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SHORT PAPER

Overexpression of Vimentin in Canine Prostatic Carcinoma

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Summary

Canine prostatic tumours exhibit similarities to those of man and may represent a useful model system to explore the mechanisms of cancer progression. Tumour progression to malignancy requires a change from an epithelial phenotype to a fibroblastic or mesenchymal phenotype. Vimentin expression is associated with the invasive phenotype of human prostate cancer cells. The aim of the present study was to characterize immunohistochemically the expression of vimentin by canine prostatic carcinomas. Primary carcinomas and metastatic tumour foci both showed vimentin expression. This finding suggests that the acquisition of the epithelial–mesenchymal transition phenotype in canine prostatic carcinoma may be characterized by the presence of mesenchymal intermediate filament (vimentin) that could lead to a higher likelihood of metastasis.

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The prevalence of prostatic carcinoma is higher in man and dogs than in other species (Cornell *et al.*, 2000; Leroy and Northrup, 2008; Zhao *et al.*, 2008), but the factors that contribute to this susceptibility are poorly identified, with the exception of ageing (Cornell *et al.*, 2000). Canine prostatic tumours arise spontaneously and have many similarities with the human counterparts. The dog may therefore represent a useful model system to explore the mechanisms of cancer progression in both species (Leroy and Northrup, 2008), as well as to study factors that regulate carcinogenesis within the aged prostate (Cornell *et al.*, 2000).

Tumour progression to malignancy requires a change from an epithelial phenotype to a fibroblastic or mesenchymal phenotype, a re-programming process recognized as an epithelial–mesenchymal transition (EMT; Wei *et al.*, 2008; Zhao *et al.*, 2008). EMT is a process in which cells undertake a developmental switch from an epithelial to a motile mesenchymal phenotype (Thiery, 2002; Natalwala *et al.*, 2008). This process has been associated with embryological morphogenesis, fibrosis and the progression and metastasis of epithelial tumours (Thiery and Sleeman, 2006).

Singh *et al.* (2003) and Zhao *et al.* (2008) demonstrated the important contribution of vimentin to the invasive phenotype of prostate cancer cells. In dogs, EMT has been demonstrated in canine kidney fibrosis (Aresu *et al.*, 2007); however, there are no reports of this process in canine prostatic cancer. In the present study, we evaluated the expression of vimentin in prostatic carcinomas in dogs in a tissue microarray (TMA).

A total of 149 samples of prostate glands from 69 dogs (aged 1–15 years and of pure and mixed breeds) were taken from the archives of the Veterinary Pathology Service, São Paulo State University (UNESP), Botucatu, Brazil and the School of Veterinary Medicine, Federal University of Goiás (UFG), Goiania, Brazil. Tissues were fixed in 10% neutral buffered

