J. Comp. Path. 2019, Vol. 170, 1–9

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NEOPLASTIC DISEASE

Lipoxygenase-5 Expression in Canine Urinary Bladder: Normal Urothelium, Cystitis and Transitional Cell Carcinoma

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Summary

Transitional cell carcinoma (TCC) is the most common canine urinary tract tumour and mimics human invasive TCC. Human TCCs overexpress lipoxygenase (LOX)-5 and the use of target inhibitors has proven effective in inhibiting neoplastic growth. In this study, we investigated the immunohistochemical expression of LOX-5 in normal canine urinary bladder, cystitis and TCC. The comparative expression of LOX-5, cyclooxygenase (COX)-1 and COX-2 among the three tissue groups was also examined. Biopsy samples from cases of cystitis and TCC were reviewed from 2012 to 2016; samples of histologically normal bladder were used as controls. Dogs were excluded if they had received glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs) and/or chemotherapy prior to tissue collection. LOX-5 was expressed in 95% of TCCs, 23% of cases of cystitis and 10% of controls. LOX-5 and COX-2 immunohistochemistry scores were significantly ($P < 0.01$) higher in TCCs versus cystitis and normal bladders. Results of this study support the rationale for further investigation of the use of NSAIDs with dual anti COX-2 and LOX-5 effect for the treatment of canine TCC.

Keywords: cystitis; dog; lipoxygenase; transitional cell carcinoma

Introduction

Tumours of the urinary bladder account for 2% of all malignant tumours in dogs, with almost 90% being malignant and of epithelial origin (Knapp *et al.*, 2014; Meuten and Meuten, 2017). Transitional cell carcinoma (TCC), also referred to as urothelial carcinoma, is known to be the most common (75–90%) of these neoplasms with the majority of TCCs (>90% of cases) having an intermediate or high histological grade and showing an invasive behaviour (Valli *et al.*, 1995; Anderson *et al.*, 2002; Patrick *et al.*, 2006).

The treatment of canine TCC can include surgery, radiation therapy and/or chemotherapy; however, the latter is used most commonly (Knapp *et al.*, 2014). Most TCCs are not amenable to surgery due to the presence of multiple lesions and their common trigone location; radiation therapy is not commonly advised as initial investigations have been discouraging due to the high incidence of side-effects; however, newer approaches have provided better results (Anderson *et al.*, 2002; Poirier *et al.*, 2004; Nolan *et al.*, 2012; Knapp *et al.*, 2014). Despite being the most common treatment, chemotherapy provides only poor to modest responses (0–58%) (Knapp *et al.*, 2014). To date, the combination of cisplatin and piroxicam remains probably the most effective (71% objective response); however, this protocol

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0021-9975/$ - see front matter
https://doi.org/10.1016/j.jcpa.2019.05.001

does carry a relatively high-risk of renal toxicity (Greene *et al.*, 2007; Knapp *et al.*, 2013). Piroxicam is a non-selective cyclooxygenase (COX) inhibitor, which has shown antineoplastic effect as a single agent and provided significant advantages when combined with standard antineoplastic agents (Knapp *et al.*, 1994, 2014). The drug's main antineoplastic effects are believed to occur through inhibition of COX-2, which is overexpressed in canine TCCs when compared with normal urothelium (Khan *et al.*, 2000; Doré, 2011). COX is a key enzyme in the biochemical pathway leading to the synthesis of prostaglandins, which are short-chain, lipid-derived metabolites involved in a wide variety of physiological processes and pathological conditions such as inflammation and the development of neoplastic processes (Doré, 2011). The oncogenic role of COX-2 may occur as a result of its direct effect on cell proliferation or indirectly due to the release and action of other cytokines as suggested by their role in mitogenesis, ovulation and development of kidney and bladder, and/or as a pro-angiogenic factor (Khan *et al.*, 2000; Doré, 2011). Conversely, COX-1 is expressed constitutively in the canine urinary bladder as it is responsible for the production of prostaglandins involved in maintaining homeostasis. Its expression appears not to be affected by neoplastic transformation; the role of COX-1 in the pathogenesis of urothelial tumours remains unknown (Khan *et al.*, 2000; Doré, 2011).

