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REVIEW ARTICLE

Cyclooxygenase-2 as a biomarker with diagnostic, therapeutic, prognostic, and predictive relevance in small animal oncology

Marta Szweda[1], Andrzej Rychlik[2],
Izabella Babińska[3], Andrzej Pomianowski[1]

[1]Department of Internal Diseases with Clinic,
[2]Department of Clinical Diagnostics, Faculty of Veterinary Medicine,
University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland
[3]Department of Pathophysiology, Forensic Medicine and Administration,
Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-718 Olsztyn, Poland
m.szweda@wp.pl

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Abstract

In canine and feline populations, the number of neoplasm cases continues to increase around the world. Attempts are being made in centres of research to identify new biomarkers that speed up and improve the quality of oncological diagnostics and therapy in human and animal tumour patients. Cyclooxygenase-2 (COX-2) is a promising biomarker with increasing relevance to human oncology, but as yet with less application in veterinary oncology. The expression of COX-2 increases significantly during pathological processes involving inflammation, pain or fever. It is also overexpressed in humans presenting various types of tumours and in selected types of tumours in animals, particularly in dogs. This article discusses the expression of COX-2 in canine and feline tumours, the importance of COX-2 as a biomarker with diagnostic, therapeutic, prognostic and predictive relevance in oncology, and the clinical significance of inhibiting COX-2 overexpression in tumours.

Keywords: dog, cat, biomarker, COX-2, tumours.

Introduction

Neoplasms are the second leading cause of death in the world after cardiovascular diseases. According to the World Health Organisation, 8.8 million people died of tumours in 2015 (http://www.who.int/cancer/en/index.html). Polish National Cancer Registry data show that the prevalence of malignant tumours has more than doubled in the last 30 years. In 2016 there were 164,140 cancer sufferers in Poland (427.1 cases/100,000 people) (http://onkologia.org.pl).

In canine and feline populations, the number of neoplasm cases also continues to increase around the world (60). According to Bronson (10) the death rate from tumours is the highest in older animals and reaches 45% in dogs older than 10 years. In cats, tumours are also most prevalent in older animals with an estimated death rate of 32% in cats older than 10 years. This can be attributed to the fact that companion animals live in the human environment and their predisposing risk factors are the same as humans. Advanced diagnostic techniques have also increased the detection rate for tumours in veterinary medicine (60).

COX-2 as a biomarker

In research centres, attempts are being made to identify new biomarkers that speed up and improve the quality of oncological diagnostics and have prognostic and predictive relevance in humans and animals with tumours (36). Rapid advances in knowledge of biochemical and especially molecular mechanisms responsible for oncogenesis and the progression of various types of tumours facilitate the development of new, more effective diagnostic methods and therapeutic

protocols. The existing methods should be integrated, and the results of clinical evaluations, post-mortem examinations, histopathological, immunohistochemical, cytological, and molecular analyses should be used to deepen our understanding of tumour development and to improve diagnosis and treatment. The development of reliable parameters with prognostic and predictive applicability poses one of the greatest challenges in the treatment of tumours. Various biological, clinical, histological, immunohistochemical, and molecular parameters have been evaluated to date, but the choice of the most effective markers remains an open issue (28, 36, 46).

Cyclooxygenase-2 (COX-2) is an important diagnostic and prognostic biomarker to which human oncology has increasing recourse, while veterinary oncology does so to a lesser extent (50). COX-2 is not yet used as a biomarker in routine cancer screening in human medicine (36). This enzyme is overexpressed in humans presenting with various types of tumours and in selected types of tumours in animals, particularly in dogs. These findings indicate that COX-2 could be a potentially effective biomarker with diagnostic, therapeutic, prognostic, and predictive relevance in oncology. However, further research is required, particularly in veterinary medicine, because researchers are divided in their opinions on the expression of COX-2 in different types of tumours (7, 41).

COX-2 function in organism and in cancerogenesis

Cyclooxygenase (COX) is an enzyme belonging to the myeloperoxidase family, which catalyses conversion of the arachidonic acid to prostanoids. These comprise prostaglandins, prostacyclin and thromboxane, which are bioactive proteins which regulate various physiological processes in human and animal organisms (14). COX-2 as an inducible isoform is found at low levels in mammalian cells and it is not generally detected in physiological conditions, although the presence of COX-2 was observed in the central nervous system, alimentary tract, heart, kidney, eye, and reproductive organs. In the beginning COX-2 was connected with the response to stress, but its overexpression is also found during fever, pain, and inflammation (50). In several studies, increased COX-2 expression was also demonstrated in neoplastic tissues, which suggests participation of this enzyme in carcinogenesis. COX-2 overexpression causes the cells' phenotype to change from benign to malignant, which is connected with disruption of their growth and proliferation and an increase in cells' ability to evade apoptosis and immune response, promote new blood vessels, and raise their invasive potential (50). More details of the function of COX-2 in the organism and the significance of COX-2 in oncogenesis were presented in our previous paper (57).

