COX-2, mPGES-1 and EP2 receptor immunohistochemical expression in canine and feline malignant mammary tumours

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Running headline: COX-2, mPGES-1 and EP2 receptor in mammary tumours

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Abstract

Prostaglandin (PG) signaling is involved in human and animal cancer development. PG E_2 (PGE$_2$) tumour-promoting activity has been confirmed and its production is controlled by Cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1). Evidence suggests that mPGES-1 and COX-2 contribute to carcinogenesis through the EP2 receptor. The aim of our study was to detect by immunohistochemistry COX-2, mPGES-1, and EP2 receptor expression in canine (n=46) and feline (n=50) mammary tumors and in mammary non-neoplastic tissues. COX-2 positivity was observed in 83% canine and 81% feline mammary carcinomas, mPGES-1 in 75% canine and 66% feline mammary carcinomas and the EP2 receptor expression was observed in 89% canine and 54% feline carcinomas. The frequency of COX-2, EP2 receptor and mPGES-1 expression was significantly higher in carcinomas than in non-neoplastic tissues and adenomas. COX-2, mPGES-1 and EP2 receptor expression was strongly associated. These findings support a role of the COX-2/PGE2 pathway in the pathogenesis of these tumors.

INTRODUCTION

Cyclooxygenase (COX) is an enzyme involved in the production of prostaglandins (PGs) from the arachidonic acid. COX-1 is constitutively expressed in most tissues, while in humans COX-2 is constitutively expressed in specific tissues as brain, spinal cord and kidney, but it is generally induced by several cytokines and mitogens.[1] COX-2 levels are elevated in a high percentage of various human tumours, such as colorectal, gastric, mammary, prostatic and pulmonary tumours.[2] COX-2 expression has also been detected in canine[3-7] and feline mammary tumours[3,8-9] (CMT and FMT, respectively) and in both species an up-regulation of COX-2 expression has been documented in malignant tumours.

Most, if not all, actions of COX-2 are mediated by Prostaglandin E_2 (PGE_2).[10] COX-2 produces Prostaglandin H_2 (PGH_2), which is converted to PGE_2 by cytosolic or membrane-associated PGE-2 synthases (PGES). The inducible form of PGES is microsomal PGES-1 (mPGES-1). COX-2 and mPGES-1 have been reported to be functionally linked,[11] raising the possibility that aberrant mPGES-1 expression could contribute to increased amounts of PGE_2 in cancer. The fact that mPGES-1 expression has been observed in human cancers that also commonly overexpress COX-2 is consistent with a role of mPGES-1 in the increase of PGE_2 concentration noted in several malignancies.[12-14] PGE_2 exerts its activity by acting on a group of G-protein-coupled receptors, designated subtypes EP1, EP2, EP3 and EP4. Each EP subtype shows differences in signal transduction, tissue localization and regulation of expression.[15] Differential expression of these EP receptors mediates the diverse and often antagonistic effects of PGE_2 on a variety of cell types.[16] PGE2 may promote malignant growth by stimulating angiogenesis, tumour invasiveness, and apoptosis resistance, and by inhibiting immune surveillance.[2] Thus, the PGE_2-EP pathway may play a role in the development of tumours. Amongst the four subtypes, EP2 receptor has been shown to be

implicated in the control of intestinal polyp angiogenesis and growth.[17]   Recently, it has been described that EP2 receptor is required for mammary epithelial hyperplasia in COX-2 transgenic mice, and that in mammary tumour cell lines the expression of EP2 receptor followed by an EP-2 specific agonist strongly induced the expression of amphiregulin, a potent growth factor.[18]

Due to the epidemiological relevance of mammary malignant tumours in the canine and feline species and the aggressive behavior of these tumours, the aim of this study was to investigate the events downstream to COX-2 that lead to an increased PGE_2 production and to provide a rationale for targeting PGE_2 rather than inhibiting the production of all the COX-derived prostanoids.   We therefore analyzed by immunohistochemistry (IHC) the expression of COX-2, mPGES and EP2 receptor in a sample of canine mammary adenomas and carcinomas and feline mammary carcinomas, as well as healthy and hyperplastic mammary tissues.

