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Histopathological and immunohistochemical characteristics of malignant adenomyoepithelioma in a cat: case report

[*Características histopatológicas e imuno-histoquímicas do adenomioepitelioma maligno em gata: relato de caso*]

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ABSTRACT

The malignant adenomyoepithelioma is a rare mammary tumor in women and uncommon in cats with only one report in this species. In this case report, the histopathological and immunohistochemical characteristics of six cases of malignant adenomyopithelioma in the feline mammary gland are described. Microscopic evaluation of tumors showed dense cellular neoplastic proliferation, composed of malignant myoepithelial and epithelial cells, formed by varied arrangements and presenting papillary, tubular and solid nest proliferation. Immunohistochemistry was performed for markers Ki67, Cox-2, RE, RP, p63 and HER-2. All cases were positive for p63, confirming the myoepithelial nature of neoplastic cells. The diagnosis of malignant adenomyopithelioma was made possible through the association between histopathological characteristics and immunohistochemical results.

Keywords: cat, malignant adenomyoepithelioma, diagnosis

RESUMO

O adenomioepitelioma maligno é uma neoplasia mamária rara em mulheres e incomum em gatas, possuindo apenas uma descrição nessa espécie. Neste relato de caso, são descritas as características histopatológicas e imuno-histoquímicas de seis casos de adenomioepitelioma maligno na glândula mamária felina. A avaliação microscópica dos tumores demonstrou proliferação neoplásica densamente celular, composta por células mioepiteliais e epiteliais malignas dispostas em padrão papilar, tubular e ninhos sólidos. Foi realizada técnica de imuno-histoquímica para os marcadores Ki67, Cox-2, RE, RP, p63 e HER-2. Todos os casos foram positivos para p63, confirmando a natureza mioepitelial das células neoplásicas. O diagnóstico de adenomioepitelioma maligno foi possível por meio da associação entre as características histopatológicas e os resultados de imuno-histoquímica.

Palavras-chave: gata, adenomioepitelioma maligno, diagnóstico

INTRODUCTION

Feline mammary tumors present the third highest incidence among feline tumors, being only behind skin and hematopoietic (Misdorp, 2002; Overley *et al.*, 2005; Cassali *et al.*, 2020). Mammary tumors in cats occur on average around 10 and 11 years old of age (Misdorp, 2002). Among these tumors, 80% to 96% are malignant, with carcinomas representing most of them. The feline mammary carcinomas have a high growth rate and often metastasize, mainly to regional lymph nodes, in lungs and liver (Overley *et al.*, 2005).

The adenomyoepithelioma is a mammary tumor characterized histologically by the proliferation of epithelial and myoepithelial cells. Most of these tumors are benign, but malignant transformation, although uncommon, may occur in one or both cellular components (Howlett *et al.*, 2003; Rosen, 2009). In human medicine, the adenomyoepithelioma is a rare histological type

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first described by Hamperl in 1970 (Hamperl, 1970). In veterinary medicine, the malignant adenomyoepithelioma is poorly described in cats, with only one case report in the literature (Campos *et al*., 2015). However, a more detailed description of the histopathological and immunohistochemical presentation of malignant adenomyoepitheliomas in felines can help in a more accurate diagnosis of animal mammary neoplasms, clarifying the definition of treatment for the disease. Thus, the purpose of this study is to describe the histopathological and immunohistochemical characteristics of six cases of feline malignant adenomyoepithelioma.

CASE REPORT

Histological sections of six feline mammary tumors were obtained from the archive of the Comparative Pathology Laboratory, at the Institute of Biological Sciences, UFMG. The neoplasms were selected for presenting similar characteristics related to tissue architecture and the presence of epithelial and myoepithelial components, being later evaluated retrospectively. The average age of the cats was 8 years old. The lesions were distributed among the thoracic (case 1), abdominal (case 2, 3, 4, 5) and inguinal mammary glands (case 6) and the diameter of the tumors varied from 0.2cm to 9.0 cm. The animals were not from a defined breed and contraceptives were applied in only one animal. The previous histopathological diagnoses were reviewed and immunohistochemistry for the markers Ki67, Cox-2, RE, RP, p63 and HER-2 was performed. The macroscopic histopathological characteristics of the cases are shown in Table 1.

