

## Clinical significance of Cox-2, Survivin and Bcl-2 expression in hepatocellular carcinoma (HCC)

Yu Yang · Jiang Zhu · Hongfeng Gou ·  
Dan Cao · Ming Jiang · Mei Hou

Received: 21 March 2010 / Accepted: 26 March 2010 / Published online: 17 April 2010  
© Springer Science+Business Media, LLC 2010

**Abstract** Cox-2, Survivin and Bcl-2 are frequently over-expressed in numerous types of cancers. They are known to be the important regulators of apoptosis. This study was designed to investigate the correlation between the clinical characteristics and the expression of Cox-2, Survivin and Bcl-2 in hepatocellular carcinoma. A total of 63 postoperative hepatocellular carcinoma (HCC) samples, 10 adjacent non-tumor samples and 10 normal liver samples were immunochemically detected for the expression of Cox-2, Survivin and Bcl-2. A median follow-up of 4 years for the 63 HCC patients was conducted. Univariate tests and multivariate Cox regression were performed for statistical analysis. The Kaplan–Meier method was used to analyze the survival. Positive expression of Cox-2 (84.3%) and Survivin (77.8%) was detected significantly more frequently in the HCC samples than in the normal liver tissues (30% and 0, respectively). Bcl-2 was highly expressed in the adjacent non-tumor tissue. Cox-2 was positively correlative to Survivin. Survivin and Bcl-2 were significantly associated with the pathological grade of HCC ( $P < 0.05$ ). Expression of

both Cox-2 and Survivin was significantly associated with the poor overall survival (OS) ( $P = 0.0141$ ,  $P = 0.0039$ ). Furthermore, multivariate analysis confirmed the independent prognostic value of Survivin expression, along with tumor size and hepatic function. Cox-2 and Survivin were highly expressed in the HCC tissue. Survivin and Bcl-2 were significantly associated with the pathological grade of HCC. The expression of Survivin was an independent prognostic factor for HCC after a hepatectomy. Treatment that inhibits Survivin may be a promising targeted approach in HCC.

**Keywords** Cox-2 · Survivin · Bcl-2 · Hepatocellular carcinoma

### Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors with the 5th incidence and 3rd mortality among all malignant tumors [1]. About 306,000 new HCC patients and 300,000 deaths occur in China every year [2]. Although surgical resection and liver transplantation are the most important methods for earlier period HCC, the recurrent rates may be about 50% at 2 years after hepatectomy [3]. HCC is not sensitive to traditional chemotherapeutic agents, the advances in the treatment of advanced HCC is targeted therapies in recent years [4, 5]. There is a need for a better understanding of the biology of HCC, the identification of potential prognostic factors and clinically relevant molecular targets for therapy.

Cox-2 is the central enzyme in the biosynthetic pathway to prostaglandins (PGs) from arachidonic acid (AA). It is composed when the cell is stimulated, and it takes part in many pathophysiologic processes, such as carcinogenesis and inflammation [6]. Cox-2 can participate in

---

Yu Yang and Jiang Zhu equally contributed to this work. Yu Yang and Jiang Zhu designed this study, analyzed the data and drafted the manuscript. Yu Yang, Hong Feng Gou and Dan Cao enrolled and followed up patients in the clinical protocol. Ming Jiang did immunohistochemistry assay. Mei Hou was in charge of the clinical protocol. All authors read and approved the final manuscript.

---

Y. Yang · J. Zhu · H. Gou · D. Cao · M. Jiang · M. Hou  
Department of Medical Oncology, Cancer Center of West China Hospital, Sichuan University, Chengdu, China  
e-mail: zhujiang1@medmail.com.cn

J. Zhu · M. Hou (✉)  
Department of Thoracic Oncology, Cancer Center of West China Hospital, Sichuan University, Chengdu, China  
e-mail: hxhoumei@gmail.com