MATERIALS AND METHODS

Animals

This study was retrospectively performed on 74 canine and 60 feline neoplastic and non-neoplastic mammary tissue samples retrieved from the archives of the Tumour Registry of the Department of Veterinary Science of the University of Pisa. Non-neoplastic tissues consisted of 6 samples of canine and 10 feline healthy mammary glands and 22 canine mammary hyperplasias. Neoplastic mammary tissues (46 canine and 50 feline) were collected from bitches and queen who had surgically treated for mammary disease at the same Department and submitted to histological diagnosis.   The mean age ±SD of the bitches bearing mammary tumours was 9.0 ± 2.4 years, range (4-16 years), while the mean age of the

queens bearing mammary tumours was 11.0 ± 2.8 years, range (4-19 years). All the patients had undergone local mastectomy for the onset of masses involving a single mammary gland. Both bitches and queens bearing carcinoma were submitted to a 2-years follow-up study to evaluate survival times. At the end of the follow-up period 26/36 (72.2%) bitches and 14/50 (28.0%) queens were still alive, while 10/36 bitches (27.8%) and 36/50 queens (72.0%) had died due to tumor-related causes.

Histology

Representative mammary specimens were fixed in buffered formalin and embedded in paraffin wax. Histological examination was performed on 4 µm-thick sections stained with hematoxylin and eosin (HE). Mammary lesions were classified according to the WHO criteria.[19] For the malignant tumours, the presence of lymphatic involvement was also recorded, and tumor grading was performed both for canine[20] and feline[21] carcinomas.

Immunohistochemistry

The expression of COX-2, mPGES-1 and EP2 receptor was investigated by IHC: COX-2 expression was analysed according to a previously described protocol[3] using a goat polyclonal antibody against COX-2 (Santa Cruz Biotechnology, Santa Cruz, California, USA; diluted 1 in 50); the mPGES-1 and EP2 receptor expression was evaluated according to previous studies on human and canine tissues[18,22,23] using a rabbit polyclonal antibody against mPGES-1 (Cayman Chemical, Ann Harbour, Michigan, USA; diluted 1 in 100) and rabbit polyclonal antibody against EP2 receptor (Cayman Chemical; diluted 1 in 100). Briefly, additional sections 4 µm-thick tissue sections were mounted on positively-charged Super Frost® Plus slides (Gerhard Menzel GmbH, Braunschweig, Germany), de-waxed in xylene, hydrated through a graded series of ethanol and rehydrated in deionised water. For EP2

receptor and m-PGES-1 an unmasking pre-treatment was achieved by microwaving the slides in citrate buffer solution pH 6.0, for three cycles of five minutes each at 650 W.   After rinsing in 0.05% Tween Tris-buffered saline solution (TBST, pH 7.6), endogenous peroxidase activity was blocked by incubation of the sections with H_2O_2 0.5% in methanol for 10 min and after this, three washes were performed in TBST.   Non-specific reactions were blocked by incubation with normal rabbit or goat serum (Dako, Glostrup, Denmark; diluted 1 in 10 in TBST) for 10 min at room temperature.   After three further washes, the sections were incubated for 1 h at room temperature in humid chambers with the primary antibodies.   After three washes in TBST, the sections incubated with anti-mPGES-1 and anti-EP2 receptor were incubated with EnVision® (Dako) at room temperature for 30 min, while tissues incubated with anti-COX-2 were incubated at room temperature for 30 min with a biotinylated horse anti-goat immunoglobulin reagent (Vectastain®, Vector Laboratories, Burlingame, California, USA).   After washing again with TBTS, the peroxidase reaction was developed for 10 min with 3, 3'-diaminobenzidine (DAB) (Impact DAB®, Vector Laboratories), blocked with deionised water and followed by light counterstaining with Mayer's haematoxylin.   Negative controls were performed by omitting the primary antibody and by replacing the antibody with species-matched unrelated primary antibodies.   Human breast cancer tissue sections known to express the three antigens (kindly provided by Dr. P. Viacava) were used as positive controls. Immunolabelling was scored in blinded fashion by two pathologists (FM and AP), and when there was disagreement (<5% of the slides), a consensus was obtained. COX-2, mPGES-1 and EP2 receptor expression was indicated by the presence of brown cytoplasmic labelling. The expression of the markers was quantified according to the same scoring system adopted for COX-2, EP2 receptor and mPGES-1 expression on the bases of previously published criteria on human[18,22,24] and feline and canine tissues.[3,23]   The method is based on the evaluation of the staining intensity and the percentage of positive cells: 0, no labelling; +1,

