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COX-2 and mPGES-1 expression in canine mast cell tumour

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Summary

COX-2 and mPGES-1 participate in the development of tumours and play a key role in their progression by impacting cell proliferation, apoptosis and neoangiogenesis. The aim of the study was to assess the expression of COX-2 and mPGES-1 in mast cell tumours. The existence of correlation between the histologic malignancy of the tumour and the expression of the proteins was verified through statistical analysis. Fifteen canine skin mast cell tumours were used in the study. Tissue sections were stained with H&E to determine their histologic grade. Subsequently, immunohistochemical staining of COX-2 and mPGES was carried out. A cytoplasmic reaction was observed in all tumours. Statistical analysis revealed a strong, positive correlation ($p < 0.05$) between the two markers and the grade of the tumour. Both markers may be used effectively in the diagnosis of skin mast cell tumours in dogs.

Keywords: COX-2, dog, mast cell tumour, mPGES-1

Mast cell tumours (MCT) are some of the most frequently diagnosed skin tumours in dogs (10, 11, 15). Tumours have a variable biological behaviour – from benign to highly aggressive and invasive. MCTs in dogs usually occur at the age of 7-9 years, and a sex predisposition has not been reported (13). According to Patnaik et al., three different degrees of malignancy can be distinguished. The scale is based on the evaluation of tumour invasiveness, the mitotic index and the morphology of mast cells. Grade I (MCT I) is characterized by round cells with multiple granules. Round, oval and, less frequently, giant cells with granular cytoplasm are visible in grade II (MCT II). The mitotic index usually corresponds to 0-2 figures per high power field (hpf). Grade III tumours (MCT III) are the most aggressive and can be identified by the presence of pleomorphic cells. Usually 3-4 figures can be observed per hpf. Oedematous, hemorrhagic and necrotic areas are present within the tumour stroma (10).

An excessive expression of cyclooxygenase-2 (COX-2) and an increase in its enzymatic product, prostaglandin E2 (PGE2), seem to be involved in the development of various tumours. Deregulation of this enzymatic pathway appears to play a key role in neoplasm progression by affecting cell proliferation, apoptosis, immune-surveillance and angiogenesis (3, 15, 16).

Enzymes important for the synthesis of prostaglandin E2 (PGE-2) include COX-2, prostaglandin E synthase (PGES) and phospholipase A_2 (PLA_2) (8). COX-2 is undetectable in most tissues, but increases during inflammatory and mitogenic stimuli. The peroxidase activity of COX can convert procarcinogens to carcinogens and initiate tumour formation (2). Furthermore, COX-2 inhibits cellular apoptosis, promotes neoangiogenesis and increases tumour cell motility and invasiveness (1). *In vitro* studies have shown that COX inhibitors may prevent tumour occurrence (2). COX-2 overexpression has previously been described in canine mast cell tumours (11, 15).

Prostaglandin is the most common prostanoid, which is produced by a variety of cells and tissues and has a broad range of biological activities (4). Three PGES enzymes have been identified: microsomal PGES-1 (mPGES-1), PGES-2 (mPGES-2) and cytosolic PGES (cPGES). mPGES-1 expression is observed in inflammatory reactions, as well as in neoplastic lesions (4). It is induced by interleukin 1 (IL-1β) and the TP53 protein (14). It has been noted that transgenic mice overexpressing both COX-2 and mPGES-1 develop metaplasia, hyperplasia, and tumorous growth in the glandular stomach with heavy macrophage infiltration (9). mPGES-1 expression has previously been described in canine tumours (6).

The aim of the study was to assess the expression levels of COX-2 and mPGES-1 and their association with the histologic grade of MCTs. The study also aimed to determine whether the two proteins can be useful in defining the prognosis for patients.

Material and methods

The studies were conducted on 15 canine neoplastic skin tumours, previously diagnosed as MCTs. The tumours were excised from dogs of various breeds, sexes and ages. The age of the dogs ranged from 5 to 12 years (in most cases n = 10 between 6 and 9 years). The neoplasms originated from 10 females and 5 males (Tab. 1).

