RESEARCH COMMUNICATION

Lack of Influence of Cyclooxygenese-2 Expression in Hepatocellular Carcinomas on Patient Survival

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Abstract

Recent studies suggest that cyclooxygenese-2 (COX-2) enzyme activation may play a role in hepatocarcinogenesis. However, the clinical significance of COX-2 expression in hepatocellular carcinoma (HCC) remains obscure. This study evaluated COX-2 expression in hepatitis B and hepatitis C virus related HCC and in HCC patients with an unknown etiology. Liver tissue samples of 31 patients with HCC (27 men and 4 women; age range, 48-75 years) were analyzed. COX-2 expression was evaluated by immunohistochemically in the tumor tissues. Patient data including age, sex, Child score, stage, grade of the tumor and survival were analyzed. Of these patients 19 were positive for hepatitis B virus (HBV), 6 were positive for hepatitis C virus (HCV) and 6 patients were negative for all viral markers and other etiologic factors. COX-2 staining were evaluated in 2 groups (group 1: COX-2 expression less than 25% (grades 1-2 COX-2 expression), and group 2: Cox-2 expression 25% or more (grades 3-5 COX-2 expression). COX-2 expression was shown in all HCC samples with positive or negative viral markers. No difference was found between degree of COX-2 expression and the etiology of HCC. COX-2 expression was not correlated with number of lesion or stage of the disease or grade of the tumor. COX-2 expression was not related with Child score of the patients. Median survival of all patients was 32 months. Median survival of patients did not differ according to patient's viral marker status. No difference was observed in median survival of patients in group 1 and 2. As a result, COX-2 system seem to be shared part in hepatocarcinogenesis regardless factors that initiate the disease. Although COX-2 expression appears to be independent of disease's characteristics', treatments that target this system appear to be feasible in the management of HCC.

Key Words: Hepatocellular carcinoma - cyclooxygenese-2 - survival

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Introduction

In Asia and Africa, hepatocellular carcinoma (HCC) is one of the most common gastrointestinal tumors. Hepatic cirrhosis is observed in up to 90% of patients with HCC (Okuda, 1992). Chronic hepatitis B (HBV) or hepatitis C (HCV) infection or chronic alcohol consumption are the most common predisposing factors. The exact mechanism of hepatocarcinogenesis has not been clarified, and the role of HBV and HCV as direct carcinogens is unclear, because the HCV genome is not integrated into human host; HBV is integrated in a seemingly random fashion (Barlett et al., 2005). HCC is characterized by hypervascularity and high metastatic potential. Because few patients are candidates for potentially curative treatment; current efforts should focus on developing new molecules which can effectively treat HCC.

COX-2, a rate-limiting enzyme involved in converting arachidonic acid to prostaglandins, is overexpressed in many malignant neoplasm. The COX-2 gene is associated with cellular growth, differentiation, and tumorigenesis (McGinty et al., 2000; Williams et al., 2000). With regard to tumorigenesis, COX-2 is implicated thusly; angiogenesis is enhanced by production of vascular endothelial growth factor (VEGF), and prostoglandins, antiapoptosis is mediated by Bcl-2 and protein kinase B signaling, and invasion is mediated via activation of matrix metalloproteinases (Dannenberg et al., 2005).

Recently COX-2 expression has been demonstrated in HBV- and HCV-related HCC (Koga et al., 1999; Cheng et al., 2004). However controversy exists with regard to COX-2 expression in HCC. One study has postulated that COX-2 upregulation is apparent in the early phases of hepatocarcinogenesis; another study suggests that COX-2 expression is a factor both in the early and in the late stages of carcinogenesis (Koga et al., 1999; Morinaga et al., 2002). Still, little is known about the degree of COX-

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2 immunostaining in HCC tissues and the disease's prognosis. The current study was designed to evaluate the relationship between tumoral COX-2 expression and clinical features of patients with HCC.