Table 1. Macroscopic and histopathological characteristics of adenomyoepithelioma in cats

Patient	Measured to its largest extent (cm)	Mitosis figures in 10 40X fields	Presence of lymphatic invasion	Regional lymph node
Case 1	0.5	2	No	No metastasis
Case 2	0.5	2	No	No metastasis
Case 3	0.5	8	No	No metastasis
Case 4	0.5	52	No	No metastasis
Case 5	0.2	3	No	No metastasis
Case 6	9.0	47	Yes	With metastasis

The histopathological evaluation of tumors showed densely cellular neoplastic proliferation, composed of myoepithelial and epithelial cells, formed by varied arrangements, and presenting papillary, tubular, and solid nest proliferation. Myoepithelial cells exhibited slightly fusiform and vacuolized cytoplasm, with high nucleus/cytoplasm ratio, ovoid nuclei, finely granular chromatin, and prominent nucleoli. Carcinomatous cells were polyhedral, with vacuolized cytoplasm, high nucleus/cytoplasm ratio, ovoid nuclei, and prominent nucleoli (Fig. 1). Also, moderate anisokaryosis and anisocytosis were observed. In general, the neoplasms presented a high mitotic count, with an average of 19 mitosis figures in 10 high power fields (40x, FN 20mm). Extensive areas of necrosis were observed in three cases (case 4, 5 and 6) and one case (case 6) presented neoplastic emboli in lymphatic vessels adjacent to neoplastic proliferation.

Histopathological evaluation of regional lymph nodes was possible in all analyzed cases. Only one case (case 6) showed macrometastasis in regional lymph node. A loss of tissue architecture was observed due to the proliferation of neoplastic cells, formed by irregular islands of epithelial and myoepithelial cells. Epithelial cells exhibited moderate nuclear pleomorphism, multiple and prominent nucleoli. Myoepithelial cells presented slightly fusiform cytoplasm, granular chromatin, and prominent nucleoli.

