Histol Histopathol (2005) 20: 1309-1312

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

COX-2 overexpression in canine tumors: potential therapeutic targets in oncology

E.P. Spugnini[1], A. Porrello[2], G. Citro[1] and A. Baldi[1,3]

[1]SAFU Department, Center for Experimental Research, Regina Elena Cancer Institute, Rome, italy,

[2]Molecular Oncogenesis Laboratory, Department of Experimental Oncology, Regina Elena Cancer institute, Rome, Italy and

[3]Department of Biochemistry, Section of Pathology, Second University of Naples, Naples, Italy

Summary. Cyclooxygenases catalyze the initial, rate-limiting steps of prostaglandin synthesis from arachidonic acid. Two isoforms of this enzyme exist in mammalian and avian species: COX-1 and COX-2. COX-1 is constitutively expressed and is the major isoform of gastrointestinal tissue. COX-2 is induced in response to inflammatory stimuli. COX-2 has been implicated in carcinogenesis of several neoplasms. Furthermore, COX-2 over-expression has been noted in many solid tumours and has been correlated with a worse prognosis in colorectal cancer, non-small-cell lung cancer, mesothelioma and gastric cancer. In this review, the most recent findings on the mechanisms by which COX-2 promote tumorigenesis are discussed, with particular emphasis on the studies involving spontaneous canine neoplasms.

Key words: COX-2, Canine tumors, Chemotherapy

The Cox enzyme and cancer

The enzyme prostaglandin G/H synthase, also named cyclooxygenase (COX), is responsible for the first rate limiting step of the synthesis of all the prostaglandins from arachidonic acid (Smith, 1992). The enzyme is a homodimer composed of two subunits of 70000 Daltons and heme group (van der Ouderaa et al., 1979) and has two catalytic activities that mediate the conversion of arachidonic acid into several different prostanoids. Two isoforms of Cox have been identified and have been named Cox-1 and Cox-2 (Williams and DuBois, 1996), they share many similarities at a protein level but are encoded by different genes and are transmitted by different RNA messengers. The expression of the two isoforms is remarkably different in that Cox-1 is present in a broad spectrum of tissues and is referred to as the constitutive form, while Cox-2 is generally undetectable in most tissues and is synthesized under the influence of a variety of agonists, thus being referred to as the inducible form, with the exceptions of the adult kidney and the central nervous system (Nasir et al., 1998; Ostrom et al., 2001; Teather et al., 2003) It is generally believed that Cox-1 is involved in maintaining tissue omeostasis, whereas Cox-2 is involved in inflammatory and immune responses (Vane et al., 1998) Cox-2 is strongly induced by proinflammatory cytokines, growth factors, carcinogens and oncogenes. (Subbaramaiah et al., 1996; Mestre et al., 1997). Of interest, Cox-2 is upregulated in the early phases of oncogenesis (Nathan et al., 2001) and is strongly expressed by several tumors in humans. In particular, it seems that epithelial neoplasms are particularly prone to express high levels of the inducible form; high levels of the enzyme have been evidenced in colorectal cancer, gastric carcinoma, squamous cell carcinoma of the neck, bladder carcinoma, carcinoma of the cervix and endometrium, thyroid carcinoma, and mammary carcinoma (Ristimaki et al., 1997; Chan et al., 1999; Hao et al., 1999; Reddy et al., 2002; Mohammed et al., 1999; Kulkarni et al., 2001; Half et al., 2002; Specht et al., 2002). Few non epithelial neoplasms have been found to express Cox-2 so far, in particular chodrosarcoma and malignant mesothelioma (Baldi et al., 2004; Sutton et al., 2004). Several studies pointed out the multiple actions played by Cox-2 overexpression in cancer such as increased production of PGE2 that contributes to the malignant phenotype (Parhar and Lala, 1988; Brock et al., 1999) and causes impairment of the immune system (Sharma et al., 2003), as well as regulation of cell cycle molecules in some tumors (Baldi et al, 2004). Cox-1 and Cox–2 are targeted by a broad variety of non steroidal anti-inflammatory inhibitors (NSAIDs). Cox-2 inhibitors, and among others, piroxicam, are currently used as anti-inflammatory drugs, primarily to treat arthritis in men (Ando and Lombardino, 1983). Of interest, anti-Cox-2 molecules have shown anti-tumor activity in chemically induced murine neoplasm,

Offprint requests to: Dr. Enrico Spugnini, SAFU Department, Regina Elena Cancer Institute, Via delle Messi d'Oro 156, 00158 Rome, Italy. e-mail: enrico.spugnini@tiscali.it

(Pollard et al., 1983; Nigro et al., 1986; Reddy et al., 1987; Pollard and Luckert, 1989) and in orthotopic mouse models of malignant mesothelioma and prostate carcinoma (Spugnini E.P., and Baldi A. unpublished). Finally, epidemiologic studies evidenced the protective effect of NSAIDs in breast, bladder and colon cancer. (Schreinemachers and Everson, 1994; Castelao et al., 1999; Garcia-Rodriguez et al., 2001; Reddy and Rao, 2002).

