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RESEARCH ARTICLE

Expression and prognostic significance of CIP2A mRNA in hepatocellular carcinoma and nontumoral liver tissues

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Abstract

Objective: This study was undertaken to determine the role of cancerous inhibitor of protein phosphatase 2A (CIP2A) in predicting prognosis of hepatocellular carcinoma (HCC).

Methods: CIP2A mRNA level of 136 pairs of tumor and nontumoral liver tissues of HCC patients after hepatectomy were investigated by quantitative real-time reverse transcription polymerase chain reaction.

Results: Intratumoral CIP2A mRNA was not associated with patients' prognosis. However, nontumoral CIP2A mRNA, which was correlated with lack of tumor encapsulation, poor tumor differentiation, intrahepatic metastasis, and high tumor-node-metastasis stage was an independent risk factor for overall survival and recurrence-free survival.

Conclusions: Nontumoral CIP2A mRNA expression might serve as a novel biomarker for HCC patients undergoing resection.

Keywords: cancerous inhibitor of protein phosphatase 2A (CIP2A), hepatocellular carcinoma, mRNA expression, Prognosis

Introduction

Hepatocellular carcinoma (HCC) is one of the largest causes of deaths from cancer worldwide, especially in East Asia and Sub-Saharan Africa (Jemal et al., 2011). The resistance of HCC to existing treatments makes it one of the world's deadliest cancers (Bruix and Llovet, 2009). The poor outcome for patients with HCC is primarily caused by late-stage disease diagnosis, metastasis, and *de novo* tumor formation (Libbrecht et al., 2001, Hoshida et al., 2008). Therefore, it is important to explore accurate prognosis prediction systems. Owing to the limitations of current staging systems, there is still a need to refine and complement outcome prediction models (Villanueva et al., 2010b). Recent data suggest that molecular alterations in both HCC tumor tissues and nontumoral liver tissues can complement clinical variables in staging systems and guide therapeutic decision making (Villanueva et al., 2010a).

It is well known that data regarding pathogenesis and signal transduction pathways in HCC are helpful for identifying novel biomarkers of cancer stage and prognosis, as well as for the development of therapeutic targets (Villanueva et al., 2010b). An increasing number of reports have highlighted that activation of Akt and c-Myc frequently occurs in hepatocarcinogenesis and is correlated with HCC prognosis (Aravalli et al., 2008,

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El-Serag and Rudolph, 2007). The importance of Akt-related PP2A activity in HCC drug therapy has been well documented (Chen et al., 2010b). Cancerous inhibitor of protein phosphatase 2A (CIP2A) is a recently characterized human oncoprotein that promotes c-Myc protein stability in human cancer cells by inhibiting PP2A activity against serine 62 of the c-Myc protein (Junttila et al., 2007). Upregulated expression of CIP2A is observed in a number of human cancers and correlates with poor disease outcome in gastric, lung, breast, and tongue cancer (Junttila et al., 2007, Li et al., 2008, Côme et al., 2009, Katz et al., 2010, Vaarala et al., 2010, Böckelman et al., 2011, Dong et al., 2011, Liu et al., 2011, Lucas et al., 2011, Wang et al., 2011, Qu et al., 2012). High expression levels of CIP2A correlate with aggressiveness of breast and gastric cancers (Come et al., 2009, Khanna et al., 2009), and CIP2A depletion decreases proliferation of cells from cancers (Junttila et al., 2007, Khanna et al., 2009). Kerosuo et al. demonstrated that overexpression of CIP2A increased mouse neural progenitor cells' self-renewal and growth (Kerosuo et al., 2010). In HCC, the CIP2A-PP2A-Akt signaling axis has been shown to mediate drug resistance (Chen et al., 2010a). Taken together, we hypothesize that CIP2A may be a useful prognostic marker for HCC.

Based on the considerations mentioned above, we have carefully analyzed CIP2A mRNA expression in HCC tissues and matched nontumoral liver tissues from resected HCC patients in order to examine the prognostic value of CIP2A.