The specimens were fixed in 7% buffered formalin for 24 h, embedded in paraffin, cut into 4 µm-thick sections, and stained with hematoxylin and eosin (H&E). Immunohistochemical analyses were conducted on 4 µm-thick paraffin sections mounted on silanized slides, deparaffinized in xylene and rehydrated in an alcohol gradient. The slides were heated in citrate buffer (pH 6.0, 97°C, 20 min) to retrieve the antigens. Then the slides were double washed with TBS and blocked in 10% normal serum with 1% BSA in TBS. The primary antibodies specific for COX-2 Clone RBT-COX2, BioSB (diluted 1 : 200) and for PTGES Catalog No.: AP07074PU-N, Acris (diluted 1 : 200) were applied. The slides were then incubated at room temperature for 20 min. Subsequently, the slides were washed in TBS. After rinsing, endogenous peroxidase was blocked in a 3% solution of hydrogen peroxide for 10 minutes. Immunohistochemical reactions were developed with 3,3-diaminobenzidine tetrahydrochloride (DAB). Finally, the slides were rinsed in distilled water, counterstained with hematoxylin, dehydrated in an alcohol gradient, passed through xylene and sealed. A positive or negative control was used for each marker. The specificity of the immunolabeling was verified by incubation with PBS instead of the specific primary antibody, and the sections of canine skin were used as positive controls.

The expression of COX-2 and mPGES-1 was evaluated on the Queiroga scale. This technique takes into account both the percentage of immunoreactive cells: no cells labelled (grade 0); 1-10% (grade 1); 11-50% (grade 2); 51-80% (grade 3); 81-100% (grade 4) and the labelling intensity: negative (0); weak (1); moderate (2) and strong (3). The final score represents a sum of the two parameters, ranging from 0 to 12 points: no reaction – 0 pts (–); scanty reaction 1-2 pts (+), moderate reaction 3-4 pts (++), intense reaction 6-12 pts (+++) (12).

Tab. 1. Characteristics of animals

Breed	Age	Sex
Alaskan Malamute	7	m
American Staffordshire Terrier	9	f
Bernese Mountain Dog	5	f
Black Russian Terrier	11	m
Boxer	9	f
Boxer	6	f
Boxer	6	f
Boxer	7	f
Bullterier	8	m
Labrador Retriever	9	f
Labrador Retriever	10	m
Mongrel	8	m
Mongrel	11	f
Polish Tatra Sheepdog	6	f
Weimaraner	12	f

The microphotographs of all the specimens examined were taken with an Olympus BX53 optic microscope (Olympus, Japan) equipped with a Color View IIIa digital camera (Olympus, Japan). The results were subjected to statistical analysis, including Spearman's correlation coefficient (rho), by the Statistica PL package (StatSoft, Poland). The significance was set at $p < 0.05$.

Results and discussion

A moderate cytoplasmic reaction (++) to COX-2 expression was observed in all MCTs I. A relatively moderate (n = 3) or intense (n = 2) reaction was visible in II grade MCTs. In III grade MCTs, a very strong (+++) reaction was detected in all cases (Fig. 1). The expression of COX-1 is summarized in Table 2.