Inflammation and cancer are closely linked by specific oxidative processes in the tumour microenvironment (Nowsheen *et al.*, 2012). Consequently, oxidative enzymes that are known to play a key role in inflammation are increasingly being investigated in connection with cancer (Morrison, 2012; Wisastra and Dekker, 2014). Lipoxygenases (LOXs) are oxidative enzymes, with a non-haem iron atom in their active site, involved in the regulation of inflammatory responses by generation of pro-inflammatory mediators known as leukotrienes or anti-inflammatory mediators known as lipoxins (Wisastra and Dekker, 2014). LOXs and their degradation products have been associated with tumour cell proliferation, differentiation and apoptosis (Wang and Dubois, 2010). Several LOXs have been scrutinised, including LOX-5, LOX-12 and LOX-15; however, recognition of the complexity of the activation mechanism of LOX-5 has exposed opportunities for further investigation into strategies of inhibition (Wisastra and Dekker, 2014). The pro-inflammatory effect of LOX-5 is predominantly associated with the activation of nuclear factor κβ (NFκβ) (Wisastra and Dekker, 2014). In particular, the activation of LOX-5 leads to sustained angiogenesis through induction of vascular endothelial growth factor (VEGF), cell migration, invasion and resistance to apoptosis, mediated by the inhibition of caspase 3 (Bishayee and Khuda-Bhuks, 2013). LOX-5 overexpression has been identified in various human neoplastic diseases including, but not limited to, urogenital tumours such as prostatic, renal and bladder cancer (Gupta *et al.*, 2001; Hennig *et al.*, 2002, 2005; Jiang *et al.*, 2003; Yoshimura *et al.*, 2003; Matsuyama *et al.*, 2004; Hoque *et al.*, 2005; Li *et al.*, 2005; Nathoo *et al.*, 2006; Soumaoro *et al.*, 2006; Faronato *et al.*, 2007; Melstrom *et al.*, 2008; Yang *et al.*, 2012; Savari *et al.*, 2013; Knab *et al.*, 2015). In man, LOX-5 overexpression has been observed when comparing TCC with normal urinary bladder tissue. Additionally, studies have reported a dose-dependent growth inhibition of cancer cell lines with the use of LOX-5 inhibitors (Yoshimura *et al.*, 2003; Hayashi *et al.*, 2006; Matsuyama and Yoshimura, 2009). In a study using transgenic mice as a model, an increase in LOX-5 expression was also correlated with more aggressive TCC phenotypes (Madka *et al.*, 2014). The same study noted a dose-dependent cancer growth reduction after treatment with licofelone, a dual LOX/COX inhibitor, confirming previous findings (Madka *et al.*, 2014). Conversely, in veterinary oncology knowledge about LOX-5 expression is limited to canine prostatic carcinoma and osteosarcoma (Goodman *et al.*, 2011; Goupil *et al.*, 2012). Naturally occurring canine TCC closely mimics human invasive TCC, having similar histopathological characteristics, molecular features and biological behaviour (Knapp *et al.*, 2014). We hypothesised that LOX-5 has the potential to be overexpressed in canine TCC relative to normal tissue, therefore suggesting a potential role in pathogenesis.

The primary aim of this study was to investigate the immunohistochemical expression of LOX-5 in normal canine urinary bladder, cystitis and TCC. Secondarily, we analysed the relationships between the expression of LOX-5, COX-1, COX-2 and tumour location.

Materials and Methods

Study Population

Tissue samples of canine cystitis and TCC, previously submitted to the Department of Veterinary Pathology and Public Health, University of Liverpool, Liverpool, UK, were retrieved through a database search. Original tissue sample collection took place from January 2012 to December 2016. Cases were considered eligible for the study only if sufficient

formalin fixed and paraffin wax-embedded (FFPE) tissue was available for review.

Patient signalment and clinical data were retrieved from the database of the Small Animal Teaching Hospital, University of Liverpool, Liverpool, UK, and via telephone calls to the referring veterinarians. Dogs were excluded from the study if they were known to have received glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs) and/or antineoplastic chemotherapy prior to the tissue collection.