carcinogenesis by inhibiting cell apoptosis, facilitating angiopoiesis and profiting tumors invasion or metastasis. During recent years, more and more studies found that Cox-2 overexpression is inversely correlated with the prognosis of several cancers such as ampullary, gastric, lung, cervical and ovarian cancers [7–11]. Survivin included in the family of inhibitor of apoptosis proteins (IAPs) is strongly associated with apoptosis, cell proliferation and cell cycle control. Survivin plays a crucial role in the genesis and progression of malignancy, and it is an important prognostic parameter in tumors [12–16]. Bcl-2 is a proto-oncogene. Bcl-2 protein blocks the apoptotic process by inhibiting the release of cytochrome C from mitochondria and blocking the destruction of cell by oxidation. Overexpression of Bcl-2 was found in some kinds of tumors [17]. Some studies reported that high expression of Bcl-2 suggests a better prognosis of some solid tumors such as breast cancer and colon cancer [18, 19]. Several authors already reported a relationship between Cox-2 and Survivin in some kinds of malignant lesions such as ovarian, breast and non-small cell lung cancer [20–22], and another relationship between Survivin and Bcl-2 in stomach cancer, cervical cancer [23, 24]. In human colon cancer cell, PGE2 inhibited apoptosis and induced Bcl-2 expression [25] and NS398 (inhibitor of Cox-2) down-regulated Bcl-2 expression in vitro [26].

Above all, Cox-2, Survivin and Bcl-2 are important regulators of apoptosis. They were reported to be expressed in many types of cancers. But the studies that investigate the clinical value of these factors in HCC have not been reported. The purpose of our study was to investigate the impact of Cox-2, Survivin and Bcl-2 expression on clinical characteristics and prognosis in HCC. Moreover, the interrelationship among these three factors was initially evaluated.

## Methods

### Patients and clinical–pathological data

This study was based on the analysis of 63 postoperative HCC samples at the Department of General Surgery, West China Hospital, Sichuan University from July 2000 to July 2003. A total of 62 patients were male, and one patient was female. Age ranged from 32 to 78 (mean age 52). None of these patients received any treatment before surgery. Ten normal liver samples of operated patients with hepatic injury or hemangiomas were collected as control. These HCC patients were staged according to UICC 5th edition: 2 stage I, 43 stage II, 15 stage III, 3 stage IV. Tumors were pathologically graded according to the criteria described by Edmondson-Steiner: well-differentiated HCC group (grades

I + II) 21 samples, poorly differentiated HCC group (grades III + IV) 42 samples. Other clinical–pathological characteristics such as tumor size, tumor numbers, capsule, hepatic cirrhosis, AFP, vaso-invasion, hepatic function (Child-Pugh grade) and HBsAg were involved. These patients were followed up for 4 years or death.

### Immunohistochemistry assay

All specimens were routinely fixed by formalin (10%), paraffin embedded and sectioned (4  $\mu$ m). The sections were baked at 56°C for one night, dewaxed in xylene two times and hydrated by passage through a graded series of ethanols (100, 95 and 85%), then washed with distilled water. After antigen retrieval by pressure cooking, sections were washed with phosphate-buffered saline (PBS) three times for total 15 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (20 min). Then, the sections were washed with PBS. These sections were, respectively, covered with rabbit anti-Survivin polyclonal antibody (1:50 dilution, Beijing Zhong Shan-Golden Bridge Biological Technology CO, LTD, China), mice anti-Cox-2 monoclonal antibody (DakoCytomation) and mice anti-Bcl-2 monoclonal antibody (DakoCytomation) for 60 min and stayed overnight in 4°C. After washing with PBS, the sections were stained with a diaminobenzidine liquid and were incubated for 30 min counterstained with hematoxylin and mounted. The primary antibody was replaced by PBS for negative control.

Intensity and percentage of positive cells were used to evaluate each tissue section. The mean percentage of positive tumor cells in five strongly staining areas at  $\times 400$  magnification was determined. Positive cell rates of 6–25%, 26–50%, 51–75% and  $>75\%$ , respectively, scored 1, 2, 3 and 4. The staining intensity was graded as follows: no staining (score 0); pale yellow staining (score 1); buffy staining (score 2); and strongly brown staining (score 3). The scores for the above parameters were summed for each section; a score of  $<3$  as low expression, and  $\geq 3$  as high expression.

### Statistics

Statistical analyses were all performed using SPSS 11.5 software. The difference of Cox-2, Survivin or Bcl-2 expression in HCC, adjacent non-tumor and normal liver tissue was assessed using Kruskal–Wallis test. To test for difference between positive and negative of Cox-2, Survivin and Bcl-2 expression scores, the chi-square analysis was performed for categorical variables. Various typical factors were considered for univariate analysis. The impact of Cox-2, Survivin and Bcl-2 expression on survival was assessed

with the Kaplan–Meier method and compared by the log-rank test. Multivariate survival analysis conducted with a forward stepwise application of Cox regression was used. Spearman correlation from ranks was used to analyze the interaction among Cox-2, Survivin and Bcl-2. The results were defined as  $P < 0.05$  for statistical significance.

