Cyclooxygenase-2 Expression in Postmastectomy Chest Wall Relapse

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Abstract

Purpose: Cyclooxygenase-2 (COX-2) expression has been shown to be associated with radiation resistance, which theoretically could be overcome with the use of COX-2 inhibitors. The purpose of this study was to assess the prognostic significance and clinical correlations of COX-2 expression (COX) in a cohort of patients treated with radiation for postmastectomy chest wall relapse.

Experimental Design: Between 1975 and 1999, 113 patients were treated for isolated postmastectomy chest wall relapse. All patients were treated with biopsy and/or excision of the chest wall recurrence followed by radiation therapy. Median follow-up was 10 years. All clinical data, including demographics, pathology, staging, receptor status, HER-2/neu status, and adjuvant therapy, were entered into a computerized database. Paraffin-embedded chest wall recurrence specimens were retrieved from 42 patients, of which 38 were evaluated, created into a tissue microarray, stained by immunohistochemical methods for COX, and graded 0 to 3+. A score of 2 to 3+ was considered positive.

Results: Overall survival from original diagnosis for entire cohort was 44% at 10 years. Survival rate after chest wall recurrence was 28% at 10 years. The distant metastasis-free survival rate after chest wall recurrence was 40% at 10 years. Local-regional control of disease was achieved in 79% at 10 years after chest wall recurrence. COX was considered positive in 13 of 38 cases. COX was inversely correlated with estrogen receptor ($P = 0.045$) and progesterone receptor ($P = 0.028$), and positively correlated with HER-2/neu ($P = 0.003$). COX was also associated with a shorter time to postmastectomy chest wall relapse. The distant metastasis-free rate for COX-negative patients was 70% at 10 years, compared with 31% at 10 years for COX-2–positive patients ($P = 0.029$). COX positive had a poorer local-regional progression-free rate of 19% at 10 years, compared with 81% at 10 years for COX negative. This was of high statistical significance with a P value of 0.003.

Conclusions: Outcome following radiation therapy for postmastectomy chest wall relapse is relatively poor. Positive COX correlated with other markers of poor outcome, including a shorter time to local relapse, negative estrogen receptor/progesterone receptor, and positive Her-2/neu status. Positive COX correlated with higher distant metastasis and lower local-regional control of disease. If confirmed with larger studies, these data have implications with respect to the concurrent use of COX-2 inhibitors and radiation for postmastectomy chest wall relapse.

Despite the increasing use of breast-conserving surgeries for breast cancer, mastectomy still plays a large role in primary and salvage treatment. Unfortunately, despite the attempted removal of all breast tissue during a mastectomy, local-regional recurrences still occur. About 10% to 20% of all patients develop a local recurrence, mostly within the boundaries of the chest wall, within a 10-year period despite achieving negative surgical margins (1–8). Prognosis is poor for patients who develop a chest wall relapse. Five-year postrecurrence survival rates range from 36% to 53% (3, 4, 8–15), whereas disease-free survival rates range from 13% to 44% (3, 9, 12, 14, 15). Thus, these women represent a high-risk subgroup with respect to systemic recurrence and mortality. Although various clinical and pathologic parameters have been indicated as prognostic factors for disease-free and overall survival after local-regional recurrence, there is a need to examine molecular markers as prognostic tools to aid clinical management.

Cyclooxygenase-2 (COX-2) has been identified to serve as a tumor marker associated with poor prognosis in various cancers based on epidemiologic, preclinical, and translational studies. COX is a well-known enzyme in the inflammatory pathway that catalyzes the conversion of arachidonic acid to prostaglandin H_2, the rate-limiting step in the formation of

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prostaglandins from membrane phospholipids. There are two forms of COX: COX-1 and COX-2. Whereas COX-1 is constituitively expressed in a variety of mammalian cells, COX-2 is thought to be up-regulated in response to growth factors, tumor promoters, cytokines, and several oncogenes (16–18).