For dogs with a diagnosis of TCC, only subjects know to have died of tumour-related causes were enrolled in the study. For dogs diagnosed with cystitis, only dogs with a histological diagnosis of inflammatory urinary bladder disease, known to be alive 2 years after collection of tissue samples, were enrolled in the study.

Due to the retrospective nature of the study and the likelihood of variation among treatments offered to each patient, data retrieved from the medical records were restricted to standard signalment (i.e. sex, age, breed and weight).

As a control group, normal urinary bladder tissue was retrieved from the necropsy archive of the same institution, enrolling only cases that died of no urinary tract related causes and where no urinary tract pathology was detected on histopathology. The study was approved by the University of Liverpool Ethics Committee (RETH000942).

Histopathology and Immunohistochemistry

Histological slides were reviewed by a board-certified veterinary pathologist (RV), blinded to the original pathology report and the patient's clinical data. The slides had been prepared from FFPE tissues, stained routinely with haematoxylin and eosin (HE) and were observed under a bright-field upright microscope. TCCs were graded according to the adapted 2012 World Health Organization (WHO) grading system (Meuten and Meuten, 2017). Mitoses were determined in an area of 2.37 mm^2 (10 high-power fields [HPFs], ×400 with an ocular field number of 22).

Representative sections of the lesions were selected for immunohistochemistry (IHC). Tissue sections were dewaxed in xylene and hydrated through a series of graded ethanol concentrations to distilled water. Antigen retrieval was performed by calibrated water bath capable of maintaining the epitope retrieval solution in 10 mM sodium citrate buffer (pH 6.0) at 97°C for 30 min. The sections were allowed to cool to room temperature for 20 min. Endogenous peroxidase was blocked using 100 µl Dako REAL™ peroxidase blocking solution for 10 min (Dako, Carpinteria, California, USA). Tissue sections were incubated overnight with the primary antibodies specific for LOX-5 (Abcam, Cambridge, Massachusetts, USA), COX-1 (Cayman Chemical, Ann Arbor, Michigan, USA) and COX-2 (BD Transduction Lab, San Jose, California, USA) in a humid chamber at 4°C; the antibodies were selected according to the published literature (Khan *et al.*, 1998; Goodman *et al.*, 2011; Campos *et al.*, 2014). Evaluation and validation of the optimal concentration of each primary antibody were performed with serial antibody dilutions (Table 1). Labelling was 'visualized' by use of peroxidase conjugated polymer (EnVision™ FLEX Target Retrieval Solution High pH, Tris/EDTA buffer pH 9; Dako) for 30 min and 3, 3′ diaminobenzidine tetrahydrochloride was used as chromogen (DAB; Fisher Scientific, Loughborough, UK). Sections were counterstained with Mayer's haematoxylin. As negative serum control, mouse and rabbit unrelated sera were used instead of the primary antibodies; control sections were treated at the same time as sample sections. Positive tissue controls are described in Table 1.

The distribution of immunohistochemical labelling was scored as: 0, no positive cells; 1, 1−33% positive cells; 2, 34−66% positive cells; 3, 67−100% positive cells. Labelling intensity was scored as: 0, no positive cells; 1, barely perceptible labelling if compared with controls; 2, positive labelling, but weaker if compared with controls; or 3, positive labelling relative to controls. An IHC score was obtained by multiplying distribution by intensity for each antibody. Immunohistochemical assessment of all three markers was performed by avoiding any positive labelling of

Table 1
Panel of antibodies used for immunohistochemistry

Antigen	*Antibody*	*Type*	*Dilution*	*Positive control*
LOX-5	ab103765	Rabbit polyclonal	1 in 100	Canine leucocyte cell pellet
COX-1	160108	Rabbit polyclonal	1 in 100	Normal canine kidney
COX-2	Clone 33	Mouse monoclonal	1 in 800	Normal canine kidney (macula densa)

LOX-5, lipoxygenase-5; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2.