Cox-2 in spontaneous canine tumors

Spontaneous neoplasms of companion animals are a formidable and still underexploited tool to make rapid advances in cancer therapy by testing new compounds and delivery systems that have shown promise in vitro (Knapp et al., 1997, 2000; Spugnini and Porrello, 2003; Porrello et al., 2004). The advantages of this model system have been reviewed (MacEwen, 1990; Hahn et al., 1994; Vail and Mac Ewen, 2000) and include: 1) similarities between specific types of canine and human cancer with regards to histopathological appearance, biological behavior and response to therapy, 2) significant similarity in drug metabolism between dogs and humans, 3) pet owners' willingness to enter their tumor bearing dogs in humanely conducted clinical trials due to the absence of a well defined "standard" therapy for many canine cancers, 4) the compressed lifespan of dogs, which allows the completion of clinical trials in a timely manner, 5) the fact that dogs share the same environment as their owners but lack their unhealthy habits such as alcohol consumption and cigarette smoking which act as "confounding" factors in many clinical and epidemiological studies, 6) the larger size of dogs compared to rodents which makes it feasible to perform many medical procedures, 7) novel interventional strategies developed in vitro or in laboratory animal studies can be tested in dogs affected by cancer, whereas similar studies might be unacceptable or less feasible in human patients, especially when a standard, yet only partially effective treatment exists.

Several studies have investigated the expression of Cox-2 in canine tumors. In table 1 data available from the scientific literature are summarized.

A phase I study on piroxicam in 62 dogs affected by a broad spectrum of neoplasms documented 8 partial remissions (3/10 transitional cell carcinoma (TCC), 3/5 squamous cell carcinoma (SCC), 1/3 mammary carcinoma, 1 transmissible venereal tumor), suggesting a Cox-2 inhibition mechanism at the base of the antitumor response (Knapp et al., 1992). A second study was performed to better assess the mechanisms of Cox-2 inhibition in a canine model of TCC. In that investigation, the levels of PGE2 were inversely related to the tumor response to therapy while a correlation with the activity of the natural killer cells was not observed (Knapp et al., 1994). Further studies confirmed the effectiveness of piroxicam at inhibiting TCC in dogs (Knapp et al., 2000; Henry et al., 2003). Prostaglandin E2 concentrations have been measured in tumor tissue samples and in the culture medium of several canine cancer cell lines; the results showed that high levels were present in TCC, prostate carcinoma and osteosarcoma derived cell lines (Mohammed et al., 2001). Histopathology studies have identified expression of Cox-2 in sections of canine primary tumors, in particular prostatic adenocarcinoma, TCC, SCC, mammary carcinoma, renal cell carcinoma, intestinal epithelial neoplasms, and epithelial nasal tumors (Tremblay et al., 1999; Khan et al., 2000, 2001; Pestili de Almeida et al., 2001; McEntee et al., 2002; Dorè et al., 2003; Kleiter et al., 2004). These studies helped to identify potential targets for anti-Cox-2 therapy, leading to hypothesize the possible use of such molecules as radiosensitizers or in synergy with chemotherapy and immunotherapy. A paper recently published suggests a possible correlation between Cox-2 levels in appendicular osteosarcomas and overall survival time (Mullins et al., 2004). Of interest, one of the authors (EPS) observed a complete remission in a mammary inflammatory carcinoma and two complete and two partial remissions (lasting 6 and 14 months, respectively) in four dogs with osteosarcoma. In all the patients piroxicam has been administered as anti-inflammatory and pain killer to palliate the symptoms related to the neoplasms; these results are very interesting, especially the response of the inflammatory carcinoma that is an incurable cancer in women. It would be worth investigating the expression of such metabolic pathways in these cancers, because it could lead to better targeted therapies. The only prospective study on the effects of piroxicam in a canine TCC model, available in literature, suggested that tumor response to anti-Cox-2 drugs was related to induction of apoptosis and reduction of angiogenic growth factors as indicated by the reduction of basic growth factors concentration in the urine (Mohammed et al., 2002). Finally, only one recent paper

Table 1. Pooling of canine neoplasms expressing positivity for Cox-2.