The mechanisms of COX-2 participation in oncogenesis are very complicated and weakly understood, particularly in animals. They comprise interactions between tumour cells and the surrounding microenvironment to create the best conditions for their growth, proliferation, and dissemination (22). A strict connection between chronic inflammatory processes and carcinogenesis was observed, when overexpression of COX-2 can contribute to the conversion of inflammation into cancer. Gandhi *et al.* (21) summarised the latest scientific achievements highlighting the significance of COX-2 and its downstream signalling effectors' role in life-cycle events of the gammaherpesviruses – Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus enabling cancer progression. Among various inflammatory mediators including pathological processes leading to cancer, COX-2 and its effector molecules are of greater significance. They generate a microenvironment highly favourable for cancer development, progression, and metastasis. Recent studies showed a link between upregulated COX-2 levels and induction of lytic reactivation in gammaherpesvirus-infected cells. There are several cases on record of patients with chronic inflammatory processes and with COX-2 overexpression showing high incidences of EBV-associated malignancies, indicating the role of increased COX-2 levels in virus-mediated tumourigenesis.

Higher COX-2 expression was found in tumours of various organs in humans (17, 28) and dogs (19, 39), and to a lesser extent in cats (7, 39), which is often connected with a higher histological grade of tumour malignancy, worse prognosis, and shortening of overall survival (OS) (13, 23). In Poland previous studies evaluating COX-2 expression in animal tumours are relatively limited, comprising mainly canine mammary (44) and mast cell tumours (27).

Expression of COX-2 in canine and feline tumours

Studies of COX-2 expression in animal populations in various countries have demonstrated that it is overexpressed in various canine epithelial tumours, including adenocarcinomas and carcinomas of the mammary gland, ovarian and prostate tumours, transitional cell (urothelial) carcinoma (TCC), colorectal and small intestine tumours, squamous cell carcinoma (SCC) of the skin and oral cavity, osteosarcoma, and melanoma (19, 20, 39, 46). The enzyme is minimally expressed or is not expressed in canine lymphoma, sarcoma, fibrosarcoma, glioma, and mastocytoma (41).

Research conducted on small populations of feline patients has revealed that unlike in dogs, COX-2 is expressed more weakly and far less frequently in cats. The enzyme was found in one study by Millanta *et al.* (39) to be overexpressed in 96% (45/47) of cats with invasive mammary carcinoma and in selected cases of urothelial TCC, skin and oral SCC, and pancreatic adenocarcinoma, but not in feline patients with intestinal, lung or mammary gland carcinomas,

lymphomas of the nasal cavity and the intestines or vaccine-induced fibrosarcomas (7, 19). These differences have been attributed to variations in the size of analytical samples, sample preparation methods, lower COX-2 levels in cats with tumours, and the absence of interspecies reactions with human antigen antibodies (7).

Several research works on COX-2 expression in canine and feline neoplasms have been written in recent years. In most of them, overexpression of COX-2 in tumours occurring in dogs and cats was found. Millanta *et al.* (38) observed COX-2 positivity in 83% (30/36) of canine and 82% (41/50) of feline mammary carcinomas. The frequency of COX-2 expression was significantly higher in canine carcinomas than in non-neoplastic tissues (18%, 4/22) or adenomas (20%, 2/10), which supports the existence of a role of the COX-2/prostaglandin E_2 (PGE_2) pathway in the pathogenesis of these tumours. Their results are very similar to earlier results obtained by Sayasith *et al.* (53), who noticed COX-2 expression in 88% (35/40 cases) of feline mammary carcinomas at low (50%, 20/40), intermediate (33%, 13/40) and high (5%, 2/40) levels. Investigations of the relationship between the expression of COX-2 mRNA level and malignancy degree in canine malignant mammary tumours were conducted by Anadol *et al.* (1). They found that the expression of COX-2 mRNA was significantly higher in both benign and malignant mammary tumours than in adjacent non-neoplastic mammary gland tissue. COX-2 mRNA levels were related to the histological grade of malignancy, being higher in grade 3 malignant mammary tumours than in grade 2 tumours and higher in these than in grade 1 tumours.

Some studies concerned cutaneous and oral SCC in dogs and cats. Millanta *et al.* (37) noticed COX-2 overexpression in 53% (8/15) of canine and 61% (22/36) of feline SCC, which was higher in cutaneous (88%, 7/8 with overexpression in canines; 70%, 19/27 in felines) than in non-cutaneous lesions (14%, 1/7 with overexpression in canines; 33%, 3/9 in felines). In both species, expression of COX-2 correlated with the progression of disease but not with lymphatic invasion, tumour grading, or tumour classification in the cutaneous tumours. Sparger *et al.* (56) revealed positive but weak COX-2 immunostaining of neoplastic epithelium and stroma in 75% (9/12) cases of feline oral SCC. In a similar study, Nasry *et al.* (42) observed that tumour cells were more likely to express COX-2 (51%, 22/43) than stroma (19%, 8/43) and adjacent oral epithelium (29%, 9/31). These results give confirmation of former results obtained by Bardagi *et al.* (6), who found COX-2 immunoreactivity in all of their 27 feline and 9 canine cases of SCC.