weak diffuse cytoplasmic labelling (may be stronger labelling of < 10% of the cancer cells); +2, moderate to strong granular cytoplasmic labelling of 10–90% of the cancer cells; +3, > 90% of the tumour cells labelled with strong intensity.   For COX-2, mPGES-1 and EP2 receptor expression samples showing +1 to +3 scores were considered as positive, and 0 as negative. As previous reported,[3] for COX-2 also the overexpression was included in the statistical analysis, and samples scored as +2 and +3 were considered as COX-2 overexpressing.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS Advanced Statistics 13.0 (SPSS Inc., Chicago, Illinois, USA).   The Chi square test was used to investigate the significance of the relationship between antigen expression and histological diagnosis and antigen expression and the following features: age, tumor size, morphology, lymphatic invasion, histological grade and overall survival.   The threshold for statistical significance was set at 5%.   Correlation between COX-2, mPGES-1 and EP2 receptor expression scores was evaluated using the Pearson correlation test.

RESULTS

Histology

The histological examination of the 74 canine mammary tissues yielded healthy mammary glands (n=6, 8.1%), lobular hyperplasia (n=22, 29.7%), simple mammary adenomas (n=10, 13.5%) and mammary carcinomas (n=36, 48.7%).   Of the malignant tumours, 23/36 (64%) were complex carcinomas, 8/36 (22%) were simple tubulopapillary

carcinomas and 5/36 (14%) were simple solid carcinomas. All the malignant tumours were invasive: 25/36 (69.4%) were locally invasive while in 11/36 cases (30.6%) the invasion of lymphatic vessels was observed. Fourteen out of 36 malignant tumours were graded as well differentiated carcinomas (WDC) (39%), 19/36 as moderately differentiated (MDC) (53%), and 3/36 as poorly differentiated carcinomas (PDC) (8%). The histologic examination of the feline mammary samples showed 10 healthy mammary gland tissues and 50 malignant tumours. Of these latter, all were classified as simple carcinomas. Thirty-five out of 50 (70%) were of tubulopapillary type, and 15/50 (30%) were solid carcinomas. All the tumour were invasive, with 29/50 (58%) carcinomas showing lymphatic invasion and 21/50 (42%) that were only locally invasive. Considering the tumour grading, 13/50 (26%) scored as WDC, 28/50 (56%) as MDC and 9/50 as PDC (18%).

