

## Cyclooxygenase-2 Expression in Human Soft-tissue Sarcomas is Related to Epithelial Differentiation

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**Abstract.** *Background:* Cyclooxygenase-2 (Cox-2) is expressed by several types of epithelial malignancies, i.e., carcinomas, and inhibition of Cox-2 may have a therapeutic role in chemoprevention and treatment of cancer. The role of Cox-2 in non-epithelial malignancies, however, is unclear. *Materials and Methods:* We investigated, by immuno-histochemistry, the expression of Cox-2 in 103 human soft-tissue sarcomas. *Results:* All 10 biphasic synovial sarcomas were positive for Cox-2, but positivity was observable only in the epithelial component of these tumours. Excluding sarcomas with epithelial differentiation, uniform staining of the tumour was observed in only 2 samples. In addition, positivity for Cox-2 appeared in tumour cells in only 18 samples around necrotic areas. *Conclusion:* In human soft-tissue sarcomas, Cox-2 expression seems to be associated with epithelial differentiation and, in some types of sarcomas, to be expressed in otherwise negative tumours at sites of necrosis.

Cyclooxygenase (Cox), also known as prostaglandin H synthase or prostaglandin endoperoxide synthase, is the key enzyme in the conversion of arachidonic acid to prostanoids (1). Two Cox genes have been cloned: Cox-1 is a constitutive enzyme produced constantly in most tissue types, and is probably responsible for the production of prostanoids under physiological conditions. Cox-2, originally cloned from chicken embryo fibroblasts transformed by Rous sarcoma virus (2), is undetectable in most normal tissues, but can be induced in various cell types by pro-

inflammatory agents, growth factors and carcinogens (3, 4). The expression of Cox-2 has been associated with chronic inflammation and carcinogenesis (1, 3-5).

Epidemiological studies indicate that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the incidence of cancer in the gastrointestinal tract (3, 5, 6). For NSAIDs, Cox-2 is an important target, and several types of carcinomas express elevated levels of Cox-2 mRNA and protein (7, 8). Cox-2 may participate in carcinogenesis through various mechanisms including stimulation of tumour growth (in many cases by inhibition of apoptosis), and promotion of angiogenesis, invasion and metastasis (9-12).

Although the expression and function of Cox-2 have been studied extensively in several carcinomas, little is known about Cox-2 in non-epithelial tumours. Soft-tissue sarcomas (STS) are a heterogenous group of mesenchymal tumours, ranging from poorly- to highly-differentiated tumours of different grades of malignancy (13). Since Cox-2 was originally cloned from fibroblasts transformed by Rous sarcoma virus, and since there have been some reports suggesting a role for Cox-2 in non-epithelial malignancies, we investigated Cox-2 expression in different types of STS. Furthermore, we evaluated whether Cox-2 expression shows any correlation with the clinical parameters and the prognosis of sarcoma patients.

### Materials and Methods

*Patients.* STS patients (n=103) were treated by the multidisciplinary STS group at Helsinki University Central Hospital, Finland, between 1988 and 1998. Tissue specimens were stored in the files of the Department of Pathology of Helsinki University. The tumours were classified according to WHO definitions for their histological classification (13): angiosarcoma (n=3), fibrosarcoma (n=2), malignant fibrous histiocytoma (n=45), leiomyosarcoma (n=19), liposarcoma (myxoid/round cell and pleomorphic; n=11), epithelioid sarcoma (n=3) and synovial sarcoma (monophasic and

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*Key Words:* Cox-2, STS, immunohistochemistry, soft tissue sarcomas, epithelial differentiation.

Table I. Clinicopathological data.