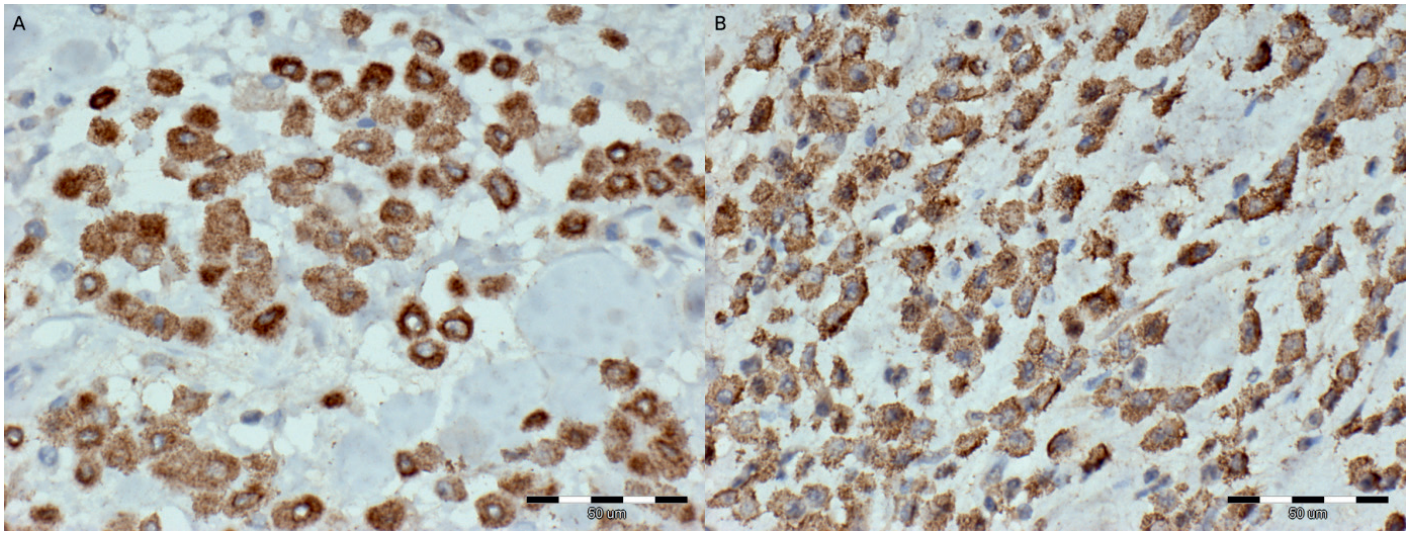

Fig. 1. The staining patterns of COX-2 (A) and mPGES-1 (B). A pronounced cytoplasmic reaction is visible in A and B. ×400

Tab. 2. Summary of the results obtained

Specimen	Histologic grade	COX-2			mPGES-1		
		percentage	intensity	final	percentage	intensity	final
1	I	3	1	3	3	1	3
2	I	3	1	3	3	2	6
3	I	4	1	4	2	2	4
4	I	2	2	4	3	1	3
5	I	3	1	3	4	2	8
6	II	4	1	4	4	1	4
7	II	2	2	4	3	2	6
8	II	4	1	4	3	3	9
9	II	2	3	6	2	2	4
10	II	3	3	9	4	3	12
11	III	4	3	12	3	3	9
12	III	3	3	9	4	3	12
13	III	3	3	9	3	3	9
14	III	3	3	9	3	2	6
15	III	4	3	12	3	3	9

Explanations: * Percentage of immunoreactive cells: no labelled cells (grade 0), 1-10% (grade 1), 11-50% (grade 2), 51-80% (grade 3), 81-100% (grade 4); and labelling intensity: negative (0), weak (1), moderate (2), strong (3). The final score represents a sum of the two parameters, ranging between 0 and 12 points.

During the examination of mPGES-1, a cytoplasmic pattern was observed. The expression was either moderate (MCT I: n = 3; MCT II: n = 2) or intense (MCT I: n = 2; MCT II: n = 3). In all cases of MCT III, a very intense reaction was observed (Fig. 1). Detailed information is presented in Table 2.

Statistical analysis showed a positive, significant ($p < 0.05$) correlation between the grade of MCT (defined in the H&E staining) and the expression of COX-2 and mPGES-1. The correlation coefficient (rho, according to Spearman) amounted to $r = 0.88$ ($p < 0.05$) in the case of H&E-COX-2 and $r = 0.64$ in the case of H&E-mPGES-1 ($p < 0.05$). A relationship between COX-2 and mPGES, amounting to 0.6, was also defined. These results demonstrated a strong relationship between the two cell markers and tumour malignancy (Fig. 2).