## Results

### Cox-2, Survivin and Bcl-2 expression

Cox-2 staining was detectable in the cytoplasm of cells (Fig. 1). Of the samples, 52/63 (84.3%) HCC, 8/10 (80%) adjacent non-tumor samples and 3/10 (30%) normal liver samples were positive for Cox-2 staining. There was no statistical difference between HCC and adjacent non-tumor samples ( $P > 0.05$ ). The expression of Cox-2 in normal liver samples was the lowest ( $P < 0.05$ ).

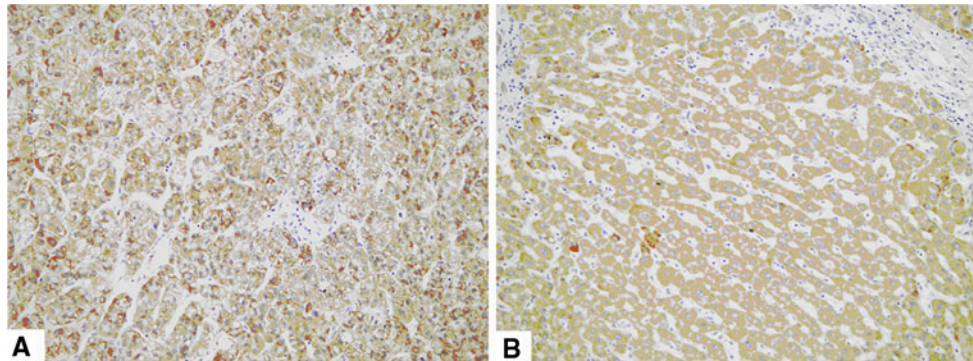
The immunostaining for Survivin was detected in the cytoplasm (Fig. 2). Of the samples, 49/63 (78%) HCC, 2/10 (20%) adjacent non-tumor samples and 0 normal

liver samples were positive for Survivin staining. The expression of Survivin in HCC was the highest ( $P < 0.05$ ). There was no statistical difference between normal liver and adjacent non-tumor samples ( $P > 0.05$ ).

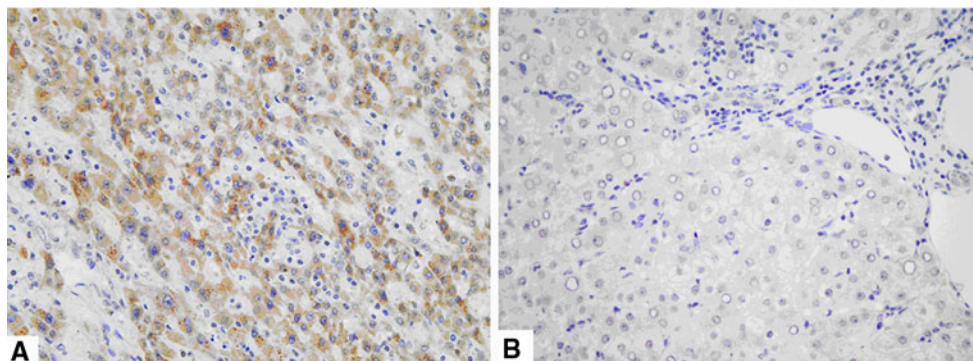
Bcl-2 was weakly stained in the cytoplasm of 13/63 (20%) HCC samples and 1/10 (10%) normal liver samples ( $P > 0.05$ ). And 6/10 (60%) expression in adjacent non-tumor samples was higher than that in HCC or normal liver samples ( $P < 0.05$ ) (Fig. 3).

