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COX-2 Expression Correlates With Survival in Patients With Osteosarcoma Lung Metastases

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Summary

The purpose of this study was to determine whether a correlation exists between tumor cyclooxygenase (COX)-2 expression and disease-specific survival in patients with osteosarcoma lung metastases. Thirty-six patients diagnosed with osteosarcoma lung metastases between the years 1990 and 2001 were included in this retrospective study. The majority of the patients (72%) presented newly -diagnosed osteosarcoma lung metastases whereas the remaining patients (28%) presented recurrent disease. Clinicopathologic parameters were obtained from patients' clinical records. Tissue samples were obtained at the time of resection of the lung metastases and stained for COX-2 using immunohistochemistry. Samples were graded according to the intensity of COX-2 staining (grade 0: negative, grade 1: very weak, grade 2: weak, grade 3: moderate, and grade 4: strong). COX-2 staining was correlated with disease-specific survival and clinicopathologic parameters using the Jonckheere-Terpstra and the Kruskal-Wallis tests. All patients with grade 3 or 4 COX-2 expression died of osteosarcoma lung metastases. Ten percent of patients with grade 2 COX-2 expression and 29% of patients with grade 1 expression were alive and free of disease at the last follow-up. By contrast, 60% of the patients with grade 0 COX-2 expression were alive and free of disease at the last follow-up. No association between COX-2 expression and clinicopathologic parameters was found. However, COX-2 expression correlated inversely with disease-specific survival in patients with osteosarcoma lung metastases. Our data indicate that COX-2 expression in metastatic osteosarcoma may have prognostic significance.

Keywords

COX-2; osteosarcoma; lung metastases

Soft tissue and bone sarcomas comprise nearly 13% of all cancers in childhood. Approximately 1500 new cases of pediatric sarcoma are diagnosed each year in the United States.¹ For osteosarcoma, the most common primary tumor site is the metaphysis of long bones, and the most common site of metastatic involvement is the lungs. Patients with

osteosarcoma lung metastases are at high risk for a poorer long-term survival. Some patients never have recurrence after surgical excision of lung metastases and are essentially cured of their disease.² However, more than 80% of the patients relapse within 1 year. Treatment for osteosarcoma is limited to surgery and chemotherapy and the prognosis depends on the extent of disease and on histologic response to chemotherapy.³ Most patients with osteosarcoma lung metastases do not survive metastatic disease, and osteosarcoma lung metastases remain the most common cause of death in patients with osteosarcoma. As a result, survival rates for osteosarcoma have remained unchanged over the last few decades.¹ There is a need to identify which patients with osteosarcoma lung metastases are at highest risk for relapse so that novel therapies can be initiated as early as possible in an effort to improve patient outcome.

Cyclooxygenase-2 (COX-2) is an inducible enzyme that is expressed in response to inflammatory stimuli such as cytokines, growth factors, endotoxins, and tumor cells.^{4–6} COX-2 overexpression has been associated with tumor proliferation and invasion, inhibition of apoptosis, and suppression of immune surveillance.^{5,7–9} COX-2 also plays an important role in angiogenesis: studies have shown down-regulation of vascular endothelial growth factor expression after treatment with COX-2 inhibitors.^{10–12} COX-2 catalyzes the conversion of arachidonic acid into multiple prostaglandin products. One of these, prostaglandin E₂, has been strongly implicated in the process of carcinogenesis. COX-2 expression has been used as a prognostic marker for several malignancies, including colorectal, lung, pancreas, and ovarian cancers.^{13–16}

The majority of sarcomas in children express COX-2 to varying degrees. Previous studies have shown a trend toward higher COX-2 expression in metastatic sarcomas than in nonmetastatic disease.^{17–18} However, a correlation between COX-2 expression and prognosis in osteosarcoma has not been established.

The purpose of this study was to determine whether a correlation exists between tumor COX-2 expression and disease-specific survival in patients with osteosarcoma lung metastases. A secondary objective was to evaluate whether COX-2 serves as a prognostic marker in this subgroup. To address these questions, tumor samples from patients with osteosarcoma lung metastases were analyzed using immunohistochemistry for COX-2 expression and expression was correlated with survival.

MATERIALS AND METHODS

Patients

Thirty-six patients initially diagnosed with osteosarcoma between January 1, 1990 and December 31, 2001, who had osteosarcoma lung metastases at initial diagnosis (26/36) or developed them later (10/36), were included in this study. Patients were diagnosed and treated at The University of Texas MD Anderson Cancer Center (Houston, TX/n=15), Mayo Clinic (Rochester, MN/n=13), or Children's Hospital and Regional Medical Center (Seattle, WA/n=8). The patient's medical records were reviewed for demographic information (age at diagnosis, sex, and ethnicity), primary tumor location, chemotherapy regimen, tumor histology, extent of tumor necrosis after preoperative chemotherapy, and survival data.