Immunohistochemistry

COX-2, mPGES-1 and EP2 receptor positivity was consistently observed in canine and feline neoplastic mammary cells, while there was no expression in feline healthy tissues and only rare expression was observed in the canine healthy (COX-2 = 17%, mPGES-1 = 17%) and hyperplastic tissues (COX-2 = 18%, EP2 receptor = 32%). Both in canine and feline mammary tissues COX-2 labelling was cytoplasmic and diffuse to granular, often with perinuclear localization (Figures 1 A and D). COX-2 expression and overexpression is summarized in Table 1. COX-2 overexpression was observed in 1/6 (16.7%) healthy mammary glands (with a +2 score), in 4/22 (18.2%) lobular hyperplasias (+2 score), in 2/10 (20%) mammary adenomas (+2 score) and in 30/36 (83.3%) mammary carcinomas. In this latter group, 17 cases scored +2, and 13 scored +3. The remaining 6 cases (16.7%) showed a weak immunostaining with less than 10% neoplastic cells with a stronger staining intensity and were recorded as non-overexpressing the enzyme. COX-2 overexpression in canine

mammary carcinomas was significantly higher than that recorded in healthy (P<0.001), hyperplastic (P<0.001) and adenoma tissues (P<0.001).   In canine carcinomas COX-2 overexpression was significantly associated to increased tumour dedifferentiation (P<0.05), but not with age, tumour size, histologic type, lymphatic invasion or overall survival, as reported in Table 2.

In feline mammary tissues the evaluation of the expression of COX-2 showed a lack of immunostaining in all the healthy mammary samples examined. Of the 50 FMTs, 9 (18%) scored negative, 17 (34%) scored +2 and 24 (48%) scored +3. COX-2 overexpression was thus observed in 41/50 carcinomas (82%). COX-2 overexpression was significantly higher in neoplastic than in healthy mammary tissues (P<0.001). No significant correlations were found between COX-2 expression and age, tumour size, tumour type, grade and lymphatic invasion, while was associated to shorter survival (P=0.001), as showed in Table 3.

The expression of mPGES-1 was observed as a diffuse to granular cytoplasmic staining, with a staining intensity ranging from weak to strong both in feline and canine tissues (Fig. 1 B and E). mPGES-1 expression in canine and feline mammary tissues is summarized in Table 1.   mPGES-1 expression was recorded in one healthy canine mammary tissue sample (16.7%).   None of the canine hyperplasias showed mPGES-1 expression, and in canine mammary adenomas, 1/10 (10%) expressed mPGES-1. In malignant tumors, 27/36 cases (75%) were positive to mPGES-1 expression, with 3/36 (8%) scoring +1, 12/36 (33%) scoring +2 and 12/36 (33%) scoring +3. The percentage of mPGES-1 expressing cases was significantly higher in carcinomas than in healthy (P<0.05), hyperplastic (P<0.001) and adenoma tissues (P<0.05). mPGES-1 expression was not associated to age, tumour size, tumour histotype or lymphatic invasion, while was associated to tumour grading (P < 0.05) and poorer survival (P < 0.05).

In the feline samples, the expression of mPGES-1 was not detected in the healthy

mammary tissues. In contrast, 33/50 (66%) FMTs scored positive for the expression of mPGES-1, with 7/50 (14%) cases scoring +1, 15 (30%) scoring +2 and 11 (22%) scoring +3. Of the 17 non mPGES-1-expressing carcinomas, 7 cases (41%) showed less than 10% (score +1) immune reactive tumour cells, while no immunoreactivity was detected in 10 (59%) samples. Microsomal PGES-1 expression was significantly higher in neoplastic than in healthy mammary tissues (P<0.001). There was no statistically significant association between the expression of the enzyme and age, tumour size, tumour histotype, grading and lymphatic invasion, while a significant association was found with poorer survival times (P = 0.001), as summarized in Table 3.

In both species EP2 receptor labelling was granular to diffuse, cytoplasmic and often with perinuclear localization with a staining intensity varying from weak to strong (Fig. 1 C and F). EP2 receptor expression in canine and feline mammary tissues is summarized in Table 1. In healthy canine tissues, no immunostaining was observed. In canine hyperplastic and neoplastic tissues, the receptor was expressed in 7/22 (32%) lobular hyperplasias, in 2/10 (20%) adenomas and in 32/36 (89%) carcinomas. Of these latter, 8 (22%) scored +1, 15 (42%) scored +2 and 9 (25%) scored +3. The percentage of EP2 receptor expressing cases was significantly higher in carcinomas than in healthy (P<0.001), hyperplastic (P<0.05) and adenoma tissues (P<0.05). EP2 receptor expression was not associated to age, tumour size, tumour histotype and grading, lymphatic invasion and survival times.