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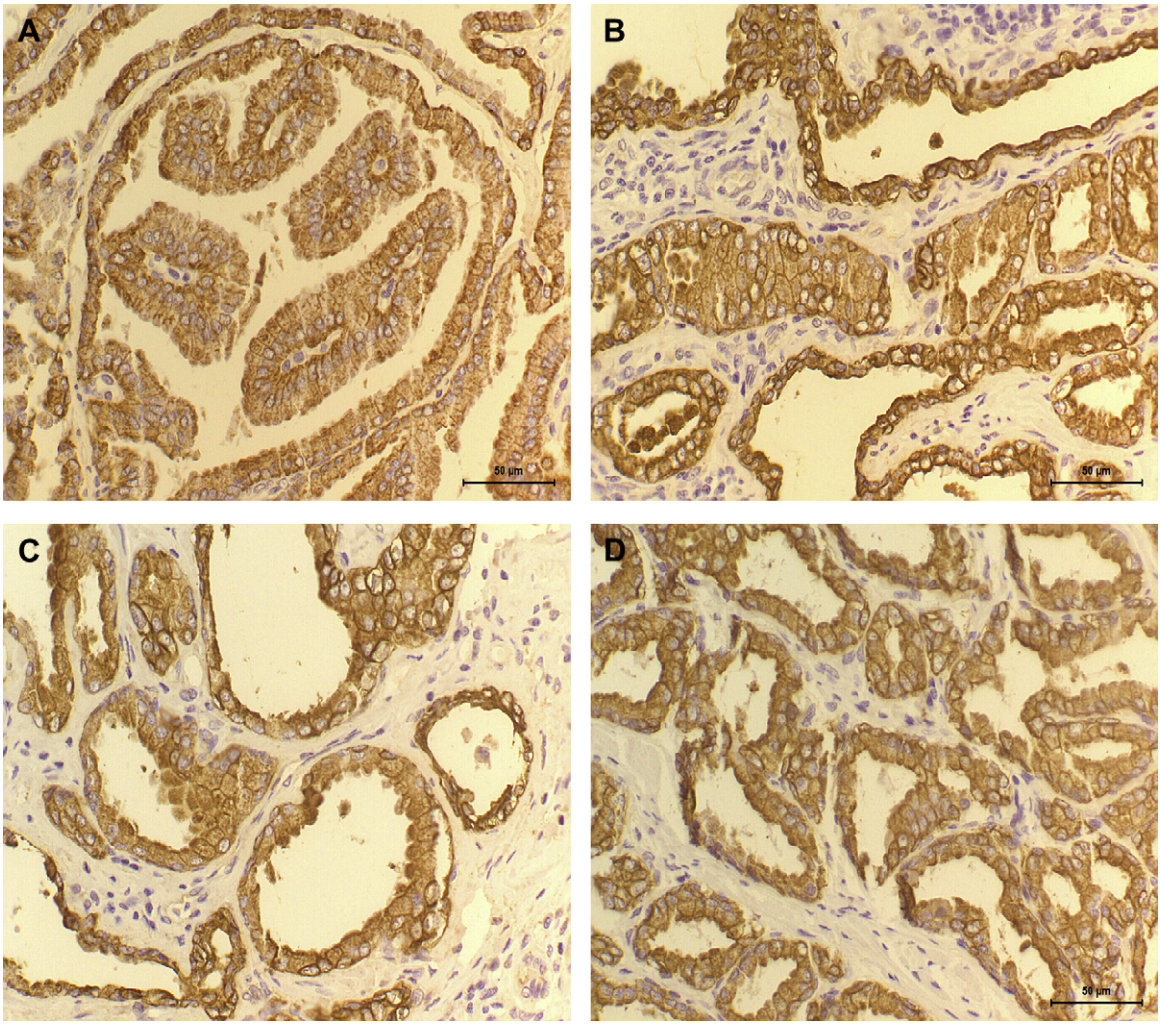

Fig. 1. Cytokeratin expression by canine prostatic lesions. (A) BPH. (B) PIA. (C) PIN. (D) Prostatic carcinoma. IHC. Bar, 50 µm.

formalin and were used to construct a TMA using a microarray construction apparatus (Beencher Instruments, Silver Spring, USA). The most representative areas of the tumours were selected by evaluation of the original section stained by haematoxylin and eosin (HE) and these areas were included in the TMA. Classification of the tumours was performed as described by Leroy and Northrup (2008). Sections (3 µm) were cut onto specific glass slides for TMA (Fischerbrand-Color Frost™; Fischer Scientific, Pittsburgh, Pennsylvania) and were kept at −20°C until used for immunohistochemistry (IHC). Two slides were used for IHC; one was labelled for expression of vimentin and the other for expression of pan-cytokeratin (AE1/AE3).

Briefly, slides were dewaxed and rehydrated. Antigen retrieval for vimentin labelling was by incubation with a commercial solution (Labvision, Fremont, California) in a water bath at 96°C for 20 min and for cytokeratin labelling the slide was incubated in citrate buffer (pH 6.0) for 30 s in a pressure cooker (Pascal®; Dako, Carpinteria, California). After cooling, the slides were immersed in H_2O_2 3% in methanol for 20 min to block endogenous peroxidase activity. Monoclonal mouse anti-vimentin clone v9 (1 in 500 dilution, Dako) and monoclonal mouse anti-cytokeratin clone AE1AE3 (1 in 50 dilution, Dako) were applied to the sections for 90 min in a humid chamber at room temperature (for vimentin labelling) and 18 h (overnight) in a humid chamber at 4°C (for cytokeratin labelling). Detection of binding of the primary antibodies was with Novolink™ Max Polymer (Novocastra, Newcastle upon Tyne, UK) for vimentin labelling and with Advance™ (Dako) for cytokeratin labelling. The slides were washed with Tris buffered saline between steps. Labelling was 'visualized' by incubation (3 min) with 3,3′diaminobenzidine (Dako) and the sections were lightly counterstained with haematoxylin, dehydrated and mounted. Adjacent mesenchymal tissue