Materials and Methods

HCC Cases

Liver tissue specimens from 31 patients with HCC (27 men and 4 women; age range 48-75 years) were obtained between 1996 and 2006 at Baskent University Hospital in Ankara, Turkey. Nineteen patients tested positive for HBV and 6 patients tested positive for HCV and 6 tested negative for both. Clinical features of patients are shown in Table 1. After the study, 14 patients (45.1 %) were alive, 15 patients (48.3%) were dead. Two patients were lost the follow-up. Six received local treatment (transarterialchemoembolisation radiofrequency ablation), 13 underwent transplantation, 4 surgical excision and 7 palliative care. Only 1 patient received systemic chemotherapy.

Immunohistochemical staining and evaluation of COX-2

All hepatectomy specimens were fixed in formalin, embedded in paraffin, and cut into serial sections of 3-4 mm thick. Specimens were then stained with hematoxylin and eosin, Masson's trichrome, Gomori's reticulin for collagen fibers and Perls' iron stain. We immunohistochemically analyzed all samples using the ABC (avidin-biotin complex) method, and applied primary COX-2 antibody (cyclooxygenase-2, rabbit, Lab Vision Corp/Neomarkres, Fremont, Calif, USA) to deparaffinized tissue sections. For COX-2, a 1/50 dilution was used, and specimens were incubated overnight at room temperature. Colon carcinoma cell immunoreactivity was used as positive control. Grading was semiquantitative from 0 to 5: grade 0 = no or very rare staining, 1 = few cells stained (approximately 1% to 5%), grade 2 = more cells stained (5% to 25%), grade 3 = a large number stained (25% to 50%), grade 4 (50% to 75%), and grade 5= diffuse staining of more than 75% of cells. COX-2 expression was evaluated only in tumor tissues.

Statistical analyses

Data were expressed as medians and range. The Mann-Whitney U test and Chi-square test were used when appropriate. Kaplan-Meier test was used for survival analysis. Survival difference of the groups was compared by the log-rank test. Values for P less than 0.05 were considered statistically significant.

Results

All HCC specimens stained positive for COX-2. For the statistical analysis COX-2 stained were evaluated in

Table 1. Clinical Features of the HCC Patients

Patient	Sex	Age(yr)	HM	Lesion	Stage	Grade	CS	Treatment	Status	Survival(m)	Cox-2
1	M	35	B	One	1	3	A	Surgery	alive	28+	2
2	M	49	C	More	1	2	A	Surgery	alive	44+	5
3	F	68	C	More	2	2	B	Local	death	16	4
4	M	44	B	More	2	2	C	Palliative	death	3	4
5	M	67	B	More	3	1	A	Surgery	death	24	3
6	M	51	B	One	2	3	A	Tx	loss	14	5
7	M	49	-	Diffuse	3	2	A	Tx	alive	20+	1
8	M	66	B	One	3	2	C	Palliative	death	1	2
9	M	68	B	One	4	3	B	Local	death	13	4
10	M	55	B	More	3	2	B	Local	death	9	1
11	M	48	B	One	2	3	A	Tx	alive	9+	2
12	M	55	-	One	3	2	B	Palliative	loss	6	4
13	F	70	C	More	3	2	C	Palliative	death	1	1
14	M	48	B	One	2	3	A	Local	death	32	1
15	M	18	-	One	3	3	A	Surgery	death	4	2
16	M	70	-	One	2	2	A	Local	alive	51+	5
17	M	75	B	More	3	3	C	Palliative	death	1	5
18	M	59	B	More	4	3	B	Chemo	death	2	4
19	M	59	C	More	3	2	A	Tx	alive	25+	4
20	M	55	C	More	3	2	A	Tx	death	32	1
21	M	51	B	More	3	2	A	Tx	alive	39+	1
22	F	54	B	More	3	2	C	Palliative	death	3	2
23	M	62	-	More	2	3	C	Palliative	death	3	1
24	M	48	B	More	2	2	A	Tx	alive	22+	3
25	M	68	C	One	3	3	B	Local	death	2	3
26	F	56	-	One	2	1	A	Tx	alive	18+	1
27	M	55	B	One	3	3	A	Tx	alive	21+	3
28	M	56	B	More	2	3	A	Tx	alive	19+	1
29	M	19	B	More	2	1	A	Tx	alive	19+	3
30	M	65	B	More	2	2	A	Tx	alive	17+	1
31	M	61	B	One	2	2	A	Tx	alive	13+	1