Materials and methods

Cell lines

The human cell lines MIHA and L-02 (two immortalized normal human liver cell lines), MHCC-97L and MHCC-97H (hepatoma cell lines with low and high metastatic properties, respectively), and other hepatoma cell lines, including QGY-7703, SMMC-7721, BEL-7402, and HUH-7 were cultured by continuous passage in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were maintained in a humidified incubator at 37°C in 5% CO_2. RNA was extracted from exponentially growing cells.

Clinical specimens

Hepatic tissues were obtained from patients who underwent hepatectomy between 2001 and 2007 in a single group at the Department of Hepatobiliary Oncology, Sun Yat-sen University Cancer Center. The fresh tissues were frozen in liquid nitrogen immediately after resection, archived in the institution's liver tumor bank, and stored at –80°C until processing. Gene expression profiles were conducted in primary tumor tissues and corresponding nontumoral liver tissues from 136 consecutive patients with HCC who had not received preoperative trans-hepatic arterial chemoembolization, radiotherapy, or chemotherapy. In this study, nontumoral liver tissues were defined as 2.0 cm from the tumor margin, which had described previously (Qiu et al., 2011). Normal liver tissues without hepatitis were obtained from 20 patients who underwent liver resection for hemangiomas or focal nodular hyperplasia and were randomly recruited. The clinicopathological variables were shown in Table 1. The diagnosis was confirmed histologically in all cases based on detailed examination of sections stained with hematoxylin and eosin (H&E). All

Table 1. Correlation between intratumoral/nontumoral cancerous inhibitor of protein phosphatase 2A (CIP2A) mRNA expression and clinicopathalogical variables in 136 patients with hepatocellular carcinoma.

		Intratumoral CIP2A			Nontumoral CIP2A		
Variables	Cases	Low ($n = 68$)	High ($n = 68$)	p value[a]	Low ($n = 68$)	High ($n = 68$)	p value[a]
Gender							
Female	14	7	7	1.000	7	7	1.000
Male	122	61	61		61	61	
Age (years)							
≤50[b]	68	31	37	0.303	34	34	1.000
>50	68	37	31		34	34	
HBsAg							
Negative	15	7	8	0.784	8	7	0.784
Positive	121	61	60		60	61	
AFP (μgL)							
≤25	47	23	24	0.857	25	22	0.589
>25	89	45	44		43	46	
Cirrhosis							
No	15	8	7	0.784	9	6	0.412
Yes	121	60	61		59	62	
Child-Pugh							
A	118	60	58	0.613	62	56	0.129
B	18	8	10		6	12	
Tumor size (cm)							
≤5	51	25	26	0.859	29	22	0.215
>5	85	43	42		39	46	
Tumor encapsulation							
Complete	32	16	16	1.000	21	11	0.043
None	104	52	52		47	57	
Differentiation							
I–II	76	21	35	0.300	45	31	0.016
III–IV	60	27	33		23	37	
Intrahepatic metastasis							
No	77	42	35	0.226	50	27	<0.001
Yes	59	26	33		18	41	
TNM stage							
I	73	40	33	0.229	48	25	<0.001
II–III	63	28	35		20	43	

[a]Chi-square or Fisher's exact test.
[b]Value is median.
AFP, α-fetoprotein; CIP2A, cancerous inhibitor of protein phosphates 2A; TNM, tumor-node-metastasis; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma.

histological assessments were made by an experienced pathologist. Written informed consent was obtained from all of the patients, and the study was approved by the Clinical Research Ethics Committee of Sun Yat-sen University Cancer Center. Hepatitis B history was defined as history with positive serum hepatitis B surface antigen (HBsAg). Tumor encapsulation was defined that presence of a clear fibrous sheath around the tumor at gross inspection. Tumor differentiation was based on the Edmondson and Steiner classification. Intrahepatic metastasis was defined as the presence of tumor thrombi in the portal vein or the presence of satellite nodules surrounding a larger main tumor (Morimoto et al., 2003). Tumor staging were determined according to the 7th edition tumor-node-metastasis (TNM) classification of the American Joint Committee on Cancer.

