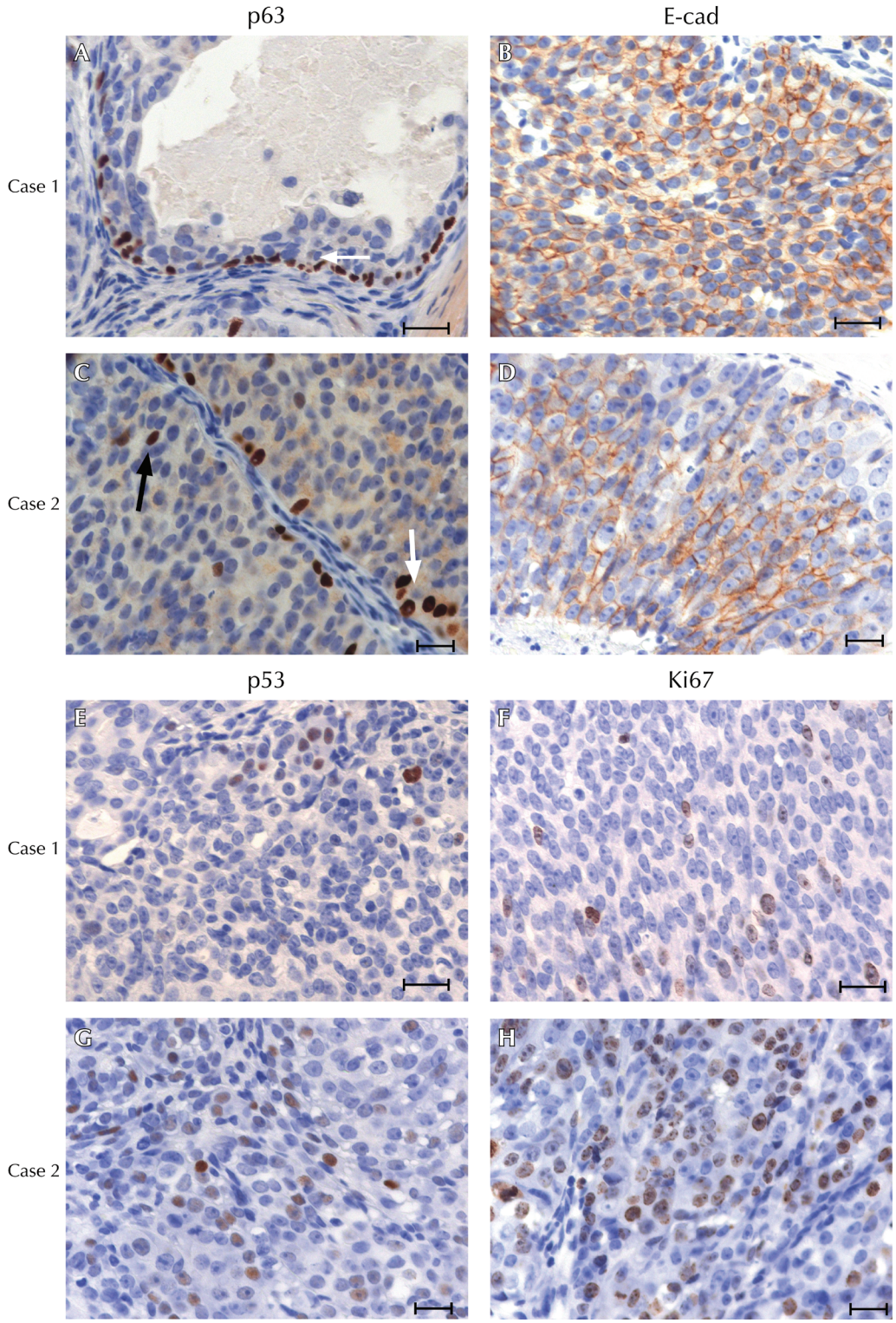

Figure 4. Immohistochemical features of the mammary tumors from cases 1 and 2. (A and C) p63 immunohistochemistry. Myoepithelial cells constituting the basal layer of neoplastic lobules were immunoreactive for p63 in both cases. Immunoreactive cells formed a discontinuous row of basally located cells (white arrows) and were rarely noted more superficially in the tumor (arrow, C). Hematoxylin counterstain; scale bar, 20 µm. (B and D) E-cadherin immunohistochemistry. Neoplastic cells exhibited strong positive membranous (and less prominent cytoplasmic) staining for E-cadherin in both cases. Hematoxylin counterstain; scale bar, 20 µm. (E and G) p53 immunohistochemistry. Scattered neoplastic cells exhibit strong positive nuclear expression of p53 in both cases. Hematoxylin counterstain; scale bar, 20 µm. (F and H) Ki67 immunohistochemistry. (F) Approximately 10% to 15% of neoplastic cells in case 1 exhibited strongly positive nuclear staining with the proliferation marker Ki67, (H) whereas a greater proportion (20% to 25%) were immunopositive in case 2. Hematoxylin counterstain; scale bar, 20 µm.