EP2 receptor expression was not detected in healthy feline mammary tissues. Of the carcinomas 6 (12%) scored +1, 11 (22%) scored +2, and 10 (20%) scored +3. The percentage of EP2 overexpressing cases was statistically higher in carcinomas than in healthy mammary tissues (P<0.001). In FMT, EP2 receptor expression was significantly higher in poorly differentiated tumours (P<0.05) and was associated with shorter survival (P=0.001).

Using the Pearson Correlation test, in the canine mammary tissues there was a

significant association between COX-2 overexpression and of mPGES-1 (r = 0.729; P < 0.0001) and EP2 receptor (r = 0.750; P < 0.0001) positivity, while a stronger association was detected between expression of mPGES-1 and EP2 receptor (r = 0.802; P < 0.0001). In the feline mammary tissues a strong association was detected between COX-2 and mPGES-1 (r = 0.701; P < 0.0001) and mPGES-1 and EP2 receptor (r = 0.699; P <0.0001), while the association between COX-2 and EP2 receptor was less evident even if still significant (r = 0.421; P < 0.0001).

DISCUSSION

It is well documented that PGE_2 is the major prostaglandin involved in the progression of several human tumours.[2] Aberrant up-regulation of COX-2 resulting in accumulation of PGE2 in a cancer cell environment is a marker of progression in many human tumours, including breast cancer.[2,25] Chemopreventive and chemotherapeutic strategies using COX-2 inhibitors are nowadays used in several human cancers.[26,27] Long-term therapies with NSAIDS are reported to significantly decrease the risk of developing colorectal carcinomas in humans.[2]

The use of non-steroidal anti-inflammatory drugs (NSAIDs) has become in the past decades a chemotherapic and chemopreventive strategy for human colorectal tumours.[2] The use of selective anti COX-2 NSAIDs has reduced the onset of adverse side effects deriving from COX-1 inhibition. However, a long term use of COX-2 inhibitors often leads to the onset of adverse cardiovascular side effects.[31,32] COX-2 inhibition in fact may lead to a decreased synthesis of both PGE_2 and PGI_2, these latter being important for normal vascular integrity.[27] mPGES-1 is not involved in PGI_2 biosynthesis and therefore its inhibition might be an useful strategy to reduce PGE_2 levels with a decreased risk of side effects.

In domestic animals, COX-2 inhibitors have shown to have antineoplastic effects on several tumour cell lines *in vitro*[29] and are already used for the adjuvant treatment of inflammatory mammary carcinomas in the canine species.[30] The traditional NSAIDs inhibit both COX-1 and COX-2, while the recently developed coxibs preferentially inhibit COX-2. In the feline and canine species the use of NSAIDs for anti-inflammatory purposes has led to the onset of adverse, mainly gastrointestinal, events.[33,34]

It has thus been suggested the need for new and more selective targets in the PGE2 synthesis pathway downstream of COX. Jimènez et al.[35] and Murakami et al.[11] demonstrated that cells overexpressing both mPGES-1 and COX-2 produced more PGE_2, grew faster and exhibited abnormal morphology when compared to cells in which either COX-2 alone or mPGES-1 alone were expressed.

