Purpose: The aim of this study was to evaluate the expression of cyclooxygenase-2 in human breast cancer using immunohistochemistry and to determine whether the expression of cyclooxygenase-2 is associated with clinicopathological factors in invasive ductal breast carcinoma.

Methods: Cyclooxygenase-2 expression was investigated by immunohistochemistry in 30 invasive ductal breast carcinoma specimens and relationships between cyclooxygenase-2 expression and age, histological grade, histological type, nodal status, and hormone receptor status were evaluated.

Results: Cyclooxygenase-2 expression was found in 56.7% of the tumor samples and was related to histological grade ($P < 0.01$) and histological type ($P < 0.001$).

Conclusions: Our results suggest that cyclooxygenase-2 expression has an important role in tumor differentiation in invasive ductal breast carcinoma.

Key words: cyclooxygenase-2, breast carcinoma, histological grade

Introduction

Epidemiological studies have suggested the chemopreventive effects of non-steroidal anti-inflammatory drugs (NSAIDs) in colorectal cancer. Cyclooxygenase (COX) is the major cellular target of NSAIDs and regulates the synthesis of prostaglandins. Two isoforms of COX are recognized; COX-1, the constitutively expressed isoform, and COX-2, the inducible type, which is induced by proinflammatory cytokines, growth factors, and mitogens. Numerous studies have emphasized that COX-2 plays an important role in the prevention of colon cancer, and may be involved in tumorigenesis of the esophagus, stomach, and other solid tumors. More recent studies indicate that COX-2 contributes to cell proliferation, mediates inhibitory effects on apoptosis, modulates the production of several proangiogenic factors, and increases cell invasiveness.

In early studies, the effects of NSAIDs on breast cancer risk were underestimated. In contrast, analysis of prospective data has recently indicated the protective effects of the regular aspirin, ibuprofen and other NSAIDs against the development of breast cancer. Animal studies have demonstrated that selective COX-2 inhibitors can reduce tumor incidence and multiplicity.

In human breast cancer, several studies have shown that expression of COX-2 mRNA and protein is elevated, and COX-2 has been implicated the pathogenesis of the disease. Ristimäki et al. have studied large numbers of breast tumor samples and showed a significant correlation between elevated COX-2 protein expression and a number of clinico-
pathological variables, including tumor stage, hormone receptor status and HER-2. However, other groups have reported that COX-2 expression is not associated with some of these factors. Thus, the relationships between COX-2 expression and tumor characteristics remain uncertain. The aim of this study was to assess whether expression of COX-2 protein is associated with clinicopathological factors in invasive ductal breast carcinoma by immunohistochemistry.

Materials and Methods

Patients

Surgical specimens from 30 patients diagnosed as having primary invasive ductal breast carcinoma and operated on between 1999 and 2001 at the Department of Surgical Oncology of Tokyo Medical and Dental University were studied. The Human Subjects Committee at Tokyo Medical and Dental University approved the research protocol. All patients gave written consent to participate in the study.

Immunohistochemistry

For immunohistochemical examination of COX-2, we used a universal immunoenzyme polymer method. Formalin-fixed, paraffin-embedded specimens were serially sectioned at a thickness of 3 μm, placed onto MAS-coating slides. Specimens were deparaffinized, rehydrated, and antigen was retrieved using an autoclave at 121°C for 20 minutes in 10 mM sodium citrate buffer (pH 6.0), then treated with 3% hydrogen peroxide in methanol solution at room temperature for 15 minutes to quench endogenous peroxidase activity. Nonspecific reactivity was blocked by treating slides with 10% normal goat serum for 10 minutes. The primary antibody used for immunohistochemical staining was anti-human COX-2 monoclonal antibody (Cayman Chemical Co., Ann Arbor, MI, USA) at a dilution of 1:500 and applied overnight at 4°C. Next, slides were incubated with labeled polymer (N-Histofine Simple Stain MAX PO MULTI, Nichirei Co., Tokyo, JAPAN) for 30 minutes at room temperature. Sections were treated with normal mouse IgG serum instead of primary antibody.

Evaluation of COX-2 immunohistochemistry

COX-2 immunohistochemical staining was scored independently by two investigators (E.T. and T.O.) who were blinded as to the sample origins. Evaluation of COX-2 expression was semiquantitatively estimated by intensity and area of positive cells, modifying the methods of Ristimäki et al. Negative staining; no staining or weak diffuse cytoplasmic staining (may contain stronger intensity in less than 10% of cancer cells); Elevated staining, moderate to strong granular cytoplasmic staining in 10-100% of cancer cells. Association between COX-2 staining and clinicopathological factors was then investigated.

Pathological factors

Histological grade was assigned according to the Nottingham modification of the Bloom and Richardson grading system. The grade was obtained by summing the scores for tubule formation, nuclear pleomorphism, and mitotic count, each of which was given 1, 2, or 3 points. Grade I invasive carcinomas had 3 or 4 points, grade II neoplasms had 5 to 7 points, and grade III tumors had 8 or 9 points. The histological type of human breast cancer investigated was invasive ductal carcinoma, which was classified as papillotubular carcinoma, solid-tubular carcinoma or scirrhous carcinoma according to the Japanese Breast Cancer Society criteria. When combined histological type was observed, predominant type was selected for evaluation.