Reverse transcription PCR analysis

A blinded reverse transcription polymerase chain reaction (RT-PCR) analysis was carried out; no clinicopathological or follow-up data were revealed to the bench researchers until the RT-PCR results had been finalized. Total RNA extraction and cDNA preparation were described in our previous study. PCR reaction was performed using the Taq PCR Kit (Promega, WI) under the following PCR cycles: 95°C for 5 min, 32 cycles at 95°C for 20 s, 60°C for 20 s, and 72°C for 1 min. For internal control 18S, 19 PCR cycles were used. Primers for CIP2A are 5′-CCATATGCTCACTCAGATGATGT-3′ (forward) and 5′-GTGTATCATCTCCACAGAGAGTT-3′ (reverse). Primers for 18S ribosomal RNA are 5′-CTCTTAGCTGAGTGTCCCGC-3′ (forward) and 5′-CTGATCGTCTTCGAACCTCC-3′ (reverse). The PCR products were analyzed on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination. All experiments were performed in duplicate.

Real-time quantitative PCR analysis

Real-time quantitative PCR (Q-PCR) was performed in the same batch of cDNA prepared for RT-PCR to quantify CIP2A mRNA. Q-PCR was performed with an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, CA) and SYBR green I Master Mix kit (Invitrogen, CA) according to the manufacturer's instructions. Quantitative RT-PCR was performed in at least triplicates and repeated once for every sample. The primers were 5′-GAACAGATAAGAAAAGAGTTGAGCATT-3′ (forward) and 5′-CGACCTTCTAATTGTGCCTTTT-3′ (reverse) for CIP2A. For 18S, the primers were identical with those used for RT-PCR. Analysis of melting curve for each pair of primers was conducted to examine the specificity of each PCR product. The relative expression level of CIP2A mRNA for each sample was calculated as follows: $\Delta\Delta Ct(\text{sample}) = \Delta Ct(\text{sample}) - \Delta Ct(\text{calibrator})$, where $\Delta Ct(\text{sample}) = Ct(\text{sample})$ of CIP2A mRNA – $Ct(\text{sample})$ of 18S RNA; $\Delta Ct(\text{calibrator})$ of CIP2A mRNA = $Ct(\text{calibrator})$ of CIP2A – $Ct(\text{calibrator})$ of 18S RNA. The fold changes in mRNAs were calculated by the equation $2^{-\Delta\Delta Ct}$. The calibrator was defined as the sample with the highest Ct value of CIP2A mRNA (sample with the lowest expression level of CIP2A mRNA) among all samples.

Follow-up

Follow-up was finished on February 26, 2011. The median length of follow-up was 39 months (range, 1–109 months). All patients were monitored prospectively by physical examination, serum α-fetoprotein (AFP), abdomen ultrasonography, and chest x-ray every 1–3 months in the 1t year, and every 3–6 months thereafter for surveillance of recurrence or metastases. For patients with test results suggestive of recurrence, computed tomography and/or magnetic resonance imaging and/or positron emission tomography were used to verify whether recurrence had occurred. Intrahepatic tumor recurrence or distant metastasis detected only by imaging diagnosis after tumor resection was designated as recurrence. During the follow-up period, 99 patients (72.8%) were found to have recurrent HCC, and 71 patients (52.2%) died of cancer-related causes.