Active research has been directed at exploring the association between COX-2 and carcinoma. As a result, COX-2 has been found to be overexpressed in various cancers, such as colon, cervical, pancreas, head and neck, and lung (19–24). Furthermore, there has been increasing evidence of the role of COX-2 in breast cancer. COX-2 is expressed in ~40% of human breast cancers, and is associated with poor patient prognosis (25). The possible role of COX-2 in breast cancer was first noted when elevated levels of prostaglandins were found in breast tumor cells (26–28). A study in rat models subsequently showed that forced overexpression of COX-2 induced breast cancer (29). Translational studies have also confirmed the positive correlation between these two parameters. Ristimaki et al. (30) found that 37.4% of invasive cancers analyzed by immunohistochemistry were found to have positive COX-2 expression. In another study, 43% of invasive cancers and 63% of ductal carcinomas *in situ* were found to stain positively for COX-2 (31). Positive COX-2 expression has also been correlated with decreased distant disease-free survival, large tumor size, high proliferation rate, and human epidermal growth factor receptor 2 (*HER2*) gene amplification. These studies suggest that COX-2 inhibition may play a potential role in breast cancer treatment.

The main objective of this analysis was to determine whether a significant correlation exists between COX-2 expression and prognosis for patients who developed a chest wall recurrence after mastectomy for breast cancer as analyzed by immunohistochemistry. Secondary objectives were to examine the relationship between COX-2 expression and estrogen receptor, progesterone receptor, and HER-2/neu status at the time of local-regional recurrence.

Patients and Methods

A retrospective review was conducted of 113 patients who were treated with radiation therapy for chest wall recurrence at the Yale University Department of Therapeutic Radiology between 1979 and 1999. All patients had an isolated chest wall recurrence without evidence of distant metastases at the time of presentation. Patients who presented with primarily lymph node metastases, patients with simultaneous distant metastases, and patients who received prior radiation therapy were excluded from the analysis.

Patients underwent surgical excision of the local-regional recurrence when feasible, followed by external beam radiation therapy. Standard radiation treatments were given, and patients were treated to a total median dose of 60 Gy to the chest wall. Nineteen patients received radiation treatment to the chest wall only, and the remaining patients received radiation to the chest wall and regional lymphatics. Adjuvant chemotherapy and hormone therapy were administered as deemed necessary by physicians and in accordance with patient preference. Forty patients received chemotherapy and 56 patients received hormonal therapy at the time of local-regional recurrence. The median follow-up after treatment for recurrence was 10.13 years.

Patient charts were reviewed for demographics and radiation therapy parameters. Other data, including clinical and pathologic staging of initial tumor, method of detection, lymph node status, histologic parameters, surgery done, adjuvant treatment for the initial tumor, estrogen receptor status, progesterone receptor status, chest wall progression, distant metastases, disease-free survival, and overall survival, were documented. All data were entered into a computerized database. The protocol for chart review and processing of tumor specimens was approved by the Yale School of Medicine Human Investigations Committee.

Tumor blocks from chest wall recurrence specimens were successfully collected from 42 patients, of which 38 were evaluable for tissue microarray analysis. The recipient paraffin block was prepared with 2-fold redundancy, which has been shown to correlate well with conventional immunhistochemical staining (32). Core depths were made at ~2 to 3 mm, whereas diameters were set at 0.6 mm. Once all cores were in place, the block was incubated at 37°C for 10 minutes to allow the cores to adhere to the walls of the holes in the array. The array block was then placed into a microtome for sectioning and cut 5 μmol/L thick with a tape-based tissue transfer system (Intrumedics, Hackensack, NJ). After each section was placed on paraffin sectioning aid (PSA-4X) adhesive–coated slides, they were radiated with UV light for 20 seconds. The slide was then placed in xylene for 60 seconds and allowed to air dry.

Analysis by immunohistochemistry was done on microarray slides after deparaffinization in xylene followed by 100% ethanol. Samples were then pretreated to promote antigen retrieval with the DAKO Target Retrieval Solution (DAKO, Carpinteria, CA). A 3% hydrogen peroxide solution was then used for endogenous peroxidase blocking. Slides were then incubated with monoclonal antibody COX-2 (Cayman Chemical, Ann Arbor, MI; dilution 1:50) After incubation, the slides were washed in PBS and a biotinylated secondary antibody was applied. Samples were then applied with DAKO streptavidin-horseradish peroxidase using LSAB+ kit. DAKO 3,3-diaminobenzidine tetrahydrochloride dehydrate was then applied as a chromogenic substrate. Tissue microarrays were also stained for HER-2/neu, estrogen receptor, and progesterone receptor from a previous study (33).