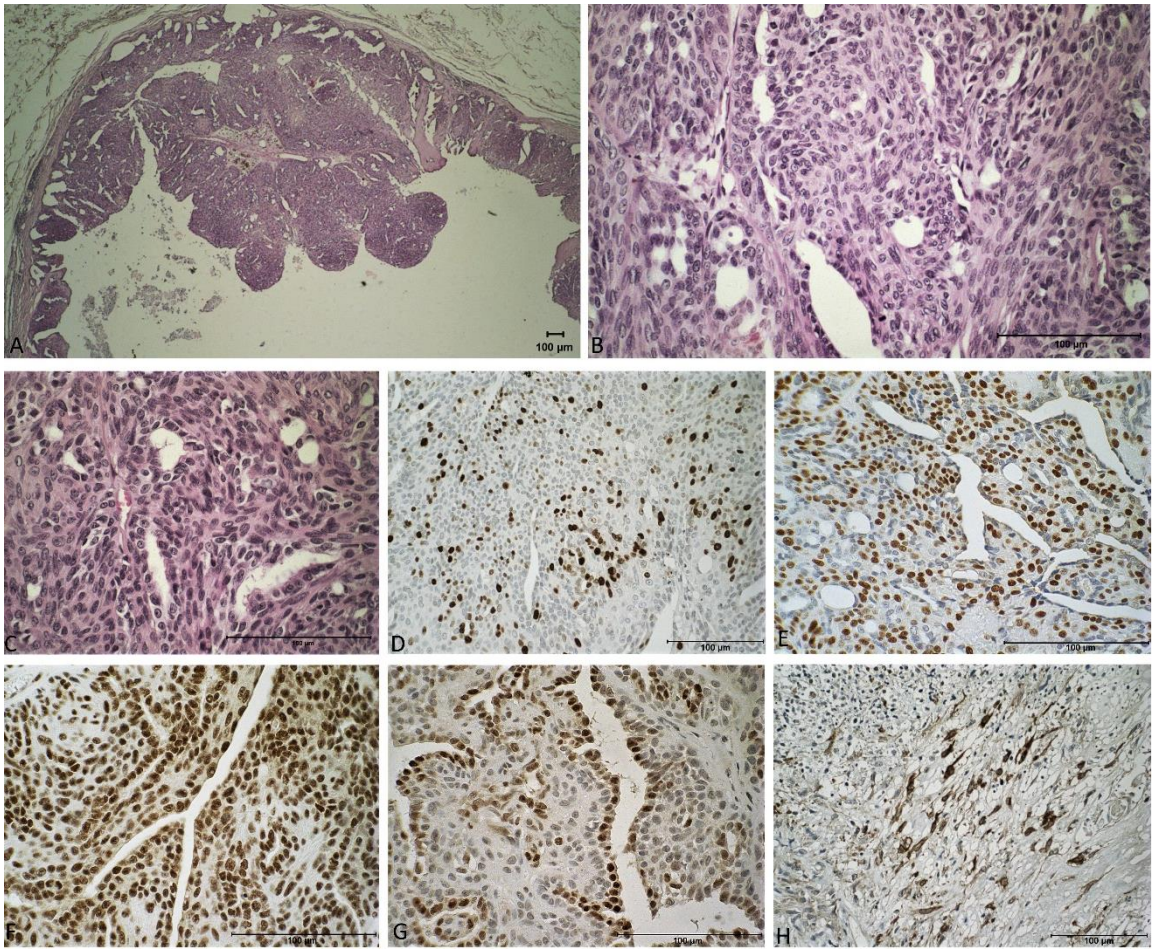

Figure 1. Cat. Adenomyoepithelioma. Mammary gland. A, B and C. HE showing epithelial and myoepithelial cells arranged in solid nests. D. Immunohistochemical staining showing strong labeling for the cell proliferation antigen Ki67. E. Immunohistochemical staining showing strong positivity for p63. F. Immunohistochemical staining showing positivity for ER. G. Immunohistochemical staining showing positivity for PR. H. Immunohistochemical staining showing positivity for COX-2.

For the immunohistochemistry technique, sections of 3 µm thickness were prepared from primary tumors and mounted on common slides for IHC analysis. The antigen was immunodetected by the detection system anti-mouse/anti-rabbit (Novolink Polymer Detection System, Leica Biosystems, Newcastle Upon Tyne, UK) according to the manufacturer's instructions. Endogenous peroxidase activity was blocked with a 10% hydrogen peroxide (H2O2) solution in methyl alcohol. Reagents were manually applied, and immunoreactivity was visualized by incubating the slides with diaminobenzidine chromogen (DAB Substrate System, Dako, Carpinteria, CA, USA) for 3 minutes. Details of the antibodies, dilutions, antigen retrieval procedures and incubation times used in the immunostaining process are shown in Table 2.

Immunoreactivity was visualized with the 3 ′-diaminobenzidine chromogen (DAB Substrate System, Dako, Carpinteria, CA, USA) and contrasted with Mayer's hematoxylin. Samples of breast tissue fragments from women positive for ER, PR, HER-2 and Ki67 were used as positive controls of the reactions. Negative controls were obtained by substitution of primary antibody by normal serum. The analysis of the slides and quantification of immunoreactions were performed according to Cassali and collaborators (2020). The antibodies are the standard ones used in our laboratory routine procedures and the antigenic specificity has already been tested

according to works published by our group (Campos *et al.*, 2015; Cassali *et al.*, 2020). The myoepithelial nature of the neoplasm was confirmed by positive staining for the p63 antibody. Other immunohistochemical results are shown in Table 03. All analyzes were evaluted in the highest magnification field (40X, FN 20mm).

Based on the histopathological and immunohistochemical findings, we were able to confirm the diagnosis of malignant adenomyoepithelioma in all selected cases. No clinical and therapeutic information was obtained after the diagnosis of most selected cases. Clinical information from one case showed that the patient remains alive 330 days after the diagnosis with no signs of metastasis or recurrences.

Table 2. Antibodies, dilutions, antigen retrieval procedures and incubation times.

Target Antigen (Clone)	Dilution	Antigen Retrieval Method	Incubation Time (h)/Temp
Ki-67 (MIB-1)	01:50	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0	14-16h/4°C
p63 (4A4)	1: 100	Water bath (98°C/20min) with citrate buffer pH 6.0	14-16h/4°C
ER (1D5)	1: 50	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0	14-16h/4°C
PR (HPRA2)	1: 50	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0	14-16h/4°C
HER-2 (polyclonal)	1:300	Water bath (98°C/20min) with citrate buffer pH 6.0	14-16h/4°C
Cox-2 (SP21)	1:50	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0	14-16h/4°C

Table 3. Results of immunohistochemical evaluation for Ki-67, estrogen receptor, progesterone receptor, cyclooxygenase-2 (cox-2) and p63.

Tumor	Ki-67 (%)	p63 (+/-)	ER (+/-)	PR (+/-)	HER-2 (+/-)	Cox-2 (+/-)
Case 1	12	+	+++	+++	-	-
Case 2	40	+	++	++++	-	+
Case 3	57	+	+++	++++	-	-
Case 4	30	+	+	++++	-	-
Case 5	12	+	++	++++	-	-
Case 6	60	+	-	+	-	+

DISCUSSION

In human medicine, adenomyoepithelioma may present a heterogeneous growth pattern due to variable proliferation of epithelial and myoepithelial cells (Lee *et al*., 2015). In general, epithelial and myoepithelial components may form solid nests or groups, trabeculae and/or papillary projections (Jones *et al*., 2003). In the cases reported, the epithelial component presented tubular and papillary growth pattern, while the myoepithelial component was in a solid pattern, surrounding the epithelial component. Thus, malignant adenomyoepithelioma in the cat histologically resembles malignant adenomyoepithelioma in women's breast.

