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Cyclooxygenase-2 Expression in Brain Metastases

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Abstract. *Background: Elevated Cyclooxygenase-2 (COX-2) expression is thought to increase metastatic potential of many tumors. Furthermore, elevated COX-2 expression correlates with radiation resistance in many tumor types. We evaluated whether: (i) the degree of COX-2 expression correlated with either metastatic tumor type or with the presence of necrosis and whether (ii) radiation-resistant tumors (renal cell and melanoma) had higher expression of COX-2 than did relatively radiation-sensitive tumors (breast and lung). Materials and Methods: Specimens from sixteen patients who underwent resection of brain metastases were analyzed for COX-2 expression using a COX-2 antibody-based immunoassay. Specimens consisted of brain metastases from lung tumors, breast adenocarcinomas, melanomas and renal cell carcinomas. All specimens were analyzed for the presence or absence of necrosis. Results: Ten of sixteen brain metastasis specimens had ten percent or less Cox-2 immunostaining. Statistical analyses showed no correlation between Cox-2 immunostaining and metastatic tumor type or between Cox-2 immunostaining and necrosis in this study. Furthermore, renal cell carcinoma and melanoma showed variable Cox-2 immunostaining. Conclusion: Cox-2 is not consistently expressed in metastases to the brain. The degree of Cox-2 expression does not correlate with metastatic tumor type or with the presence of necrosis. Radioresistant tumors did not have statistically different expression of Cox-2 than radiosensitive specimens studied in this analysis.*

Cyclooxygenase-2 enzyme expression may have a role as a prognostic marker in some metastatic cancers. The metastatic potential of primary tumors has been shown to correlate with Cox-2 expression in both animal and human models (1, 2). Higher levels of Cox-2 expression are seen in malignant *versus* benign breast tumors (3). Mechanisms of Cox-2-mediated carcinogenesis may be related to Cox-2-mediated angiogenesis (4-6). Cox-2 expression in brain lesions correlates with expression in primary non-small cell lung tumors (7). In this paper, we analyze resection specimens from sixteen patients with brain metastases to determine whether: (i) the specimens from the brain lesions had high expression of Cox-2 and (ii) the degree of Cox-2 expression in these specimens correlated with metastatic tumor type or with necrosis and (iii) the radioresistant tumors (renal cell and melanoma) had higher expression of Cox-2 than did the radiosensitive tumors (breast and lung).

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Key Words: COX-2, brain metastasis, necrosis, radiation.

Materials and Methods

Specimens from sixteen patients with metastatic brain disease who underwent surgical resection were studied for degree of Cox-2 expression and necrosis. The tissue sections were cut at 4 microns in thickness and mounted on Fisher positive-charged (+) slides, heat-dried at 70˚C for 30 minutes. The slides were deparaffinized in two changes of xylene, 5 minutes each. The slides were hydrated to distilled water, 3 changes each and in to PBS for 5 minutes. The slides were antigen retrieved in citra plus antigen retrieval solution (Biogenex Laboratories, San Ramon, CA, USA) in a HEIR steamer for 25 minutes, allowed to cool at room temperature and rinsed in distilled water. Dual enzyme endogenous blocker was applied to the slides for 10 minutes then rinsed off with PBS, 3 changes. Cox-2 (Transduction Labs, Lexington, KY, USA) antibody was applied to the slides, 1:250 dilutions at room temperature for 30 minutes, then rinsed in 4 changes of PBS. LSAB 2 secondary antibody (Dako Corp., Carpinteria, CA, USA) was applied to the slides for 15 minutes, then rinsed off with PBS, 4 changes. LSAB 2 label (Dako Corp.) was applied to the slides for 15 minutes, then rinsed off with PBS, 4 changes. DAB chromogen substrate (Dako Corp., Carpinteria, CA, USA) was applied to the slides for 5 minutes, then rinsed off with PBS, 4 changes. Hematoxylin (Dako Corp.) was applied to the slides for 3 minutes, rinsing in warm distilled water. The slides were placed in the PBS buffer to blue the background for 2 minutes. The slides were dehydrated, cleared and coverslipped in 95% alcohol, 100% alcohol and xylene. Both positive and negative controls for the Cox-2 staining assays were established prior to specimen analyses.