In mammary tumours of domestic animals the overexpression of COX-2 has already been described in several studies.[3-5] As we have previously reported,[3] COX-2 overexpression may have a prognostic relevance in canine and feline malignant mammary tumours. A recent study also describes a strong COX-2 expression in a high percentage of metastatic mammary lesions.[6] As previously described,[5] COX-2 expression was higher in malignant CMTs than in benign ones, and COX-2 overexpression was more frequently observed in poorly differentiated CMTs and FMTs, but was not associated to tumour type or lymphatic invasion, as previous reported in our study.[3] In the available current literature there is only one study describing a correlation between COX-2 expression and tumour histotype.[6]

To our knowledge, our study describes for the first time the profiles of expression of mPGES-1 and EP2 receptor in canine and feline mammary tissues. In a previous study evaluating the expression of mPGES1 and its receptors in the canine corpum luteum by RACE PCR and RT PCR, alignment against the canine genomic sequence identified three sequence-fragments on chromosome 9, showing a 100% similarity with the cloned sequence

and corresponding to other known mammalian exon-homologs.[36] Furthermore the expression of EP2 was detected in the canine ovarian tissue.[36] The antibodies that we used in our study, commonly adopted for the immunohistochemical investigation on PGES-1 and EP2 receptor expression in human tissues, and already tested on canine non neoplastic and neoplastic bone tissues,[23] have shown to cross react also with the canine and feline mammary tissues. In canine healthy mammary tissues, mPGES-1 was expressed only in one case, with a low percentage of immunoreactive cells, even though above the established cut off value. This finding may reflect a phisiological role of mPGES-1, but due to the low percentage of positive cells detected in only one case of healthy mammary tissue, further evaluations are required to support this hypothesis. None of the hyperplastic lesions scored positive, however, 8/22 cases showed a faint cytoplasmic immunoreactivity below the threshold of the scoring system. In human literature the data in this respect are still conflicting. It has been described that mPGES-1 can be overexpressed also in healthy skin and premalignant epithelial lesions, especially of the epidermis, suggesting that the COX-2 mediated prostaglandin signaling can be related to epithelial carcinogenesis.[37,38] In another study on mammary tissues by Mehrotra et al., mPGES-1 was undetectable in normal breast epithelial cells.[22] In our study population of bitches, both mPGES-1 and EP2 receptor were statistically more expressed in carcinomas than in adenomas, with a high percentage of carcinomas (67%) showing mPGES-1 and EP2 receptor overexpression. The percentage of mPGES-1 positive carcinomas (75%) recorded in our study is quite similar to that reported in human literature. Mehrotra et al.[22] observed the immunohistochemical expression of mPGES-1 in >10% neoplastic cells in 79% breast cancers. In the feline species 52% of the examined neoplastic tissues have shown to overexpress mPGES-1. This value is lower compared to that recorded in the canine and human species, but it's noteworthy that feline healthy mammary gland were always negative, suggesting an increase in mPGES-1 expression, and, consequently, in PGE_2 production,

during the neoplastic progression.

In agreement with the human literature mPGES-1 did not correlate with tumour histologic type also in canine and feline malignant tumours. Of interest, mPGES-1 was more frequently expressed in PDC CMTs, graded according to Peña and colleagues.[20] Despite a low sample size, our data is consistent with previously described higher overexpression of COX-2 described in less differentiated CMTs[3] and in anaplastic carcinomas compared to adenocarcinomas.[39]

The fact that not all the feline and canine COX-2 overexpressing tumours also express mPGES-1 reflects previous human findings. According to Mehrotra et al,[22] the expression of mPGES-1 in breast cancer did not correlate with that of COX-2. Yoshimatsu et al.,[40] suggest that, although mPGES-1 and COX-2 are up-regulated in colorectal cancers, the mechanisms controlling the expression of these enzymes are not identical. Recently, a difference of regulation of COX-2 and mPGES-1 in non-small cell lung cancer has been described.[41] In this study IL-4 inhibited the formation of PGE_2 predominantly via a decrease in mRNA transcription, whereas the expression of mPGES-1 was unaffected. Another study showed increased mPGES-1 immunoreactivity in neoplastic cells both in colorectal adenomas and cancers compared with adjacent normal epithelium. Also in this report, differences in COX-2 and mPGES-1 expression were observed, with COX-2 but not mPGES-1 induced by chenodeoxycolate, and tumour necrosis factor-α inducing both COX-2 and mPGES-1 but with different time course and magnitude of induction.[40]