Statistical analysis

For statistical comparisons, the chi-square test or Fisher's exact test was performed to compare qualitative variables, and Student's *t*-test was applied to compare quantitative variables. Overall survival (OS) was defined as the interval between HCC resection and death; patients alive at the end of follow-up were censored. The time to recurrence was calculated from HCC resection to the first radiological evidence of recurrence. For analysis of recurrence, the data were censored for patients without signs of recurrence. Using 12 months as the cutoff value, all incidences of recurrence were divided into early recurrence ($n = 67$) and late recurrence ($n = 31$) (Poon et al., 2000). For early recurrence prediction, late recurrences were treated as censored observations and vice versa. Kaplan–Meier survival curves were generated, and comparisons between survival times were made with log-rank statistics. Multivariate analysis was carried out to identify the independent predictors for recurrence and survival using the Cox proportional hazards model. Only significant prognostic factors found by univariate analysis were entered into multivariate analysis. The expression level of the CIP2A gene in liver tissues was categorized as low or high according to its median value. SPSS 13.0 software (SPSS Inc., Chicago, IL) was employed to analyze all data, and a $p < 0.05$ was considered to be statistically significant.

Results

Expression of CIP2A mRNA in liver cell lines and tissues

We firstly examined CIP2A expression in liver tissues by RT-PCR analysis. Of 136 paired HCC patients' specimens, the frequency of CIP2A expression was

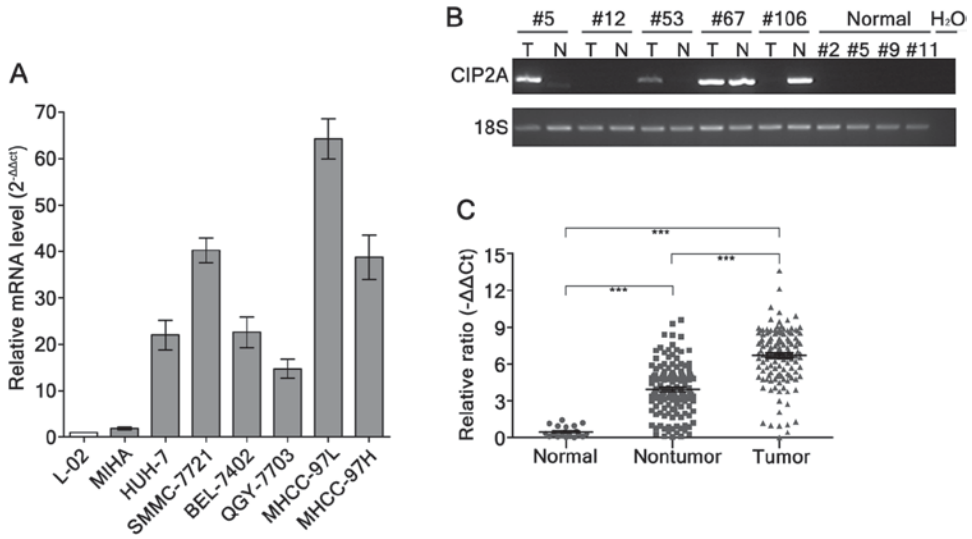

Figure 1. Analysis of cancerous inhibitor of protein phosphatase 2A (CIP2A) mRNA expression in a panel of human liver cell lines and liver tissues. (A) CIP2A mRNA was overexpressed in all six hepatoma cell lines when compared with the immortalized normal human liver cell lines. (B) Representative results of reverse transcription polymerase chain reaction showed the expression of CIP2A mRNA in hepatocellular carcinoma (HCC) tumor tissues (T), matched nontumoral liver tissues (N) and normal liver tissues (Normal). (C) CIP2A mRNA levels were measured with Q-PCR in 136 paired HCC samples and their nontumor counterparts, and 20 normal liver tissues. Gene expression results were normalized to internal control 18S rRNA. ***$p < 0.001$.

significantly elevated in tumor tissues (106/136, 77.9%) compared with their nontumor counterparts (43/136, 31.6%, $p = 0.004$), and none of the normal liver tissue samples yielded a positive CIP2A signal (Figure 1B). To further confirm the mRNA level of CIP2A in liver cell lines and clinical samples, Q-PCR was employed to examine CIP2A mRNA expression in a panel of liver cell lines and the same batch of liver tissues. High CIP2A mRNA expression was detected in all of the six hepatoma cell lines, whereas there was low expression in the immortalized liver cell lines L-02 and MIHA (Figure 1A). Analysis from Student's *t*-test showed that the expression level of CIP2A mRNA was significantly higher in the tumor tissues than that in nontumoral liver tissues ($p < 0.001$) and the normal liver tissues ($p < 0.001$). However, CIP2A mRNA level was significantly higher in nontumoral liver tissues than that in normal liver tissues ($p < 0.001$) (Figure 1C).