Treatment

Before primary tumor resection, all patients received either 3 or 4 courses of chemotherapy. Preoperative chemotherapy regimens consisted of doxorubicin, high-dose methotrexate, and ifosfamide with or without cisplatin^{19–20} or doxorubicin, cisplatin, and high-dose methotrexate with or without ifosfamide.²¹ Four patients also received etoposide. Intravenous chemotherapy was administered at the following total doses per course:

doxorubicin, 75 mg/m²; cisplatin, 120 mg/m²; methotrexate, 12 g/m²; ifosfamide, 9 g/m²; and etoposide, 500 mg/m². In addition to surgical resection of the primary tumor, patients underwent unilateral or bilateral thoracotomy, depending on whether the lung metastases involved one or both lungs. Salvage chemotherapy was administered to patients with unresectable lung metastases and/or recurrent lung disease. The agents most commonly used for this purpose were a combination of etoposide and ifosfamide, or either agent alone.

Immunohistochemistry

Formalin-fixed, paraffin-embedded samples of osteosarcoma lung metastases tissue were obtained for all 36 patients. The diagnosis was confirmed by analyzing hematoxylin-eosin-stained section and immunohistochemical staining.

To determine COX-2 protein expression, formalin-fixed, paraffin-embedded metastasis samples were sectioned at 5- μ m intervals. Tissues were deparaffinized in xylene and rehydrated in ethanol. Antigen retrieval with 0.1 M citrate buffer (pH 6.0) was performed using microwave heat for 5 minutes. Tissues were incubated for 15 minutes with 3% H₂O₂ to block endogenous peroxides. Nonspecific protein binding was blocked for 30 minutes with 6% normal horse serum. Tissues were then incubated overnight at 4°C with primary COX-2 antibody (1 μ g/mL in blocking buffer) (Santa Cruz Biotechnology Inc, Santa Cruz, CA). Secondary antibody labeled with horseradish peroxidase was applied for 1 hour at room temperature. Immunoreactive complexes were detected using 3, 3'-diaminobenzidine as a chromogen. Sections were counterstained with hematoxylin. Negative control slides were obtained by omitting COX-2 primary antibody. As the human colon adenocarcinoma cell line HT-29 is well known to overexpress COX-2, murine tumor tissue after subcutaneous injection of HT-29 cells was obtained to serve as a positive control. COX-2 expression was graded by 3 independent investigators who were blinded to the samples and clinical data. Samples were graded according to the proportion of stained tumor cells and the staining intensity, and grade was assigned as follows: grade 0, negative (no COX-2 expression by osteosarcoma cells); grade 1, very weak expression (5% to 10% COX-2 expression); grade 2, weak (more than 10% but <30% COX-2 expression); grade 3, moderate (at least 30% but <50% COX-2 expression); and grade 4, strong (at least 50% COX-2 expression) (Fig. 1). Grading assigned by each independent investigator was identical in the majority of the samples. However, if a discordant grading was identified, samples were reviewed by each investigator and the final grading was assigned on the basis of concordance of at least 2 investigators. After grade was assigned, samples were classified into 1 of 2 categories: group 1 (COX-2 grade 0 to 1) or group 2 (COX-2 grade 2 to 4).

Statistical Analysis

Statistical analysis was performed to analyze survival data. This analysis was based on disease-specific survival and was focused on testing for differences between groups defined by COX-2 expression (Jonckheere-Terpstra test). A *P* value <0.05 was considered statistically significant. Overall survival and disease-specific survival were calculated from the date of diagnosis to the date of last follow-up or death.

The correlation between clinicopathologic parameters (primary tumor site, histologic subtype, presence or absence of lung metastases at diagnosis, and extent of tumor necrosis after preoperative chemotherapy) and COX-2 expression was also examined.