Statistical Analysis

The correlations between COX-2 expression and several clinicopathological parameters were analyzed by χ² test. P values of less than 0.05 were considered significant. For statistical evaluation, we used the StatView software package (version 5.0; SAS Institute Inc., Cary, NC).

Results

Patient characteristics

Mean patient age was 55 (range, 31-75) years. Patient characteristics, including histological type, histological grade, hormone receptor status, and nodal status are shown in Table 1.
Elevated COX-2 expression was found in 57% (17 out of 30) of breast cancer samples. Representative examples of COX-2 expression are shown in Fig. 1 (1a-b). COX-2 staining was granular and localized predominantly in the cytoplasm of the tumor cells. Negative staining was shown in Fig. 1b. In some tumor samples, enhanced COX-2 staining was observed in the perinuclear area (Fig. 1c). Staining in normal-appearing epithelia was of no intensity (Fig. 1d). Within the same tissue sections, stromal staining for COX-2 in endothelial cells, lymphocytes, and fibroblasts was weakly detected adjacent to COX-2 expressing tumor epithelia (Fig. 1e).

COX-2 Expression and Tumor Characteristics

Elevated COX-2 expression was more frequent in high grade tumors (grade III) than in lower grade tumors (grade I and grade II) \((P<0.01, \text{ Table 1})\). Histological type (papillotubular and solid-tubular carcinoma vs. scirrhous carcinoma) was also associated with elevated COX-2 expression \((P<0.001, \text{ Table 1})\). All samples of scirrhous carcinoma, grade III shows elevated expression of COX-2 (1a, X40). Example of negative expression: solid tubular carcinoma, grade II (1b, X40). COX-2 protein is strongly expressed in perinuclear area of the tumor cells (1c, X400). Normal breast epithelia are shown with negative staining (d, X200). Immunoreactive COX-2 is detected in stromal cells surrounding the tumor cells (e, X200).

Discussion

In the present study, we found that elevated expression of COX-2 was associated with higher histological grade. This suggests that COX-2 has important role in tumor differentiation in invasive ductal breast carcinoma. Elevated COX-2 expression was previously found to be associated with tumor size and depth in colorectal and gastric cancer\(^{34,35}\) and with grade of dysplasia in colorectal adenoma\(^{36}\). In those studies, however, no evaluation was conducted on COX-2 expression and tumor differentiation.

In breast cancer studies, our analyses of correlation between COX-2 expression and histological factors are consistent with the results of others\(^{27,37,38}\), but they also contradict some reports\(^{28,30,39,40,41}\). In a recent study,
Shim et al. showed that COX-2 expression was detected in almost all ductal carcinoma in situ specimens, with increased COX-2 staining correlating with higher nuclear grade. In the present findings, association between lower histological grade (I and II) versus higher grade (III) was compared to COX-2 expression. Based on O’Reilly’s description, histological grades I and II were combined to one category of “well-differentiated” tumors, and only grade III tumors were analyzed as a separate, single group. The presence of significant correlations in our study group may have resulted from the close relationship between histological grade and histological type of invasive ductal carcinoma; almost all scirrhous carcinoma samples exhibited elevated COX-2 expression and most scirrhous carcinoma belonged to grade III.

According to the classification by the Japanese Breast Cancer Society, there are three types of invasive ductal carcinoma and prognosis of patients with papillotubular type is known to be the best, while scirrhus is the worst. Elevated COX-2 expression would therefore be a marker of poor differentiation, and may reflect the prognosis of invasive ductal carcinoma. Some investigators have reported that elevated COX-2 expression is associated with significantly worse disease-free survival and overall survival, and have evaluated COX-2 as one of prognostic factors.

COX-2 in malignant tumors is thought to be involved in tumorigenesis. Shattuck-Brandt et al. have reported that COX-2 expression was observed in both epithelial and tumor stromal cells. In our samples, COX-2 immunoreactivity was detected with weak intensity in stromal cells surrounding the tumor cells. Moreover mastopathy and adenosis adjacent to COX-2 positive carcinomas also expressed COX-2 otherwise mastopathy alone didn’t show COX-2 positivity (data not shown). This observation supports the notion that COX-2 in stromal cells acts to promote tumors and exerts paracrine effects on nearby carcinoma cells.

One study has emphasized the significant roles of fibroblasts and endothelial cells in the intestinal polyp development through COX-2 expression, while little COX-2 expression was seen in macrophages. In contrast, other groups found that COX-2 was expressed predominantly by macrophages in the stroma. COX-2 expression in breast cancer stromal cells has not been clarified. One reason may be that it is difficult to distinguish stromal cells from tumor cells because of the unique morphology of breast cancer.

The potential value of COX inhibitors for breast cancer is currently under examination. However, it is not known which group of patients could gain the most benefit from COX inhibitors. Our results showing the positive correlation between elevated COX-2 and higher histological grade suggest that COX-2 inhibitors have a potential therapeutic effect on grade III scirrhous invasive ductal breast carcinoma.

Acknowledgments

We thank Ms. Yoko Takagi for valuable technical assistance.

References