Morphological and immuno-histochemical characterization of five phyllodes mammary gland tumors in dogs

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ABSTRACT

Araújo MR, Damasceno KA, Gamba CO, Campos CB, Campos LC, Reis DC, Souza CM, Cassali GD., Morphological and immuno-histochemical characterization of five phyllodes mammary gland tumors in dogs, Onl J Vet Res., 18(8): 688-695, 2014. In bitches, mammary tumors represent 52% of all neoplasms. The phyllodes tumor is a rare fibro-epithelial neoplasm found in humans characterized by epithelial and mesenchymal (stromal) cell proliferation creating elaborate leaf-like structures. Morphological and immune-histochemical characteristics in 5 canine phyllodes tumors are described. Four benign and one malignant phyllodes tumors were diagnosed. Epithelial and stromal tumor cells were positive for cytokeratin AE1AE3 and vimentin, respectively, and all were positive for estrogen and progesterone receptors. Benign tumors were negative for p63 whereas the malignant tumor was positive for p63. Cell proliferation index was <1% in benign tumors tissue but 69.5% in the malignant one. All tumors were negative for c-KIT. The morphological and immune-phenotypical aspects observed in this study suggest similarities between phyllodes tumors diagnosed in women and in bitches. However, new studies are necessary to enhance knowledge regarding the biological behavior of these neoplasms.

Key words: dog; immunohistochemistry; mammary neoplasm; phyllodes tumor
INTRODUCTION

In bitches, mammary neoplasms represent 52% of all diagnosed neoplasms of which 50% are malignant. Many mammary tumors are characterized by the proliferation of epithelial, myoepithelial, and mesenchymal components such as the mixed tumors, frequently diagnosed in canine mammary tumors. Neoplasms characterized by an association of epithelial and mesenchymal (fibroepithelial) components, such as the phyllodes tumor (PT), are still rarely described in the canine species.

In humans, PT represent a rare histological subtype that is histo-morphologically characterized by an epithelial and mesenchymal (stromal component) cell proliferation that, when associated, form leaf-like structures. Clinically, PTs resemble fibroadenomas but tend to occur in older women. Most PT behave in a benign fashion with local recurrences occurring in a small proportion of cases. Very rarely the tumor may metastasize mainly in malignant grade cases. Ancillary diagnostic tools may confirm the histogenesis and help to identify tumors with potentially aggressive behavior, e.g., immunohistochemistry assessment of vimentin, Ki-67 and c-KIT.

On account of insufficient data for the occurrence and biological behavior of canine mammary gland PTs we describe clinical-pathological and immune-histochemical aspects of five canine phyllodes tumors.

Case reports

Between 2011 and 2013, five bitches (Dogs 1, 2, 3, 4, and 5) with abdominal and inguinal mammary gland masses were submitted for surgery and diagnosed with PTs. The dogs ranged from 7 to 14 years old with a mean of 11. Tumor lesions were solitary measuring 6 to 8 cm in diameter (Table 1). Four tumor lesions were well demarcated with two partially encapsulated tumors. The cut surface revealed multinodular (2/4) or solid (2/4) lesions of heterogeneous consistency, varying from soft to firm and friable, and with a whitish to brownish color. Two of four lesions had cystic cavities containing a transluclid or brownish gelatinous liquid and 5th lesion was poorly demarcated, soft consistency and whitish tint with a cystic cavity containing a brownish gelatinous liquid.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Age (years)</th>
<th>Tumour Location (mammary gland)</th>
<th>Tumor size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cocker Spaniel</td>
<td>14</td>
<td>Abdominal Caudal</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Poodle</td>
<td>11</td>
<td>Abdominal Caudal</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>SRD</td>
<td>7</td>
<td>Abdominal Caudal</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Poodle</td>
<td>12</td>
<td>Inguinal</td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>Daschund</td>
<td>11</td>
<td>Abdominal Cranial</td>
<td>6x4x3</td>
</tr>
</tbody>
</table>