### Interrelationship among Cox-2, Survivin and Bcl-2 expression

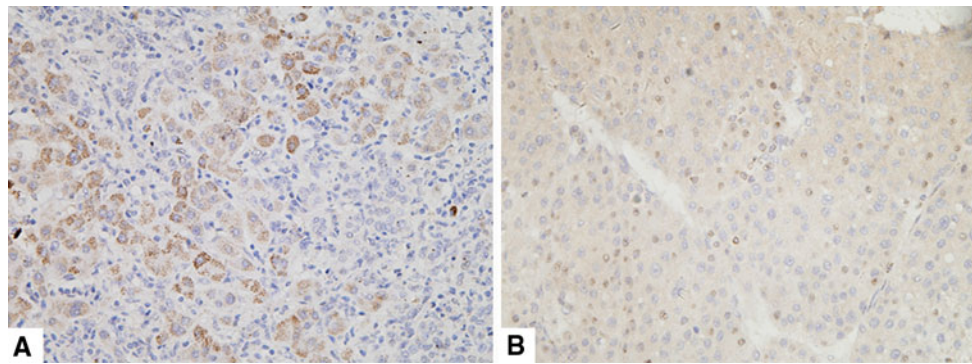
In Cox-2-positive group, 46/52 (88%) showed Survivin-positive expression. While in Cox-2-negative group, 9/11 (82%) samples were detected of Survivin negative. Cox-2 expression showed strong significant correlation with Survivin ( $r = 0.659$ ,  $P < 0.001$ ). No association was observed between Cox-2 expression and Bcl-2 expression ( $r = 0.028$ ,  $P = 0.828$ ), nor was found between Survivin expression and Bcl-2 expression ( $r = 0.084$ ,  $P = 0.513$ ) by Spearman Rank Correlation test.



**Fig. 1** Immunohistochemical cytoplasmic staining of Cox-2 in HCC (a) and adjacent non-tumor sample (b)



**Fig. 2** Immunohistochemical cytoplasmic staining of Survivin in HCC (a) and in adjacent non-tumor sample (b)



**Fig. 3** Immunohistochemical cytoplasmic staining of Bcl-2 in HCC (a) and adjacent non-tumor sample (b)

#### Cox-2, Survivin, Bcl-2 and clinical characteristics of HCC

In univariate analysis, none of Cox-2, Survivin and Bcl-2 expression was associated with the following clinical parameters: age, gender, tumor size, tumor numbers, capsule, hepatic cirrhosis, hepatic function, HBsAg AFP, vaso-invasion age, TNM stage ( $P > 0.05$ ) (Table 1). Survivin expression in poorly differentiated HCC samples was higher than that in well-differentiated samples ( $P < 0.001$ ). Bcl-2 expression in poorly differentiated HCC samples was lower than that in well-differentiated samples ( $P = 0.002$ ). Cox-2 expression in HCC had no association with pathological grade ( $P > 0.05$ ).

#### Survival analysis

##### *Univariate prognostic analyses*

With a total follow-up of 48 months, 22 of the 63 assessable HCC patients were still alive, and 41 patients were known to have died. The 4-year survival rate for all patients was 34.83%. Analysis of the impact of negative or positive Cox-2 expression composite score on OS is shown in Fig. 4. The 1- and 4-year survival rates were 96.4 and 81.4%, respectively, in HCC patients with Cox-2-negative expression and 71.2 and 31.4% in patients with Cox-2-positive expression. Patients with Cox-2-positive expression had poorer prognosis than that with Cox-2-negative expression (log-rank:  $\chi^2 = 6.02$ ,  $P = 0.0141$ ). The 1- and 4-year survival rates of patients with Survivin negative were 96.3 and 78.6%, respectively, significantly higher than that of the patients who were Survivin positive (69.4 and 29.1%) ( $P = 0.0039$ ) (Fig. 5). The survival of patients with Bcl-2 negative and positive expression was showed in Fig. 6. The difference had no statistical significance ( $P = 0.8909$ ).

##### *Multivariate analysis of survival*

According to our multivariate analysis (Cox regression), using all clinical–pathological characteristics except gender (1/63 was female), Cox-2, Survivin and Bcl-2. Survivin expression ( $P = 0.000$ ) remained significantly associated with OS (RR = 7.967, 95% confidence interval, 2.806–22.616). Multivariate analysis identified that Survivin was a statistically significant independent prognostic factor for HCC patients after a hepatectomy along with tumor size ( $P = 0.003$ ) and hepatic function ( $P = 0.002$ ) (Table 2).

#### Discussion

The present study referred to three molecular markers (Cox-2, Survivin and Bcl-2) correlative to inhibition of the cell apoptosis and found that Cox-2 and Survivin expression were significantly associated with each other and implied poor prognosis in patients with HCC after hepatectomy. Moreover, in multivariate analysis, overexpression of Survivin was an independent prognostic factor for OS of HCC.

