

The status of cyclooxygenase-2 expression in ductal carcinoma in situ lesions and invasive breast cancer correlates to cyclooxygenase-2 expression in normal breast tissue

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Abstract

Objectives: There is a paucity of data on cyclooxygenase (COX)-2 expression in normal breast tissue and on the changes in COX-2 expression from normal tissue via ductal carcinoma in situ (DCIS) lesions to invasive cancer. The aim of this study, therefore, was to investigate COX-2 protein expression in normal breast tissue, DCIS, and invasive breast cancer in samples from the same patients.

Methods: In 39 patients, we investigated and compared COX-2 expression in paired samples of invasive cancer and normal adjacent breast epithelium by immunohistochemistry with a monoclonal COX-2 antibody. Furthermore, in 29 of these cases, we also analyzed a concomitant DCIS lesion.

Results: Patients without COX-2 expression in normal breast tissue also do not express COX-2 in invasive breast cancer and in DCIS lesions, respectively. Conversely, COX-2 expression in normal breast tissue was an indicator for COX-2 expression in the paired breast tumors. There was no significant correlation between COX-2 expression and pathologic tumor stage, nodal status, hormone receptor status, tumor size, grading, and lymphovascular space involvement.

Conclusions: This is the largest study to date investigating COX-2 in paired samples of breast tumors and normal adjacent breast tissue. Our data are consistent with the hypothesis that COX-2 overexpression is an early event in breast carcinogenesis.

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Keywords:

Breast cancer; Ductal carcinoma in situ; Normal adjacent breast tissue; Cyclooxygenase-2; Immunohistochemistry

1. Introduction

Over the past years, several studies demonstrated upregulation of cyclooxygenase (COX)-2 in a wide variety of solid tumors including colon cancer, gastric cancer, cervical cancer, and breast cancer [1–5]. Cyclooxygenase-2 upregulation is induced by numerous extracellular stimuli such as growth factors, cytokines, hypoxia, and tumor promoters [6,7]. Cyclooxygenase-2 overexpression leads to increased production of prostaglandins that are involved in different physiologic and pathophysiologic processes [8]

including proliferation [9], apoptosis [10,11], angiogenesis [12], and invasion [13].

In breast cancer, studies have shown that COX-2 is overexpressed in invasive cancers [1,4,14] and in ductal carcinoma in situ (DCIS) lesions [1,14–16]. This observation suggests that COX-2 expression is an early event in breast carcinogenesis. Studies regarding the clinical implications of COX-2 overexpression in breast cancer have come to conflicting results. On one hand, Ristimäki et al [4] demonstrated a positive correlation between COX-2 expression and histopathologic parameters associated with an aggressive tumor phenotype. Furthermore, COX-2 overexpression correlated significantly with a shorter disease-free survival. Similar findings concerning the association with histopathologic parameters and disease-free survival were reported by Denkert et al [17]. On the other hand,

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some studies could not find a relation between COX-2 expression and histopathologic parameters [14,18,19] or disease-free survival [20].

Based on epidemiologic data [21–23] and COX-2 expression patterns, evidence is rising that suggests that COX-2 inhibitors may function as chemopreventive agents in breast cancer and DCIS lesions. However, there is a paucity of data on COX-2 expression in normal breast tissue and on the changes in COX-2 expression from normal tissue via DCIS lesions to invasive cancer.

The aim of this study, therefore, was to investigate COX-2 expression in normal breast tissue, DCIS, and invasive breast cancer in samples from the same patient. In 39 patients, we investigated and compared COX-2 expression in paired samples of invasive cancer and normal breast epithelium. Furthermore, in 29 of these cases, we also analyzed the concomitant DCIS lesion. We demonstrate that patients without COX-2 expression in normal breast tissue also do not express COX-2 in invasive breast cancer. However, COX-2 expression in normal breast tissue was an indicator for COX-2 expression in invasive breast cancer. We found no relation between COX-2 expression in invasive breast cancer and clinical or pathologic parameters.

2. Materials and methods

2.1. Tissue specimens

Tissue samples were selected for a sufficient amount of normal adjacent breast epithelium and an extensive DCIS component. Archival tissue samples of primary breast cancer ($n = 39$) and paired normal breast epithelium from the same patients ($n = 39$) that were obtained after cancer surgery from the gynecologic pathology laboratory at Leipzig University were investigated (Table 1). The tissue specimens were formalin fixed and paraffin embedded. Of these primary breast cancer samples, 29 exhibited extensive concomitant DCIS lesions that were also analyzed. The normal breast epithelium, generally, had a distance of at least 10 mm from the invasive cancer or the DCIS lesion, respectively.