Analysis of the tissue staining was done by a single pathologist (V.B.) and one of the authors who were both blinded to patient outcome. Both distribution (percentage of tumors stained) and intensity of the cytoplasmic staining were documented. Intensity was recorded on a scale of 0 to 3+, with 3+ having the strongest intensity. The specimens were then given a positive or negative based on the intensity and distribution scores. Any score with 2 or 3+ with a distribution of >10% was considered positive in this study, in accordance with standard clinical practices (34). These data were added to the computerized database with all clinical and pathologic outcome variables.

The Prodas Data Base System (Conceptual Software, Houston, TX) was used to assess patient data and statistics. The COX regression model was used to test clinical and pathologic factors by both univariate and multivariate analysis. The life table method was used to calculate survival curves and the Mantel-Hanszel χ^2 test to measure differences in survival curves.

Results

One hundred thirteen patients presented to the Department of Therapeutic Radiology at Yale-New Haven Hospital between January 1979 and December 2000 for radiation treatment to their chest wall for local-regional recurrence of breast cancer. The mean ± SD age at initial diagnosis for these 113 patients was 52.3 ± 13.7 years. The mean ± SD age at presentation with a first isolated chest wall recurrence was 56.9 ± 14.5 years. The mean time to the initial chest wall recurrence was 4.6 years. The median follow-up after initial diagnosis was 13.7 years, and the follow-up after treatment for local-regional recurrence was 10.1 years.

Of 113 patients, 98 were Caucasian, 10 were African American, and 5 were from other racial groups. All patients received simple, modified, or radical mastectomy for their

initial tumor. The mean ± SD size of the tumor at initial diagnosis of breast cancer was 3.3 ± 2.1 cm. The mean number of axillary lymph nodes sampled was 14.4 ± 9.2 lymph nodes, with a mean ± SD of 3.7 ± 6.9 positive lymph nodes. Half of the patients (n = 57) received adjuvant chemotherapy and 33 patients received adjuvant hormone therapy at the time of diagnosis of their original breast carcinoma. The mean ± SD time between initial diagnosis and chest wall recurrence was 4.6 ± 4.6 years. Within 5 years, 66% of patients had experienced their recurrence; and, by 10 years, 90% of patients had developed a recurrence. At the time of chest wall relapse, the mean ± SD size of the recurrence tumor was 1.6 ± 1.0 cm (median, 1.5 cm; range, 0.5-5.0 cm; n = 78). Clinical outcomes for entire patient population are summarized in Table 1. Overall survival after original diagnosis was 69% at 5 years and 44% at 10 years. Overall survival after chest wall recurrence was 46% at 5 years and 28% at 10 years. The distant metastasis-free rate for all patients after chest wall recurrence was 49% at 5 years and 40% at 10 years.

Table 2 summarizes the demographic, staging, receptor status, and treatment parameters at both initial diagnosis and time of chest wall relapse for the cohort of 38 patients included in the tissue microarray. There were no significant differences between this selected cohort and the overall group of 113 patients with respect to the major clinical characteristics and outcomes.

COX-2 staining was predominantly cytoplasmic. Using the criteria of 2 to 3+ staining in >10% of cells, 13 of 38 (34%) were considered positive. The majority of patients underwent complete excision of their recurrence tumors (33 of 38). Those who underwent biopsy were in complete remission following external beam radiation therapy. Only 8 of 38 patients received adjuvant chemotherapy, whereas 25 of 38 patients were known to receive hormone therapy at the time of chest wall relapse. There were no significant differences in treatment (surgery, chemotherapy, or hormonal therapy) as a function of COX-2 status (Table 3).

Correlation of COX-2 expression with various molecular markers, disease-free interval, and chest wall progression is summarized in Table 4. COX-2 expression was found to be positively correlated with HER-2/neu status, early time to chest wall recurrence, and chest wall progression. All were statistically significant. When HER-2/neu status was analyzed, 20 of 24 patients (83%) with negative HER-2/neu status had negative COX-2 expression. This compares with only 4 of 24 patients (17%) with negative HER-2/neu status who had positive COX-2 expression. With regard to time to chest wall recurrence, patients were divided into two groups of early recurrence and late recurrence. Early recurrence was defined as <24 months. Seven of 11 patients (64%) who tested positive for COX-2 showed early recurrence, whereas only 6 of 27 patients (22%) who tested positive for COX-2 showed late