Samples of mammary lesions and inguinal lymph nodes from the 5 dogs were taken and fixed in 10% neutral buffered formalin for 24 hours and processed using routine methods for histological evaluation at the Laboratory of Comparative Pathology, Federal University of Minas Gerais, Brazil. Sections were stained with hematoxylin and eosin, and immunohistochemistry (IHC) staining was performed in mammary lesions.
using the ‘Advance HRP’ polymer (Dako North America Inc., Carpinteria, CA) visualization system and diaminobenzidine (Dako North America Inc., Carpinteria, CA) as the chromogen. Antigen retrieval was heat induced (98ºC, 20 minutes) in a citrate-buffered solution (Dako North America Inc., Carpinteria, CA) at pH 6.0 (for cytokeratin-CK, vimentin, Ki67, p63 and c-KIT) or in an ethylenediamine tetra-acetic acid (EDTA) buffered solution (Dako North America Inc., Carpinteria, CA) at pH 9.0 (for the estrogen receptor-ER and progesterone receptor-PR ). To block endogenous peroxidase activity, the slides were incubated in a solution of H2O2 (3%) in methyl alcohol. Reagents were applied manually, with 1 hour incubation for the monoclonal primary antibodies, except c-KIT (16 hours) and a 30-minutes incubation for the other reagents, except for the diaminobenzidine chromogen, which was applied for 5 min. Sections were counterstained using Harris hematoxylin. The IHC antibody panel is described in Table 2. Sections from canine tissues previously recognized as positive for each marker were used as positive controls. For the negative controls, the primary antibodies were replaced with normal serum (Ultra V Block) (NeoMarkers Inc., Fremont, CA). The evaluation of CK, vimentin, p63, and c-KIT expression was qualitative. Immunostaining for ER and PR was performed according to ASCO/CAP guidelines (Hammond et al., 2010). The proliferation index (Ki-67) was evaluated according to Dutra et al., 2008. 

RESULTS

Four benign phyllodes tumors (BPT) Dogs 1, 3, 4, and 5) and one malignant phyllodes tumor (MPT) (Dog 2) were diagnosed by microscopy. Inguinal lymph nodes were found to have reactive hyperplasia. BPT were characterized by benign proliferation of the double layer associated with stromal/mesenchymal cell proliferation forming leaf-like projections (Figure 1A). Variable grades of epithelial hyperplasia (two cases presenting up to five layers of epithelial cells) were observed in all BPT. The stromal component was loose and characterized by a discrete proliferation of fusiform cells with a colagenous and/or myxoid aspect, discrete pleomorphism and low mitotic index.

The malignant tumor presented an exuberant stromal proliferation poorly delimited characterized by fusiform cell proliferation forming interwoven bundles occasionally with a myxoid aspect (Figure 2A next page). Accentuated cellular pleomorphism and elevated mitotic index (6 mitosis in 10 fields of 40x) (Figure 2B) were observed in the stromal component. The epithelial component was discrete and characterized by a benign epithelial component proliferation in a double layer.

Immuno histochemical analysis of the epithelial components in all PTs was positive for ER and PR. The epithelial and stromal cells of all PTs were positive for CK (Figure 1B) and vimentin (Figure 2D), respectively. Stromal cells of the MPT were positive for p63 (Figure 2E), while negative in the BPT.
Figure 1. Benign phyllodes tumor. A: Epithelial benign proliferation in multiple layers (epithelial hyperplasia, arrowhead) associated with a stromal/mesenchymal cell proliferation forming leaf-like projections. Hematoxylin and eosin (HE). Bar = 50 µm. B: Neoplastic epithelial cells were positive for pan-cytokeratin (cytoplasm). Bar = 50 µm. C: Immunohistochemistry for Ki-67 presenting <1% of positive stromal cells (nuclear, arrows). Bar = 25 µm.