Other canine and feline neoplasms were also the object of studies. Gregorio *et al.* (23) evaluated 50 cases of canine mast cell tumours (MCT) immunohistochemically for the expression of several biomarkers including COX-2. They found an association between COX-2 expression and higher grades of malignancy on the Patnaik and Kiupel grading scales. COX-2 expression was also associated with higher cell proliferative antigen (Ki-67) scores, higher mitotic index, and higher microvascularisation density, suggesting an active role of COX-2 in MCT oncogenesis mainly through proliferation and angiogenesis stimulation. Therefore in the authors' opinion, COX-2 is a potentially relevant clinical prognostic marker and therapeutic target. Samarini *et al.* (51) investigated COX-2 expression in 15 cases of feline meningioma for any possible association between COX-2 immunoreactivity and tumour grade. They noticed that all tumour cases were immunoreactive to COX-2. No significant correlation between COX-2 expression and tumour grade was found, but some was found between COX-2 expression and necrosis. The results of these studies indicate COX-2 expression in feline meningiomas, but without any difference between low- and high-grade tumours, however the association between COX-2 expression and the presence of necrosis indicates the possibility for therapy with selective COX-2 inhibitors. The very recent study of Santelices Iglesias *et al.* (52) to determine COX-2 expression in 117 cases of feline injection site sarcoma (FISS) showed that COX-2 immunolabelling was positive in 56.4% (66/117) of FISS cases. There was a significant association between COX-2 expression by neoplastic cells and a higher degree of inflammation, but COX-2 expression was lower in tumours with a higher degree of anaplasia. The authors conclude that these findings may be useful in predicting the sensitivity of FISS to treatment with COX-2 inhibitors, but their potential therapeutic use could be restricted to tumours with a lower degree of anaplasia. These results were in accordance with those of the investigations of Magi *et al*. (35) and Carneiro *et al.* (11), who found COX-2 expression in 97.0% (30/31) and 61.9% (13/21) of FISS cases, respectively, but in contrast to those of Beam *et al*. (7), who did not find COX-2 expression in any FISS cases.

COX-2 as a biomarker with diagnostic, therapeutic, prognostic, and predictive relevance

The recent very considerable progress in molecular techniques for tumour diagnosis, including the emergence of DNA and RNA sequencing tools, single-nucleotide-polymorphism–based genotyping, and evaluation of mRNA, ncRNA, and miRNA profiles, as well as the achievements in proteomics, metabolomics, and bioinformatics have expanded our understanding of the molecular mechanisms responsible for tumour initiation, progression, and response to treatment, in particular in combination with histopathological and clinical evaluations (28, 36, 50). The results of intensive large-population studies are rapidly implemented in tumour treatment, albeit with varying degrees of success. However, they are less often used to develop

sensitive and specific markers with diagnostic, prognostic, and predictive relevance in cancer medicine. Despite the subordinacy of this goal, the number of identified biomarkers with potential use in cancer diagnosis and treatment as well as prognostic and predictive biomarkers continues to increase (36).

Prognostic biomarkers such as β-tubulin, carbohydrate antigen 19-9 (CA19-9), cell-surface antigen CD44 (CD44), carcinoembryonic antigen, ColoPrint, circulating tumour cell, cyclin D1, E-cadherin, epidermal growth factor receptor (EGFR), inhibitor of growth protein 3 (ING3), Ki-67, matrix metalloproteinase-2, p-21, retinoblastoma gene, ribonucleotide reductase M1, and vascular endothelial growth factor (VEGF) are used to evaluate the malignant potential of tumours and measure the patients' OS and progression-free survival (PFS) without treatment and after conventional treatment. These biomarkers are applied to qualify patients for treatment, but they do not support the prediction of treatment outcomes (Table 1) (36).

Predictive biomarkers such as epidermal growth factor receptor 1 (EGFR1), excision repair cross-complementation group 1, O(6)-methylguanine-DNA methyltransferase, thymidyne phosphorylase, and phosphatase and tensin homolog support objective identification of individuals who are more likely to benefit from a given treatment or aid the evaluation of differences in the outcomes of two or more treatment procedures in view of their toxicity (Table 2) (36).

Some biomarkers, including breast cancer gene (BRCA1), carbonic anhydrase IX (CAIX), oestrogen receptor (ER), progesterone receptor (PR), tumour suppressor protein (p53), human epidermal growth factor receptor 2 (HER2/neu), and Kirsten rat sarcoma oncogene, have both prognostic and predictive relevance (36). Multigene panel tests are also used to identify groups of up to several dozen genes, mainly in the diagnosis of breast cancer, for which application MammaPrint (59) or Mammostrat (49) are examples of available assays.