Table 1. Outcomes and survival data for 113 patients

Endpoint	Actuarial rate at 5 y ± SE (%)	Actuarial rate at 10 y ± SE (%)
Overall survival after chest wall recurrence	46 ± 4.9	28 ± 4.8
Distant metastasis-free survival after chest wall recurrence	49 ± 5.3	40 ± 5.9
Local progression-free survival after chest wall recurrence	83 ± 3.6	79 ± 4.1

Table 2. Clinical characteristics of 38 patients in tissue microarray

Characteristic	No. patients	Mean	Range
Age at diagnosis (y)	38	56.7	35-92
Follow-up from original diagnosis (y)	38	21.1	7-45
T status at original diagnosis			
Pathologic T status			
T_1	7		
T_2	17		
T_3	8		
T_4	1		
Unclear original clinical T status	5		
Lymph node status at original diagnosis			
No. with positive lymph nodes	14		
No. with negative lymph nodes	23		
Unknown	1		
Receptor status at original diagnosis			
Estrogen receptor			
Positive	13		
Negative	14		
Unknown	11		
Progesterone receptor			
Positive	12		
Negative	12		
Unknown	14		
Surgery done			
Total mastectomy	3		
Modified radical mastectomy	34		
Radical mastectomy	1		
Adjuvant systemic therapy			
Hormone therapy			
Given	11		
None given	27		
Chemotherapy			
Given	12		
None given	26		
Surgery at CWR			
Excisional biopsy	33		
Incisional biopsy	5		
Chemotherapy at CWR			
Given	8		
None given	30		
Hormone therapy at CWR			
Given	25		
None given	10		
Unknown	3		

Abbreviation: CWR, chest wall recurrence.

Table 3. Correlation of COX-2 with clinical characteristics of 38 patients

Characteristic	COX-2 Positive	COX-2 Negative
Surgery at CWR		
Excisional biopsy	12/13	21/25
Incisional biopsy	1/13	4/25
Hormone therapy at CWR		
Given	9/13	16/22
Not given	4/13	6/22
Chemotherapy at CWR		
Given	3/13	5/25
Not given	10/13	20/25

Abbreviation: CWR, chest wall recurrence.

recurrence. In chest wall progression analysis, 7 of 11 (64%) of patients who tested positive for COX-2 expression developed another chest wall recurrence after treatment for their original chest wall relapse, whereas 6 of 27 (22%) of positive COX-2 patients did not.

COX-2 expression was negatively correlated with both estrogen receptor and progesterone receptor status, both with statistical significance. When estrogen receptor status was analyzed, 5 of 23 (22%) of patients with positive estrogen receptor status showed positive COX-2 expression. This was in contrast to 18 of 23 (78%) of patients with positive estrogen receptor status demonstrating negative COX-2 expression. With regard to progesterone receptor status, only 2 of 15 (13%) of patients with positive progesterone receptor status showed positive COX-2 expression, whereas 13 of 15 (87%) showed negative COX-2 expression.

Table 4. Correlation of COX-2 with estrogen receptor, progesterone receptor, HER-2/neu, time to chest wall recurrence, and chest wall progression

	COX-2 Positive	COX-2 Negative	*P*
ER status			
Positive	5/23 (22%)	18/23 (78%)	0.045
Negative	8/15 (53%)	7/15 (47%)	
PR status			
Positive	2/15 (13%)	13/15 (87%)	0.028
Negative	11/23 (48%)	12/23 (52%)	
HER-2/neu			
Positive	9/14 (64%)	5/14 (36%)	0.003
Negative	4/24 (17%)	20/24 (83%)	
Time to CWR			
Early (<2 y)	7/11 (64%)	4/11 (36%)	0.015
Late (>2 y)	6/27 (23%)	21/27 (77%)	
Chestwall progression			
Positive	7/11 (64%)	4/11 (36%)	0.015
Negative	6/27 (22%)	21/27 (78%)	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

Evaluations of COX-2 expression in relation to outcomes are summarized in Table 5. It is worth noting that the 5-year distant metastases-free rate after primary diagnosis for COX-2 negative patients was 79%, whereas the rate for COX-2 – positive patients was 41% (Fig. 1). This was significant with a *P* value of 0.029. The 5-year local progression-free rate for COX-2 – negative patients was 87%, whereas the rate for COX-2 – positive patients was 38% (Fig. 2). This was highly significant with a *P* value of 0.003.