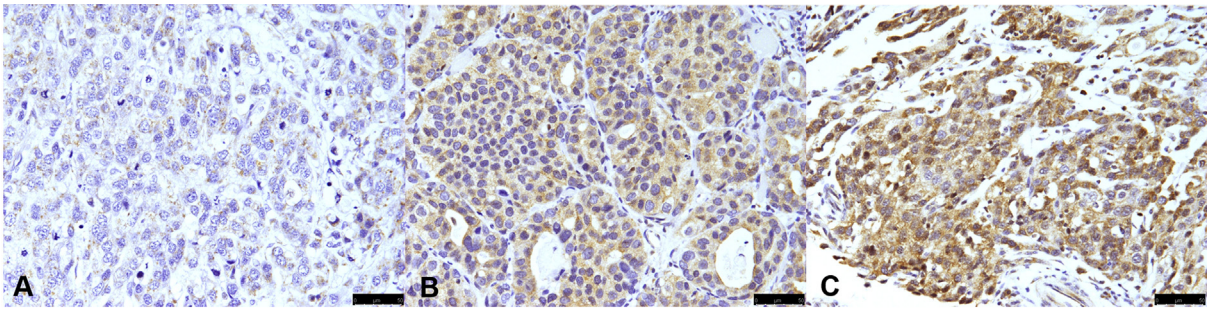

Fig. 1. Examples of LOX-5 immunohistochemical labelling intensity grades 1 (A, mild), 2 (B, moderate) and 3 (C, marked) in canine urinary bladder TCC. Bars, 50 μm.

inflammatory cells. Immunohistochemical results were compared between normal epithelium, cystitis samples and TCCs.

Statistical Analysis

Correlations between LOX-5, COX-2 and COX-1 intensity and distribution, LOX-5, COX-2 and COX-1 IHC score (score = intensity × distribution) and age, sex or weight between groups were analysed by use of the Spearman's test. Associations between LOX-5, COX-2 and COX-1 IHC score and study groups, as well as antibody expression and biopsy site (i.e. body, apex or neck), were investigated by use of the Mann–Whitney *U* test. Statistical analysis was performed using SPSS 13 Software (SPSS 13.0, SPSS Inc., IBM Chicago, Illinois, USA).

Results

Study Population

Twenty-nine TCCs of the urinary bladder, 13 cases of cystitis and 10 normal urinary bladders fulfilled the group-specific inclusion criteria and were included in the study. For the purpose of the study, groups were named as: 'TCC', 'cystitis' and 'normal bladder'.

In the TCC group ($n = 29$) there were eight crossbred dogs, four Scottish terriers, three Labrador retrievers, two cocker spaniels and one each of golden retriever, Rhodesian ridgeback, dachshund, miniature schnauzer, fox terrier, Airedale, keeshond, doberman pincher, Bernese Mountain dog, Lhasa Apso, English bulldog and Jack Russell terrier. Twenty dogs were female (13 neutered, seven entire) and nine were male (six neutered, three entire). With regards to the site of biopsy, 18 were collected from the neck, three from the apex and three from the body of the urinary bladder; in five cases this information was not specified and only 'urinary bladder' was listed as the site of biopsy. The median age was 10 years (range 4–16 years) and the median body weight was 27 kg (range 6–42 kg). In eight cases, tissue samples were collected by means of cystotomy/partial cystectomy; the remaining 21 samples were collected by means of cystoscopy ($n = 12$) or ultrasound-guided urinary catheter suction biopsy ($n = 9$).

In the cystitis group ($n = 13$) there were three crossbred dogs, three cocker spaniels and one each of boxer, corgi, golden retriever, Scottish terrier, Shih Tzu, Labrador retriever and bearded collie. All dogs were female (four entire and nine neutered). The median age was 6 years (3–11 years) and the median body weight was 20 kg (9–30 kg). All samples were collected by means of cystoscopy.

In the normal bladder group there were two crossbred dogs and one each of cocker spaniel, golden retriever, American Staffordshire terrier, pit bull, fox terrier, doberman pincher, English bulldog and Labrador retriever. Five dogs were female (three neutered, two entire) and five were male (three neutered, two entire). The median age was 6 years (3–11 years) and the median body weight was 20 kg (9–30 kg).