2.2. Immunohistochemistry

Immunohistochemical staining was performed according to standard procedures. With a monoclonal antibody, clone CX229 (Cayman Chemical, Ann Arbor, Mich), 5- μ m sections were stained. Briefly, slides were boiled in Target retrieval solution (Dako Cytomation, Glostrup, Denmark) for 20 minutes in a pressure cooker for antigen demasking and incubated overnight with the anti-COX-2 antibody (dilution, 1:1000) at 4°C. This was followed by incubation with a biotinylated antirabbit secondary antibody and CSA system (CSA Rabbit Link, Dako Cytomation). Staining was visualized by using DAB chromogen (Dako Cytomation). For control of antibody specificity, blocking experiments were performed with a COX-2-specific blocking peptide

Table 1
Patient and tumor characteristics

	No. of patients
pT stage	
pT1mic	3
pT1a	4
pT1b	10
pT1c	10
pT2	9
pT3	3
pN stage	
N0	26
N+	11
ND	2
Grade	
1	1
2	18
3	20
LVSI	
L0	19
L1	20
Tumor diameter (mm), median (range)	12 (1–60)
Patient age (y), median (range)	59 (32–92)
Estrogen receptor	
Positive	29
Negative	8
ND	2
Progesterone receptor	
Positive	27
Negative	10
ND	2

pN stage indicates pathologic node stage; ND, not documented.

(Cayman Chemical), which resulted in complete suppression of COX-2 staining. Negative controls were performed by omitting the anti-COX-2 antibody in the primary antibody incubation.

2.3. Evaluation of COX-2 immunostaining

For the evaluation of cytoplasmic staining results for COX-2, a predefined scoring system based on the product of staining intensity and percentage of positive tumor cells was used [14]. Staining intensity was evaluated as negative (0), weak (1), moderate (2), strong (3), and the percentage of positive tumor cells was categorized as follows: 0 = 0% to 5%, 1 = 6% to 25%, 2 = 26% to 50%, 3 = 51% to 75%, and 4 = 76% to 100%. By multiplying both components, a score (0–12) was obtained. Cyclooxygenase-2 expression was categorized as negative (0–3), moderate (4–8), or strong (9–12) using this calculated score. Evaluation of the samples was performed by 2 independent investigators who were blinded to the clinicopathologic parameters. In cases of discrepant assessment, an agreement was obtained after collegial revision. The nonmalignant breast epithelium was analyzed analogously.

2.4. Statistical analysis

Spearman rank correlation (r_s) was calculated to analyze the association between COX-2 expression in different

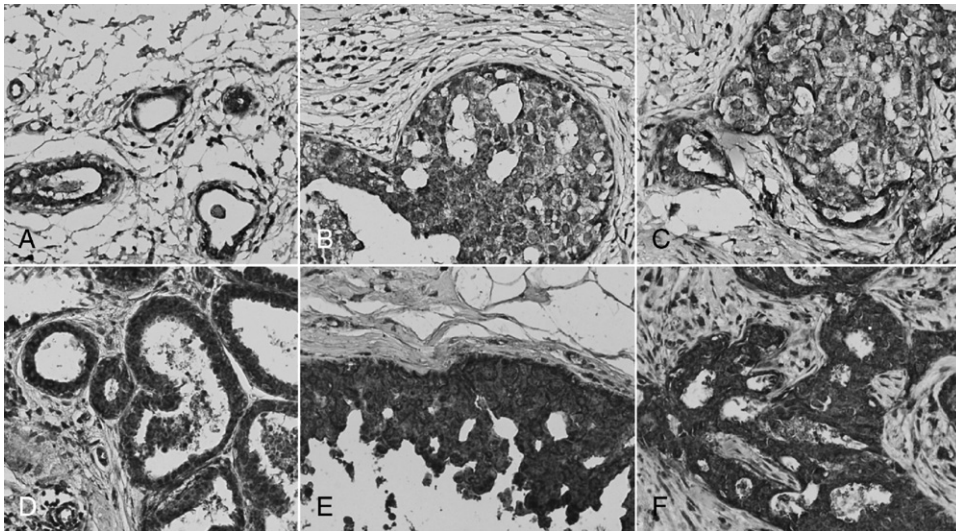


Fig. 1. Immunohistochemical analysis of COX-2 protein in paired samples of adjacent normal breast tissue, DCIS, and invasive cancer in a patient with weak expression (A-C) and another patient with strong COX-2 expression (D-F) (original magnification $\times 400$).

tissues. The Mann-Whitney U test, Kruskal-Wallis test, Wilcoxon test, and Friedman test were used to compare different groups. The McNemar test was applied to assess discrepancies in COX-2 expression in paired tissues. P values of less than .05 were considered significant. Statistical analysis was performed using SPSS version 11.5 for Windows (SPSS GmbH, Munich, Germany).