A multivariate model, taking into account all molecular markers, as well as time to recurrence, was done on the 38 patients. COX-2 overexpression was a significant predictor of chest wall progression (hazard ratio, 4.91; confidence interval, 1.09-22.1; $P = 0.038$). In the analysis for distant metastases-free survival, significant predictors were COX-2 expression (hazard ratio, 5.25; confidence interval, 1.44-19.2; $P = 0.01$) and positive HER-2/neu status (hazard ratio, 0.22; confidence interval, 0.054-0.92; $P = 0.038$). Progesterone receptor status showed borderline significance (hazard ratio, 0.29; confidence interval, 0.075-1.12; $P = 0.073$).

Discussion

Despite receiving treatment for local-regional recurrence after mastectomy, prognosis is poor for these patients and much research has been directed at determining the factors that lead to this poor prognosis. The location, extent, and size of recurrence; number of recurrences; number of recurrence nodules; primary lymph node status; estrogen receptor status of primary; estrogen receptor and progesterone receptor status of recurrence; and age at time of recurrence have all been reported to be indicators of prognosis (3, 4, 8, 9, 13 – 15). A large number of studies have reported that the most significant factor for distant metastases and survival is the disease-free interval, or interval to recurrence from primary diagnosis. Decreased disease-free interval or early recurrence has been associated with ultimately poor outcome in the majority of these studies (3, 4, 8, 9, 13 – 15, 35, 36). Despite these clinical and pathologic prognostic parameters, there is a need to determine molecular markers to more accurately guide clinical management. Unfortunately, it has been difficult to accrue an adequate number of patients for large prospective randomized studies following women after

Table 5. Correlation of COX-2 with outcomes

Endpoint	COX-2 Positive	COX-2 Negative	*P*
Five-year survival after CWR (%)	47	56	0.32
Five-year distant metastasis-free rate after primary diagnosis (%)	41	79	0.029
Five-year local progression-free rate after CWR (%)	38	87	0.003

Abbreviation: CWR, chest wall recurrence.

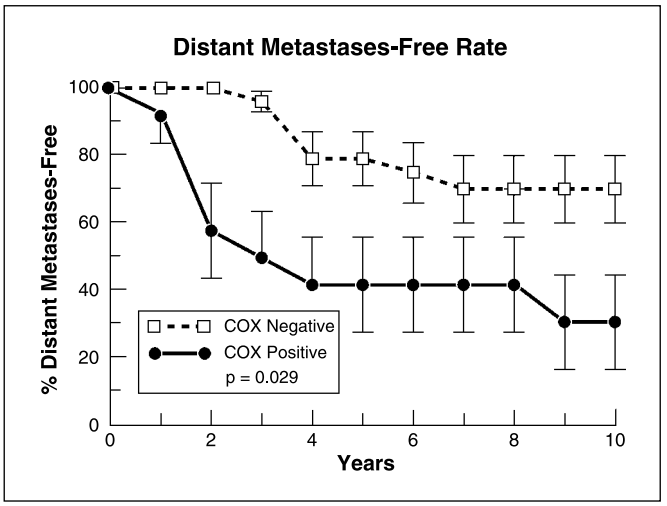

Fig. 1. Distant metastasis-free rate following chest wall relapse by COX-2.

chest wall recurrence. As a result, Haffty et al. (33) conducted a retrospective study to explore the prognostic significance of estrogen receptor, progesterone receptor, p53, HER-2/neu, and cyclin D at chest wall relapse. Significant factors for distant metastasis after local recurrence were time to recurrence and progesterone receptor status, and for local-regional disease progression was HER-2/neu status. Additional biological markers need to be assessed to more clearly define prognosis and assist the physician in selecting therapy regimens catered for high-risk patients. One marker that has been the focus of active investigation is COX-2.