The increased expression of COX-2 in various types of tumours, in particular in dogs, but also in cats, suggests possibilities for its utilisation in practice. Its introduction may be feasible into routine evaluation as a diagnostic, therapeutic, prognostic, and predictive biomarker in small-animal veterinary oncology especially, in like manner to how it is exploited to a certain extent in human oncology (5).

In human medicine, COX-2 overexpression in tumour patients is often associated with poor prognosis and reduced OS and/or PFS (30). The applicability of COX-2 in the diagnosis of canine tumours requires further research because the results of studies evaluating these associations and another between the overexpression and response to treatment are contradictory (19, 20). Correlations with poor prognosis and reduced OS have been observed in canine mammary gland carcinoma (47), whereas no such relationships have been reported in canine prostatic carcinoma (55). Queiroga *et al*. (47) evaluated COX-2 expression in canine mammary tumours to assess its prognostic significance and any connection with clinical and pathological parameters. They examined 129 mammary tumour samples from 57 bitches of various breeds aged 6–14 years, including 22 from dysplastic lesions, 40 from benign and 57 from malignant tumours, and 10 from inflammatory carcinomas. Thirteen samples from normal tissues were examined for comparison. COX-2 expression was found in all samples, but with various intensities – the lowest in normal tissues and the highest in inflammatory carcinomas. COX-2 expression increased with tumour malignancy. Directly proportional relationships were found between COX-2 expression and various clinico-pathological parameters – tumour size, skin ulceration, adherence to the tissues and skin, histological type, time of metastases and relapses, worse prognosis, and shorter PFS and OS, especially in inflammatory carcinomas. In a similar study, Millanta *et al*. (39) determined COX-2 expression in invasive mammary carcinomas in 47 queens aged 8.8 ± 2.5 years and 28 bitches aged 10.9 ± 2.7 years and also measured the expression of this enzyme in normal tissues to assess the relationship to clinico-pathological features and explore its prognostic aptitude. COX-2 expression was evaluated in relation to age, tumour size, histological type, blood vessel density, expression of ER and PR receptors, expression of Ki-67, HER-2 and VEGF, and OS. In both animal species COX-2 expression was not observed in normal tissues, but it was found in tumour cell cytoplasm in 100% (28/28) of bitches and 96% (45/47) of queens; in 79% of bitches and 81% of queens the COX-2 expression was rated as average to strong. In bitches, an increase in COX-2 expression was significantly correlated with overexpression of HER-2 and weak differentiation of tumour cells, but in queens with the status ER(−) and PR(+) and increased VEGF expression, it indicated higher malignancy. Heller *et al.* (25) selected 50 bitches and performed an evaluation of the interaction of COX-2 expression and various histological types of canine mammary carcinomas – adenocarcinoma, solid carcinoma, and anaplastic carcinoma. Expression of COX-2 was found in 56% (28/50) of cases, including 47% (17/37) of adenocarcinomas and 100% (11/11) of anaplastic carcinomas, while there was no expression (0/2) in solid carcinomas. The intensity of COX-2 expression (the immunohistochemical score – IHS) varied within the range 1–3 (average IHS 1.0) in adenocarcinomas and 2–12 (average IHS 5.1) in anaplastic carcinomas. These studies demonstrated that weakly differentiated tumours show stronger COX-2 expression than well-differentiated ones, *e.g.* adenocarcinomas. Queiroga *et al.* (48) examined the prognostic value of COX-2 expression in malignant canine mammary tumours in 27 bitches also through evaluation of correlation with clinico-pathological parameters such as tumour size, histological type, presence of necrosis, metastases to lymph nodes, and

PFS and OS. COX-2 expression was found in all cases over a broad range of IHS scores (3–12), but was predominantly noted at scores for stronger expression intensity (average IHS 8.8). Expression of COX-2 was significantly higher in tumours with metastases to lymph nodes, but a correlation between COX-2 expression and tumour size, histological type, or presence of necrosis was not noticed. However, the study showed a statistically significant correlation between strong COX-2 expression and shorter PFS and OS and worse prognosis.