EP characterization on tumour cells is only beginning and the precise role of each EP in malignant behavior has yet to be determined. Much of the data regarding EP come from animal models of colon cancer, where all the receptors are implicated, but the specific subtype differs depending upon the model examined.[17] EP2 receptor has however been shown to be linked to growth stimulation,[42] to be implicated in promoting early carcinogenesis.[16] EP2

receptor has been also found to be increased in Barret's esophageal metaplasia and esophageal carcinoma.[35] Our results show that the receptor is not expressed in healthy tissues both in dogs and cats, and that the percentage of positive cases increases in the canine hyperplastic lesions. This is in agreement with previous studies in human literature, where it has been described that EP2 receptor is involved in COX-2-induced mammary hyperplasia.[18] From our data, the statistically higher percentage of the malignant neoplastic lesions positive to m-PGES-1 and EP2 receptor compared to healthy, hyperplastic and adenoma tissues suggest an increased PGE_2 production in carcinoma tissues and support the results of an early study by Mohammed et al.,[43] in which increased levels of PGE_2 were found in canine mammary tumours. Our data also confirm the role of the COX-2/PGE_2 pathway in the neoplastic progression, with the expression of the three markers that significantly increase in malignant tissues, and provide a rationale for further investigating the role of PGE_2 in canine and feline mammary tumours development.

Moreover, as mPGES-1 is downstream to COX-2 and not involved in the synthesis of other prostanoids, it would be an excellent target for therapeutic approaches also in the canine and the feline species. The higher percentage of poorly differentiated feline tumours positive to EP2 receptor would be of interest in view of an adjuvant therapeutic approach to tumours with a poorer prognosis. Although further investigation will be required to assess the EP2 receptor status in feline mammary hyperplastic lesions, the finding of EP2 receptor positive canine mammary hyperplasia may also suggest the possibility of EP2 receptor targeting also for chemo preventive purposes at least in the canine species. Further, due to the aggressive biologic behavior of FMTs, with poor post-surgical overall survival times,[44,45] the fact that PDCs are frequently EP2 receptor positive support the investigation of the role of COX-2/PGE_2 signaling pathway and the possibility of antagonizing the COX-2-derived PGE_2 by targeting the EP2 receptor.

On the basis of the data on current literature on the role of COX-2 in several solid neoplasms of the small animals, and of the therapeutic effects of COX-2 inhibition, further studies may have broad implications for the prevention and treatment of canine mammary tumours by antagonising COX/PG signaling inhibiting the EP2 pathway or targeting mPGES-1.

Conflict of interest

No conflicts of interest have been declared.

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Table 1. Immunohistochemical expression of COX-2, mPGES-1 and EP2 receptor in the canine and feline mammary tissues examined.

	COX-2		COX-2		mPGES-1		EP2 receptor	
	Positive	%	Over-expression	%	Positive	%	Positive	%
DOG								
Healthy (n=6)	1	17	1	17	1	17	0	0
Hyperplasia (n=22)	4	18	4	18	0	0	7	32
Adenomas (n=10)	2	20	2	20	1	10	2	20
Carcinomas (n=36)	36*	100	30†	83	27°	75	32§	89
CAT								
Healthy (n=10)	0	0	0	0	0	0	0	0
Carcinomas (n=50)	48**	96	41††	82	33°°	66	27§§	54

