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Elevated Cyclooxygenase-2 Expression Correlates with Distant Metastases in Breast Cancer

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Abstract. *Background: Cyclooxygenase-2 (COX-2) overexpression clearly plays an important role in the pathogenesis of breast cancer. In this study, we analysed the relationship between COX-2 expression and various clinicopathological factors in human breast cancer. Materials and Methods: Using immunohistochemistry, we analysed archival specimens of human breast cancer (n=29) using antibodies to COX-2, ER, PgR and HER2 and, from medical records, obtained clinicopathological data. Results: We observed a significant association between COX-2 overexpression and distant metastasis. COX-2 expression was not significantly associated with any other clinical or pathological variable. Conclusion: These findings lend support to the hypothesis that COX-2 overexpression represents an adverse prognostic event in human breast cancer and are encouraging for proposed strategies of COX-2 suppression to treat the disease.*

Approximately 1 in 10 women worldwide will develop breast cancer during their lifetimes, this condition being the leading cause of death in females between the ages of 40 and 50 years (1).

Recent epidemiological studies have indicated that long-term administration of non-steroidal anti-inflammatory drugs (NSAIDs), which target the enzyme cyclooxygenase (COX), may reduce the risk of developing breast cancer by nearly 18 percent (2, 3). We recently critically appraised the evidence supporting a role for COX-2 in the pathogenesis of breast cancer (4). A number of studies have examined the expression of COX-2 in human breast cancer specimens. In one of the most recent, Ristimaki *et al.* observed elevated expression of COX-2 in 37.4% of breast cancers (5). Elevated expression was significantly associated with factors such as unfavourable distant disease-free survival, large tumour size, high histological grade, negative hormone receptor status and HER-2 oncogene amplification. In other studies, Soslow *et al.* (6) found immunohistochemical evidence of COX-2 expression in 56% of breast tumours. Moderate-to-strong expression was found in tumour cells, with almost negligible expression in adjacent non-cancerous tissue (ANCT). Additionally, ductal carcinoma *in situ* (DCIS) was found to be more likely to express COX-2 than invasive carcinoma, and poorly-differentiated features were associated with low COX-2 expression. Half *et al.* (7) found elevated COX-2 expression in 43% of invasive cancers and also noted increased COX-2 expression in adjacent non-cancerous tissue. This lends support to our previously described findings of elevated COX-2 mRNA in human breast cancer and ANCT (8).

In the present study, we investigated the expression of COX-2 in human breast cancer specimens and determined the correlations between COX-2 expression and various clinicopathological factors such as hormone receptor expression, HER2 expression, tumour grade, stage of cancer and long-term survival.

Materials and Methods

We collected archival formalin-fixed and paraffin-embedded (FFPE) breast cancer specimens from 29 patients who underwent potentially curative cancer surgery between January 1991 and December 1994, at St. George's Hospital, London, U.K. Local Ethical Committee approval was granted for a retrospective review of the histological and medical records. The mean length of follow-up for these patients was 10.8 years (range: 9-12 years). There were 27 invasive ductal carcinomas in the sample and 2 invasive lobular carcinomas.

Immunohistochemistry. Four-micron-thick sections were cut from the FFPE blocks and mounted onto polylysine-coated slides. After dewaxing in xylene and rehydration in descending alcohols, the slides were treated with a solution of 3% methanolic peroxide to inactivate endogenous peroxidase. They were then incubated with a polyclonal rabbit IgG antibody to COX-2 (Oxford Biosciences, Oxford, UK) for one hour, before incubation with a peroxidase-

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labelled secondary antibody for thirty minutes. Control slides were incubated with normal rabbit serum. Diaminobenzidine (DAB) was used as a chromogenic substrate, with the presence of a brown precipitate used to identify areas of positive staining. After counterstaining with haematoxylin, the slides were rehydrated, mounted with histomount and subjected to histopathological assessment.

In order to determine hormone receptor expression and HER2 status, primary antibodies to oestrogen receptor, progesterone receptor and HER2 were also used for each archival specimen with the above protocol.

Histopathological evaluation. A consultant histopathologist (VT) reviewed all the stained slides and scored them according to recognised working protocols for hormone receptors and HER2 recommended by the National Health Service Breast Screening Programme, UK (9, 10). With regards to COX-2, the Soslow scoring system was used (6). An immunohistochemical score from 0-12 was calculated from the product of scores for quantity and intensity of staining. A score of 0=negative immunoreactivity, 1-4=weak immunoreactivity, 5-8=moderate immunoreactivity and 9-12=strong immunoreactivity. The specimens were also re-analysed for grade of tumour. Scores of greater than or equal to 5 were taken as evidence of elevated COX-2 expression.

Statistical analysis. Correlations between COX-2 and hormone receptor / HER2 expression were studied using Kendalls tau statistics.

The relationship between patient age and COX-2 expression was studied using the unpaired *t*-test. The other clinicopathological features were analysed in relation to COX-2 expression using Fisher's exact test and the Chi-squared test. Differences were considered statistically significant at $p<0.05$.