Sarcoma type	No.	Gender (M/F)	Age at operation (years) <sup>1</sup>	Tumor site (superficial / deep)	Tumor size (cm) <sup>1</sup>	Grade (II / III / IV)	Local recurrence	Metastases	Follow-up time (years) <sup>1</sup>	Survival (alive / dead of disease / dead of other cause)
Total	80	31 / 49	63±17	23 / 53	8.2±5.8	7 / 31 / 39	20 / 77	33 / 76	6.3±3.4	55 / 23 / 2
SS biphasic	4	2 / 2	48±7	1 / 2	4.8±1.7	0 / 4 / 0	2 / 4	1 / 4	8.8±3.4	4 / 0 / 0
SS monophasic	5	2 / 3	43±18	0 / 4	6.8±3.0	0 / 4 / 0	1 / 5	2 / 5	5.2±4.4	4 / 1 / 0
AS	3	1 / 2	76±6	3 / 0	1.7±1.2	0 / 1 / 2	2 / 3	0 / 3	6.7±4.2	2 / 0 / 1
FS	2	0 / 2	59±12	0 / 2	6.0±1.4	1 / 1 / 0	1 / 2	0 / 2	10.0±2.8	2 / 0 / 0
MFH	39	16 / 23	66±15	11 / 26	8.2±6.0	1 / 8 / 28	9 / 38	16 / 37	5.6±3.1	24 / 15 / 0
LMS	16	5 / 11	67±14	7 / 9	8.9±4.8	2 / 9 / 5	3 / 15	9 / 15	6.4±3.3	10 / 6 / 0
LS	9	5 / 4	60±12	1 / 8	12.2±8.0	3 / 2 / 4	1 / 8	4 / 8	6.1±3.2	7 / 1 / 1
ES	2	0 / 2	37±5	0 / 2	5.0±0	0 / 2 / 0	1 / 2	1 / 2	11.7±4.2	2 / 0 / 0

Clinicopathological data were available from 80 of the 103 soft-tissue sarcoma patients. SS: synovial sarcoma, AS: angiosarcoma, FS: fibrosarcoma, MFH: malignant fibrous histiocytoma, LMS: leiomyosarcoma, LS: liposarcoma, ES: epitheloid sarcoma. <sup>1</sup>Data given as mean±SD.

biphasic; n=20). All samples were re-evaluated by a pathologist specialised in STS (T.B.). Clinicopathological data were available from 80 of the 103 STS patients (Table I).

**Immunohistochemistry.** Formalin-fixed, paraffin-embedded specimens were sectioned (4 µm), deparaffinized and microwaved for 4 x 5 minutes at 700 W in 0.01 M sodium citrate buffer (pH 6.0) for antigen retrieval. The slides were then immersed in 0.6 % hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity and in blocking solution (1.5:100 normal horse serum in PBS) for 15 minutes to block unspecific binding sites. Immunostaining was performed with a mouse monoclonal antibody to human Cox-2 (160112, Cayman Chemical Co., Ann Arbor, MI, USA), at a dilution of 1:200 in PBS containing 0.1% sodium azide and 0.5% bovine serum albumin, at room temperature overnight. Then, the sections were treated with biotinylated horse anti-mouse immunoglobulin (1:200; Vector Laboratories Inc., Burlingame, CA, USA) and antibody-binding sites were visualised by an avidin-biotin peroxidase complex (Vectastain ABCComplex, Vector Laboratories, Inc.) and 3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, MO, USA). The counterstaining was performed with Mayer's haematoxylin. The specificity of the antibody was determined by preadsorption of the primary antibody with human Cox-2 control peptide (10 µg/ml, Cayman Chemical) for one hour at room temperature prior to the staining procedure. The intensity of the immunoreaction – either negative, weakly-positive, or strongly-positive – was graded (0 to 2), and the amount of positive tumour cells were estimated (% of positive tumour cells among all tumour cells) by two investigators (T.B. and P.L.) in consensus. Some tumours showed single scattered Cox-2-positivity (less than 1% positive tumour cells). These cases were considered negative. A Cox-2-positive sample of transitional cell carcinoma of the urinary bladder served as a positive control to minimize interassay variation (14).

The patient data are presented as mean±SD, and the results as mean±SEM. A staining index was calculated for each sample (staining intensity x percentage of cells stained). The mean staining index for each sarcoma group was used in all analyses. The Student's *t*-test and one-way ANOVA served for analyses between staining indices in various sarcoma groups. The Student's *t*-test and simple regression analyses were used for analyses between clinical data and staining indices. Cox-Hazard analysis served for survival analysis. *P*-values less than 0.05 were considered statistically significant. All calculations were done with StatView 5.0® (Abacus Concepts Inc.).