Correlation of intratumoral/nontumoral CIP2A mRNA expression with clinicopathological variables

To better understand the clinical relevance of CIP2A mRNA in HCC, we next analyzed the correlation of the expression status of CIP2A mRNA in HCC tumor tissues and nontumoral liver tissues with clinicopathological variables of HCC, including patient gender, age, hepatitis B surface antigen, α-fetoprotein (AFP) values, degree of underlying cirrhosis, Child-Pugh classification, tumor size, tumor encapsulation, tumor differentiation, intrahepatic metastasis, and TNM stage (Table 1). No correlation was found between intratumoral CIP2A mRNA level and the clinicopathological variables, whereas patients with high nontumoral CIP2A mRNA were more prone to have absence of tumor encapsulation ($p = 0.043$), poor tumor differentiation ($p = 0.016$), presence of intrahepatic metastasis, ($p < 0.001$), and high TNM stage ($p < 0.001$).

CIP2A mRNA expression and patient outcome

For the entire study population, the OS and recurrence-free survival (RFS) rates were 73.9% and 50.0% at 1 year, 58.5% and 35.7% at 3 years, and 41.5% and 22.3% at 7 years, respectively. To confirm that the expression of CIP2A mRNA correlates with HCC prognosis, OS and RFS rates were compared between CIP2A-high and CIP2A-low groups. On univariate analysis, tumor size, tumor number, tumor encapsulation, differentiation, intrahepatic metastasis, and TNM stage were prognostic factors for OS and/or RFS (Table 2). Intratumoral CIP2A mRNA was not associated with either OS or RFS ($p = 0.654$ and $p = 0.687$, respectively; Table 2, Figure 2A). However, patients with high CIP2A expression in their nontumoral liver tissue had a poor prognosis; the 3- and 7-year OS and RFS in the CIP2A high group were significantly lower than that in the CIP2A low group ($p < 0.001$ and $p < 0.001$, respectively; Table 2, Figure 2B). As the TNM stage was associated with several clinical indexes, such as tumor size, tumor number, and vascular invasion, we did not enter the TNM stage into multivariate Cox proportional hazards analysis with these indexes to avoid potential bias. On multivariate analysis, mRNA in nontumoral liver tissues was identified as an independent prognostic indicator for both OS ($p < 0.001$) and RFS ($p = 0.001$) (Table 3). With respect to time to recurrence, univariate analysis indicated that the presence of CIP2A mRNA in nontumoral liver tissues was associated with both early ($p = 0.021$) and late recurrence ($p < 0.001$) (Figure 3A and 3B). It was further confirmed

Table 2. Univariate analysis of prognostic factors associated with overall and recurrence-free survival.

