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Diagnostic Utility of Cytokeratin-5 for the Identification of Proliferative Inflammatory Atrophy in the Canine Prostate

C. Palmieri[*], M. Story[*], F. Z. X. Lean[*], S. H. Akter[*,†], V. Grieco[‡] and A. M. De Marzo[§]

[*] *School of Veterinary Science, The University of Queensland, Gatton Campus, Gatton, Queensland, Australia,* [†] *Faculty of Veterinary Science, Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensing, Bangladesh,* [‡] *Department of Veterinary Science and Public Health, Universita' degli Studi di Milano, via Celoria 10, Milan, Italy and* [§] *Departments of Pathology, Urology and Oncology, The Johns Hopkins School of Medicine, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins and the Brady Urological Research Institute, Baltimore, Maryland, USA*

Summary

Proliferative inflammatory atrophy (PIA), which is comprised of highly proliferative but atrophic prostate epithelial cells in association with chronic inflammation, is considered a risk lesion for prostate cancer in men, while its role in canine prostate carcinogenesis is still unknown. We evaluated the value of immunohistochemical labelling for the basal cell marker cytokeratin-5 (CK5) in identifying PIA lesions in 87 samples of formalin-fixed and paraffin wax-embedded canine prostate. Canine PIA showed cytological features identical to the human counterpart and in most cases was associated with chronic lymphoplasmacytic inflammation. PIA lesions were identified in a higher number of CK5-labelled slides (43 out of 87) compared with slides stained by haematoxylin and eosin (HE) (24 out of 87). This lesion was frequently present in normal, hyperplastic and neoplastic canine prostates, although it was underestimated on evaluation of HE-stained slides. Therefore, CK5 can be considered a useful basal cell marker with high sensitivity and specificity for PIA.

Keywords: cytokeratin 5; dog; proliferative inflammatory atrophy; prostate

The causal connection between inflammation and carcinoma of the prostate has been investigated extensively in men (De Marzo *et al.*, 1999; Perletti *et al.*, 2010). Chronic prostatitis is often associated with atrophic epithelium that exhibits an unexpected increase in proliferation and a low apoptotic rate (De Marzo *et al.*, 1999). These lesions have been defined as proliferative inflammatory atrophy (PIA) (De Marzo *et al.*, 1999). In this condition, atrophic epithelial cells appear to be regenerating in response to cellular damage (De Marzo *et al.*, 1999) and they are considered targets of neoplastic transformation in the prostate. Cells in PIA lesions give rise to carcinoma either indirectly via development of the well-recognized preneoplastic lesion called high-grade prostatic intra-epithelial neoplasia (HGPIN) (Putzi and De Marzo, 2000), or at times directly. While a number of somatic genomic changes have been reported to occur in atrophic cells in PIA lesions (Perletti *et al.*, 2010), clonal alterations in loci that are commonly involved in adenocarcinoma (and some HGPIN lesions), such as chromosome 8p loss (encompassing *NKX3-1*) and *TMPRSS2-ERG* gene fusions (or other ETS gene fusions), have not been found to date in prostatic atrophy (Valdman *et al.*, 2008). Epigenetic changes in the CpG island of the upstream regulatory region of the *GSTP1* gene, which

Correspondence to: C. Palmieri (e-mail: c.palmieri@uq.edu.au).

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occur in roughly 90% of human prostatic adenocarcinomas and 70% of human HGPIN lesions, also occur in atrophic epithelial cells in PIA in approximately 6% of cases (Nakayama *et al.*, 2003).

Since focal atrophy lesions/PIA can occupy extensive regions in the human prostate, these lesions may be a fertile ground for prostate cancer development and so their clinical significance should not be underestimated. Prostatic epithelium contains two major cell types: basal epithelial cells, characterized by the expression of 'basal-specific' cytokeratins, such as cytokeratin 5 (CK5) and cytokeratin 14 (CK14), and columnar luminal epithelial cells characterized by the absence of CK5/CK14 and high expression of luminal keratins, such as cytokeratins 8 and 18, and high levels of androgen receptor and NKX3.1 protein (De Marzo *et al.*, 2010).