Because limited veterinary literature is available regarding prognostic biomarkers for canine renal cell carcinoma (CRCC), Carvalho *et al.* (13) retrospectively evaluated COX-2 expression and histological and clinical features associated with the prognosis of CRCC in 64 cases in which nephrectomy had been required. COX-2 expression was significantly associated with overall median survival time (MST) – 420 days if the COX-2 score was > 3 versus 1176 days if it was < 3. The authors concluded that the addition of COX-2 immunostaining to standard histopathological evaluations would help to predict outcomes in CRCC patients. De Campos *et al*. (15) investigated several prognostic factors including COX-2 in feline mammary gland neoplasms, correlating them with OS. Immunoreactivity for COX-2 was higher in metastases than in primary tumours and was directly correlated with OS. The authors suggest that COX-2 inhibition may represent a therapeutic option for malignant feline mammary gland neoplasms. COX-2 scores should be analysed in primary tumours and metastases for a better understanding of disease outcome in patient conditions characterised by a poor prognosis. The recent study of Gregorio *et al*. (23) revealed a strict connection of COX-2 overexpression to OS in canine MCT. Confirmation of these findings could also be recognised in the results obtained by Carvalho *et al.* (12) on 109 cases of canine mammary tumours. High COX-2 expression was associated with more serious grades of malignancy, lymph node metastasis, and shorter OS. In a similar study, during investigation of several biomarkers including COX-2, Araujo *et al*. (2) found a concordance of COX-2 expression, worse prognosis and shorter OS in canine mammary primary carcinomas with lymph node metastasis. Nobrega *et al*. (43) evaluated the five biomarkers - factor VIII (FVIII), COX-2, vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA), and caspase-3 (casp-3) in relation to OS in 60 cases of canine cutaneous haemangiosarcoma (cHSA). Marker expression was positive in 80–100 % of samples, with weak to moderate labelling intensity for FVIII, COX-2, and VEGF and strong for PCNA and casp-3, but without any relationship to OS. The authors concluded that although expression of COX-2 and VEGF is frequent in canine cHSA, these possible therapeutic targets need further investigations for greater clarity about their potential in treatment.

Table 1. Prognostic biomarkers for survival in cancer medicine (36)

Prognostic biomarker	Type of cancer	Clinical significance	Detection	Clinical use
Beta-tubulin	NSCLC	High expression of β-tubulin confers worse prognosis	IHC	No
BRCA1	Breast	High expression of BRCA1 confers worse prognosis in untreated patients	IHC	Yes
	NSCLC	High expression of BRCA1 confers worse prognosis in untreated patients	qRT-PCR	No
CA19-9	Pancreatic	Higher preoperative CA19-9 levels are associated with lower resectability, more advanced stage and inferior survival I	IHC	No
CAIX	RCC	High expression of CAIX is associated with a better prognosis	IHC	No
CD44	Bladder	Expression of CD44 is associated with poor prognosis	qRT-PCR	No
CEA	CRC	Elevated preoperative CEA levels in resectable colorectal cancer is associated with poor prognosis	IHC	Yes
c-KIT	GIST	GIST patients have a better prognosis if they harbour a mutation in exon 11 of the c-KIT gene	Pathway detection via FDG-PET	Yes
ColoPrint	CRC	Prognosis for colorectal cancer patients	Microarray	Yes
CTC (*e.g.* CellSearch)	Melanoma	Increased number of circulating melanoma cells is associated with poor prognosis	Circulating tumour cells	No
	CRC	Colorectal patients with 3 CTC/7.5 ml of peripheral blood were associated with shorter PFS and OS, *i.e*. poor prognosis	Circulating tumour cells	Yes
	Breast	Breast cancer patients with 5 CTC/7.5 mL of peripheral blood are associated with shorter PFS and OS, *i.e*. poor prognosis 5 CTC/7.5 ml of peripheral blood is associated with poor prognosis	Circulating tumour cells	Yes
	Prostate		Circulating tumour cells	Yes
Cyclin D1	Bladder	Expression of Cyclin D1 is associated with low grade, low stage and recurrence	IHC	No
Cyclin E	Bladder	Expression of Cyclin E is associated with low stage and survival	IHC	No
E-Cadherin	Bladder	E-Cadherin is associated with poor prognosis	IHC	No

Continued on next page

Table 1 (continued)