## Results

Cox-2 immunoreactivity existed in 49% (50/103) of the STS. Of the Cox-2-positive tumours, staining was visible only around necrotic areas in 36% (n=18) (Figure 1C). In 32 tumours, sarcoma cells not adjacent to necrosis showed positivity for Cox-2. Nine of these stainings were strong and the rest weak. These 9 strongly-stained samples were: 5 biphasic synovial sarcomas, 1 epitheloid sarcoma (Figure 1B), 1 leiomyosarcoma and 2 malignant fibrous histiocytomas.

All 10 biphasic synovial sarcomas were positive for Cox-2, the expression being restricted to the epithelial component (Figure 1A). Moderate intensity staining was seen in 5, and strong staining in another 5. In 2 specimens, staining was seen in 50%, and in the other 8, in 90 to 100% of the cells of the epithelial component of the tumour. Only 1 of the monophasic synovial sarcomas was positive for Cox-2 (Table II).

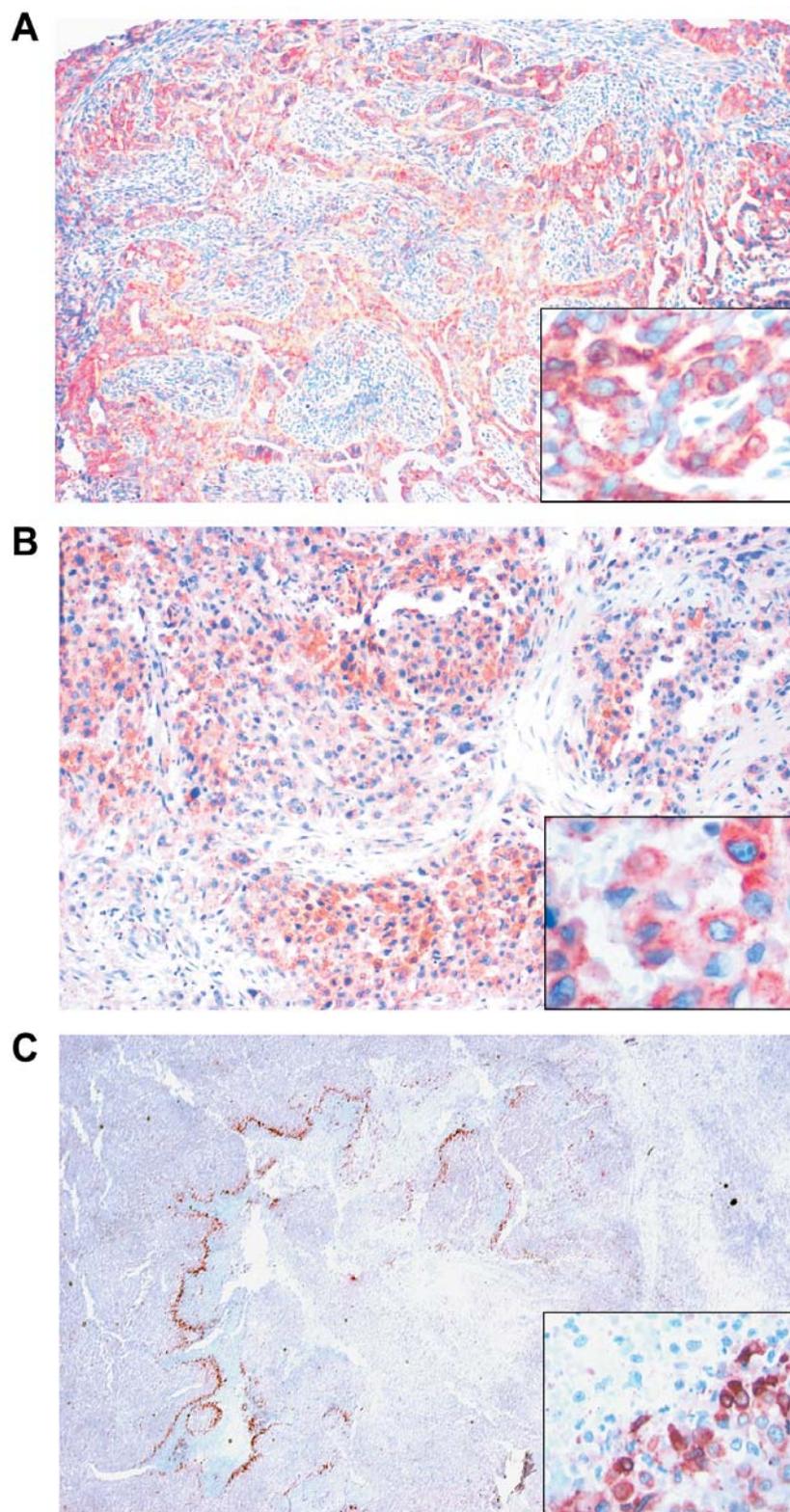


Figure 1. Cox-2 immunohistochemistry in human soft-tissue sarcomas. A) Biphasic synovial sarcoma, expression in the epithelial component. B) Epithelioid sarcoma. C) Malignant fibrous histiocytoma, expression in tumour cells bordering a necrotic area in an otherwise negative tumour.

Table II. *Cox-2 immunohistochemistry.*

Sarcoma type	No.	Cox-2 + only around necrosis	Cox-2 + diffusely in tumour cells	Staining intensity (0-2) <sup>2</sup>	% of cells stained	Staining index <sup>1</sup>	
Total	103	18	32	0.38±0.07	13±3	0.20±0.05	
SS biphasic	10	0	10	1.50±0.17	87±6	1.35±0.20	<i>p</i> =0.0001 <sup>3</sup>
SS monophasic	10	0	1	0.10±0.10	5±5	0.05±0.05	<i>p</i> =0.0001 <sup>4</sup>
AS	3	1	1	0.33±0.33	7±7	0.07±0.07	NS <sup>5</sup>
FS	2	0	0	0	0	0	NS <sup>5</sup>
MFH	45	10	10	0.31±0.09	10±3	0.14±0.05	NS <sup>5</sup>
LMS	19	5	3	0.21 ±0.12	7±5	0.12±0.10	NS <sup>5</sup>