CK5 is a large polypeptide that is found in complex epithelium such as epidermis, hair follicles and epithelia of the trachea, apocrine sweat glands and mammary gland (Moll *et al.*, 1982). CK5 is also expressed in the basal cells of benign prostate glands, where it usually demonstrates continuous cytoplasmic labelling (Trpkov *et al.*, 2009).

Human focal atrophy/PIA lesions have been found to contain a relatively high number of cuboidal luminal epithelial cells with an 'intermediate' phenotype (a phenotype between basal and luminal cells) that express CK5 (van Leenders *et al.*, 2003; Wang *et al.*, 2009). In the present study, we explored the utility of CK5 as an aid in the identification of PIA in canine prostatic samples. We comment on the benefits of using a highly sensitive, basal cell-specific antibody to detect these lesions, which are potentially implicated in prostatic carcinogenesis, and we report on our experience performing CK5 immunohistochemistry (IHC) on routine diagnostic submissions. Antibodies to CK5 are available commercially, are reliable and consistent and perform optimally in formalin-fixed paraffin wax-embedded tissues. Although PIA has been variably reported in canine prostates (Fonseca-Alves *et al.*, 2013; Rodrigues *et al.*, 2013; Palmieri *et al.*, 2014a,b), to our knowledge, a systematic study of this type, with CK5 in a large cohort of cases, has not been performed.

Eighty-seven formalin-fixed and paraffin wax-embedded samples of canine prostate were retrieved from the archives of the School of Veterinary Science of the University of Queensland ($n = 73$) and the Department of Veterinary Science and Public Health of the University of Milan ($n = 13$). The archival tissue specimens were comprised of both clinically procured needle or wedge biopsy sections and specimens collected at the time of necropsy examination. Specimens containing non-prostatic tissue or gland-free prostatic stroma, as well as cases with significant tissue autolysis, were discarded. Furthermore, any cases where there was evidence of neoplastic invasion of the prostate by either metastasis or extension of cancer from adjacent organs were excluded. The haematoxylin and eosin (HE)-stained slides were re-analysed and classified as normal prostate, benign prostatic hyperplasia (BPH), prostatic carcinoma (PC) or prostatitis (i.e. suppurative, lymphoplasmacytic or histiocytic). In most cases, only a single large transverse section of prostate tissue was available for review. Where possible (depending on availability of paraffin wax blocks), three sections per slide were obtained. The first and third levels were stained with HE and the second intervening level was subjected to IHC as described by Green and Epstein (1999).

For each sample we recorded: (1) PIA lesions: acini composed of a two-layered epithelium consisting of flat basal cells and cuboidal luminal cells, the latter having scant basophilic cytoplasm often in the majority of cells, and a small round to oval central nucleus with inconspicuous nucleolus; the epithelial lining does not form papillary projections, but it is either rounded or angular (van Leenders *et al.*, 2003); (2) chronic inflammatory foci: mononuclear inflammatory infiltrates in the periglandular stroma composed predominantly of lymphocytes and plasma cells.

IHC was performed using a primary mouse monoclonal antibody against CK5 (NCL-L-CK5; Novocastra, Newcastle-upon-Tyne, UK; dilution 1 in 300) with an indirect avidin–biotin–peroxidase procedure (Vector Labs, Burlingame, California, USA), heat-induced antigen retrieval in 0.1 M sodium citrate buffer pH 6.0 and 3,3′ diaminobenzidine (Abcam, Cambridge, UK) as a chromogen, as previously described (Akter *et al.*, 2015). Labelling specificity was assessed in negative controls by omitting the primary antibody from the reaction sequence. Canine skin was used as a positive control.

To evaluate the diagnostic usefulness of CK5 for the identification of PIA lesions, the following parameters were assessed: (1) prevalence of PIA lesions in different sample types (i.e. normal prostate, BPH, PC, prostatitis); (2) prevalence of PIA lesions diagnosed on HE-stained slides; (3) immunohistochemical features and prevalence of PIA lesions diagnosed on CK5-labelled slides; and (4) association between chronic lymphoplasmacytic inflammation and PIA. Selected foci on HE-stained slides were matched to the CK5-labelled slides and the immunolabelling characteristics (i.e. continuous versus disrupted labelling of basal cells; uniformity and intensity of labelling) were evaluated.