		OS (%)			RFS (%)		
Variables	Cases	3 year	7 year	*p* value	3 year	7 year	*p* value
Age (year)							
≤50[a]	68	57.5	44.4	0.830	33.4	22.9	0.318
>50	68	59.6	40.5		37.9	22.6	
Gender							
Female	14	85.4	40.3	0.354	50.0	33.3	0.206
Male	122	55.5	41.3		34.0	21.0	
HBsAg							
Negative	15	80.0	50.4	0.322	46.7	35.0	0.185
Positive	121	55.7	40.0		34.3	20.8	
AFP (μg/L)							
≤25	47	63.0	46.8	0.334	38.3	21.1	0.572
>25	89	56.2	38.9		34.3	22.9	
Cirrhosis							
No	15	73.3	65.2	0.133	40.0	40.0	0.248
Yes	121	56.6	38.0		35.2	20.0	
Child-Pugh							
A	118	59.8	44.0	0.237	37.7	25.4	0.090
B	18	50.0	26.8		22.2	–	
Tumor size (cm)							
≤5	51	79.7	69.4	<0.001	62.0	40.2	<0.001
>5	85	45.3	25.8		20.0	11.9	
Tumor encapsulation							
Complete	32	74.1	57.7	0.029	49.2	26.1	0.156
None	104	53.7	37.2		31.5	20.4	
Differentiation							
I–II	76	67.6	53.8	0.002	42.8	24.6	0.047
III–IV	60	46.8	27.7		26.7	19.0	
Intrahepatic metastasis							
No	77	71.9	59.1	<0.001	47.8	37.1	<0.001
Yes	59	39.7	17.9		18.6	3.2	
TNM stage							
I	73	74.5	61.2	<0.001	43.0	38.1	<0.001
II–III	63	40.1	18.2		20.3	3.6	
Intratumoral CIP2A							
Low	68	57.2	41.7	0.654	30.5	22.6	0.687
High	68	58.2	39.9		39.7	17.8	
Nontumoral CIP2A							
Low	68	77.4	66.8	<0.001	52.8	42.1	<0.001
High	68	38.1	14.2		17.3	–	

[a]Value is median.
AFP, α-fetoprotein; CIP2A, cancerous inhibitor of protein phosphates 2A; TNM, tumor-node-metastasis; HBsAg, hepatitis B surface antigen; OS, overall survival; RFS, recurence-free survival.

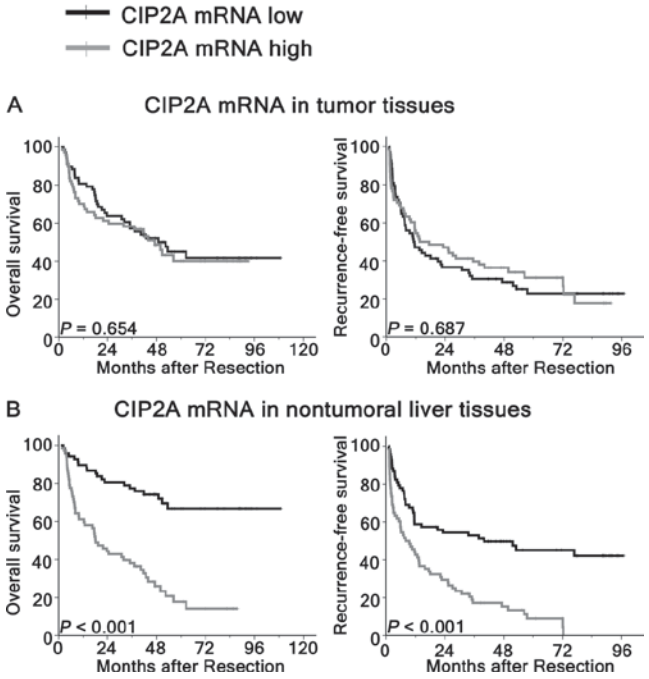

Figure 2. Overall survival and recurrence-free survival assessed by Kaplan–Meier analysis in the entire cohort of hepatocellular carcinoma patients according to intratumoral (A) and nontumoral (B) cancerous inhibitor of protein phosphatase 2A (CIP2A) mRNA expression.

Table 3. Multivariate analysis of prognostic factors associated with overall and recurrence-free survival.

