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Short Communication

Immunohistochemical expression of cyclooxygenase-2 (COX-2) is not associated with tumor grade in feline meningiomas

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ABSTRACT

Meningioma is the most common primary brain tumor in cats and occurs less frequently in the spinal cord. This study aimed to investigate cyclooxygenase-2 (COX-2) expression in feline meningiomas, and the possible association between COX-2 immunoreactivity and tumor grade using eight low-grade and seven high-grade meningiomas. All tumors ($n = 15/15$) were immunoreactive to COX-2. The expression of COX-2 was not significantly correlated with tumor grade ($P = 0.22$ and 0.34 for staining and intensity, respectively) but was significantly associated with necrosis ($P = 0.04$ and 0.01 for staining and intensity, respectively). The findings in this study suggest that feline meningiomas express COX-2, but there were no differences in COX-2 immunoreactivity patterns between low- and high-grade meningiomas. However, the association between COX-2 expression and the presence of necrosis indicates a potential area for therapeutic intervention with selective COX-2 inhibitors.

Meningioma is the most common primary brain tumor in cats. Feline meningiomas show less aggressive behaviour than human and canine meningiomas, thus their classification is not adaptable to the human 2007 WHO classification (Mandara et al., 2010; Higgins et al., 2017).

Several studies have reported an association between cyclooxygenase-2 (COX-2) expression in human meningiomas with cell proliferation or tumor grade (Lin et al., 2003). In one canine study, significant correlation between COX-2 immunoreactivity patterns and morphologic features of meningioma malignancy were not detected (Rossmeisl et al., 2009).

The objectives of this study were to: (1) evaluate COX-2 expression in meningiomas in cats and the possible association between COX-2 immunoreactivity and tumor grade; and (2) to identify whether histopathological features, such as the presence of necrosis, cholesterol clefts and calcium deposits were associated with COX-2 expression and meningioma grade.

Formalin-fixed, paraffin-embedded tissue sections of meningiomas diagnosed between 2006 and 2016 were retrieved from the archives of the Veterinary Pathology Diagnostic Service of the Universitat Autònoma de Barcelona and the Veterinary Neuropathology Diagnostic Service of the University of Perugia.

Clinicopathological information obtained from all cases included breed, sex, age, site of tumor, histological subtype and grade, presence of necrotic foci and presence of calcium or cholesterol clefts. Hematoxylin and eosin (H and E) slides were evaluated by certified pathologists to confirm the diagnosis and to grade all tumors based on the recently published classification of nervous system tumors (Higgins et al., 2017). Meningiomas were considered low-grade tumors if they did not show the traditional criteria of malignancy, including frequent mitosis, high cellularity, uninterrupted patternless growth, extensive necrosis, brain invasion and metastasis.

Immunohistochemistry was performed using a rabbit polyclonal anti-murine COX-2 antibody (Cayman Chemical; Bardagí et al., 2012) using a dilution of 1:200. An isotype-specific immunoglobulin was used as a substitute for the primary antibody as a negative control. Feline mammary adenocarcinoma and a normal feline brain were used as positive controls.

Immunostaining of COX-2 was evaluated for proportion and staining intensity of tumor cells. The percentage of positive cells and staining intensity was determined by evaluating 20 high power fields (HPF; 40× objective lens) within each tumor. The scoring systems used were the same as those described previously for canine meningiomas (Rossmeisl et al., 2009).

Statistical analysis was performed with SAS software (SAS, version 9.3) and statistical significance was set at $P < 0.05$. Relationships between COX-2 expression and tumor grade and

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Table 1
Clinicopathological and histopathological characteristics of 15 meningioma samples.

Gender	Age	Breed	Sample collection	Lesion localization	Meningioma grade	Histological classification	Calcium deposits	Cholesterol clefts	Necrosis
Male	9y	DSH	Biopsy	Intracranial	Low	Fibroblastic	+	–	+
Female	12y	DSH	Biopsy	NA	Low	Psammomatous	+	–	–
Male	11y	DSH	Biopsy	Intracranial	High	Transitional	+	+	+
Female	12y	DSH	Necropsy	Intracranial	Low	Psammomatous	+	+	–
Male	7y	DSH	Biopsy	Intracranial	Low	Transitional	+	–	+
Male	11y	DSH	Necropsy	Intracranial	Low	Fibroblastic	+	–	–
Male	11y	DSH	Biopsy	Intracranial	Low	Transitional	+	+	+
Male	10y	DSH	Biopsy	Intracranial	Low	Fibroblastic	+	–	–
Female	15y	DSH	Biopsy	Intracranial	Low	Transitional	+	–	+
Male	12y	DSH	Biopsy	Spinal cord	High	Syncytial	–	–	–
Female	12y	DSH	NA	Intracranial	High	Malignant	+	+	+
NA	11y	NA	NA	NA	High	Malignant	+	+	+
Male	8y	DSH	NA	Intracranial	High	Malignant	+	–	+
Male	13y	DSH	NA	Intracranial	High	Malignant	+	+	+
Male	8y	DSH	NA	Intracranial	High	Malignant	–	–	+

DSH, domestic shorthair; NA, not available; +, present; –, absent.

between COX-2 expression and the presence of cholesterol clefts, calcium or necrosis were tested using one-sided Mann–Whitney *U* tests. Chi-square tests were used to investigate possible relationships between the presence of cholesterol clefts, calcium or necrosis and tumor grade.