RESULTS

Patients and Disease Characteristics

Table 1 summarizes patient and disease characteristics for the 36 patients in the study. The ages of the patients ranged from 9 to 75 years; the median age was 17 years. Ten patients had osteosarcoma lung metastases at initial diagnosis. The remaining 26 patients had localized disease at diagnosis but subsequently developed osteosarcoma lung metastases either during or after chemotherapy. In 26 patients, the lung metastases developed at a mean of 22 months after initial diagnosis (range: 2mo to 7 y). The primary tumors were located in the long bones in 33 cases (femur, n=21; tibia, n=7; humerus, n=3; fibula, n=2) and in the axial skeleton in 3 cases (pelvis, n=2; clavicle, n=1). Twenty-eight patients died of osteosarcoma lung metastases, 1 patient (no. 2) was cured but died of another cause, and 7 patients were alive and cured at last follow-up. (Table 1).

Table 2 shows the correlation between clinicopathologic parameters and COX-2 expression. There was no correlation between COX-2 expression and any of the parameters analyzed.

Immunohistochemical Analysis

Grade 4 COX-2 expression was seen in 2 (5.6%) of the 36 patients, grade 3 expression in 5 (13.9%), grade 2 expression in 10 (27.8%), grade 1 expression in 14 (38.9%), and grade 0 expression in 5 (13.9%) patients.

Correlation Between COX-2 Expression and Disease-specific Survival

Analysis of the data suggested that patients with grade 2 to 4 COX-2 expression had a poorer disease-specific survival (Table 3, $P=0.0103$). All patients with grade 3 or grade 4 COX-2 expression and 9 of 10 patients with grade 2 COX-2 expression died of osteosarcoma lung metastases. In contrast, among the patients with grade 1 COX-2 expression, 29% (4/14) were disease free at the time of last follow-up. However, 1 patient from this group died after last follow-up for reasons unrelated to osteosarcoma. At the time of patient's death, there was no evidence of disease. Among those with grade 0 COX-2 expression, 60% (3/5) were disease free at the time of last follow-up. These data indicated an inverse correlation between COX-2 expression and disease-specific survival in patients with osteosarcoma lung metastases.

DISCUSSION

In this study, patients with osteosarcoma lung metastases with absent or very weak (grade 0 or grade 1) COX-2 expression had better disease-specific survival than patients with stronger COX-2 expression (grade 2 to 4).

The correlation between COX-2 expression and clinical outcome has previously been investigated for other types of solid tumors. A strong association between higher COX-2 expression and worse prognosis has been reported for chondrosarcoma,²² breast cancer,²³ colorectal cancer,¹³ pancreatic cancer,¹⁵ and nonsmall cell lung cancer.¹⁴ However, the converse has also been reported in other studies for breast cancer: stronger COX-2 expression correlated with a more favorable outcome.²⁷

Using immunohistochemistry, Endo et al²² recently showed that COX-2 overexpression in primary chondrosarcoma was significantly associated with a higher histologic grade and decreased disease-specific survival. Their finding suggested that COX-2 overexpression in primary chondrosarcoma not only correlated with histologic grade but also could be used as a prognostic factor.

Similarly, our findings suggest that COX-2 expression in osteosarcoma lung metastases can be used as a prognostic factor. Of the 16 patients with grade 2 to 4 COX-2 expression, only 1 (6%) remained disease free at last follow-up, whereas 4 of 14 (29%) patients with grade 1 and 3 of 5 (60%) patients with grade 0 COX-2 expression were alive and free of disease. These data suggest that a cut point of only 10% difference in COX-2 expression between grades 1 and 2 seems important, which may be secondary to the presence of more chemotherapy-resistant cells.

Dickens et al reported a trend toward higher COX-2 expression in metastatic sarcomas than nonmetastatic sarcomas, including in osteosarcoma, rhabdomyosarcoma, and Ewing sarcoma.^{17–18} Trends toward higher COX-2 expression in older individuals and in females were also noted. Osteosarcoma lung metastases samples were more likely to express COX-2 than samples taken from diagnostic biopsies. However, no correlation between COX-2 expression at the time of diagnosis and clinical outcome could be established. Therefore, Dickens et al concluded that COX-2 expression did not seem to be a reliable prognostic marker for osteosarcoma.

Our current study focused on a single type of sarcoma (osteosarcoma) in a selected patient population (patients with osteosarcoma lung metastases) and evaluated whether COX-2 expression could be used as a prognostic marker for this patient population. Patients with osteosarcoma lung metastases are at high risk for a poorer long-term survival. Some patients never have recurrence after surgical excision of lung metastases and are essentially cured of their disease.² Most patients, however, relapse within 1 year from diagnosis. It would, therefore, be advantageous to identify the group of patients at high risk for relapse and individualize therapy on the basis of the expression of molecular markers in the tumor. The ability to identify high risk patients may allow novel therapies to be considered early and may also spare individuals who are unlikely to have recurrence from the toxicity of further therapy.