3. Results

3.1. Patient characteristics and clinicopathologic features

Archival breast tissue of 39 women with invasive breast cancer was evaluated. No cases were excluded. Of these cases, 92% were infiltrating ductal adenocarcinomas, 5% represented lobular carcinomas, and 3% were mucinous carcinomas. Of these primary breast cancer samples, 29 exhibited an extensive concomitant DCIS lesion (median, 50%; range, 1%–98%). Clinicopathologic parameters, in-

cluding pathologic tumor (pT) stage, nodal status, hormone receptor status, tumor size, grading, and lymphovascular space involvement (LVSI), are summarized in Table 1.

3.2. Cyclooxygenase-2 protein expression in invasive breast cancer, DCIS lesions, and normal breast epithelium

Cyclooxygenase-2 protein expression was assessed by immunohistochemistry. Positive tumor cells and benign ductular epithelial cells presented a granular cytoplasmic staining (Fig. 1). Some peritumoral mononuclear inflammatory and stromal cells also displayed cytoplasmic staining. Of invasive breast cancers, 59% exhibited moderate or strong COX-2 expression, whereas 41% were negative. In DCIS, 55% of the lesions showed a moderate or strong COX-2 staining, whereas 45% had negative COX-2 expression. In normal breast epithelium, 54% exhibited moderate or strong COX-2 expression, whereas 46% were negative for COX-2 (Fig. 2A-C). The degree of COX-2

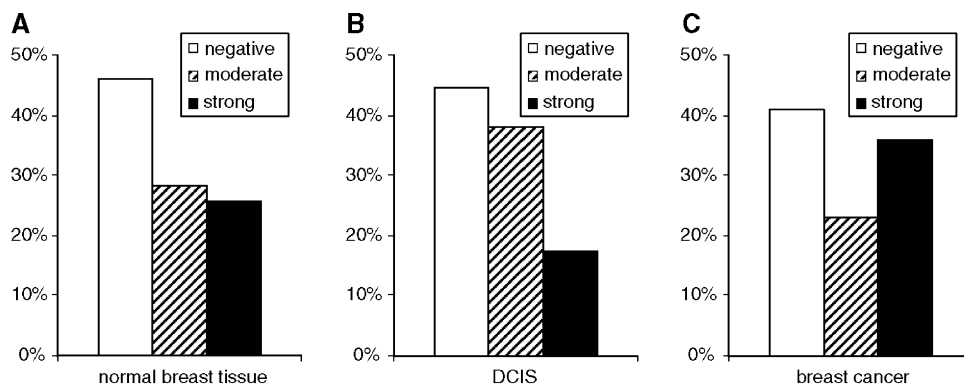


Fig. 2. Levels of COX-2 protein expression in (A) normal adjacent breast tissue, (B) DCIS, and (C) invasive breast cancer.

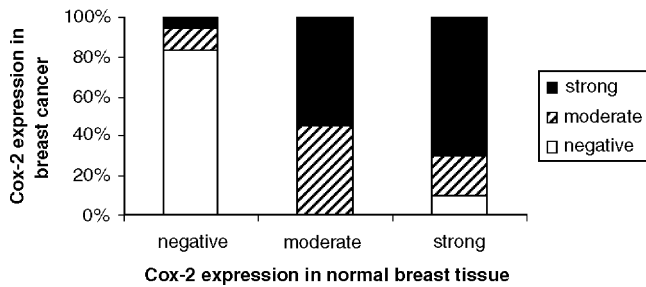


Fig. 3. COX-2 protein expression in invasive breast cancer as a function of COX-2 expression in paired normal adjacent breast epithelium.

expression did not significantly differ between these tissue entities but, generally, the COX-2 score in the normal breast tissue was 2 score points less than that in invasive cancer.