TUMOR TYPE	TUMOR EXPRESSING COX-2	REFERENCES
TCC	54/61	Mohammed 1999, 2001; Khan, 2000
SCC oral	47/50	Pestili de Almeida, 2001
Intestinal adenoma	13/20	McEntee, 2002
Intestinal carcinoma	7/15	McEntee, 2002
Nasal carcinoma	17/21	Kleiter, 2004
Renal cell carcinoma	3/3	Khan, 2001
Prostatic carcinoma	23/30	Tremblay, 1999
Mammary adenoma	15/63	Dore, 2003
Mammary carcinoma	55/92	Mohammed, 2001; Dore, 2001
Osteosarcoma	38/59	Mohammed, 2001; Mullins, 2004
Melanoma	5/10	Mohammed, 2001

TCC: Transitional cell carcinoma; SCC: squamous cell carcinoma.

investigated the expression of Cox-2 in feline tumors, of interest, very few tumor types were positive for the enzyme, positivity was restricted to 7 out of 19 TCC and 2 out of 21 SCC. Pulmonary, mammary, and intestinal carcinomas were all negative as well as sarcomas, this might reflect interspecies differences or could be ascribed to the smallness of the sample tested (Beam et al., 2003)

In conclusion, studies on the expression of Cox-2 in tumors of companion animals and its inhibition should warrant further investigation in order to gain insights into the mechanisms involved at a gene level (e.g. p53 expression) and to have better therapeutic protocols.

Acknowledgements This work was supported by Grant "Project 2004" of the Italian Ministry of Health to G.C. and by an AIRC grant to A.B.

References

Ando G.A. and Lombardino J.G. (1983). Piroxicam: a literature review of new results from laboratory and clinical studies. Eur. J. Rheumatol. Inflamm. 6, 3-23.

Baldi A., Santini D., Vasaturo F., Santini M., Vicidomini G., Di Marino M.P., Esposito V., Groeger A.M., Liuzzi G., Vincenzi B., Tonini G., Piccoli M., Baldi F. and Scarpa S. (2004). Prognostic significance of cyclooxygenase-2 (COX-2) and expression of cell cycle inhibitors p21 and p27 in human pleural malignant mesothelioma. Thorax 59, 428-433.

Beam S.L., Rassnick K. M., Moore A.S. and McDonough S.P. (2003). An immunohistochemical study of cyclooxygenase-2 expression in various feline neoplasms. Vet. Pathol. 40, 496-500.

Brock T.G., McNish R.W. and Peters-Golden M. (1999). Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E2. J. Biol. Chem. 274, 11660-11666.

Castelao J.E., Yuan J.M., Gago-Dominguez M., Yu M.C. and Ross R.K. (2000). Nonsteroidal anti-inflammatory drugs and bladder cancer prevention. Br. J. Cancer 82, 1364-1369.

Chan G., Boyle J.O., Yang E.K., Zhang F., Sacks P.G., Sha J.P., Edelstein D., Soslow R.A., Koki A.T., Woerner B.M., Mansferrer J.L. and Dannenberg A.J. (1999). Cycloxoygenase-2 expression is up-regulated in squamous cell carcinoma of head and neck. Cancer Res. 59, 991-994.

Dore M., Lanthier I. and Sirois J. (2003). Cyclooxygenase-2 expression in canine mammary tumors. Vet. Pathol. 40, 207-212.

Garcia-Rodriguez L.A. and Huerta-Alvarez C. (2001). Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal anti-inflammatory drugs. Epidemiology 12, 88-93.

Hahn K.A., Bravo L., Adams W.H. and Frazier D.L. (1994). Naturally occurring tumors in dogs as comparative models for cancer therapy research. In Vivo 8, 133-143.

Half E., Tang XM., Gwyn K., Sahin A., Wathen K. and Sinicrope F.A. (2002). Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma in situ. Cancer Res. 62, 1676-1681.

Hao X., Bishop A.E., Wallace M., Wang H., Willcocks T.C., Maclouf J., Polak J.M., Knight S. and Talbot I.C. (1999). Early expression of cyclooxyenase-2 during sporadic colorectal carcinogenesis. J. Pathol. 187, 295-301.

Henry C.J., McCaw D., Turnquist S.E., Tyler J.W., Bravo L., Sheafor S., Straw R.C., Dernell W.S., Madewell B.R., Jorgensen L., Scott M.A., Higginbotham M.L. and Chun R. (2003). Clinical evaluation of mitoxantrone and piroxicam in a canine model of human invasive urinary bladder carcinoma. Clin. Cancer Res. 9, 906-911.