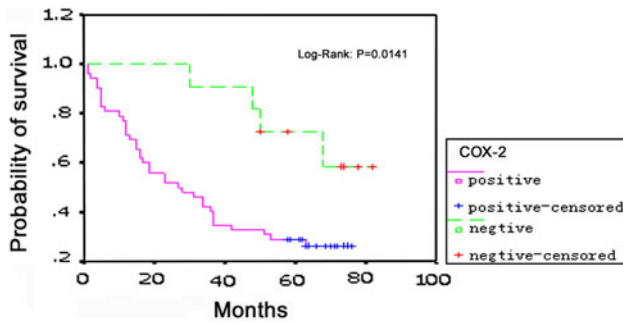
Our study shows 84.3% of HCC, 80% of adjacent non-tumor samples and 30% of normal liver samples were positive for Cox-2 staining that is consistent with previous findings [27]. The expression of Cox-2 in different age, tumor size, tumor numbers, capsule, hepatic cirrhosis, hepatic function, HBsAg, AFP, vaso-invasion age, TNM stage and differentiation had no difference ( $P > 0.05$ ). Cox-2 expression is generally higher in well-differentiated HCC compared with poorly differentiated HCC [28, 29]. This difference with our data may be because of our small sample size or the different cause of HCC. In China, HBV is the main cause of HCC, and Cox-2 takes part in many pathophysiologic processes, such as inflammation [6]. Increased expression of Cox-2 in noncancerous liver tissue was significantly associated with shorter disease-free

**Table 1** Association of Cox-2, Survivin and Bcl-2 expression with clinical–pathological characteristics of HCC

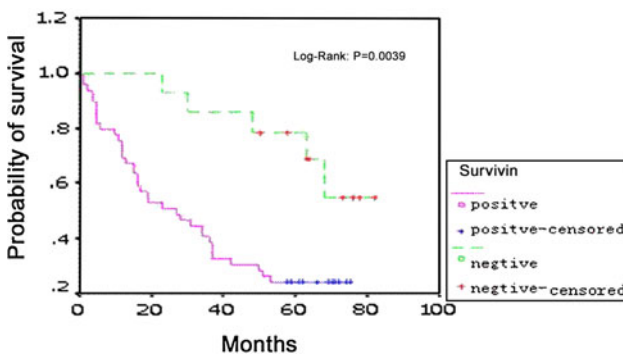
Parameter	No. of patients	Cox-2		Survivin		Bcl-2	
		No. of positive	<i>P</i> value	No. of positive	<i>P</i> value	No. of positive	<i>P</i> value
<b>Age</b>							
≤52	28	24(86%)	0.892	22(79%)	0.892	5(18%)	0.600
>52	35	28(80%)		27(77%)		8(23%)	
<b>Sex</b>							
Male	62	51(82%)	0.643	48(77%)	0.590	13(21%)	0.607
Female	1	1(100%)		1(100%)		0	
<b>Tumor number</b>							
Single	50	41(82%)	0.825	38(76%)	0.506	11(22%)	0.506
Multitude	13	11(85%)		11(85%)		2(15%)	
<b>Tumor size</b>							
≤5 cm	25	21(84%)	0.804	22(88%)	0.113	4(16%)	0.461
>5 cm	38	31(82%)		27(71%)		9(24%)	
<b>Capsule</b>							
No	34	30(88%)	0.197	28(82%)	0.344	7(21%)	0.992
Yes	29	22(76%)		21(72%)		6(21%)	
<b>Cirrhosis</b>							
No	29	24(83%)	0.966	23(79%)	0.787	8(28%)	0.208
Yes	34	28(82%)		26(76%)		5(15%)	
<b>AFP</b>							
<400 ng/ml	39	32(82%)	0.896	31(79%)	0.677	8(21%)	0.976
≥400 ng/ml	24	20(83%)		18(75%)		5(21%)	
<b>HBsAg</b>							
Negative	17	14(82%)	0.981	13(76%)	0.879	2(12%)	0.290
Positive	46	38(83%)		36(78%)		11(24%)	
<b>Pathological grade</b>							
Poor	42	38(90%)	0.051	40(95%)	<0.001	4(10%)	0.002
Well	21	15(71%)		9(43%)		9(43%)	
<b>Hepatic function</b>							
Grade A	51	42(82%)	0.136	42(82%)	0.072	11(22%)	0.706
Grade B	12	10(83%)		7(58%)		2(17%)	
<b>Vaso-invasion</b>							
Negative	54	43(80%)	0.136	41(76%)	0.386	11(20%)	0.899
Positive	9	9(100%)		8(89%)		2(22%)	
<b>Tumor stage</b>							
I + II	45	35(78%)	0.115	33(73%)	0.180	10(22%)	0.623
III + IV	18	17(94%)		16(89%)		3(17%)	

survival in patients with HCC [30]. This result suggests that Cox-2 expression reflects more aggressive clinical behavior in HCC. High recurrence rate after curative treatment might be the reason for poor prognosis. Our results show that the 4-year OS of patients with negative and positive Cox-2 expression was 81.4 and 31.4%, respectively. Patients with positive Cox-2 HCC had a statistically significant poorer prognosis in comparison with patients with negative Cox-2 HCC (log-rank:  $\chi^2 = 6.02$ ,  $P = 0.0141$ ). Similar association of Cox-2 overexpression