We also analyzed the relationship between COX-2 expression and clinicopathologic parameters (primary tumor site, tumor histologic subtype, presence or absence of lung metastases at diagnosis, and extent of tumor necrosis) in an attempt to identify other factors that could be important in assessing prognosis. However, no correlation was found.

Several studies have addressed whether age is an important factor when assessing prognosis in patients with osteosarcoma, as the tumor biology may differ between age groups and could affect response to chemotherapy. In a study published by Bacci et al,²⁸ preadolescent patients with osteosarcoma (<13 y) were compared with older patients (13 to 40 y of age) in terms of 5-year event-free survival, overall survival, rate of amputation, time/type of first relapse, and histologic response to preoperative chemotherapy. The authors concluded that even though there may be differences in osteosarcoma tumor biology between preadolescent and older patients, the prognosis for both groups was not different. Our analysis included mostly pediatric patients (<18 y of age). However, as the data supports no difference in prognosis according to age, a few young adults (18 to 35 y of age) were included in our study along with a 75-year-old patient.

COX-2 inhibitors have been shown to inhibit the growth of osteosarcoma cell lines in vitro. Meloxicam, a selective COX-2 inhibitor, suppressed the growth of osteosarcoma cells that expressed high levels of COX-2 by both COX-2–dependent and COX-2–independent mechanisms.²⁹ COX-2 inhibitors were also shown to prevent occurrence and recurrence of solid tumors, such as colorectal cancer.³⁰ Our data showing high COX-2 expression in metastatic osteosarcoma tumor samples suggest that the use of selective COX-2 inhibitors may be of therapeutic benefit for these patients.

In summary, our results suggest that COX-2 expression in patients with osteosarcoma lung metastases may be prognostic for disease-specific survival and may identify those patients that are most likely to benefit from the early initiation of novel therapies to prevent relapse. A prospective trial is needed to validate this finding. This and other investigations identifying new therapeutic targets are critical if we are to improve survival in patients with metastatic osteosarcoma.

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FIGURE 1.

COX-2 expression in representative human samples of oosteosarcoma lung metastases. A, grade 0 (negative COX-2 expression). B, grade 1 (very weak). C, grade 2 (weak). D, grade 3 (moderate). E, grade 4 (strong). Negative and very weak expressions were classified as group 1. Weak and moderate and strong expressions were classified as group 2.

TABLE 1

Patient and Disease Characteristics

Institution	Dx	Sex	Race	Primary Tumor	Histo	Postop TN (%)	Lung Mets at Dx	Time to Mets	Outcome	Time to Death or Last f/u	COX-2 Grade	
1	MDA	11-94	F	W	Femur	O	99	No	7 y	A, NED	01-06	0
2	MDA	02-99	M	A	Femur	O	95	No	2 y	D, NED	04-05	1
3	MDA	10-99	M	H	Femur	Ch	96	No	3 y	D	11-02	4
4	MDA	06-99	M	W	Femur	O	86	Y	0	D	08-01	3
5	MDA	04-95	M	Ar	Femur	O	99	No	3 y	D	02-01	4
6	MDA	06-91	M	W	Tibia	Ch	65	No	4 y	D	11-04	3
7	MDA	07-99	F	W	Femur	Ch	65	No	2 y	D	09-03	2
8	MDA	01-98	F	A	Femur	U	90	No	1 y	D	03-00	1
9	MDA	12-98	F	A	Pelvis	Fi	83	No	1 y	D	11-01	1
10	MDA	09-97	F	W	Clavicle	O	10	No	4 mo	D	05-01	2
11	MDA	01-00	F	A	Femur	Fi	90	Y	0	D	10-01	1
12	MDA	11-99	M	A	Humerus	O	99	No	2 y	D	04-05	2
13	MDA	10-97	M	Ar	Tibia	O	99	No	3 y	D	04-03	2
14	MDA	04-00	F	H	Femur	Ch	80	No	3 y	D	04-03	2
15	MDA	08-02	M	H	Femur	O	80	No	1 y	D	07-04	2
16	CHR	02-92	-	-	Tibia	Ch	90	Y	0	A, NED	03-03	1
17	CHR	08-96	-	-	Femur	U	67	Y	0	A, NED	01-02	2
18	CHR	04-98	-	-	Femur	Ch	10	No	7 mo	D	07-99	1
19	CHR	03-96	-	-	Humerus	U	90	Y	0	D	07-98	2
20	CHR	06-92	-	-	Femur	U	40	Y	0	D	03-97	2
21	CHR	03-92	-	-	Tibia	U	95	No	3 y	D	01-96	3
22	CHR	10-91	-	-	Femur	U	75	No	1 y	D	09-93	1
23	CHR	01-98	-	-	Tibia	U	75	No	2 y	D	10-01	3
24	Mayo	06-90	-	-	Femur	U	100	Y	0	A, NED	09-02	1
25	Mayo	11-90	-	-	Tibia	T	-	No	2 y	A, NED	08-01	1
26	Mayo	02-95	-	-	Fibula	U	80	No	1 y	A, NED	05-02	0
27	Mayo	03-98	-	-	Humerus	U	82	No	6 mo	A, NED	05-01	0
28	Mayo	08-91	-	-	Pelvis	U	98	Y	0	D	07-93	3
29	Mayo	09-94	-	-	Fibula	Fi	50	No	2 y	D	05-98	0