Results

Immunostaining. In all cases, COX-2 expression was predominantly localised to tumour cells, with weak staining in some stromal cells. In neoplastic cells, COX-2 expression was observed only in the cytoplasm and nucleus. Normal breast tissue showed no COX-2 immunoreactivity.

Patients were divided into low and high COX- expression groups according to the extent of COX-2 expression from the Soslow score (high expression: Soslow score 5-12, low expression: Soslow score 0-4). High COX-2 expression was observed in 11 (37.9%) cases and low expression in 18 cases (62.1%).

Relationship between COX-2 expression and clinicopathological variables. No significant relationships were detected between the degree of COX-2 expression and age, tumour grade, hormone receptor expression, tumour size, lymphovascular invasion, lymph node metastasis or local recurrence. There was, however, a significant relationship between elevated COX-2 expression and recurrence by distant metastasis ($p=0.02$, Fisher's exact test, Table I).

Table I. *Association between COX-2 expression and clinicopathological factors in invasive breast cancers.*

	High COX-2 Group	Low COX-2 Group	*p* value
Number of cases	11	18	
Age	62.4±12.8	67±12	0.34
Local recurrence			
+	3	6	
			1.00 NS*
-	4	6	
Distant metastases			
+	8	4	
			0.02
-	3	14	

*NS – not significant

Significant positive correlations were observed between ER / PR ($\tau=+0.328$, $p=0.004$) and negative correlations between ER / HER2 ($\tau=-0.344$, $p=0.012$) and PR / HER2 ($\tau=-0.398$, $p=0.003$).

Discussion

We examined COX-2 expression in invasive breast cancer using immunohistochemistry and demonstrated that COX-2 overexpression occurred in 37.9% of our sample, and was significantly associated with distant metastasis. COX-2 expression was not associated significantly with any other clinicopathological variables.

Ristimaki *et al.* (5) found moderate to strong COX-2 expression in 37.4% of tumours (n=1576), with correlation of elevated expression to large tumour size, high histological grade, high proliferation rate and HER2 amplification. In their study, 68.9% of tumours studied were ER-positive, whilst 55% of tumours were PgR-positive. In our study, 86.2% of tumours had a ER score of over 2 (n=25), and 72.4% of tumours had a PgR score of over 2 (n=21). Of these, 32% of ER-positive tumours and 38% of PgR-positive tumours over-expressed COX-2. There was a strong positive correlation between ER and PgR receptor expression in our sample The proportions described are comparable to those found by Ristimaki *et al.*, who additionally noted COX-2 overexpression was significantly associated with hormone receptor-negative status. In our study, we did not find a significant association between elevated COX-2 protein expression and hormone receptor negativity, or HER2 status. We have previously demonstrated that a significant relationship exists between COX-2 and PR expression in human breast cancer at the mRNA level, and these current findings indicate that this relationship is not established at the level of protein expression (11)

HER2 was detected in only 4 tumours in our sample. Although recent studies seem to indicate that there is paucity of association between HER2 status and COX-2 overexpression (7), this could be a consequence of the small sample size in our study. Other studies may also have utilised different working assessments or definitions of ER/PR positivity. The possibility that there is a subset of breast cancers which overexpress HER2 and COX-2, initially suggested by Subbaramaiah *et al.* (13), clearly needs to be investigated further.

The only significant correlation of elevated COX-2 expression with metastasis supports the findings of previous studies, which suggest that elevated COX-2 expression is an adverse prognostic factor in breast cancer. This association has also been described in colorectal cancer (5, 14).

Tumour growth and metastasis require the development of new blood vessels. Angiogenesis requires the development of new blood vessels from existing, quiescent vascular endothelium. COX-2 may have a functionally significant role in the promotion of angiogenesis. Prostaglandins are pro-angiogenic (15), and are derived from arachidonic acid by the action of COX-1 and COX-2. Inhibition of prostaglandin synthesis by NSAIDs has been shown to retard the development of mammary tumours in an animal model (16), which additionally show reduced vascularity. *In vivo* and *in vitro* studies have shown inhibition of COX-2 expression results in suppression of angiogenesis, and abrogation of endothelial cell spreading and migration (17, 18). More recently, a significant correlation was observed between the expression of COX-2 and CD31, an endothelial cell marker of angiogenesis, in human breast cancer (12). Moreover, we have recently described a significant association between vascular endothelial growth factor - 189 (VEGF-189) and COX-2 mRNA levels in human breast cancer. VEGF is a potent stimulator of angiogenesis and is considered to be an endothelial, specific mitogen, inducing shape change, protease production and migration in these cells, all pre-requisites for new blood vessel formation (8).

In summary, this report confirms that elevated COX-2 expression is an adverse prognostic factor in human breast cancer and lends support to the hypothesis that COX-2 inhibition would be a viable strategy for the chemoprevention and treatment of the disease.

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