J. Comp. Path. 2011, Vol. 144, 303–307

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SHORT PAPER

Canine Invasive Lobular Carcinoma of the Mammary Gland: Morphological and Immunohistochemical Characterizations of Three Cases

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Summary

Invasive lobular carcinoma (ILC) represents 15% of invasive human breast tumours. This report describes the morphological and immunohistochemical features of three canine mammary tumours comparable with human ILC. These tumours were composed of a non-delimited proliferation of discrete cells infiltrating fibrous connective tissue. Multifocal in-situ carcinoma associated with invasive lesions was present. Invasive tumour cells and in-situ lesions expressed cytokeratin and CK34betaE12, but not E-cadherin. Based on these morphological and immunohistochemical characteristics, the tumours were classified as canine ILC.

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Keywords: CK34betaE12; dog; invasive lobular carcinoma; mammary gland

Canine mammary gland tumours (CMGTs) are one of the most common neoplasms of bitches (Vail and MacEwen, 2000) and their classification is based on histomorphological features, with simple and complex carcinomas recorded as the most common type of malignant CMGTs (Misdorp *et al*., 1999). Several histotypes that are uncommon and show distinct and unique features are included in the World Health Organization (WHO) system of classification. Invasive lobular carcinoma (ILC) represents 5–15% of invasive breast tumours in women (Cocquyt and Van, 2005; Singletary *et al*., 2005). The classical histological pattern of ILC is characterized by a proliferation of small, non-cohesive epithelial cells that are individually dispersed in fibrous connective tissue or arranged in linear cords, which invade the stroma. This tumour is often (in 90% of cases) associated with a lobular carcinoma *in situ* (LCIS), which immunohistochemically expresses CK34betaE12, but not E-cadherin (Tavassoli and Devilee, 2003; Hanby and Hughes, 2008). In this report we describe the morphological and immunohistochemical findings of three canine mammary tumours that are comparable with human ILC.

Three canine mammary tumours, morphologically resembling human ILC, were identified from 3,948 canine mammary lesions submitted over a period of 6 years (2002–2008) to the Department of Veterinary Pathology, University of Pisa. The identification criteria were based on morphological similarities to human ILC as described by Tavassoli and Devilee (2003). Original diagnoses made at the time of sample submission were invasive anaplastic undifferentiated tumours. Immunohistochemistry (IHC) was not performed at the time of the diagnosis. Clinical information was obtained from the clinical case history recorded by the referring veterinary surgeons.

Case 1 was a 10-year-old, entire female German shepherd dog, case 2 was a 9-year-old neutered female boxer and case 3 was an entire female crossbred dog. The dogs presented with neoplastic masses located in the left inguinal (case 3) or second abdominal (case 2) mammary gland. Case 1 presented with two masses located in the first and second left abdominal mammary glands: the mass in the first left mammary gland

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0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2010.08.015

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was the lesion comparable with human ILC and the mass located in the second was a concomitant complex carcinoma. All cases had a surgical mastectomy. In case 3, thoracic radiography performed at the time of diagnosis revealed an appearance consistent with pulmonary metastasis. Cases 1 and 2 also had pulmonary metastasis diagnosed 63 days (case 1) and 80 days (case 2) after the diagnosis of the primary tumour.

In case 2, cytological aspirates from the mass were available and were stained with a modified Romanowsky stain. Samples from the primary tumours were fixed in 10% neutral buffered formalin, processed routinely and sections were stained with haematoxylin and eosin (HE). In cases 1 and 2, regional lymph nodes were also available for histological examination. To confirm the epithelial nature and rule out a mesenchymal origin of the discrete cells observed, rabbit polyclonal anti-pan-cytokeratin (CKp; Novocastra Laboratories Ltd., Newcastle upon Tyne, UK) and mouse monoclonal anti-vimentin (Clone V-9; Novocastra Laboratories) antibodies were used in IHC. As described for human ILC (Moriya *et al*., 2009), mouse monoclonal anti-CK34betaE12 (Leica Microsystems, Wetzlar, Germany) and anti-E-cadherin (Clone 36; Transduction Laboratories, Lexington, Kentucky) antibodies were used to assess the lesions. Additional labelling with mouse monoclonal anti-oestrogen receptor (OR; Zymed Laboratories Inc., San Francisco, California), progesterone receptor (PR; clone PR4-12; Oncogene™ Research Products, Boston, Massachusetts) and rabbit polyclonal anti-HER-2 (Dako Inc., Glostrup, Denmark) was also performed. All antibodies have previously been used with canine tissues in published studies (Sarli *et al*., 2004; Millanta *et al*., 2005; Hsu *et al*., 2009; Ressel and Poli, 2010) except for anti-CK34betaE12. Anti-CK34betaE12 is directed against high molecular weight cytokeratins (cytokeratins 1, 5, 10 and 14) that are expressed by the myoepithelial-basal layer as defined by Gusterson *et al*. (2005) in normal mammary glands and are also highly expressed in LCIS (Tavassoli and Devilee, 2003).