The main histological criteria of malignancy for adenomyoepitheliomas include cellular and nuclear pleomorphism, high mitotic activity, presence of necrosis and invasion in the periphery of the tumor (Ahmadi *et al*., 2015). In all analyzed cases, both cellular components presented malignant characteristics with cellular atypia and high mitotic index, confirmed by the high immunohistochemical expression of Ki67.

The diagnosis of adenomyoepithelioma in human medicine is based on the morphological characteristics of epithelial and myoepithelial cells in HE associated with the use of immunohistochemical markers (Mclaren *et al*., 2005). In general, cytokeratins are used for epithelial components, while smooth muscle

actin-alpha, smooth muscle actin, specific muscle actin, calponin, cytokeratin-14, S-100, vimentin, and p63 are used for myoepithelial components (Ahmadi *et al*., 2015; Awamleh *et al*., 2012).

In the selected cases, the diagnosis of malignant adenomyoepithelioma was possible through histopathological analyses and confirmed by immunohistochemistry results. All cases presented positive immunostaining for p63, a selective marker for myoepithelial cells. All tumors showed positive estrogen and progesterone receptor labeling in at least 10% of neoplastic cells. Invasive breast carcinomas in the felines often exhibit steroid hormone receptor expression and the prevalence of ER and PR positive tumors using the recommendations of the American Society of Clinical Oncology and the American College of Pathologists is hight (Cassali *et al*, 2020).

The cell proliferation rate was considered high, with an average of 35% positive nuclear labeling for Ki67, being that the results ranged from 12 to 60%. According to Cassali *et al.* (2020), the evaluation of the cell proliferation index by immunostaining with Ki-67 is important in determining the rate of growth of mammary in felines. Studies of mammary lesions in cats have shown a progressive increase in the proliferative index of normal mammary glands, non-neoplastic lesions, benign tumors, carcinomas in situ, invasive and metastatic carcinomas. When the proliferation rate in neoplastic cells was less than 25.2 (mean value of all cases, calculated as positive nuclei in 1000 neoplastic cells), a significant association with increased survival in one was observed in a post follow-up study (Cassali *et al.*, 2020). Thus, the Ki67 immunostaining index can be used as a prognostic biomarker in feline breast carcinomas when there are values greater than 14% (Cassali *et al.*, 2020).

In general, the analyzed cases presented negativity for Cox-2, inferring carcinomas with better prognosis and longer survival time for patients (Cassali *et al*., 2020. The immunohistochemical results obtained in the analyzed cases are similar to those reported in the first description of malignant adenomyoepithelioma in cats, except that, in the previous report, the immunostaining for Ki67 was 3% and the HER-2 marker showed positivity (Campos *et al*., 2015). COX-2 expression levels in feline breast carcinomas vary widely between studies (Cassali *et al.*, 2020). Although it appears that COX-2 expression may have prognostic potential in feline breast tumors, as discrepancies between studies due to different use of data interpretation needs, such as assessment of distribution and/or intensity. A study designed by Campos *et al*. (2015) evaluated some molecular markers in feline breast tumors, including a COX-2. The evaluation criterion was the multiplication of the distribution and the labeling intensity in the neoplastic cells according to the present study. This allows the identification of 12 scores, with a neoplasm being considered positive when previous results $\geq$ 6 scores. These criteria were adopted by another study performed on canine mammary tumors and are adaptable for the predictive and prognostic classification of these neoplasms in cats, being indicated as a prognostic factor (Cassali *et al.*, 2020). The immunohistochemical results obtained in the analyzed cases are similar to those reported in the first description of malignant adenomyoepithelioma in cats, except that, in the previous report, the immunostaining for Ki67 was 3% and the HER-2 marker showed positivity (Campos *et al*., 2015).

In women, the malignant adenomyoepithelioma presents high metastatic potential, often for lungs, brain, liver, and lymph nodes (Awamleh *et al*., 2012). In the present study, clinical information of a patient shows no signs of metastasis or recurrences. However, complementary studies, as well as clinical and therapeutic follow-up of a larger number of patients are indispensable for the definition of a better prognosis for feline malignant adenomyoepithelioma.

CONCLUSION

The malignant adenomyoepithelioma of the feline mammary gland is still little described in the literature. Studies on the prognosis of this new histological type in female cats are important to define the biological behavior of these tumors, in addition to establishing more specific and appropriate treatments for this species.

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