LS	11	1	6	0.55±0.16	13±6	0.13±0.06	NS <sup>5</sup>
ES	3	1	1	0.67±0.67	17±17	0.33±0.33	NS <sup>6</sup>

SS: synovial sarcoma, AS: angiosarcoma, FS: fibrosaroma, MFH: malignant fibrous histiocytoma, LMS: leiomyosarcoma, LS: liposarcoma, ES: epitheloid sarcoma. <sup>1</sup>Staining index: staining intensity X% of tumour cells stained. Samples with positivity only around necrotic areas were considered negative. <sup>2</sup>Data given as mean±SEM. <sup>3</sup>Biphasic SS vs all other sarcomas. <sup>4</sup>Monophasic SS vs biphasic SS. <sup>5</sup>vs all other sarcomas. <sup>6</sup> ES vs all other sarcomas excluding biphasic SS. NS: nonsignificant.

In 3 angiosarcomas, Cox-2 positivity was apparent only around necrotic areas in 1, and weak staining in 20% of tumour cells not adjacent to necrosis in another. Two fibrosarcomas showed no expression. In 45 malignant fibrous histiocytomas, staining for Cox-2 was evident only around necrosis in 10 samples, and not adjacent to necrosis in another 10. Two of these tumours showed strong and the rest weak staining. Only 1 sample showed positivity in more than 50% of the tumour cells. Of the 19 leiomyosarcomas, Cox-2 staining occurred only around necrosis in 5 samples, and diffusely in tumour cells in 3. Two of these showed moderate staining in 20% of the tumour cells, and 1 showed strong staining in 90% of the tumour cells. In 1 of the 11 liposarcomas, staining for Cox-2 occurred only around necrosis. Moderately-intense staining not adjacent to necrosis was detected in 6 samples, in 5 of these in less than 30% of tumour cells. In 1 of the 3 epitheloid sarcomas, expression was detectable around necrosis, and in another sample, strong staining was seen in 50% of the tumour cells. The biphasic synovial sarcomas had a higher mean staining index than the other sarcomas (*p*=0.0001) (Table II).