that have malignant architectural and cytologic features, but these cells have not breached the basement membrane.[34] As mentioned earlier, foci of DCIS are often located within IDC lesions at the time of diagnosis.[24] However, extensive invasion of neoplastic cells into surrounding stroma was seen histopathologically in both of the cases we present here, indicating loss of basement membrane integrity and progression to IDC. Invasive lobular carcinoma is the second most common histologic type of breast cancer in women and is more likely to have a better prognosis than is IDC.[4,14,31] Histopathologically, there is no tubule formation with invasive lobular carcinoma.[13] Instead, neoplastic cells tend to infiltrate in small loose groups, trabeculae, sheets, or even in single file, which is the hallmark feature.[13] Signet-ring cells with intracytoplasmic mucin are often a prominent feature as well.[13]

For diagnostic purposes, it was important to determine the site of origin of the tumors as ductal or lobular. Rhesus macaques have 5 to 7 lactiferous ducts exiting each nipple, with varying degrees of communication between the corresponding ductal and lobular units.[4] In humans, lobular mammary carcinomas often lose expression of E-cadherin, whereas carcinomas of ductal origin do not; therefore, the presence of positive E-cadherin staining within a neoplastic focus may suggest the origin of the mass as ductal.[34] Both masses presented here exhibited strong positive membrane staining for E-cadherin, consistent with ductal origin. In addition, in case 1, the neoplasm extended into a large lactiferous duct within the nipple, thus aiding in its classification of ductal origin (Figure 5).

In normal tissue, humans and macaques share similar patterns of sex steroid receptors (ER and PR).[34] In general, expression of ER and PR in human breast cancers is associated with a better prognosis than that of those that do not stain positive for the receptors.[15] In previously described cases of IDC in macaques, 43% of the cases were completely negative for sex steroid receptor expression, and only 1 of 5 baboon mammary carcinomas was reported to stain strongly positive for ERα and PR.[15,34] Both cases of IDC that we present here were positive for ERβ1 immunohistochemically. Unfortunately, we were unable to elicit positive immunohistochemical staining in control or neoplastic primate mammary tissue with the 3 different PR clones attempted.

Immunohistochemical staining for c-ErbB2 (to assess expression of HER2) was strong and positive for both of the carcinomas described here. c-ErbB2 is a transmembrane glycoprotein involved in the control of cell growth and is overexpressed in as many as 25% to 30% of breast cancers in women, including both DCIS and infiltrating carcinomas.[12,23] As mentioned earlier, amplification or overexpression is associated with poor prognosis and low disease-free survival rates in women with breast cancer, and increased HER2 oncogene expression was present in select higher grade lesions in a set of primate mammary carcinomas.[7,15,21,23,29,34]

Ki67 is a nuclear protein expressed in cells during proliferative phases of the cell cycle (G1, S, G2, M phases) and thus can be used as an indicator of cellular proliferation in neoplasms.[15,29] In human breast carcinomas, increased expression is considered a negative prognostic indicator and is associated with increased histologic grade, poor clinical response to endocrine therapy, and deceased overall survival.[15,23,34] In the 2 neoplasms we evaluated, approximately 10% to 15% of neoplastic cells were positive for Ki67, compared with approximately 20% to 25% in case 2, thus suggesting that case 2 was the more proliferative lesion. This assignment is in accordance with the results from the modified Scarff–Bloom–

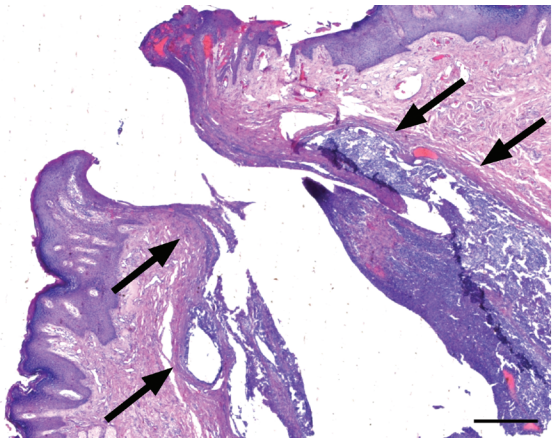

Figure 5. Case 1. The neoplasm extends into a large lactiferous duct (arrows) and is continuous with the nipple. Hematoxylin and eosin; scale bar, 200 µm.