Table I. *Degree of Cox-2 immunostaining in brain metastasis resection specimens. The metastatic tumor type and degree of Cox-2 expression is noted. Presence or absence of necrosis is recorded. Note the key for the 0-3 scale for Cox-2 expression. Statistical analyses showed no significant correlation between Cox-2 immunostaining and metastatic tumor type or Cox-2 immunostaining and necrosis.*

Pt.	Age	Gender	Source	Pathology	Necrosis	Cox-2-staining (Brain metastasis)
1	73y	Male	Lung	Adenocarcinoma	y	3
2	62y	Male	Lung	Squamous Cell	y	0
3	46y	Male	Lung	Small Cell	y	0
4	53y	Female	Lung	Squamous cell	y	2
5	60y	Male	Lung	Adenocarcinoma	n	2
6	60 y	Male	Renal	Renal Cell Carcinoma	n	0
7	40y	Female	Renal	Renal Cell Carcinoma	n	3
8	39y	Female	Renal	Renal Cell Carcinoma	n	0
9	28y	Female	Skin	Melanoma	n	0
10	47y	Female	Skin	Melanoma	n	2
11	54y	Male	Skin	Melanoma	y	0
12	38y	Male	Skin	Melanoma	y	1
13	54y	Female	Skin	Melanoma	y	1
14	62y	Female	Breast	Ductal adenocarcinoma	y	3
15	56y	Female	Breast	Ductal adenocarcinoma	y	0
16	29y	Female	Breast	Ductal adenocarcinoma	y	1

Cox-2 staining scale:
0 = no staining of cells
1 = 1-10% of cells staining
2 = 10-49% of cells staining
3 = >50% of cells staining

A two-tailed *t*-test assuming equal variance was performed based upon the standardized scale for degree of immunostaining, with respect to the following variables: type of tumor (lung *vs.* breast *vs.* melanoma *vs.* renal, using $p=0.0125$ for significance); radioresistance *vs.* radiosensitivity (lung + breast *vs.* melanoma + renal, using $p=0.05$ for significance); and presence *vs.* absence of necrosis (using $p=0.05$ for significance).

Results

Statistical analyses of the results in Table I showed that the metastatic brain lesions studied did not demonstrate a statistical correlation between Cox-2 expression and tumor type. There was also no statistical correlation between Cox-2 expression and necrosis ($p=0.918$). Seven of the sixteen brain metastasis specimens studied demonstrated no Cox-2 immunostaining on analysis. Ten of the sixteen brain metastasis specimens studied had ten percent or less Cox-2 immunostaining. Furthermore, melanoma and renal cell carcinoma, clinically radioresistant tumors, did not have significantly different expression of Cox-2 than did the breast or lung specimens studied ($p=0.425$).

Discussion

Prognostic markers can provide invaluable information that guides the diagnosis, prognosis and treatment of cancer. Such markers may be especially useful if they predict the likelihood of distant metastases from a primary cancer. Cox-2 enzyme expression has been proposed as a marker for metastatic potential in certain tumors. In this study, we assessed whether resection specimens from patients with brain metastasis expressed Cox-2. Furthermore, we determined whether the degree of Cox-2 expression correlated with metastatic tumor type or with the presence of necrosis. The results shown here suggest that Cox-2 expression was not consistently expressed in metastases to the brain, as seven of the sixteen specimens studied showed no Cox-2 immunostaining. Furthermore, no correlation was seen between Cox-2 expression and metastatic tumor type or necrosis.

We speculate that Cox-2 expression is not necessary for brain metastasis. This hypothesis is based on the assumption that the primary tumor and its subsequent metastasis both have comparable expression of Cox-2. Of the sixteen patients with brain metastases described in this paper, we had primary specimens from only four patients, two with lung cancer and two with breast cancer. When these four primary specimens were stained, the degree of Cox-2 expression varied (in the two patients with breast cancer) by no more than one point on our 0-3 scale described (data not shown). Others have also reported that primary and metastatic specimens had a comparable degree of Cox-2 expression (7).

Clinical experience suggests that certain metastatic tumors are more responsive to radiation than others. Cyclooxygenase enzyme expression has been shown to correlate with radioresistance. Furthermore, inhibition of Cox-2 has been shown to radiosensitize otherwise radioresistant tumors (8). In this study, we found that renal cell carcinoma and melanoma, generally radiation-resistant tumors, did not have significantly different expression of Cox-2 than did the breast and lung tumors studied, suggesting an alternative (non-Cox-2 mediated) pathway for radioresistance in these tumor types.

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