Histopathology

Transitional Cell Carcinoma Group. In the eight cases collected by means of cystotomy/partial cystectomy, TCC grading could be performed and all tumours were graded as high-grade and invasive TCCs. Among these, four had papillary and four had a non-papillary pattern. In the remaining 21 samples, collected by means of cystoscopy/ultrasound-guided urinary catheter suction biopsy, cellularity was considered as moderate to high in all samples, but grade and invasiveness could not be assessed accurately on the superficial biopsy samples. However, two or more mitotic figures per HPF, mild to moderate cellular atypia, including anisocytosis and anisokaryosis, nuclear abnormalities and prominent nucleoli were observed in all samples; these features

were considered as a likely predictor of high-grade morphology. Of these 21 TCCs, differentiation was attempted in 13 cases (10 papillary and three non-papillary pattern).

Cystitis Group. All samples showed mild to moderate inflammatory infiltration; this consisted of a mixed inflammatory population composed of lymphocytes, plasma cells and macrophages with no single inflammatory cellular component predominating. Occasional presence of hyperplastic lymphoid follicles with well-developed germinal centres (follicular cystitis) was noted. In two samples, the mucosa was ulcerated multifocally and this was associated with the presence of both viable and degenerate neutrophils admixed with the inflammatory infiltrates.

Normal Bladder Group. No histological abnormalities were noted as per inclusion criteria.

Immunohistochemistry

Transitional Cell Carcinoma Group. Twenty-one of the 29 samples were suitable for immunohistochemical investigation. LOX-5 was expressed in 20 out of 21 samples (95%), with a median IHC score of 2 (range 1−6). COX-2 was expressed in 19 out of 21 samples (90%) with a median IHC score of 2 (range 1−9). COX-1 was expressed in 20 out of 21 samples (95%) with a median IHC score of 4 (range 1−6). None of the samples was negative for more than one antibody. Examples of LOX-5 labelling intensity can be seen in Fig. 1.

Cystitis Group. LOX-5 was expressed in three out of 13 samples (23%) with a median IHC score of 1 (range 1−3). COX-2 was expressed in four out of 13 (31%) samples with a median IHC score of 1.5 (range 1−4). Cases that were found to be positive for LOX-5 were negative for COX-2 and *vice versa*. COX-1 was expressed in 12 out of 13 samples (92%) with a median IHC score of 3 (range 2−9). Only one sample was negative for all three antibodies.

Normal Bladder Group. LOX-5 and COX-2 were expressed in only one out of 10 cases (10%; same case) and both showed an IHC score of 1. COX-1 was expressed in all 10 cases (100%) with a median IHC score of 2 (range 1−3). IHC findings of the three groups are summarized in Table 2.

Comparative Analysis. LOX-5 and COX-2 immunolocalization was similar among samples from the cystitis and TCC groups, being mainly cytoplasmic (>90% of positive neoplastic cells) with occasional nuclear and cytoplasmic immunoreactivity (<10%). COX-1 followed the same pattern with the majority of cells (>90%) showing cytoplasmic immunolabelling.

LOX-5 and COX-2 IHC scores were significantly higher (P <0.01) in the TCC group compared with the cystitis and normal bladder group (Fig. 2). The LOX-5, COX-1 and COX-2 IHC score was not associated with any signalment variable analysed, nor there was correlation between LOX-5, COX-2 and COX-1 distribution and intensity. The LOX-5 IHC score was not affected by the biopsy site (apex versus body versus neck).