Richardson histologic grading, in which case 1 was considered to be a low- to intermediate-grade tumor, whereas case 2 was classified as intermediate grade. A previous study of mammary neoplasms in macaques similarly indicated that, among proliferative lesions of the breast, epithelial expression of Ki67 increased with lesion severity and that higher Ki67 expression was significantly associated with increased carcinoma grade and decreased overall survival.[34]

Mutations in p53, a tumor suppressor gene, have been identified in a variety of human carcinomas, including 20% to 35% of sporadic human breast cancers.[19,23] One method of detecting these mutations is immunohistochemistry. Although the typical half-life of the p53 protein is very short (and thus not usually detectable by immunohistochemistry), mutations tend to alter the conformational of the protein.[19,23,26] The altered structure increases the stability, allowing for immunohistochemical recognition of the mutant p53 protein that accumulates in the nucleus of the neoplastic cells.[19,23,26] Mutations in the p53 gene have been associated with poor overall and disease-free survival rates in breast cancer, independent of other factors, and immunohistochemical detection of the mutant p53 protein has been reported to be an independent marker of shortened survival time in women with breast cancer.[19,23,26] Scattered neoplastic cells in both of the cases reported in the current study exhibited strong positive nuclear staining by p53, indicating the likely presence of an underlying mutation. This finding is similar to that in a description of 5 cases of spontaneous mammary carcinomas in baboons.[15] However, the previous report[15] does not discuss the possible implications of this identified staining pattern in the baboons, nor do any other publications address the significance of p53 staining in mammary tumors of nonhuman primates.

We used both smooth-muscle actin and p63 to evaluate the presence of myoepithelial cells, which form a continuous layer between the epithelium and the basal lamina.[30] Whereas smooth-muscle actin also stains myofibroblasts (which lie just beneath the basement membrane), p63 is extremely sensitive and specific for myoepithelium and exhibits alterations in its expression pattern that correlate with progressive neoplastic transformation.[3,30] In normal mammary tissue, p63 is immunoreactive in most myoepithelial cells, thus presenting as a continuous rim of positively stained cells.[3] In DCIS, this staining retains its original location

(rim) but becomes discontinuous, and in invasive carcinomas, its expression is reduced further, suggesting an association of loss of p63 expression with progression of ductal breast cancer.[3,30] In both of our cases, p63 highlighted a discontinuous rim of myoepithelial cells. However, case 2 showed less intense staining and was given a slightly higher histologic grade. In addition, when neoplastic cells breach the periductular layer of myoepithelial cells, the rim of p63-stained myoepithelial cells often becomes discontinuous and therefore is considered to be an indicator of local invasion.[32] In our cases, however, because positivity for p63 was intermittent, it did not greatly enhance our ability to evaluate for invasion. Instead, we relied on the presence of the breached basement membrane, as visualized on histopathologic examination and highlighted with periodic acid–Schiff stain. In addition, there were multiple foci of cytokeratin-positive nests of neoplastic cells within the stroma, indicating invasion of the tumor into the surrounding tissue after breaching of the basement membrane.

In general, breast carcinomas in nonhuman primates tend to develop as a single subcutaneous nodule or multiple nodules near the nipple; these masses can be detected visually or palpated.[5,10,25,27,28] It is interesting to note that in humans, breast cancer is approximately 5% to 10% more likely to be diagnosed in the left breast,[33] as occurred with both monkeys reported here. With progression, tumors exhibit invasive local growth, with eventual spread to local lymph nodes, and metastasis to the lungs is possible at later stages.[11,27] At the time of mass removal, neither macaque described here was noted to have grossly enlarged lymph nodes. Thoracic radiography to rule out potential pulmonary metastasis was not performed on either animal but will constitute one aspect of future monitoring.

In humans, postmenopausal use of estrogen–progesterone hormone therapy has been associated with a 2- to 3.9-fold increased risk of ILC but has little effect on the risk of IDC.[14] There have been several case reports of mammary gland tumors arising in nonhuman primates that were exposed to excessive endogenous or exogenous hormones, although a definitive causal link was not established.[5,11,32] In our monkeys, that of case 1 had no history of exposure to exogenous hormones, whereas case 2 received 40 mg medroxyprogesterone acetate, a derivative of progesterone, each month. Although exposure to exogenous progesterone cannot be ruled out as a contributing cause in case 2, it is considered less likely in light of the histologic subtype of the tumor (IDC). However, both macaques were intact females, so it is possible that the presence of endogenous hormones from the ovary may have contributed to tumor formation.

In summary, this report describes 2 cases of invasive ductal mammary carcinoma in nonhuman primates. Both cases were diagnosed initially through histopathologic examination, with further characterization by immunostaining. Although the immunoprofiles of the 2 neoplasms were very similar, the results of staining for Ki67, a proliferation marker, and the modified Scarff–Bloom–Richardson histologic grading system both indicated that the neoplasm from case 2 was likely a more proliferative lesion than was that from case 1. At this time, both macaques are being monitored closely, and neither has shown evidence of recurrence or spread of the disease.

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