	OS		RFS	
Variables	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Tumor size (>5 vs. ≤5 cm)	2.761 (1.504–5.069)	0.001	2.240 (1.412–3.554)	0.001
Tumor encapsulation (none vs. complete)	1.197 (0.593–2.418)	0.616	n.a.	
Differentiation (III–IV vs. I–II)	1.309 (0.794–2.161)	0.291	1.072 (0.711–1.617)	0.739
Intrahepatic metastasis (yes vs. no)	1.783 (1.030–3.85)	0.039	1.921 (1.247–2.959)	0.003
Nontumoral CIP2A (high vs. low)	3.202 (1.843–5.562)	<0.001	2.112 (1.360–3.279)	0.001

95% CI, 95% confidence interval; CIP2A, cancerous inhibitor of protein phosphates 2A; HR, hazard ratio; n.a., not applicable; OS, overall survival; RFS, recurrence-free survival.

by multivariate analysis that nontumoral expression of CIP2A mRNA correlated with higher risk for late recurrence ($p < 0.001$) rather than early recurrence ($p = 0.504$) (Supplementary Table 1).

We further investigated the prognostic value of CIP2A within subgroups, which were stratified according to tumor size (Supplementary Figure 1A and 1B), grade of tumor differentiation (Supplementary Figure 1C and 1D), and TNM classification (Supplementary Figure 1E and 1F). The prognostic significance of nontumoral CIP2A mRNA for OS and RFS was retained in all of the subgroups.

Discussion

Accurate prognostic prediction is crucial in modern oncology. To provide a useful framework for decision making in HCC treatment, intense efforts have been made to develop genetic profiling tools obtained from

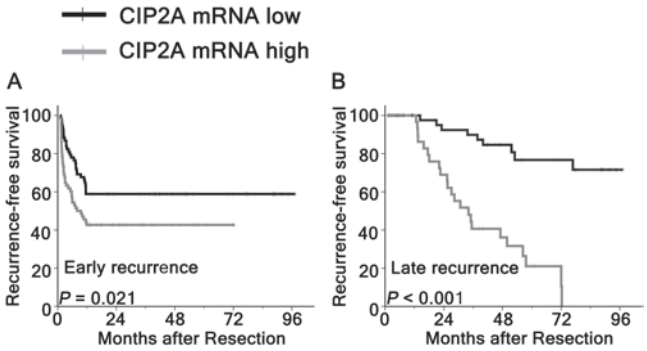

Figure 3. Early (A) and late (B) recurrence assessed by Kaplan–Meier analysis in the entire cohort of hepatocellular carcinoma patients according to nontumoral cancerous inhibitor of protein phosphatase 2A (CIP2A) mRNA expression.

tumor and nontumoral liver tissues to stratify patients with respect to prognosis and response to therapy (Villanueva et al., 2010b). The prognostic significance of CIP2A with diverse cancers and its predictive value of responsiveness to drug therapy in HCC led us to explore its expression and clinical significance in HCC.

To date, overexpression of CIP2A in many types of solid and hematological cancer compared to nonmalignant cells has been reported (Junttila et al., 2007, Li et al., 2008, Come et al., 2009, Katz et al., 2010, Vaarala et al., 2010, Cristóbal et al., 2011, Liu et al., 2011, Wang et al., 2011, Qu et al., 2012). In our study, we found that CIP2A mRNA was more prevalent in hepatoma cell lines and HCC tissues than in normal liver cell lines and nontumoral liver tissues. The lowest expression of CIP2A mRNA was detected in normal liver tissues. Those data suggest that CIP2A is activated in a wide variety of cancers. Furthermore, the difference in CIP2A expression between tumor, nontumoral and normal liver tissues may indicate its role in hepatocarcinogenesis. The mechanism underlying overexpression of CIP2A seems to be regulation of CIP2A via a positive feedback loop between CIP2A and c-Myc in gastric cancer (Khanna et al., 2009). In human HCC, c-Myc has been reported to be activated (El-Serag and Rudolph, 2007); nevertheless, whether CIP2A is activated in this feedback loop warrants future investigation.