Khan K.N., Venturini C.M., Bunch R.T., Brassard J.A., Koki A.T., Morris D.L., Trump B.F., Maziars J.J. and Alden C.L. (1998). Interspecies differences in renal localization of cyclooxygenase isoforms; implication in nonsteroidal anti-inflammatory drug-related nephrotoxicity. Toxicol. Pathol. 5, 612-620.

Khan K.N., Knapp D.W., Denicola D.B. and Harris R.K. (2000). Expression of cyclooxygenase-2 in transitional cell carcinoma of the urinary bladder in dogs. Am. J. Vet. Res. 61, 478-481.

Khan K.N., Stanfield K.M., Trajkovic D. and Knapp D.W. (2001). Expression of cyclooxygenase-2 in canine renal cell carcinoma. Vet. Pathol. 38, 116-119.

Kleiter M., Malarkey D.E., Ruslander D.E. and Thrall D.E. (2004). Expression of cyclooxygenase-2 in canine epithelial nasal tumors. Vet. Radiol. Ultrasound 45, 255-260.

Knapp D.W. and Waters D.J. (1997). Naturally occurring cancer in pet dogs: important models for developing improved cancer therapy for humans. Mol. Med. Today 3, 8-11.

Knapp D.W., Richardson R.C., Bottoms G.D., Teclaw R. and Chan T.C. (1992). Phase I trial of piroxicam in 62 dogs bearing naturally occurring tumors. Cancer Chemother. Pharmacol. 29, 214-218.

Knapp D.W., Richardson R.C., Chan T.C., Bottoms G.D., Widmer W.R., De Nicola D.B., Teclaw R., Bonney P.L. and Kuczek T. (1994). Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. J. Am. Vet. Med. Assoc. 8, 273-278

Knapp D.W., Glickman N.W., Widmer W.R., De Nicola D.B., Adams L.G., Kuczek T., Bonney P.L., De Gortari A.E., Han C. and Glickman L.T. (2000a). Cisplatin versus cisplatin combined with piroxicam in a canine model of human invasive urinary bladder cancer. Cancer Chemother. Pharmacol. 46, 221-226.

Knapp D.W., Glickman N.W., De Nicola D.B., Bonney P.L., Lin T. and Glickman L.T. (2000b). Naturally-occurring canine transitional cell carcinoma of the urinary bladder. Urol. Oncol. 5, 47-59.

Kulkarni S., Rader J.S., Zhang F., Liapis H., Koki A.T., Mansferrer J.L., Subbaramaiah K. and Dannenberg A.J. (2001). Cyclooxygenase-2 is overexpressed in human cervical cancer. Clin. Cancer Res. 7, 429-434.

Mestre J.R., Subbaramaiah K., Sacks P.G., Schantz S.P., Tanabe T., Inoue H. and Danneberg A.J. (1997). Retinoids suppress epidermal growth factor-induced transcription of cyclooxygenase-2 in human oral squamous carcinoma cells. Cancer Res. 57, 2890-2895.

Mac Ewen E.G. (1990). Spontaneous tumors in dogs and cats: models for the study of cancer biology and treatment. Cancer Met. Rev. 9, 125-136.

McEntee M.F., Cates J.M. and Neilsen N. (2002). Cyclooxygenase-2 expression in spontaneous intestinal neoplasia of domestic dogs. Vet. Pathol. 39, 428-436.

Mohammed S.I., Knapp D.W., Bostwick D.G., Foster R.S., Khan K.N., Masferrer J.L., Woerner B.M., Snyder P.W. and Koki A.T. (1999). Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. Cancer Res. 59, 5647-5650.

Mohammed S.I., Coffman K., Glickman N.W., Hayek M.G., Waters D.J., Schlittler D., De Nicola D.B. and Knapp D.W. (2001). Prostaglandin E2 concentrations in naturally occurring canine cancer.

Prostaglandins Leukot. Essent. Fatty Acids 64, 1-4.

Mohammed S.I., Bennett P.F., Craig B.A., Glickman N.W., Mutsaers A.J., Snyder P.W., Widmer W.R., De Gortari A.E., Bonney P.L. and Knapp D.W. (2002). Effects of the cylooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. Cancer Res. 62, 356-358.

Mullins M.N., Lana S.E., Dernell W.S., Ogilvie G.K., Withrow S.J. and Ehrhart E.J. (2004). Cyclooxygenase-2 expression in canine appendicular osteosarcoma. J. Vet. Intern. Med. 18, 859-865.

Nathan C.A., Leskov I.L., Lin M., Abreo F.W., Shi R., Hartman G.H. and Glass J. (2001). COX-2 expression in dysplasia of the head and neck. Cancer 92, 1888-1895.