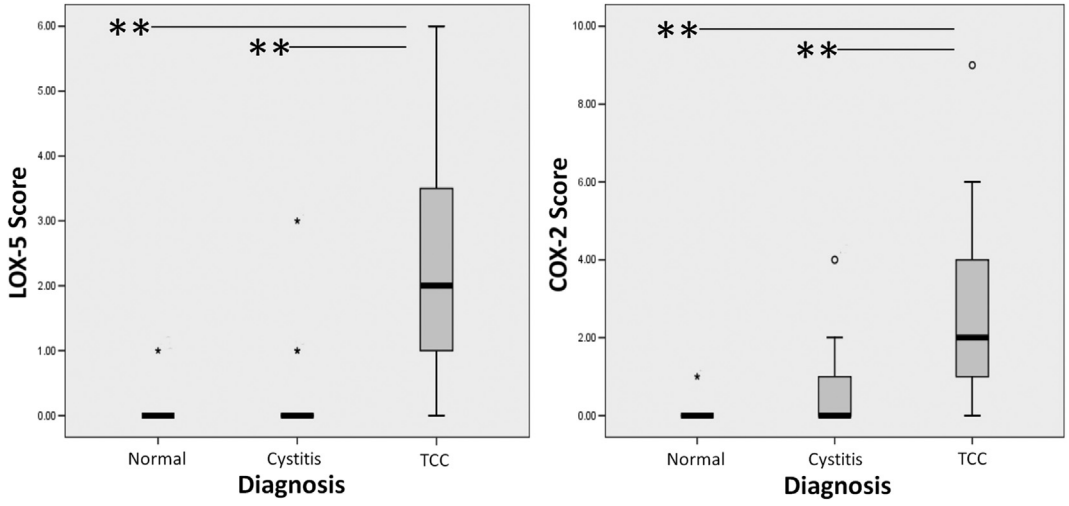

Fig. 2. Boxplots representing comparison of LOX-5 and COX-2 expression between groups. Mann−Whitney U test; central value: median. **P <0.01.

Table 2
Immunohistochemistry findings

Population		*LOX-5*				*COX-2*				*COX-1*			
Group	*Total cases*	*Positive cases*	*Intensity*	*Distribution*	*IHC score*	*Positive cases*	*Intensity*	*Distribution*	*IHC score*	*Positive cases*	*Intensity*	*Distribution*	*IHC score*
TCC	21	20	1 (1–3)	2 (1–3)	2 (1–6)	19	2 (1–3)	1 (1–3)	2 (1–9)	20	2 (1–3)	1 (1–3)	4 (1–6)
Cystitis	13	3	1	1 (1–3)	1 (1–3)	4	1 (1–2)	1 (1–3)	1.5 (1–4)	12	1 (1–3)	3 (2–3)	3 (2–9)
Normal bladder	10	1	1	1	1	1	1	1	1	10	1	2 (1–3)	2 (1–3)

IHC score = intensity × distribution.
COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; LOX-5, lipoxygenase-5; TCC, transitional cell carcinoma.

Discussion

There has been exponential growth of research investigating the role of lipoxygenases in epithelial cancer over the past two decades, both in human oncology and to a lesser extent in canine models. Human studies have demonstrated overexpression of LOX-5 in TCC compared with normal urinary bladder tissue, where the degree of immunolabelling appears to positively correlate with more aggressive TCC phenotypes (Matsuyama and Yoshimura, 2009; Madka *et al.*, 2014).

In the present study we investigated the immunohistochemical expression of LOX-5 in normal canine urinary bladder epithelium and in cases of cystitis and urothelial carcinoma, as these data were lacking in the veterinary oncology literature. Results suggest that normal canine urinary bladder epithelium usually lacks LOX-5 expression and that expression of this enzyme is not constitutive for this organ. The same lack of expression was observed for COX-2, while COX-1 expression was found to be positive in all normal samples; this is in agreement with previous literature (Khan *et al.*, 2000). In one case, all three molecules were expressed weakly. This was an unexpected finding; however, it could be explained by an aberrant expression of COX-2 and LOX-5 unique to this patient or due to undetected urinary bladder pathology. Aberrant enzymatic expression would be most likely, given the normal histological findings and the lack of urinary signs in this patient's history. This occurrence has not been reported previously in dogs and is described rarely in man (Khan *et al.*, 2000; Yoshimura *et al.*, 2001); however, normal urinary bladder tissues are usually only used as controls and the number of patients screened is extremely low; it is therefore probable that aberrations occur, but are less likely to be reported. A predisposition towards developing urinary bladder pathology could be hypothesized in this scenario; however, as this was a necropsy sample, assumptions are only speculative; moreover, no follow-up information can be found in human literature (Yoshimura *et al.*, 2001).

LOX-5 was expressed only in 23% of cases of cystitis; COX-2 was also detected in a minority of patients (31%). Interestingly, these two enzymes were not expressed simultaneously, suggesting a variable activation of the arachidonic acid (AA) degradation pathway in this condition. The findings in respect to COX-2 are in disagreement with those reported by Azuma *et al.* (2007) where COX-2 expression was observed in the epithelium from 27 of 33 samples (82%) of dogs with naturally occurring cystitis; conversely, our results appear in alignment with findings in cases of human chronic cystitis (Li *et al.*, 2014). The disparity between the findings of Azuma *et al.* (2007) and the current study may be due to differences in patient selection and the possibility that COX-2-positive inflammatory cells may have been counted in the IHC analyses, contributing to the greater number of positive patients.