M: Male; F: Female; Tx: Transplantation; HM: Hepatitis marker; CS: Child Score

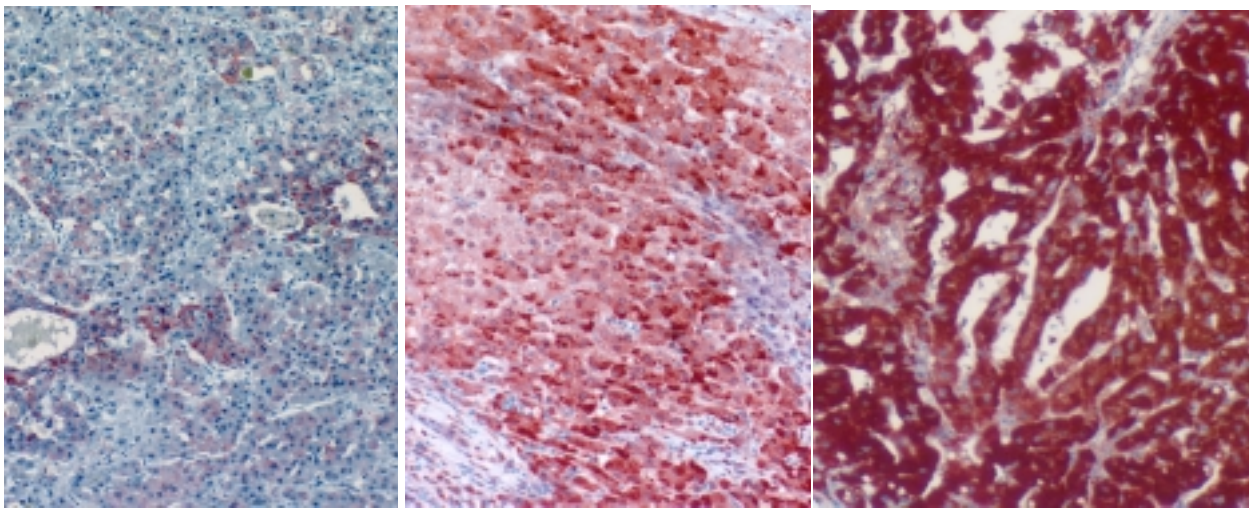

Figure 1. COX-2 Expression in Hepatocellular Carcinomas, a) Grade 1, b)Grade 2, c) Grade 3 (x10, immunoperoxidase)

2 groups (group 1: COX-2 expression less than 25% (grades 1-2 COX-2 expression), and group 2: Cox-2 expression 25% or more (grades 3-5 COX-2 expression). No correlation was found between degree of COX-2 expression and number of lesions (p=.26). Although most of the specimen with HBV-related HCC showed COX-2 staining less than 25%, this difference was not significant (p=.53) (Table 2). No difference was found between degree of COX-2 staining and HCV- or HBV-related HCC tissues or HCC with negative viral markers. No correlation was found between Child score of the patients and COX-2 staining degrees. Degree of COX-2 staining did not differ depending on stage of the disease. Also no correlation was found between alfa-fetoprotien and COX-2 expression. Degree of COX-2 expression did not differ in well-differentiated and less differentiated HCC specimens. Overall survival of whole group was 32 months. Patients in group 1 had a median survival of 32 months, whereas median survival of patients in group 2 COX-2 expression was 24 months (p>.05) (Table 2). No significant difference was found in median survival of patients that underwent transplantation or surgery in group 1 and those of group 2 (p>.05). Median survival of patients did not differ according to patient's viral marker status (p>.05). Patients who underwent surgical excision or transplantation significantly survive longer compared with patient who received local treatments (p=.00).