EGFR	Bladder	Overexpression of EGFR is associated with high grade and high stage	IHC	No
	NSCLC	High gene copy number of EGFR in NSCLC patients is associated with poor prognosis EGFR mutation in NSCLC patients is associated with better prognosis in untreated patients	FISH / SA	No
	Rectal	Overexpression of EGFR in rectal cancers is also associated with poor prognosis	IHC	No
ER	Breast	Patients with ER-positive breast tumours have better survival than patients with hormonal negative tumours	IHC	Yes
eXageneBC	Breast	Provides prognosis in node-positive or node-negative breast cancer patients	FISH	Yes
Her2/neu	Breast	Patients with Her2/neu-positive breast tumours are more aggressive and have a worse prognosis compared to Her2/neu-negative tumours	FISH	Yes
	Bladder	Overexpression of Her2/neu is associated with high grade, high stage, poor survival and metastasis in bladder cancer	IHC	No
	GIST	Overexpression of Her2/neu in advanced gastric cancer patients is associated with poor prognosis	IHC	No
Her3	Melanoma	Correlation with increased cell proliferation, tumour progression and reduced survival in melanoma patients	IHC	No
ING3	Melanoma	Reduced nuclear expression associated with poor disease-specific survival in melanoma patients	IHC	No
ING4	Melanoma	Reduced levels of ING4 in melanoma patients is associated with melanoma thickness, ulceration and poor disease-specific survival and overall survival	IHC	No
Ki-67	Bladder	Expression of Ki-67 is associated with progression and recurrence in bladder cancer	IHC	No
	Breast	Expression of Ki-67 is associated with proliferation and progression in breast cancer	IHC	No
K-ras	NSCLC	K-ras mutation is associated with poor prognosis in NSCLC patients	SA	Yes
LOH at 18q	CRC	Associated with metastasis and poor prognosis in colorectal tumours	PCR	No
MammaPrint	Breast	A 70-gene prognostic assay used to identify breast cancer cases at the extreme end of the spectrum of disease outcome by identifying patients with good or very poor prognosis	Microarray	Yes
Mammostrat	Breast	This standard purely prognostic test uses five antibodies with manual slide scoring to divide cases of ER-positive, lymph node negative breast cancer tumours treated with tamoxifen alone into low-, moderate- or high-risk groups	IHC	Yes
MMP-2	Bladder	Expression of MMP-2 is associated with poor prognosis in bladder cancer patients	PCR	No
MSI status	CRC	High frequency MSI colorectal tumours are associated with better prognosis and show improved relapse-free survival	IHC	No
NCOA3	Melanoma	Increased levels in melanoma patients correspond to poor relapse-free survival and disease-free survival	IHC	No
Oncotype DX	Breast	A 21-gene multiplex test used for prognosis to determine 10-year disease recurrence for ER-positive, lymph node negative breast cancers using a continuous variable algorithm and assigning a tripartite recurrence score	qRT-PCR	Yes
p21	Bladder	Overexpression of p21 is associated with poor prognosis	IHC	No
p53	Bladder	Overexpression of p53 is associated with poor prognosis	IHC	No
	NSCLC	High expression of p53 in NSCLC patients confers worse prognosis in untreated patients	IHC	No
	NSCLC	p53 mutation in NSCLC patients is associated with worse prognosis	SA	No
PR	Breast	Patients with PR-positive breast tumours have better survival than patients with hormonal-negative tumours	IHC	Yes
Rb	Bladder	Overexpression of Rb is associated with poor prognosis	IHC	No
RRMI	NSCLC	High expression of RRMI in NSCLC patients confers better prognosis in untreated patients	AQUA	No
VEGF	RCC	Overexpression of VEGF is associated with poor prognosis in clear cell renal carcinoma patients	IHC	Yes

AQUA – automated quantitative analysis; CA19-9 – carbohydrate antigen 19-9; CAIX – carbonic anhydrase IX; CEA – carcinoembryonic antigen; CRC – colorectal tumour; CTC – circulating tumour cells; EGFR – epidermal growth factor receptor; ER – oestrogen receptor; FDG – 18F-fluorodeoxyglucose; FISH – fluorescent in situ hybridisation; GIST – gastrointestinal stromal tumour; IHC – immunohistochemistry; LOH – loss of heterozygosity; MMP-2 – matrix metalloproteinase-2; MSI – microsatellite instability; NSCLC – non-small cell lung cancer; OS – overall survival; PET – Positron emission tomography; PFS – progression-free survival; PR – progesterone receptor; qRT-PCR – quantitative real time polymerase chain reaction; Rb b – retinoblastoma; RCC – renal cell carcinoma; RRMI – ribonucleotide reductase messenger 1; SA – sequence analysis; VEGF – vascular endothelial growth factor

Table 2. Predictive biomarkers for treatment selection in cancer medicine (36)