In our study, a uniform cohort of patients who developed chest wall relapse and received radiation therapy were selected to assess the prognostic significance of COX-2 after relapse. Although there was no significant difference in overall survival between COX-2–positive and COX-2–negative patients, patient numbers were relatively small to detect survival differences. The resulting analysis showed that patients who overexpressed COX-2 had a lower chance of being free of distant metastases and higher chance of chest wall progression both on univariate and multivariate analysis. Patients with positive COX-2 expression had a 5-year distant metastases-free rate of 41%, whereas those with negative COX-2 expression had a rate of 79% after primary diagnosis. The results of the 5-year local progression-free rate were even more significant at 38% with positive COX-2 expression and 87% with negative expression. These poor prognostic findings are consistent with a previous study that found the distribution and intensity of COX-2 expression from tissue microarray analysis to correlate significantly with diminished disease-free survival in breast cancer patients, although in this study COX-2 also correlated with decreased overall survival (37). A significant association has also been found between COX-2 overexpression and distant metastasis (38). Previous studies have also shown that positive COX-2 expression is likely associated with poor outcome due to its correlation with poor prognostic factors, such as large tumor size, high histologic grade, axillary node metastases (30), and lymphovascular invasion (39). Furthermore, in our study, COX-2 positivity more likely resulted in early local-regional recurrence or time to recurrence in <2 years (P = 0.015). This is significant because as stated before, early recurrence has been associated with poor prognosis. COX-2 was also found to be negatively correlated with estrogen receptor (P = 0.045) and progesterone receptor status (P = 0.028) at recurrence. This finding is consistent with two recent studies that also analyzed COX-2 expression using tissue microarray analysis in breast cancer (30, 39). Because positive estrogen receptor (40) and progesterone receptor status (41) has been found to be associated with good prognosis, this inverse association with these two markers most likely indicates poor prognosis for this cohort of patients.

A marker that occurs in 20% to 30% of human breast cancers and has been correlated with poor prognosis is HER-2/neu (42–46). Our results showed that COX-2 expression was positively correlated with HER-2/neu status (P = 0.003), thereby indicating poor prognosis. Other studies have also confirmed a positive correlation between these two markers. Two recent studies showed that elevated COX-2 expression was correlated with the presence of HER-2 oncogene amplification in human breast cancers (30, 47). Howe et al. (48) showed that treatment with celecoxib, a COX-2 inhibitor, significantly reduced the incidence of mammary tumors in mice overexpressing wild-type neu protein and caused about a 50% reduction in mammary prostaglandin E2 (PGE2) levels. Benoit et al. (49) further showed that COX-2 inhibition reduced HER-2/neu proteins levels and acted synergistically with trastuzumab, an anti–HER-2 monoclonal antibody in breast cancer cell lines. Concurrent administration of COX-2 inhibitors and HER2/neu antibodies for treatment experimental colorectal cancer inhibited tumor growth more effectively than when either was administered alone (50).

More pertinent to our study is the possibility that COX-2 expression may be involved with decreased radiosensitivity during treatment of recurrence. Other studies have shown this relationship between COX-2 and radiosensitivity. *In vitro* studies showed that a selective COX-2 inhibitor, SC-236, in glioma tumor cell culture medium enhanced cell killing by ionizing radiation. When administered in combination with local radiation, SC-236 caused a greater than additive increase in tumor growth delay (51). Two recent reports concluded that SC-236 markedly increased tumor radioresponse in NFSA

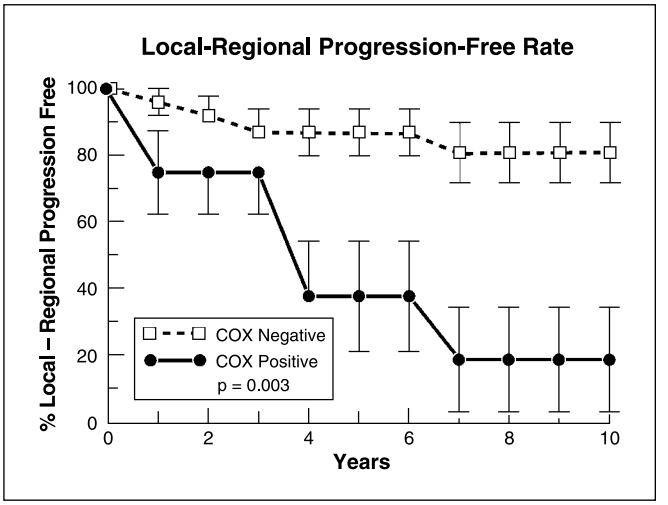

Fig. 2. Local chest wall progression-free rate by COX-2.