The results of the present study demonstrate a positive immunohistochemical reaction of COX-2 in all tumours. The percentage of immunoreactive cells in most specimens ranged between 51 and 100% (grade 3 and 4). Between 11 and 50% of cells were positively labelled in one case of MCT I and in two cases of MCT II. In terms of the intensity of the reaction, significant differences were noted in individual groups. Labelling increased with the MCT grading. The final score also indicates an increase of the results with an increasing grade of the tumour. Prada et al. noted an association between the expression of COX-2 and the histologic grade in II and III grade MCTs (11). According to Vascellari et al., COX-2 was detected in 78% of cells (15). Both authors observed that the expression increased with the tumour grade. Heller et al. performed similar research, in which they found positively labelled II grade MCTs in only 1/17 cases (5). On the basis of our investigations and the results of statistical analysis, we confirm that COX-2 is a useful biomarker of tumour progression.

The second aim of the study was to determine whether mPGES-1 could be successfully used in MCTs. As in the case of the other protein examined, a reaction was found in 100% of the tumours. The percentage of labelled cells was also similar. Most of the specimens had from 51 to 100% of labelled cells. In two cases, there were between 11 and 51% immunoreactive cells. Reaction intensity increased slightly with the tumour grade. The final score also indicated an increase with tumour malignancy. No significant differences were noted between any of the tumours

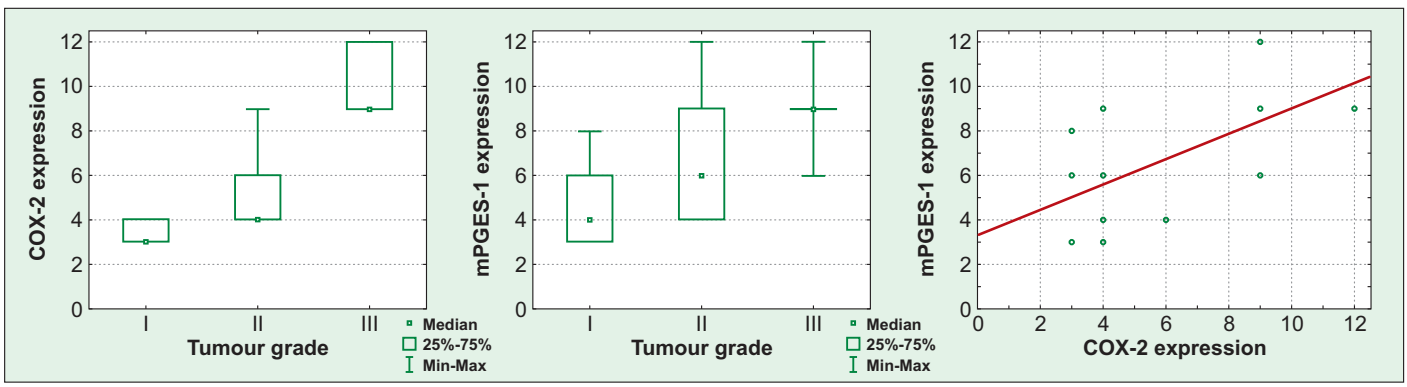

Fig. 2. Statistical analysis. A strong, positive correlation between COX-2 and mPGES-1 and MCT grading is presented. A correlation between COX-2 and mPGES-1 is also visible

examined. Millanta et al. examined mPGES-1 in normal and reactive bone and in canine osteosarcoma. They observed no reaction in healthy bone. However, they found that the expression increased in reactive and neoplastic lesions. A significantly higher expression was observed in osteosarcoma (7). In canine and feline mammary tumours, the expression of mPGES-1 was also significantly higher in carcinomas than in non-neoplastic tissues and adenomas (6). The obtained results and statistical data suggest that mPGES-1 may be a good prognostic marker in canine MCT.

In conclusion, the present study demonstrates the expression of COX-2 and mPGES-1 in canine MCTs. Statistical data confirm a strong, positive correlation between the two cell markers and MCT grading. Both proteins could be useful prognostic markers. However, further examinations are necessary to determine the influence of COX-2 and mPGES-1 on MCTs.

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