Table 2. Comparison of HCC tumors Expressing COX-2 <25% Versus ≥25%

		<25%(%)	≥25% (%)
Number		16	15
Mean age (years)		53	57
Child Score	A	11 (68.8)	8 (53.4)
	B	1 (6.25)	5 (33.3)
	C	4 (25.0)	2(13.3)
Stage	I	1 (6.25)	1(6.8)
	II	7 (43.8)	7(46.6)
	III	8 (50.0)	5(33.3)
	IV	0	2(13.3)
Viral markers	HBV	10 (62.5)	9(60)
	HCV	2 (12.5)	4(26.7)
	Negative	4 (25.0)	2(13.3)
Treatment	Resect/Tx	10 (62.5)	7(46.8)
	Local	2 (12.5)	4(26.6)
	Other	4 (25.0)	4(26.6)
Survival (months)		32	24 (n.s.)

Tx: Transplantation, n.s: not significant

Discussion

Although in many instances, HCC is the result of chronic viral hepatitis, in other instances its etiology is unknown. However, clinical manifestations are similar. The prognosis is poor, and appears to be independent of etiological factors. The mechanism of hepato-carcinogenesis initiated by viral or other factors has not been defined. Activation of the COX-2 enzyme system has been shown in the carcinogenesis of different neoplasm. In Japan, COX-2 expression was investigated in HCV-related HCC cases, and in China, investigators have investigated COX-2 in HBV- related HCC cases (Koga et al., 1999; Kondo et al., 1999; Tang et al., 2005). The pattern of COX-2 expression in the process of hepatocarcinogenesis differs (McGinty et al., 2000; Roayaie et al., 2000; Chen et al., 2001). However, the degree of COX-2 expression in cryptogenic HCC cases remains unknown. In this study, we showed that all HCC cases regardless of etiologic factors, stained immunohistochemically by COX-2. This demonstrates that COX-2 enzyme activation seems to be one of the common pathways involved in the hepatocarcinogenesis that result from either viral factors or from unknown causes. Also the degree of COX-2 expression did not differ in samples with viral marker positive and that of negative.

In some studies COX-2 expression has been shown in early stages of HCC (Koga et al., 1999; Bae et al., 2001), butin this study no difference was found in degree of COX-2 expression between patients with early and late stage of the disease. COX-2 expression did not differ in well-differentiated and poorly differentiated HCC samples and in patients with solid or multiple nodules. Similarly, in 2 trials no difference was found in COX-2 expression in well differentiated and poorly differentiated HCC (10, 13). Also no correlation was shown between clinico-

pathological characteristics of the tumors and COX-2 expression.

In the literature, there is paucity of research regarding COX-2 expression and prognosis of patients with HCC (Koga et al., 1999; Iwamoto et al., 2006). In a study of Kondo and colleagues, no correlation was found between COX-2 expression in the tumor and patient's prognosis but COX-2 expression in the nontumoral liver tissue was significantly related with relapses after surgical excision (Kondo et al., 1999). In another study, COX-2 gene expression in the tumoral tissues was significantly related with relapse free survival (Iwamoto et al., 2006).

The most important factor with regard to survival is a tumor's resectability. Patients who undergo transplantation or surgical excision live longer than others do. As it is suggested, in our study, median survival of patients underwent surgery or transplantations were longer than others. However no difference was found in this group of patients regarding to COX-2 expressions. In our study, although patients with COX-2 expression less than 25% had longer survival times this result was not statistically significant. There was no statistically significant difference in survival between patients with positive or negative viral marker.

Taken together, these data point to the fact that COX-2 expression is independent of tumor mass and tumor stage, and COX-2 system appears to be active both in the early and in the late stages of hepatocarcinogenesis. This suggest that targeted therapy against COX-2 system might be effective in all stage of the disease and also might be effective after resection in the prevention of recurrences; however; varying degrees of COX-2 expression might limit the effect of this type of therapy.

In summary, HCC has a poor prognosis. By understanding hepatocarcinogenesis, more effective treatment modalities may be developed. The COX-2 system seems to be a factor in hepatocarcinogenesis regardless of etiology of the disease. Treatments that target this system appear to be effective in managing of HCC.

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