Institution	Dx	Sex	Race	Primary Tumor	Histo	Postop TN (%)	Lung Mets at Dx	Time to Mets	Outcome	Time to Death or Last f/u	COX-2 Grade
30	Mayo 10-94	-	-	Tibia	U	90	No	1 y	D	09-98	0
31	Mayo 01-92	-	-	Femur	U	30	Y	0	D	09-98	2
32	Mayo 03-90	-	-	Femur	Ch	-	No	2 mo	D	10-93	1
33	Mayo 11-99	-	-	Femur	Ch	30	No	1 y	D	05-02	1
34	Mayo 02-96	-	-	Femur	U	10	No	1 y	D	04-01	1
35	Mayo 05-92	-	-	Femur	U	18	No	2 y	D	01-97	1
36	Mayo 12-97	-	-	Femur	U	85	Y	0	D	01-99	1

A indicates African-American; A, NED, alive no evidence of disease; Ar, Arabic; Ch, chondroblastic; CHR, Children's Hospital and Regional Medical Center; COX, cyclooxygenase; D, died of disease; D, NED, dead no evidence of disease; Dx, diagnosis; F, female; Fi, fibroblastic; f/u, follow-up; H, Hispanic; histo, histology; M, male; MDA, MD Anderson Cancer Center; mets, metastases; O, osteoblastic; Postop TN, postoperative tumor necrosis; T, telangiectatic; U, unclassified; W, white; Y, Yes.

TABLE 2

Correlation of Clinicopathologic Parameters With COX-2 Expression

Parameter	No. Cases	COX-2 Expression								P	
		Grade 0				Grade 1					
		1	2	3	4	1	2	2	2		
All cases	36	5	14	10	5	2					0.325
Primary Site											
Femur	21	1	11	6	1	2					
Tibia	7	1	2	1	3	0					
Humerus	3	1	0	2	0	0					
Fibula	2	2	0	0	0	0					0.197 (excluding unclassified)
Pelvis	2	0	1	0	1	0					
Clavicle	1	0	0	1	0	0					
Histology											
Osteoblastic	8	1	1	4	1	1					
Chondroblastic	8	0	4	2	1	1					0.325
Fibroblastic	3	1	2	0	0	0					
Telangiectatic	1	0	1	0	0	0					
Unclassified	16	3	6	4	3	0					
Lung metastases at diagnosis											
Yes	10	0	4	4	2	0					0.485 (excluding N/A)
No	26	5	10	6	3	2					
Extent of tumor necrosis (%)											
95	9	1	2	2	2	2					
90-94	5	1	3	1	0	0					
80-89	7	2	2	2	1	0					0.485 (excluding N/A)
50-79	6	1	1	2	2	0					
<50	7	0	4	3	0	0					
N/A	2	0	2	0	0	0					

Kruskal-Wallis test was used for primary site, histology, and lung metastases at diagnosis. Jonckheere-Terpstra was used for extent of tumor necrosis.

COX indicates cyclooxygenase; N/A, not available.

TABLE 3

Relationship Between COX-2 Expression and Disease-specific Survival in Patients With Osteosarcoma Lung Metastases Outcome at Last Follow-up

COX-2 Expression	Dead of Disease	No Evidence of Disease	Total No. Patients
Grade 4	2 (100%)	0 (0%)	2
Grade 3	5 (100%)	0 (0%)	5
Grade 2	9 (90%)	1 (10%)	10
Grade 1	10 (71%)	4 (29%)	14
Grade 0	2 (40%)	3 (60%)	5

Jonckheere-Terpstra for ordered alternatives: $P=0.0103$.

COX indicates cyclooxygenase.