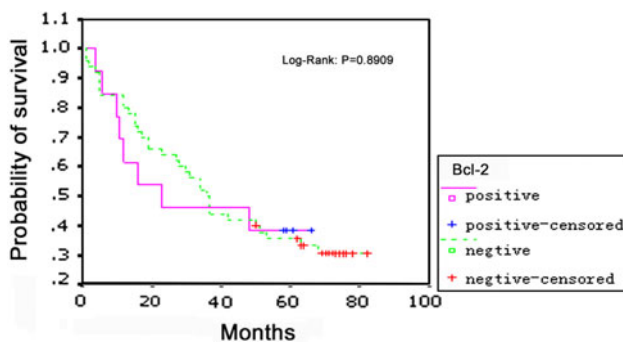
with poor prognosis has been reported in hepatocarcinoma [31] and other malignances [7–11]. In multivariate analysis of Cox model, Cox-2 expression had no statistically significant association with prognosis. This different finding about Cox-2 in univariate and multivariate analysis should logically encourage us to continue our investigations and enlarge sample size. But in a recent study, KJ Schmitz et al. reported Cox-2 overexpression was a feature of early and well-differentiated hepatocellular carcinoma with a favorable prognosis [32]. This article included 196 HCC patients



**Fig. 4** Kaplan–Meier regression analysis showing that patients with Cox-2-positive expression had significantly shorter survival time than those with negative expression



**Fig. 5** Kaplan–Meier regression analysis showing that patients with Survivin-positive expression had significantly shorter survival time than those with negative expression



**Fig. 6** Kaplan–Meier regression analysis showing that patients with Bcl-2 expression had no association with survival time of HCC

**Table 2** Multivariable analysis for overall survival and final model

Parameter	P value	Hazard ratio	95% CI
Tumor size >5 cm	0.003	2.880	1.437–5.776
Hepatic function grade B	0.002	3.678	1.639–8.253
Survivin positive	0.000	7.967	2.806–22.616

treated by operation or liver transplantation who were mostly early-stage patients. So a further study with enough samples and subset analysis is needed to identify Cox-2 contribution to different stages of HCC.

Survivin is undetectable in terminally differentiated adult tissues, but expressed in some precancerous changes such as colonic polyps [33], premalignant lesions of Bowen’s disease (SCC in situ) and hypertrophic actinic keratosis (HAK) [34], and the mostly common human cancers including colorectal, ovarian, breast carcinomas [12, 14, 16] and so on. Prior study showed the sensibility was 68.6%, and specificity was 100% in diagnosis of the bladder transitional cell cancer by detecting Survivin in urine, but the sensibility was 31.4% and specificity was 97.1% by cytological examination [35]. In our investigation, Survivin-positive expression was 77.8% in HCC that was significantly higher than 20% in adjacent non-tumor tissues or 0 in normal liver. Higher expression in poorly differentiated samples was found than that in well-differentiated samples. The 1- and 4-year OS of patients with Survivin-negative and Survivin-positive expression were 96.3, 78.6, 69.4 and 29.1%. Compared to Survivin negative HCC, Survivin-positive HCC had poorer OS ( $P = 0.0039$ ). In multivariate analysis of Cox model, Survivin was statistically significant as an independent prognostic factor for OS (RR = 7.967) along with tumor size and hepatic function. Survivin seemed to reflect more aggressive histological and clinical behaviors and was an independent prognostic factor of HCC. Survivin could be a potential biomarker to evaluate prognosis and a promising target to treat HCC.

Bcl-2 was found to be weakly stained in the cytoplasm of 20% HCC cells and 10% normal liver cells. Sixty percent of expression in adjacent non-tumor samples was higher than that in HCC or normal liver samples. Positive correlation between Bcl-2 and HBsAg was not found. And this result is not consistent with the previous data about the positive relationship between Bcl-2 expression and HBsAg [36]. The reason might be Bcl-2-positive expression of HCC in our study was 13 cases that were too few. Lower expression in poorly differentiated HCC was found than that in well-differentiated samples like in some other tumors reported [18, 19]. In our study, there was no association between Bcl-2 expression and prognosis of HCC. And the positive relationship between Bcl-2 and Cox-2 or Survivin did not exist. Abnormal expression of Bcl-2 may be a phenomenon in early HCC generation. Bcl-2 and the other two molecular markers maybe block apoptosis by different mechanisms and have roles in different stages of hepatocarcinogenesis.