Predictive biomarker	Type of cancer	Clinical significance	Detection	Clinical use
BRCA1	NSCLC	High expression of BRCA1 in NSCLC patients predicts resistance to cisplatin-based chemotherapy	qRT-PCR	No
	Breast	High expression of BRCA1 in breast cancer can predict response to chemotherapy	IHC	Yes
CAIX	RCC	Expression of CAIX in renal cell carcinoma is predictive of sensitivity of treatment with interleukin-2 therapy	IHC	No
c-KIT	GIST	GIST patients carrying the mutation on exon 11 of the c-KIT gene benefit from imatinib and sunitinib treatment, however most patients develop resistance to these over time	SA	Yes
EGFR1	NSCLC	EGFR1 mutations in patients with NSCLC are predictive for response to either gefitinib or erlotinib treatment	IHC	Yes
	CRC	EGFR1 gene amplification appears to be a predictive factor for response to anti-EGFR1 antibody treatment in CRC	PCR	Yes
ER	Breast	High cellular expression of ER predicts benefit from tamoxifen-based chemotherapy	IHC	Yes
ERCC1	NSCLC	High expression of ERCC1 in NSCLC patients predicts resistance to cisplatin-based chemotherapy	IHC	No
Her2/neu	Breast	Breast cancer patients with Her2/neu overexpressing tumors benefit from treatment with trastuzumab in the metastatic as well as in the adjuvant setting	FISH	Yes
	Gastric	Expression of Her-2/Neu in gastric cancer is predictive of patient sensitivity towards treatment with 5-FU, doxorubicin, trastuzumab and platinum-based chemotherapy	FISH	No
K-ras	NSCLC	K-ras mutation positivity in NSCLC patients predicts lack of benefit from adjuvant chemotherapy in early disease and resistance to treatment with EGFR TKI in advanced disease	SA	Yes
	CRC	K-ras mutation positivity in stage IV CRC patients predicts considerably less benefit from EGFR-specific antibody like cetuximab and panitumumab	PCR	Yes
LOH at 18q	CRC	Useful in identifying patients with resected stage III colon cancer most likely to benefit from 5-FU based adjuvant chemotherapy	PCR	No
MGMT	Glioblastoma	Methylation of MGMT promoter is predictive of sensitivity of glioblastoma to temozolomide	PCR	No
NuvoSelect	Breast	A combination of several pharmacogenomic genesets used primarily to guide selection of therapy in breast cancer patients. This test also provides the ER and HER2 mRNA status	Microarray	Yes
p53	NSCLC	High p53 expression in NSCLC patients predicts sensitivity to cisplatin-based chemotherapy, however p53 mutation is predictive of resistance to cisplatin-based chemotherapy	IHC/SA	No
PR	Breast	High cellular expression of PR predicts benefit from tamoxifen-based chemotherapy	IHC	Yes
Roche AmpliChip	Breast	Low expression of CYP2D6 predicts resistance to tamoxifen-based chemotherapy in breast cancer patients	Microarray	Yes
Rotterdam Signature	Breast	A 76-gene assay used to predict recurrence in ER-positive breast cancer patients treated with tamoxifen	Microarray	Yes
RRMI	NSCLC	High expression of RRM1 in NSCLC patients predict resistance to cisplatin-based chemotherapy	qRT-PCR	No
TP	GIST	Predictive of sensitivity of treatment to 5-FU- and capcetabine-based chemotherapy in gastric cancer patients	IHC/PCR	No
	CRC	Expression of TP in metastatic colorectal patients is predictive of sensitivity of treatment to 5-FU and capcetabine based chemotherapy	IHC/qRT-PCR	No
PTEN	Breast	PTEN mutation can result in reduced sensitivity of treatment with trastuzumab in breast cancer patients	IHC	No

CAIX – carbonic anhydrase IX; CRC – colorectal tumour; EGFR – epidermal growth factor receptor; ER – oestrogen receptor; ERCC1 – excision repair cross-complementation group 1; FISH – fluorescent *in situ* hybridisation; GIST – gastrointestinal stromal tumour; IHC – immunohistochemistry; LOH – loss of heterozygosity; MGMT – O6-methylguanine-DNA methyltransferase; NSCLC – non-small cell lung cancer; PCR – polymerase chain reaction; PR – progesterone receptor; RRMI – ribonucleotide reductase messenger 1; qRT-PCR – quantitative real-time polymerase chain reaction; RCC – renal cell carcinoma; SA – sequence analysis; TK1 – tyrosine kinase inhibitor; TP – thymidine phosphorylase

Clinical significance of inhibition of COX-2 overexpression in tumours

Experimental, clinical, and epidemiological studies have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs), in particular selective COX-2 inhibitors (coxibs), effectively inhibit tumour progression and improve chemotherapy outcomes in human patients (16). Specific COX-2 inhibitors, including celecoxib and rofecoxib, have been developed to minimise some mainly gastrointestinal side-effects of NSAIDs. In addition to anti-inflammatory, analgesic

and antipyretic effects, these compounds also deliver anticarcinogenic effects by inhibiting the production of prostanoids. In some cases, however, anticarcinogenic effects were observed independently of COX-2 inhibition. Tamura *et al.* (58) examined the antitumour effects of celecoxib in an AZACB canine mammary tumour cell line and utilised a cell line expressing low levels of COX-2 to minimise its effect on celecoxib's activity. They revealed that celecoxib downregulated COX-2 expression, induced cell apoptosis and inhibited cell proliferation mainly *via* COX-2-independent mechanisms. The results of these studies suggest that celecoxib might be used in the treatment of canine mammary tumours regardless of COX-2 expression, also in combination with other antitumour agents. This discovery has led to the development of structural analogs such as dimethyl-celecoxib (DMC), which effectively inhibits cell proliferation and induces apoptosis through the downregulation of survivin and cyclins A and B and the ensuing loss of cyclin-dependent kinase activity. DMC does not provoke the side-effects associated with COX-2 inhibition; however, further research on it and the compounds of its type is required (29).

The discovery that coxibs possess anticarcinogenic properties laid the groundwork for clinical research in human oncology, which initially focused on coxibs' chemopreventive and subsequently on its chemotherapeutic effects. Initial studies demonstrated that coxibs are effective in the treatment of familial adenomatous polyposis (FAP), but subsequent large-population research programmes revealed that coxibs have significant cardiovascular side-effects. Due to safety concerns, rofecoxib has been withdrawn from the pharmaceutical market, and celecoxib is presently prescribed only as a chemopreventive agent for FAP (3). However, a review of 72 research programmes carried out by Harris (24) did not confirm those concerns and found that coxibs caused side-effects only in patients with a higher risk of cardiovascular diseases.