(52) and FSA sarcoma (53) bearing mice without greatly affecting normal tissue radioresponse. Other studies have shown that prostaglandins may serve as radioprotectors (54). Although the mechanism is not well understood, it is thought that prostaglandins may protect cellular repair mechanisms (55) or inhibit radiation-induced apoptosis. Additional studies should be done to elucidate the association of COX-2 expression and diminished radiosensitivity.

The methods in our study included use of tissue microarray, which was composed of 0.6 mm cores from original tissue specimens. The validity of using these cores to represent larger tissues has been confirmed in a variety of cancers, such as head and neck, lung, and breast (32, 56, 57). Tissue specimens collected at chest wall relapse were relatively homogeneous. Chest wall specimens were only collected from patients who did not present with simultaneous distant metastases. A monoclonal COX-2 antibody was used, which may have improved specificity for COX-2 compared with a polyclonal antibody. There were also some limitations to this study. Although the method of immunohistochemistry is relatively fast and readily available in most pathology departments, it is a test subject to interobserver variability. The reading of stained microarray slides is also subjective and may result in variability among pathologists. Other limits of this study included its retrospective design and small sample size. Additional studies need to be carried out to validate these findings.

Due to the poor prognosis associated with increased COX-2 expression in tumors, COX-2 inhibitors as potential therapeutic targets remain an active area of research. Various selective COX-2 inhibitors have been shown to slow tumor growth in experimental animals (58, 59). Kishi et al. (53) reported that SC-236 was found to inhibit tumor growth on its own, decrease PGE2 levels in sarcoma FSA tumors, and inhibit neoangiogenesis. One study showed that NS-398, a COX-2–selective inhibitor, induced apoptosis in colorectal tumor cells that was independent of COX-2 protein expression (60). Celecoxib has been shown to decrease tumor size when compared with control groups in rat models (61). In another study by Masferrer et al., COX-2 was found to suppress tumor growth by inhibiting angiogenesis (62). COX-2 was detected in the angiogenic vasculature in most of human colon, prostate, lung, and breast tumors. In addition, celecoxib dose-dependently inhibited tumor growth and the number and size of lung metastases in two animal models of Lewis lung carcinoma and the human colon carcinoma HT-29. It is noteworthy that the expression of COX-2 in these models was mainly limited to the angiogenic blood vessels, the preexisting vasculature adjacent to the primary tumor, and the blood vessels invading the metastatic lesions, and not the cancer cells themselves. In the same study, celecoxib was also a potent inhibitor of angiogenesis in the rat corneal model.

Numerous mechanisms have been proposed attempting to elucidate the correlation between COX-2 expression and tumorigenesis. One theory suggests that COX-2 catalyzed products such as prostaglandins may directly break down into a mutagen and form adducts with deoxynucleosides (62). Prostaglandins, more specifically PGE2, are also known to be potent immunosuppressants. PGE2 blocks the antitumor activity of macrophages and natural killer cells (63, 64) and inhibits production of cytotoxic lymphokines. These activities may block natural surveillance mechanisms to inhibit tumor growth and spread. COX-2 has also been hypothesized to induce carcinogenesis through regulation of apoptosis. Rat intestinal epithelial cells transfected with COX-2 showed increased adhesion to extracellular matrix, resistance to butyrate-induced apoptosis, and elevated expression of bcl-2, a protein that inhibits apoptosis (65). COX-2 may also affect tumorigenesis by dysregulating cell growth. It has been proposed that increased duration of G_1 phase of the cell cycle may cause resistance of cells that permanently express COX-2 to undergo programmed death (66). It has also been suggested that COX-2 is correlated with angiogenesis. COX-2 showed a significant linear correlation (P = 0.001) with staining of CD31, an endothelial cell marker of angiogenesis (67).

In conclusion, our study showed that for patients who develop a local-regional recurrence after mastectomy, positive COX-2 expression at the time of recurrence is a sign of poor prognosis. These patients have a lower chance of expressing favorable prognostic markers, such as estrogen receptor and progesterone receptor, and a higher chance of expressing poor prognostic markers such as HER-2/neu. In addition, they have a greater possibility of local progression and distant metastases. These results indicate that COX-2 may play a role in decreased radiosensitivity at the time of local-regional relapse and that COX-2 inhibition with or without anti-HER2/neu therapy during chest wall radiation may improve prognosis. Prospective randomized trials with a large cohort of patients are needed to further assess these results.

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