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SHORT PAPER

Myoepithelial Cell Layer Integrity in Canine Mammary Carcinoma

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Summary

The aim of this study was to determine whether the myoepithelial (ME) cell marker calponin could be used to analyze the integrity of the ME cell layer as a means of identifying canine mammary carcinoma in situ. Tissue from 74 canine mammary lesions was evaluated (two dysplasia, eight benign tumours and 64 carcinomas including one carcinoma in situ). The 63 carcinomas included examples of histological grade 1 ($n = 32$), grade 2 ($n = 23$) and grade 3 ($n = 8$). Expression of calponin was determined by immunohistochemistry. The percentage of proliferating cells surrounded by a single layer of calponin-positive cells formed the basis of classification as type I (≥90%), type II (70–90%) and type III (≤70%). Expression of Ki67 was used to determine the proliferation index (PI). The malignant tumours comprised of an approximately equal mixture of type I, II and III lesions. The two examples of dysplasia, the carcinoma in situ and two thirds of the benign tumours were classified as type I lesions. Some overlap in the level of calponin expression was observed between benign and malignant tumours. Positive correlations between the degree of calponin expression and the type of lesion (i.e. benign versus malignant; $R = +0.3$, $P = 0.08$) and the histological grade of malignancy ($R = +0.54$, $P = 0.000001$) were found. A negative correlation between the degree of calponin expression and PI ($R = +0.027$, $P = 0.016$) was found. The ME cell marker calponin may be used as an aid in the identification of canine carcinoma in situ, but the study of the ME cell layer integrity is not definitive for the diagnosis of malignancy in canine mammary tumours.

Keywords: calponin; canine; carcinoma in situ; mammary myoepithelium

Carcinoma in situ is an epithelial tumour with malignant features that has not invaded the basement membrane (BM) and is included in the World Health Organization (WHO) histological classification of canine mammary tumours (Misdorp *et al*., 1999). Other grading systems including carcinoma in situ have been proposed (Gilbertson *et al*., 1983; Antuofermo *et al*., 2007); however, none of these schemes indicate clearly how to recognize an intact BM or the structural boundaries of the ductal system. Studies of human breast carcinoma have shown high interobserver variability in determining whether the BM is intact on examination of tissue sections stained by haematoxylin and eosin (HE; Yaziji *et al*., 2000). In the evaluation of human breast cancer, the integrity of the myoepithelial (ME) cell layer is used to identify non-invasive malignant neoplasms (i.e. carcinoma in situ) and invasion is associated with loss of immunoreactivity to ME cell markers (Tavassolli and Devilee, 2003; Moriya *et al*., 2009). The aim of the present study was to determine whether the ME cell marker calponin could be used to evaluate the integrity of the ME cell layer in canine mammary carcinoma as a means of identifying carcinoma in situ.

Seventy-four samples of canine mammary gland were studied and these were a mixture of biopsy and surgical resection specimens. Follow-up physical and

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radiological examinations were conducted for each dog undergoing surgical excision every 6 months post surgery. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax for preparation of HE-stained sections. The tumours were classified (Misdorp *et al.*, 1999) and graded based on the sum of individual scores for tubule formation, nuclear pleomorphism and mitotic activity as described by Lagadic and Estrada (1990). When submitted, sections of lymph nodes were stained by HE for assessment. For immunohistochemistry (IHC), sections from at least one representative block (with the highest histological grade) from each case were selected. Primary antibodies were monoclonal mouse anti-human calponin (clone CALP; isotype IgG1; Dako, Glostrup, Denmark; diluted at 1 in 400) and monoclonal mouse anti-human Ki67 (clone MIB-1; isotype IgG1; Dako; diluted at 1 in 75). Detection of antibody binding was by the avidin–biotin–peroxidase complex (ABC) method (Vector Laboratories; Burlingame, California) as described by Espinosa de los Monteros *et al.* (2002) and Domingo *et al.* (2008). Normal mammary gland tissue served as an internal positive control and for a negative control the primary antibodies were replaced by mouse IgG1 (Dako). Labelled sections were evaluated separately by two observers followed by joint evaluation and consensus.