Chi square test analysis: * COX-2 expression was significantly higher ($P < 0.05$) in canine mammary carcinomas than in healthy, hyperplastic and adenoma tissues. ** COX-2 expression was significantly higher ($P < 0.05$) in feline neoplastic mammary tissues than in healthy tissues. † COX-2 overexpression was significantly higher ($P < 0.05$) in canine mammary carcinomas than in healthy, hyperplastic and adenoma tissues. †† COX-2 overexpression was significantly higher ($P < 0.05$) in feline neoplastic mammary tissues than in healthy tissues. ° mPGES-1 expression was significantly higher ($P < 0.05$) in canine mammary carcinomas than in healthy, hyperplastic and adenoma tissues. °° mPGES-1 expression was significantly higher ($P < 0.05$) in feline mammary tumours than in healthy mammary tissues. § EP2 receptor expression was significantly higher ($P < 0.05$) in canine mammary carcinomas than in healthy, hyperplastic and adenoma tissues. §§ EP2 receptor expression was significantly higher ($P < 0.05$) in feline mammary tumours than in healthy mammary tissues.

Table 2. Comparison between COX-2, mPGES-1 and EP2 receptor immunohistochemical expression and canine carcinomas features. All comparisons were conducted using a chi square test.

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n = 36	COX-2 overexpression			m-PGES-1 expression			EP2 receptor expression		
	positive	negative	*P*	positive	negative	*P*	positive	negative	*P*
Age									
< median	13	5	*0.07*	13	5	*0.70*	15	3	*0.29*
> median	17	1		14	4		17	1	
Tumor size									
< 3 cm	13	5		15	5		17	3	
3 to 5 cm	10	2	*0.89*	10	2	*0.41*	12	0	*0.27*
> 5 cm	3	2		2	2		3	1	
Morphology									
Complex	24	4	*0.47*	6	2	*1*	7	1	*0.89*
Simple	6	2		21	7		25	3	
Lymphatic invasion									
Positive	10	1	*0.42*	10	1	*0.14*	11	0	*0.15*
Negative	20	5		17	8		21	4	
Differentiation									
WDC	9	5		7	7		11	3	
MDC	18	1	***0.04***	17	2	***0.02***	18	1	*0.28*
PDC	3	0		3	0		3	0	
Overall survival									
Alive	23	3	*0.18*	17	9	***0.03***	22	4	*0.19*
Dead	7	3		10	0		10	0	

Table 3. Comparison between COX-2, mPGES-1 and EP2 receptor immunohistochemical expression and feline carcinomas features. All comparisons were conducted using a chi square test.

n = 50	COX-2 overexpression			m-PGES-1 expression			EP2 receptor expression		
	positive	negative	*P*	positive	negative	*P*	positive	negative	*P*
Age									
< median	18	7	*0.16*	17	8	*0.77*	14	11	*0.78*
> median	22	3		16	9		13	12	
Tumor size									
< 2 cm	13	5		9	9		8	10	
2 to 3 cm	16	2	*0.45*	13	5	*0.19*	9	9	*0.29*
> 3 cm	11	3		11	3		10	4	
Lymphatic invasion									
Positive	24	5	*0.57*	19	10	*0.93*	17	12	*0.44*
Negative	16	5		14	7		10	11	
Differentiation									
WDC	11	2		9	4		7	13	
MDC	22	6	*0.89*	17	11	*0.62*	11	28	***0.01***
PDC	7	2		7	2		9	9	
Overall survival									
Alive	7	7	***0.001***	2	12	***0.001***	1	13	***0.001***
Dead	33	3		31	5		26	10	

Figure Legends

Figure 1.   Immunohistochemistry for COX-2, mPGES-1 and EP2 receptor expression in canine and feline mammary carcinomas.   There is moderate to intense cytoplasmic positivity in neoplastic epithelial cells in all the sections shown.   A, B and C: canine mammary malignant tumour showing COX-2 overexpression (A) and mPGES-1 and EP2 receptor expression (B and C, respectively). DAB, hematoxylin countestain, Bar, 100 μm. D, E and F: a feline mammary carcinoma showing COX-2 overexpression (D) and mPGES-1 and EP2 receptor expression (E and F, respectively).   DAB, hematoxylin counterstain, Bar = 100 μm.