In the TCC group, COX-2 was expressed in 90% of the cases, in alignment with the present literature, and LOX-5 was expressed in 95% of the samples (Khan *et al.*, 2000). For both antibodies the IHC score was significantly higher in TCC compared with the cystitis group and dual enzyme expression was a consistent finding. Interestingly, LOX-5 and COX-2 expression were not affected by the site of tumour biopsy nor signalment data.

This study has several limitations, mainly due to its retrospective nature and the collection of the majority of cystitis and TCC samples by means of minimally invasive procedures. It is possible, especially in the TCC group, that the tissues collected were not fully representative of the underling pathology; however, given the strict inclusion criteria and the similarity of our results with the human literature, chances of misdiagnoses would appear low. As the majority of the TCC samples were of the papillary subtype, association between tumour morphology and LOX-5 expression could not be investigated. Moreover,

most TCC biopsy samples had been collected by cystoscopy or ultrasound-guided urinary catheter suction biopsy, resulting in diagnostic, yet small, tissue samples that made evaluation of mitotic index (among other tumour features) less accurate; this represents an undeniable limitation. Performing this study on only cases of TCC diagnosed via surgical biopsies would have potentially provided more information. Such an approach is uncommon and can only be reserved for selected cases (i.e. solitary lesions amenable to resection, non-trigonal location) due to the significant risk of bladder wall dehiscence and neoplastic seeding of the surgical incision or abdominal cavity (Knapp *et al*., 2014). Cystoscopy usually provides better diagnostic samples compared with traumatic catheterization. Such a difference was not perceived in this study; however, this likely reflects the inclusion criteria. It is the authors' opinion that cystoscopy biopsy sampling remains the most effective method of establishing a diagnosis of TCC in canine patients, as has been reported previously (Childress *et al*., 2011).

Although the labelling intensity appeared relatively similar to that reported in human studies, the pattern of labelling observed was mainly cytoplasmic in our cases compared with the more uniform cytoplasmic and nuclear distribution observed in human tissues (Yoshimura *et al*., 2003; Matsuyama and Yoshimura, 2009). This might have biological relevance, as nuclear localization of LOX-5 is considered necessary for production of the lipid mediators associated with proliferative activities, such as leukotriene B4 and 5-oxo-eicosatetraenoic acid. The lack/paucity of nuclear LOX-5 immunolabelling has been observed previously in canine osteosarcoma and can be deduced from a study conducted on canine prostatic pathologies (Goodman *et al*., 2011; Goupil *et al*., 2012). Although at this stage we cannot confirm the extent of the role of LOX-5 in TCC development and progression, its (mainly) cytoplasmic localization could still represent a target for therapy. In canine osteosarcoma, neoplastic cells undergo apoptosis through caspase-3 activation, and apoptosis is preceded by an increase in the formation of reactive oxygen species, which appear to be related to direct LOX-5 inhibition regardless of the cellular localization (Loftus *et al*., 2016).

In conclusion, this study provides evidence that LOX-5 is overexpressed in canine TCC and its inhibition should be evaluated by future studies as a possible adjunctive treatment strategy. The therapeutic use of LOX-5 inhibitors is supported by previous studies conducted on human TCC, in which dose dependent growth inhibition of cancer cell lines has been achieved after treatment with LOX inhibitors (Yoshimura *et al*., 2003; Hayashi *et al*., 2006). The same study demonstrated a dose dependent cancer growth reduction in transgenic mice treated with licofelone, a dual LOX-5/COX-2 inhibitor (Madka *et al*., 2014).

Acknowledgments

This study was funded by the AKC Canine Health Foundation (RF). L. Schiavo's current address is the Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, UK.

Conflict of Interest Statement

The authors declare no conflict of interest with respect to publication of this manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2019.05.001.

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[*Received, February 26th, 2019*
Accepted, May 6th, 2019]