The percentage of proliferating cells surrounded by a single layer of calponin-positive cells formed the basis of classification as type I (≥90%), type II (70–90%) and type III (≤70%). Ki67 expression was used to determine the proliferation index (PI). Images were captured (×40 microscope objective) from four randomly-selected neighbouring, non-overlapping fields. The number of Ki67-positive and -negative cells was counted with a digital pen tablet (Volito 2, Wacom Europe GmbH, Germany). The PI was calculated with Image-Pro Plus 4.5 and expressed as the percentage of positively labelled cells. A minimum of 1,000 tumour cells was counted per case. Statistical analysis was performed with Statistica v.6 (Statsoft Inc., Tulsa, Oklahoma). Ki67 data were not normally distributed (Kolmogoroff–Smirnov test), so non-parametric procedures (Kruskal–Wallis ANOVA by ranks test and the Mann–Whitney U test) were used to compare differences between groups. The Spearman correlation test was used to establish correlations between variables. $P < 0.05$ was regarded as significant.

The follow-up period from surgery to the last clinical examination ranged from 10 to 24 months. All animals with malignant tumours ($n = 28$) were free of recurrence and lung metastasis at that time. In four cases, one or more nodules appeared in other mammary gland/s within 4 months of surgery. One case was humanely destroyed when a vaginal carcinoma was diagnosed 7 months after surgery.

The mammary lesions investigated included examples of dysplasia ($n = 2$) and benign ($n = 8$) and malignant neoplasia ($n = 64$) (Figs. 1a, 2a and 3a) including one carcinoma in situ. The carcinomas

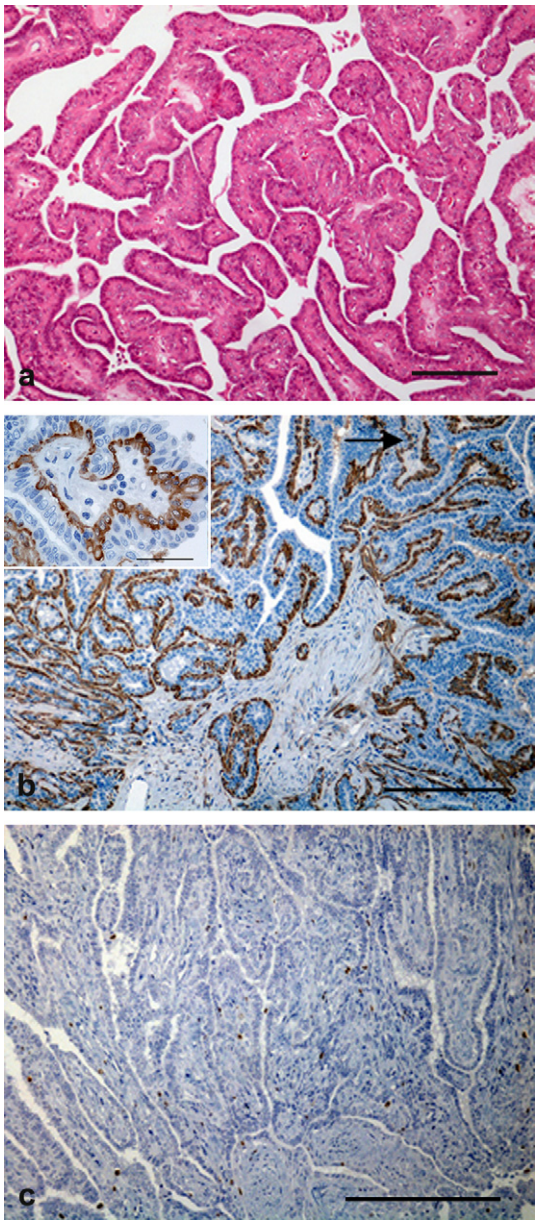

Fig. 1. (a) Simple tubulopapillary carcinoma grade 1. HE. Bar, 100 μm. (b) Simple tubulopapillary carcinoma grade 1 classified according to calponin expression as a type I lesion. Calponin-positive cells form a complete layer around all tubules and papillae. The majority of the labelled cells are elongate, but polygonal cells are also present (arrow and inset). IHC. Bar, 100 μm (inset 20 μm). (c) Simple tubulopapillary carcinoma classified as grade 1 and calponin type I with a low percentage of $Ki67^{+}$ nuclei. IHC. Bar, 100 μm.