The therapeutic effects of NSAIDs in cancer treatment have been confirmed by numerous studies which investigated the combined application of NSAIDs, radiotherapy, and chemotherapy in human patients (34). Overexpression of COX-2 has also been observed in some canine and feline tumours, and research findings indicate that this enzyme could be more widely used as a biomarker in veterinary medicine, in the diagnosis and treatment of cancer with the use of COX-2 inhibitors (39). This biomarker could be applied to identify patients where the use of non-selective and, in particular, selective COX-2 inhibitors could reduce COX-2 overexpression, limit tumour progression and increase survival rates (16, 34).

The use of NSAIDs in the treatment of canine and feline tumours has been investigated by relatively few studies, which, nevertheless, produced interesting results. Boria *et al*. (9) found that cisplatin administered in combination with piroxicam induced remission in five out of nine dogs with oral SCC and in two out of eleven dogs with oral malignant melanoma. Schmidt *et al*. (54) observed that piroxicam administered *per os* at 0.3 mg/kg/day induced remission in 3 out of 17 dogs and inhibited tumour growth in 5 out of 17 dogs with oral SCC. In a similar study, which was conducted to assess COX-2 expression in feline oral SCC and the COX-2-inhibitory activity of piroxicam in carcinoma-afflicted cats, Di Bernardi *et al*. (18) found that piroxicam at a dose of 0.3 mg/kg b.w. daily was a potentially beneficial treatment option for cats with oral SCC and with COX-2 overexpression in cancer cells and would be a notable improvement to current therapy. In a study performed on a canine model of human invasive urinary bladder cancer, Knapp *et al*. (33) demonstrated that cisplatin administered intravenously at 60 mg/m^2 every 21 days in combination with piroxicam (0.3 mg/kg/day *per os*) induced remission in 10 out of 14 dogs with invasive TCC of the urinary bladder but none was observed in the animals administered cisplatin only. Piroxicam was found to reduce tumour size, induce apoptosis, and reduce angiogenesis in 12 out of 18 dogs with urothelial TCC in research by Mohammed *et al.* (40). Feline TCC of the urinary bladder improved clinically when treated with meloxicam in findings made by Bommer *et al.* (8), who saw reduction of haematuria and/or dysuria with MST of 311 days. COX-2 expression was associated with MST, which in COX-2-positive cats was 123 days and for COX-2-negative cases was 375 days. Itturiaga *et al*. (26), examining the influence of low-dose meloxicam (0.25 µg/mL) on CF41.Mg canine mammary carcinoma cells, noticed that cell migration and invasion were significantly reduced and suggested that meloxicam has a potential adjunctive therapeutic application useful in controlling the invasion and metastasis of canine mammary carcinoma. Similarly, Pang *et al*. (45) compared the *in vitro* action of the short-acting non-selective COX inhibitor carprofen with that of the long-acting selective COX-2 inhibitor mavacoxib on cancer cells and cancer stem cell survival. They observed that mavacoxib increases apoptosis in cancer cells and has an inhibitory effect on cell proliferation and migration, but they suggest that these anti-tumour effects of mavacoxib warrant further study. King *et al*. (31) evaluated the safety of NSAID COX-2 inhibitor robenacoxib in healthy young beagle dogs and found no adverse effects of this highly selective COX-2 inhibitor administered orally once daily even at a highest dose of 40 mg/kg b.w. for one month and 10 mg/kg b.w. for six months. They also found that application of robenacoxib was associated with marked inhibition of COX-2. Similar results were obtained by King *et al*. (32) in healthy young short-haired cats in a study concerning the safety of oral robenacoxib and inhibition of COX-2. Arenas *et al.* (4) researched adjuvant therapy and evaluated the disease – free survival (DFS) and OS of COX-2 inhibitor firocoxib *versus* those of chemotherapy with mitoxantrone in dogs with highly malignant canine mammary tumours (HM-CMTs) and those of control dogs in a case-control prospective study. They noticed that dogs receiving

firocoxib treatment had statistically higher DFS and OS than control dogs. The DFS and OS of dogs medicated with mitoxantrone were, however, not statistically different from those of the controls. The authors concluded that their study supported the use of firocoxib for the treatment of HM-CMTs, but that further studies were needed to compare the efficacy of chemotherapy drugs with that of COX-2 inhibitors as adjuvant treatment in such cases in dogs.

Conclusion

Although there are several pieces of evidence supporting an important role of COX-2 in tumour development and progress in humans and animals, further studies are necessary to explore its significance. Subsequent investigation will elucidate the agency of COX-2 in oncogenesis, determine COX-2 expression levels in various types of canine and, in particular, feline tumours, assess the diagnostic, therapeutic, prognostic, and predictive relevance of this biomarker precisely, and evaluate the usefulness of NSAIDs in the chemoprevention and chemotherapy of canine and feline tumour patients.

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