Nigro N.D., Bull A.W. and Boyd M.E. (1986). Inhibition of intestinal carcinogenesis in rats: effects of difluoromethylornithine with piroxicam or fish oil. J. Natl. Cancer Inst. 77, 1309-1313.

Ostrom R.S., Gregorian C., Drenan R.M., Gabot K., Rana B.K. and Insel P.A. (2001). Key role for constitutive cyclooxygenase-2 of MDCK cells in basal signaling and response to released ATP. Am. J. Physiol. Cell. Physiol. 281, C524-C531.

Parhar R.S. and Lala P.K. (1988). Prostaglandin E2 mediated inactivation of various killer lineage cells by tumor-bearing host macrophages. J. Leuk. Biol. 44, 474-484.

Pestili de Almeida E.M., Piche C., Sirois J. and Dore M. (2001). Expression of cyco-oxygenase-2 in naturally occurring squamous cell carcinomas in dogs. J. Histochem. Cytochem. 49, 867-875.

Pollard M. and Luckert P.H. (1989). Prevention and treatment of primary intestinal tumors in rats by piroxicam. Cancer Res. 49, 6471-6473.

Pollard M., Luckert P.H. and Schmidt M.A. (1983). The suppressive effect of piroxicam on autochtonous intestinal tumors in the rat. Cancer Lett. 25, 57-61.

Porrello A., Cardelli P. and Spugnini E.P. (2004). Pet models in cancer research: general principles. J. Exp. Clin. Cancer Res. 23, 181-193.

Reddy B.S., Maruyama H. and Kelloff G. (1987). Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. Cancer Res. 47, 5340-5346.

Reddy B.S. and Rao C.V. (2002). Novel approaches for colon cancer prevention by cyclooxygenase-2 inhibitors. J. Environ. Pathol. Toxicol. Oncol. 21, 155-164.

Ristimaki A., Honkanen N., Jankala H., Sipponen P. and Harkonen M. (1997). Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res. 57, 1276-1280.

Schreinemachers D.M. and Everson R.B. (1994). Aspirin use and lung, colon and breast cancer in a prospective study. Epidemiology 5, 138-146.

Sharma S., Stolina M., Yang S., Baratelli F., Li J., Atianzar K., Luo J., Zhu L., Lin Y., Huang M., Dohadw A., Batra R. K. and Dubinett S.M. (2003). Tumor cyclooxygenase-2 dependent suppression of dendridic cell fuction. Cancer Res. 9, 961-968.

Smith W.L. (1992). Prostanoid biosynthesis and mechanisms of action. Am. J. Physiol. 263, F181-F191

Specht M.C., Tucker O.N., Hocever M., Gonzales D., Teng L. and Fahey T.J. (2002). Cycooxyenase-2 expression in thyroid nodules. J. Clin. Endocrinol. Metab. 87, 358-363.

Spugnini E.P. and Porrello A. (2003). Potentiation of chemotherapy in companion animals with spontaneous large neoplasms by application of biphasic electric pulses. J. Exp. Clin. Cancer Res. 22, 571-580.

Subbaramaiah K., Telang N., Ramonetti J.T., Araki R., de Vito B., Weksler B.B. and Dannenberg A.J. (1996). Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res. 56, 4424-4429.

Sutton K.M., Wright M., Fondren G., Towle C.A. and Mankin H.J. (2004). Cyclooxygenase-2 expression in chondrosarcoma. Oncology 66, 275-280.

Teather L.A. and Wurtman R.J. (2003). Cyclooxygenase-2 mediates platelet-activating factor-induced prostaglandin E2 release from rat primary astrocytes. Neurosc. Lett. 340, 177-180.

Tremblay C., Dore M., Bochsler P.N. and Sirois J. (1999). Induction of prostaglandin G/H synthase-2 in a canine model of spontaneous prostatic adenocarcinoma. J. Natl. Cancer Inst. 91, 1398-1403.

Vail D.M. and Mac Ewen E.G. (2000). Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest. 18, 781-792.

van der Ouderaa F.J., Buytenhek M., Slikkerveer F.J. and van Dorp D.A. (1979). On the haemoprotein character of prostaglandin endoperoxide synthetase. Biochim. Biophys. Acta 572, 29-42.

Vane J.R., Bakhle Y.S. and Botting R.M. (1998). Cyclooxygenases 1 and 2. Annu. Rev. Pharmacol. Toxicol. 38, 97-120.

Williams C.S. and Du Bois R.N. (1996). Prostaglandin endoperoxide synthase: why two isoforms? Am. J. Physiol. 270, G393-G400.

Accepted May 30, 2005