Figure 2. Malignant phyllodes tumor. A: Discrete benign epithelial proliferation in a double layer (arrowhead) associated exuberant stromal proliferation (asterisk). HE. Bar = 50 µm. B: Magnification of the stromal component presenting a fusiform cellular proliferation forming interwoven bundles, accentuated cellular pleomorphism and elevated mitotic index (arrows). HE. Bar = 25 µm. C: Immunohistochemistry for Ki-67 presenting >69% of positive stromal cells (nuclear, arrows). Bar = 25 µm. D: Neoplastic stromal cells were positive for vimentin (cytoplasm, arrows). Bar = 25 µm. E: Neoplastic stromal cells were positive for p63 (nuclear, arrows). Bar = 25 µm.
Table 2. Panel of antibodies used for immunohistochemical analysis

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Sourcea</th>
<th>Clone</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor</td>
<td>Dako</td>
<td>1D5</td>
<td>1:20</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>Neomarkers</td>
<td>hPRA2</td>
<td>1:20</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>Dako</td>
<td>AE1/AE3</td>
<td>1:100</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Dako</td>
<td>V9</td>
<td>1:100</td>
</tr>
<tr>
<td>Ki67</td>
<td>Dako</td>
<td>MIB-1</td>
<td>1:25</td>
</tr>
<tr>
<td>P63</td>
<td>Neomarkers</td>
<td>4A4</td>
<td>1:80</td>
</tr>
<tr>
<td>c-KIT</td>
<td>Dako</td>
<td>policlonal</td>
<td>1:800</td>
</tr>
</tbody>
</table>

aDako North America Inc., Carpinteria, CA; NeoMarkers Inc., Fremont, CA.

The BPT and MPT presented proliferation index of at least 1% (Figure 1C) and of 69.5% (Figure 2C), respectively. The stromal cells of all PT were negative for c-KIT (Table 3).

Table 3. Results of panel of antibodies

<table>
<thead>
<tr>
<th>Animal</th>
<th>ERa</th>
<th>PRA</th>
<th>P63b</th>
<th>MIBc</th>
<th>CKAE1AE3</th>
<th>Vimentin</th>
<th>c-KIT**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>&lt;1%</td>
<td>Ep+ Es-</td>
<td>Ep- Es+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>69.5%</td>
<td>Ep+ Es-</td>
<td>Ep- Es+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>&lt;1%</td>
<td>Ep+ Es-</td>
<td>Ep- Es+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>&lt;1%</td>
<td>Ep+ Es-</td>
<td>Ep- Es+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>&lt;1%</td>
<td>Ep+ Es-</td>
<td>Ep- Es+</td>
<td>-</td>
</tr>
</tbody>
</table>

ER: estrogen receptor; PR: progesterone receptor; Ep: epithelial cells; Es: stromal cells; +: positive; -: negative; samples with > 1% of neoplastic epithelial cells labeled were considered positive; qualitative evaluation performed solely in the stromal component; cellular proliferation index estimated by the evaluation of 1000 neoplastic stromal cells

Overall survival (OS) time in days was defined as the period between surgery and death due to the tumor. Patient follow-up was performed for 1018 days. Dog 1 was diagnosed with a metastatic solid carcinoma and treated with adjuvant chemotherapy. The treatment was aborted before conclusion of 1018 days with the dog alive until the last follow-up evaluation. Dog 2 had radiographic images of pulmonary metastasis and was treated with adjuvant chemotherapy but died due to the tumor with a OS of 374 days. Histopathological analysis of distant metastasis was not done. Dog 3 died one day after excision of the mammary gland lesion. Dog 4 had an OS of 513 days and was still alive at the end of our patient follow-up. Animal 5 was simultaneously diagnosed with carcinomas in mixed tumors and died 25 days following the surgical procedure, and cause of death could not be determined.