For IHC, sections (4 μm) were dewaxed in xylene, passed through a series of graded alcohols and rehydrated. Antigens were retrieved by boiling in citrate buffer (pH 6.0) in a microwave oven for 20 min at 650 W and cooled at room temperature (RT) for 20 min (for labelling with vimentin, CK34betaE12, HER-2, OR and PR) or by incubation with proteinase-K (Dako) for 10 min at 37°C (for pan-cytokeratin). Endogenous peroxidase was blocked with H_2O_2 0.5% in distilled water for 10 min, followed by three washes in 0.05% Tween Tris buffered saline (TBST; pH 7.6). Primary antibodies, diluted 1 in 100 (for vimentin, pan-cytokeratin, HER-2 and PR) or 1 in 40 (for OR) in TBST, or prediluted ready to use (CK34betaE12), were incubated for 1 h at RT. After three washes, Envision® anti-rabbit or anti-mouse (Dako) was added and incubated for 30 min at RT. The peroxidase reaction was developed for 10 min with 3, 3′-diaminobenzidine (DAB; Impact DAB®; Vector Laboratories Inc., Burlingame, California) and stopped with deionized water. Negative controls were performed by omitting the primary antibodies and by replacing them with mouse isotype matched or rabbit unrelated primary antibodies. Labelling was judged as positive by the presence of a distinct brown cytoplasmic (for cytokeratin, vimentin and CK34betaE12), membranous (for E-cadherin and HER-2) or nuclear (for OR and PR) colouration. Three normal mammary glands from healthy dogs were used as positive controls for all the antibodies. For CK34betaE12 the myoepithelial-basal layer of a normal mammary gland was considered as positive control.

Immunohistochemical labelling was scored semi-quantitatively by examining 10 representative non-overlapping high-power fields (HPFs; ×40 objective field) for each lesion. The number of positively labelled cells for each marker was expressed as the percentage of labelled cells among the total number of neoplastic cells observed in each field. The mean percentage of 10 HPFs was then obtained. Tumours without positively labelled neoplastic cells were scored as negative (−). Tumours with 1−25% positive cells were scored as (+), with 26−50% positive cells as (++), with 51−75% positive cells as (+++) and with 75−100% positive cells as (++++). For HER-2 expression the standard HercepTest® grading method was used (Millanta *et al*., 2006) in which scores of 0 and 1 indicate that the tumour does not overexpress the marker (−) and where scores of ++ and +++ indicate that the tumour does overexpress the marker (+).

Microscopically all three tumours were characterized by an unencapsulated proliferation of discrete cells infiltrating fibrous connective tissue (Fig. 1). Neoplastic cells were often arranged in a concentric pattern around normal entrapped ducts. Infiltrating cells were individually dispersed through the connective tissue or arranged in single linear cords that invaded the stroma (Fig. 1, arrow). Occasional clear intracytoplasmic vacuoles were observed (Fig. 1, arrowhead). Multifocally in cases 1 and 3, carcinoma *in situ* was present and characterized by a proliferation of neoplastic cells confined within the lobular structures. The subcapsular and medullary sinuses of the lymph nodes in cases 1 and 2 were infiltrated by discrete cells morphologically comparable with those of the primary lesions, but difficult to distinguish from sinusoidal macrophages as described in human patients

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