Clinicopathological data are summarized in Table 1. There were eight low-grade meningiomas (fibrous, $n = 3$; psammomatous, $n = 2$; transitional, $n = 3$) and seven high-grade meningiomas (Figs. 1A, C and E). Lesion localization was available for 13 samples. In 12 cats the tumor was intracranial, and in one cat it was intraspinal.

All meningiomas displayed positive immunoreactivity for COX-2. COX-2 expression was patchy and appeared as multifocal foci in the cytoplasm and in the perinuclear area of neoplastic cells. The percentage of positive cells stained was low in three samples (score 1), moderate in seven (score 2), and high in five meningiomas (score 3). A high concentration of COX-2 positive cells was detected in whorls (Fig. 1B) and in the periphery of necrotic foci (Fig. 1D). The immunoreactivity intensity was weak in one sample (score 1), moderate in three (score 2) and strong in 11 meningiomas (score 3). In fibroblastic meningiomas, immunoreactivity was stronger in bundles (Fig. 1F).

The percentage and staining intensity of immunopositive cells was not significantly correlated with meningioma grade ($P = 0.22$ and 0.34, for the percentage of positive cells and for staining intensity, respectively). There was a significant positive correlation between COX-2 immunopositivity and presence of necrosis ($P = 0.04$ and $P = 0.01$ for % positive cells and staining intensity, respectively), but no correlation was identified with presence of calcium deposits ($P = 0.46$ and 0.38 for % positive cells and staining intensity, respectively) or cholesterol clefts ($P = 0.18$ and 0.44 for % positive cells and staining intensity, respectively). No correlation was identified between presence of necrosis, calcium deposits or cholesterol clefts and tumor grade ($P = 0.14$, 0.10 and 0.20, respectively; Table 2).

In our study, all meningiomas displayed positive immunoreactivity to COX-2. However, as previously reported for canine meningiomas (Rossmeisl et al., 2009), the expression of COX-2 was not significantly correlated with tumor grade. Strong COX-2 immunostaining was frequently observed near areas of necrosis where inflammatory cells were also detected, and this has previously been observed in human meningiomas (Lee et al., 2013), canine osteosarcomas (Mullins et al., 2004) and feline pancreatic adenocarcinomas (Newman and Mrkonjich, 2006). It is possible that COX-2 expression protects the tumor cells from apoptosis under hypoxic conditions, or that COX-2 induction is higher in areas of inflammation (Mullins et al., 2004), or that COX-2 immunoreactivity may reflect increased angiogenesis (Lee et al., 2013). In humans, it is known that immune cells can be activated to favour tumor growth and progression, most probably influenced by the tumor microenvironment. Tumor-associated macrophages (TAM) and tumor-associated neutrophils can exert pro-tumoral functions, enhancing tumor cell invasion and metastasis, angiogenesis, and extracellular matrix remodelling, while inhibiting anti-tumor immune surveillance (Kim and Bae, 2016). In humans, COX-2 is abundantly expressed in breast TAM and is a negative prognostic indicator in women with breast cancer (Li et al., 2015). Additionally, increased expression of COX-2 in the tumor microenvironment greatly affects cancer progression (Li et al., 2015). Further work is required to investigate these aspects of feline meningiomas.

In humans, increased COX-2 expression has been described in more malignant meningiomas and has been associated with increased necrosis. It is hypothesized that COX-2 expression could be an indicator of ischemia resulting from necrosis (Kang et al., 2014). In our cohort, $n = 5/7$ (71.4%) of high-grade meningiomas had necrotic foci, as did $n = 4/8$ (50%) of low-grade tumors.

In one murine study, overexpression of COX-2 contributed to tumor growth in mouse meningioma xenograft models by increasing angiogenesis, cellular proliferation and decreasing apoptosis. However, colecoxib, a selective COX-2 inhibitor, decreased meningioma growth and there was evidence of decreased microvascular density, increased apoptosis and decreased COX-2 expression (Ragel et al., 2007). In domestic animals, COX-2 inhibitors have been shown to have antineoplastic effects on several tumor cell lines in vitro and are widely used as adjuvant treatments in several tumors in dogs and cats (Bommer et al., 2012).

The main limitations of this study are the small sample size and the absence of information on the clinical use of nonsteroidal anti-inflammatory drugs (NSAIDs). However, the administration of COX-2 inhibitors is not expected to suppress COX-2 expression, since NSAIDs only decrease COX-2 activity (Mullins et al., 2004).

Although further studies are necessary to confirm and build on this study, our findings could indicate potential therapeutic utility for selective COX-2 inhibitors in feline meningiomas.