DISCUSSION

PTs are rare fibro-epithelial mammary neoplasms in women and bitches. Shahzamani et al (2013) reported one case of a BPT in a bitch. To the author’s knowledge, this is the first report of the immune-phenotypical aspects of this neoplasm, in association to anatomo-pathological characteristics of five PTs diagnosed in bitches.
All PTs diagnosed in the present study were solitary, with a mean size of 6.9 cm in diameter. In woman, these tumors usually occur as solitary unilateral masses and average size is 4 to 5 cm, ranging from 1 cm to larger than 20 cm. Shahzamani et al., 2013 described a solitary PT in a bitch measuring 7.2 cm in diameter. In women, the definitive diagnosis of PTs is achieved through histopathological analysis and three variants may be observed: benign phyllodes, low grade (“borderline”), and high grade malignant phyllodes. The differentiation among the three subtypes should be based on stromal cellularity and exuberance, cellular pleomorphism, mitotic index and tumor delimitation. BPT present a stromal component with a discrete to moderate cellularity, low mitotic index, pseudo-angiomaticous hyperplasia, osseous, cartilaginos and lipomatous metaplasia in various degrees, and myxoid alterations may be observed. In the present study, the four diagnosed BPT presented similar histomorphologic characteristics, with a predominance of low cellularity and myxoid alterations in the stromal component.

High grade MPTs are characterized by a marked stromal cellularity, elevated mitotic index, and accentuated stromal cellular pleomorphism. Pseudoangiomaticous stromal hyperplasia may also be observed in high grade MPTs. Rarely, the stroma contains heterologous sarcomatous elements such as angiosarcoma, liposarcomas, chondrosarcoma, myosarcoma or osteosarcoma. Low grade (“borderline”) MPTs present a stroma with moderate cellularity and moderate mitotic index. The spindle cell stroma in many of these lesions resembles low-grade fibrosarcomas or it may display pseudoangiomatous hyperplasia. Infrequent instances of cartilaginous, osseous and lipomatous metaplasia have been encountered in borderline PTs. The histomorphological results and high cellular proliferation index found in the current study suggest that the MPT diagnosis was reliable.

In humans, lymph node and distant metastasis are rarely observed (Lee, 2008; Barrio et al., 2007). In the current study, the malignant phyllodes tumor did not produce regional metastasis and pulmonary metastasis was not observed. Due to limited sample size the OS reported here may not relate with the prognosis for histological subtypes in humans. Bitches diagnosed with BPT presented a superior OS compared to the MPT, and the two dogs diagnosed with BPT that presented shorter OS were due to causes not related with the tumor. The OS of 374 days of the animal with the MPT may be due to the use of adjuvant chemotherapy.

In bitches, mixed tumors are the most frequent characterized by a proliferation of epithelial cells and a stromal component with a myoepithelial and a mesenchymal cell proliferation revealing myxoid, chondroid or osseous matrix. Considering that PTs present epithelial, mesenchymal, and even associated myoepithelial components, similar to mixed tumors, veterinarian pathologists should be attentive towards the recognition of this neoplasm. The definitive diagnosis of PTs is enabled with the identification of leaf-like structures in the epithelial component in association to the exuberant intratumoral stroma, which is rarely observed in mixed tumors.
CK positivity of the epithelial component and vimentin positivity of the stromal component in all cases confirms the double (epithelial and mesenchymal) origin of the tumor, similar to cases in women. In the present study, BPT cases were negative for p63 and the MPT was positive. This indicates that, in canines, the stromal component may also be composed by myoepithelial cells. In women, studies related to p63 immunolabeling of the stromal component of PTs are conflicting. Some demonstrate that p63 may be occasionally expressed in these tumors.

In canine mammary tumors, expression of hormone receptors is associated with an improved prognosis. Both BPT and MPT tumors had a well differentiated epithelial component with estrogen and progesterone receptors according to the literature. Immunolabeling for c-KIT was not observed in the stromal component of the tumors. This findings is in opposition to an increase in c-KIT expression found by Tse et al (2004). The stroma of high grade mammary gland MPTs in women may have a different biological behavior compared with dogs. Research involving the expression of the c-KIT proto-oncogene and the development of canine PTs may elucidate this conundrum. The morphological and immunophenotypical aspects observed in this study suggest similarities between phyllodes tumors diagnosed in bitches and in women. The presence of this neoplasm in dogs may serve as a model for women.

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