3.3. Cyclooxygenase-2 expression and clinicopathologic parameters

In the investigated series of invasive breast cancers, there was no significant correlation between the extent of COX-2 expression and pT stage, nodal status, hormone receptor status, tumor size, grading, or LVSI.

3.4. Association of COX-2 expression in paired samples of normal breast epithelium and invasive breast cancer

The extent of COX-2 expression in normal breast epithelium correlated significantly to that in invasive breast cancer of the same patient ($r_s = 0.69, P < .001$).

In 83% (15/18) of cases with a negative COX-2 expression in normal breast epithelium, the paired invasive breast cancer lesion was also negative. Conversely, in 95% (20/21) of cases with a moderate or strong COX-2 expression in normal breast epithelium, this was matched by a moderate or strong COX-2 expression in the invasive breast cancer of the same patient (Fig. 3). Thus, in 90% (35/39) of all women investigated, the COX-2 expression in normal breast epithelium indicated the COX-2 expression level in the invasive cancer of the same patient. We demonstrated that the discrepancy in COX-2 expression observed in 4 of the 39 cases was nonsignificant (McNemar test, $P = .625$).



Fig. 4. COX-2 protein expression in DCIS lesions as a function of COX-2 expression in paired normal adjacent breast epithelium.

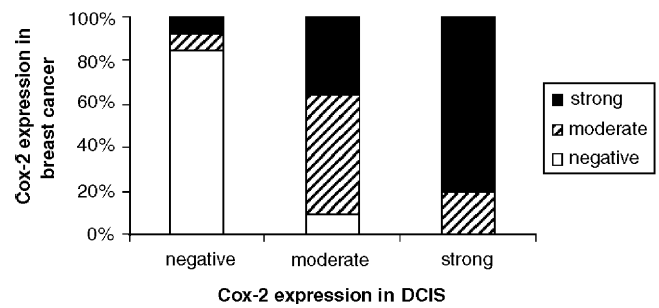


Fig. 5. COX-2 protein expression in invasive breast cancer as a function of COX-2 expression in paired DCIS lesions.

3.5. Association of COX-2 expression in paired samples of normal breast epithelium and DCIS

In the 29 cases with DCIS, we also found a significant correlation between the COX-2 expression in DCIS and normal breast epithelium ($r_s = 0.74, P < .001$).

In 92% (11/12) of the cases, a negative COX-2 expression in the normal breast tissue was matched by a negative expression in the DCIS lesion, and in 88% (15/17), a moderate or strong COX-2 expression in normal breast coincided with a similar expression level in the paired DCIS samples (Fig. 4). Thus, in 90% (26/29), the COX-2 expression in normal breast tissue was equal to that in the DCIS lesion. The 3 cases of different COX-2 expressions in the paired tissue samples are nonsignificant (McNemar test, $P = 1.00$).

3.6. Association of COX-2 expression in paired samples of DCIS and invasive breast cancer

In the 29 cases with DCIS, there was also a significant correlation between the COX-2 expression in DCIS and invasive breast cancer ($r_s = 0.72, P < .001$).

In 85% (11/13) of the cases with a negative COX-2 expression in DCIS, the paired invasive cancer lesions were also negative. Conversely, 94% (15/16) of DCIS lesions with moderate or strong COX-2 expression were matched by a similar expression level in the paired invasive breast cancer samples (Fig. 5). In total, COX-2 expression in DCIS equaled that in invasive cancer in 90% (26/29) of all cases. The 3 cases with discrepant COX-2 expression in the paired tissue samples are nonsignificant (McNemar test, $P = 1.00$).

4. Discussion

To our knowledge, this is the largest study to date comparing COX-2 protein expression in paired samples of preinvasive and invasive breast cancer and adjacent normal breast tissue.

Investigating COX-2 expression levels in paired samples of invasive breast cancer, DCIS lesions, and normal breast epithelium, we found moderate or strong COX-2 expression in 59%, 55%, and 54% of samples, respectively. There was no significant difference in COX-2 expression levels

between the 3 groups. The COX-2 positivity rate observed in invasive breast cancer, and DCIS lies within the range of that reported by other groups who have documented expression in 36% to 63% of invasive tumors [1,4,14,15,17] and in 55% to 85% of DCIS [15,16,19]. The finding regarding similar COX-2 expression levels in invasive breast cancer and DCIS is consistent with a study by Boland et al [15], which also showed no significant differences between these tissue entities. In contrast, Soslow et al [1] and Half et al [14] demonstrated a higher frequency of COX-2 expression in DCIS lesions compared with invasive breast cancer. Interestingly, the study by Boland et al [15] demonstrated a significant difference between invasive breast cancer and normal breast tissue. This normal breast tissue, however, was not paired but derived from breast reduction surgery.