Clinical data were available from 80 cases. Statistical analyses were performed for each sarcoma group separately and for all sarcomas together. No associations existed between the mean Cox-2 staining index and clinical data (gender, tumour location, tumour size, tumour grade, local recurrence, metastases). Moreover, in Cox-Hazard analysis, we found no correlations between survival or metastasis-free survival and mean Cox-2 staining index.

## Discussion

The most consistent finding was a strong Cox-2 expression in the epithelial component in all biphasic synovial sarcomas, whereas monophasic synovial sarcomas expressed Cox-2 only at low levels or it was undetectable. The epithelial component of synovial sarcoma expresses many epithelial markers, such as various keratins, epithelial membrane antigen and glycodefin, as well as epithelial adhesion molecules, those usually not expressed by the spindle cell component (15, 16). Among other sarcomas, epitheloid sarcomas also express epithelial markers and, interestingly, 1 of the 3 epitheloid sarcomas in this study showed strong and uniform Cox-2 expression. Strong Cox-2 expression may thus serve as a further marker for epithelial differentiation of sarcoma cells. Cox-2 expression has been detected in several types of carcinomas (7, 8). Cox-2-derived prostaglandins contribute to tumour growth by inducing angiogenesis (12). Moreover, Cox-2 inhibitors can inhibit the proliferation of cancer cell lines, and Cox-2 expression in gastric carcinoma correlates with tumour invasion into the lymphatic vessels and metastasis to lymph nodes (17, 18). Whether Cox-2 has the same biological functions in sarcomas with epithelial differentiation as in carcinomas remains to be studied.

In 36% of the Cox-2-positive samples, the expression was seen only around necrotic areas. Similarly, in lung carcinomas and in gliomas, Cox-2 expression accumulated in the immediate vicinity of necroses (19, 20). Acute and

chronic hypoxia induces Cox-2 expression in rat lungs (21), and hypoxia induces Cox-2 protein and mRNA expression in human vascular endothelial cells (22). The ability to express Cox-2 seems to be present in STS cells, and it is possible that the Cox-2 expression around necrotic areas seen in STS samples is induced by hypoxia. On the other hand, Cox-2 is involved in the necrotic process induced by cell-cell contacts in cell culture, suggesting that the Cox-2 expression seen in STS may be involved in the development of necrosis (23).

Excluding biphasic synovial sarcomas, the expression of Cox-2 in sarcoma cells not adjacent to necrosis was detectable in 23% of the positive STS samples. In most tumours considered Cox-2-positive, the staining was often weak and large areas were negative for Cox-2. Excluding sarcomas with epithelial differentiation, uniform staining of the tumour, as in many carcinomas, was observed in only 2 samples. In several of the positive liposarcomas, the expression was evident in large vacuolated lipoblasts, whereas spindle cell areas were usually negative. Altogether, Cox-2 expression was much less frequent in STS than in carcinomas. Moreover, no associations existed between Cox-2 positivity and clinical data. In line with our data, uterine sarcomas and most uterine carcinosarcomas do not express Cox-2 (24). Some chondrosarcomas and pediatric rhabdomyosarcomas express Cox-2, but the expression does not associate with tumour behaviour (25-27). In *in vitro* studies, a moderate degree of cyclooxygenase activity appears in human fibrosarcoma and osteosarcoma cells (28). Cox-2 inhibitors have been shown to have an effect on sarcoma cells *in vitro*, as shown by increased apoptosis in a human osteosarcoma cell line, inhibition of cell growth in a malignant fibrous histiocytoma cell line, and enhancement in tumour radio-response, as well as in reduced tumour growth, in mice implanted by osteosarcoma or rhabdomyosarcoma cells (29-32). Cox-2 has been suggested to have a role in the regulation of invasiveness in rhabdomyosarcoma cells *in vitro* (33).

In conclusion, it seems that, in human STS, Cox-2 expression is related to epithelial differentiation and, in some instances, Cox-2 is expressed at sites of necrosis in otherwise negative tumours.

### Acknowledgements

We thank Kristina von Boguslawski and Elina Laitinen for their excellent technical assistance. Funded by Finska Läkaresällskapet, Helsinki University Central Hospital Research Fund, and the Finnish Cancer Foundation.

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*Received February 16, 2005*

*Accepted May 6, 2005*