Spearman analysis shows the expression of Cox-2 was positively correlative to Survivin ( $r = 0.659, P < 0.001$ ). In vitro celecoxib (inhibitor of Cox-2) could induce

apoptosis of hepatic cholangiocellular carcinoma by activating Caspase-3 and Caspase-7 [37]. Caspase-3 and Caspase-7 were the targets of Survivin. So Cox-2 may block apoptosis by up-regulating Survivin and down-regulating Caspase-3 and Caspase-7. Efforts of further research are required to determine the precise correlation between Cox-2 and Survivin.

## Conclusions

Cox-2 and Survivin were highly expressed in the HCC tissue. Survivin and Bcl-2 were significantly associated with the pathological grade of HCC. The expression of Survivin was an independent prognostic factor for HCC after hepatectomy. Treatment that inhibits Survivin may be a promising targeted approach in HCC.

**Acknowledgments** The authors would like to thank all their colleagues who participated in this study. Special thanks to Dr WeiWang, Dr WeiJiang (West China 2nd University Hospital, Si Chuan University).

**Competing interests** The author(s) declare that they have no competing interests.

## References

- Bruix J, Sherman M, Llovet J, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the study of the liver. *J Hepatol*. 2001;35(3):421–30.
- Parkin DM, Bray F, Ferlay J, et al. Estimating the world cancer burden: Globocan 2000. *Int J Cancer*. 2001;94(2):153–6.
- Thomas MB, Zhu AX. Hepatocellular carcinoma: the need for progress. *J Clin Oncol*. 2005;23:2892–9.
- Kelley RK, Alan P, Venook. Sorafenib in hepatocellular carcinoma: separating the hype from the hope. *J Clin Oncol*. 2008;26:5845–8.
- Zhu AX, Sahani DV, Duda DG, et al. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol*. 2009;27:3027–35.
- Koki AT, Masferer JL. Celecoxib: a specific Cox-2 inhibitor with anticancer properties. *Cancer Control*. 2002;9(2Suppl):28–35.
- Santini D, Vincenzi B, Tonini G et al. Cyclooxygenase -2 overexpression is associated with a poor outcome in resected ampullary cancer patients. *Clin Cancer Res*. 2005;11(10):3784–9.
- Shi H, Xu JM, Hu NZ, Xie HJ. Prognostic significance of expression of cyclooxygenase-2 and vascular endothelial growth factor in human gastric carcinoma. *World J Gastroenterol*. 2003;9:1421–6.
- Khuri FR, Wu H, Lee JJ, et al. Cyclooxygenase-2 overexpression is a marker of poor prognosis in stage I non-small cell lung cancer. *Clin Cancer Res*. 2001;7:861–7.
- Ferrandina G, Ranelletti FO, Legge F, et al. Prognostic role of the ratio between cyclooxygenase-2 in tumor and stroma compartments in cervical cancer. *Clin Cancer Res*. 2004;10:3117–23.
- Raspollini MR, Amunni G, Villanucci A, et al. Expression of inducible nitric oxide synthase and cyclooxygenase-2 in ovarian cancer: correlation with clinical outcome. *Gynecol Oncol*. 2004;92:806–12.
- Kawasaki H, Toyoda M, Shinohara H, et al. Expression of Survivin correlates with apoptosis, proliferation, and angiogenesis during human colorectal tumorigenesis. *Cancer*. 2001;91:2026–32.
- Salz W, Eisenberg D, Plescia J, et al. A survivin gene signature predicts aggressive tumor behavior. *Cancer Res*. 2005;65:3531–4.
- Sui L, Dong Y, Ohno M, et al. Survivin expression and its correlation with cell proliferation and prognosis in epithelial ovarian tumors. *Int J Oncol*. 2002;21:315–20.
- Caldas H, Jaynes FO, Boyer MW, et al. Survivin and granzyme B-induced apoptosis, a novel anticancer therapy. *Mol Cancer Ther*. 2006;5:693–703.
- Ryan BM, Konecny GE, Kahlert S, et al. Survivin expression in breast cancer predicts clinical outcome and is associated with HER2, VEGF, urokinase plasminogen activator and PAI-1. *Ann Oncol*. 2006;17:597–604.
- Reed JC, Miyashita T, Takayama S, et al. Bcl-2 proteins: relators of cell death in the pathogenesis of cancer and resistance to therapy. *J Cell Biochem*. 1996;60(1):23–32.
- Martínez-Arribas F, Alvarez T, Del Val G, et al. Bcl-2 expression in breast cancer: a comparative study at the mRNA and protein level. *Anticancer Res*. 2007;27:219–22.
- Leahy DT, Mulcahy HE, O'Donoghue DP, et al. Bcl-2 protein expression is associated with better prognosis in colorectal cancer. *Histopathology*. 1999;35(4):360–7.
- Athanassiadou P, Grapsa D, Athanassiades P, et al. The prognostic significance of Cox-2 and survivin expression in ovarian cancer. *Pathol Res Pract*. 2008;204:241–9.
- Barnes N, Haywood P, Flint P, et al. Survivin expression in situ and invasive breast cancer relates to Cox-2 expression and DCIS recurrence. *Br J Cancer*. 2006;94:253–8.
- Krysan K, Merchant FH, Zhu L, et al. Cox-2-dependent stabilization of Survivin in non-small cell lung cancer. *FASEB J*. 2004;18:206–8.
- Lu CD, Altieri D C, Tanigawa N, et al. Expression of a novel Antiapoptosis gene, Survivin, correlated with tumor cell apoptosis and p53 accumulation in gastric carcinomas. *Cancer Res*. 1998;58(9):1808–12.
- Wang M, Wang B, Wang X. A novel antiapoptosis gene, survivin, Bcl-2, p53 expression in cervical carcinomas. *Zhonghua Fu Chan KeZaZhi*. 2001;36(9):54–68.
- Sheng HM, Shao J, Morrow JD, et al. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res*. 1998;58(2):362–6.
- Liu XH, Yao S, Kirschenbaum A, et al. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates Bcl-2 expression in LNCaP cells. *Cancer Res*. 1998;58(19):4245–9.
- Cheng J, Imanishi H, Iijima H, et al. Expression of cyclooxygenase-2 and cytosolic phospholipase A2 in the liver tissue of patients with chronic hepatitis and liver cirrhosis. *Hepatol Res*. 2002;23(3):185–95.
- Koga H, Sakisaka S, Ohishi M, et al. Expression of cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation. *Hepatology*. 1999;29:688–96.
- Bae SH, Jung ES, Park YM, et al. Expression of cyclooxygenase-2 (Cox-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell lines by a Cox-2 inhibitor, NS-398. *Clin Cancer Res*. 2001;7:1410–8.
- Kondo M, Yamamoto H, Nagano H, et al. Increased expression of Cox-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. *Clin Cancer Res*. 1999;5:4005–12.