In our analysis, we did not observe a correlation between COX-2 expression in breast cancer and various clinicopathologic parameters including tumor stage, nodal status, and hormone receptor status. This is in line with reports by Half et al [14], Kelly et al [18], Ranger et al [19], and Watanabe et al [24]. By contrast, a large study on invasive breast cancers by Ristimäki et al [4] demonstrated a significantly positive correlation between COX-2 expression and histopathologic parameters associated with an aggressive tumor phenotype such as large tumor size, presence of axillary node metastases, high histologic grade, negative hormone receptor status, high proliferation rate, high p53 expression, and HER2 amplification [4]. In addition, a study by Costa et al [25] demonstrated a significant association between COX-2 expression and a positive nodal status.

We report here that there is a significant correlation between the COX-2 expression levels in normal breast tissue and the invasive cancer of the same patient. In fact, in 90% of cases, we found a concordant COX-2 expression in the invasive cancer and the adjacent normal breast epithelium of the same patient. The same holds true for the COX-2 expression in DCIS lesions and paired normal breast epithelium. To date, this is the largest study concerning COX-2 expression in paired tissue samples of patients with breast cancer. The few published data on COX-2 expression in normal breast tissue are conflicting. Half et al [14] used reverse transcriptase–polymerase chain reaction for detection of COX-2 messenger RNA (mRNA) in 9 paired samples and reported lower COX-2 mRNA levels in adjacent normal tissue in 8 of 9 cases. Watanabe et al [24] found no COX-2 mRNA in normal breast tissue by reverse transcriptase–polymerase chain reaction in 6 investigated paired samples. Using Western blotting, Costa et al [25] did not find COX-2 protein in 2 nonmalignant breast tissues. Most likely, these observations can be explained by the paucity of ductal units in normal breast tissue as compared with malignant breast tissue. Using immunohistochemistry, Boland et al [15] investigated 60 normal breast tissues from reduction surgery and compared it with normal ductal tissue adjacent to DCIS lesions. There was COX-2 positivity in 23% and 22%,

respectively. The authors did not report paired normal adjacent breast tissue from invasive cancer patients. Shim et al [16] investigated normal tissue adjacent to DCIS lesions and reported that in high-grade lesions, the COX-2 expression in the adjacent breast tissue was equal to or greater than that in the lesion itself. Half et al [14] showed that within the same tissue sections, COX-2 expression in invasive breast tumors and adjacent DCIS were highly correlated. The authors found COX-2 expression in 81% of benign adjacent tissue and described it to be of similar or reduced intensity relative to the malignant tissue.

There are several possible interpretations of our reported data. Firstly, the observed COX-2 expression in the adjacent nonmalignant tissue could be the result of paracrine effects deriving from the malignant epithelial cells. This hypothesis is consistent with an observation by Shim et al [16] who reported that the observed increase in COX-2 expression in normal adjacent epithelium diminished with increasing distance from the lesion. Secondly, COX-2 expression in the normal breast tissue could be an early event during carcinogenesis and precede the changes in DCIS and invasive breast tumors. This might be explained by the presence of field cancerization in surrounding normal tissue. There is evidence that genetic abnormalities, potentially critical to breast tumorigenesis, accumulate before histopathologic detection of high-risk lesions or cancer is possible [26]. Recent molecular studies support a carcinogenesis model in which the development of a field with genetically altered cells plays a central role [27]. Cyclooxygenase-2 overexpression, however, could be just a marker of field cancerization without a direct involvement in carcinogenesis. Thirdly, the observed COX-2 overexpression in normal breast tissue could be independent of the neoplastic transformation and merely maintained during this process. On the basis of clinical [1,14] and experimental studies [28,29], it has been hypothesized that COX-2 overexpression is an early event in breast carcinogenesis. As a consequence, COX-2 inhibitors have been proposed as potential chemopreventive agents in breast cancer and DCIS lesions [30,31]. Although our analysis of synchronous breast tissue samples is consistent with this hypothesis, further studies in a diachronous setting are needed to dissect the temporal and causal relationship between COX-2 expression and breast carcinogenesis.

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