31. Tang TC, Poon RT, Lau CP, et al. Tumor cyclooxygenase-2 levels correlate with tumor invasiveness in human hepatocellular carcinoma. *World J Gastroenterol*. 2005;11(13):1896–902.
32. Schmitz KJ, Wohlschlaeger J, Lang H, et al. Cyclo-oxygenase-2 overexpression is a feature of early and well-differentiated hepatocellular carcinoma with a favourable prognosis. *J Clin Pathol*. 2009;62:690–3.
33. Gianani R, Jarboe E, Orlicky D, et al. Expression of Survivin in normal, hyperplastic, and neoplastic colonic mucosa. *Hum Pathol*. 2001;32(1):119–25.
34. Grossman D, McNiff JM, Li F, et al. Expression of the apoptosis inhibitor, Survivin, in non melanoma skin cancer and gene targeting in a keratinocyte cell line. *Lab Invest*. 1999;79(9):1121–6.
35. Weikert S, Christoph F, Schrader M, et al. Quantitative analysis of Survivin mRNA expression in urine and tumor tissue of bladder cancer patients and its potential relevance for disease detection and prognosis. *Int J Cancer*. 2005;116(1):100.
36. Osman HG, Gabr OM, Lotfy S, et al. Serum levels of Bcl-2 and cellular oxidative stress in patients with viral hepatitis. *Indian J Med Microbiol*. 2007;25:323–9.
37. Wu T, Leng J, Han C, et al. The cyclooxygenase inhibitor celecoxib block sphosphory lationos Akt and induces apoptosis in human cholangiocarcinoma cells. *Mol Cancer Ther*. 2004;3(3):299–307.