Current evidence has established the requirement of CIP2A for malignant transformation and tumor proliferation in various cancers (Junttila et al., 2007, Li et al., 2008, Khanna et al., 2009). Clinically, CIP2A expression has been shown to be associated with the aggressive characteristics of tumors, such as poor differentiation, higher stage and metastasis (Come et al., 2009, Dong et al., 2011, Lucas et al., 2011). However, we didn't find any association between intratumoral CIP2A mRNA and the clinicopathological variables. In our study, overexpression of CIP2A mRNA in nontumoral liver tissues was correlated with a more aggressive phenotype.

Recently, data regarding the genomic profiling of nontumoral liver tissues from HCC patients in predictive prognosis have been growing. Many reports have indicated that the genomic alterations in nontumoral liver tissues suggest the potential for future malignant transformation. Budhu et al. found that a unique inflammation/immune response-related signature obtained from nontumoral tissues was associated with intrahepatic venous metastasis (Budhu et al., 2006). Another gene expression signature of nontumoral liver tissues associated with late recurrence was also identified by Hoshida et al (2008). A recent study showed that nontumoral placental growth factor expression was correlated with tumor size, intrahepatic metastasis, TNM stage, and poor survival (Xu et al., 2011). In the present study, the correlation between CIP2A expression in nontumoral tissues and clinicopathological variables indicated a positive correlation between nontumoral CIP2A mRNA and intrahepatic metastasis, thereby suggesting that an elevated expression of nontumoral CIP2A may be induced by intrahepatic metastasis or that elevated CIP2A expression in nontumoral tissues is able to prepare an environment primed for intrahepatic metastasis. In addition, the positive association of nontumoral CIP2A mRNA expression with HCC differentiation supported the impact of microenvironment on tumor differentiation.

To identify a new cancer biomarker in addition to the common clinicopathological risk factors that could assist follow-up management after surgery, the clinical significance of CIP2A in nontumoral hepatic tissues was examined and characterized, but not in tumor tissues, as an independent prognostic factor in determining the decreased OS rate and an increased likelihood of tumor recurrence. These results support and highlight the potential role of nontumoral liver tissues in predicting the OS and recurrence in patients with HCC. In addition, the significant prognostic value of nontumoral CIP2A mRNA for late recurrence supports the notion that CIP2A expression may lead to an increased potential for future malignant transformation.

HCC patients with early-stage disease are established as well selected candidates for primary resection or liver transplantation (Agrawal and Belghiti, 2011, Rahbari et al., 2011). The presence of large tumor size, intrahepatic metastasis is associated with high risk of recurrence after resection; therefore, resection for these patients remains controversial (Rahbari et al., 2011). However, the clinical outcomes for HCC patients with identical clinical-pathological characteristics are heterogeneous (Villanueva et al., 2010b). Many studies have tried to acquire molecular information to complement clinical staging for clinical decision making (Villanueva et al., 2010a). When we stratified the patient cohort by tumor size, grade of tumor differentiation, and TNM stage, we found that the prognostic significance of nontumoral CIP2A mRNA still existed in all the subgroups. Together, our data imply that combining CIP2A mRNA expression and clinicopathological variables may more accurately predict which HCC patients are at higher risk of recurrence and death. However, further investigation with larger sample size is needed. Our data suggest that nontumoral

tissue profiling could capture complementary biological signals essential for prognosis. Despite the lack of control trials, CIP2A expression can identify subgroups of patients with good outcomes among advanced cases and patients with poor outcomes among early cases and may complement the criteria for patient selection in liver resection treatment. Taken together, it will be interesting to perform a presurgical assessment of OS and recurrence by detecting CIP2A expression in needle biopsies. However, percutaneous biopsies should be taken with caution because of the 2–3% risk of tumor seeding along the biopsy needle track (Silva et al., 2008). Owing to this drawback of tumor biopsy, the prognostic value of nontumoral CIP2A mRNA led us to consider needle biopsy of nontumoral liver tissues prior to surgical resection. Further clinical validation of CIP2A mRNA expression will be needed before this method is introduced to clinical practice for treatment decision making and surveillance programs.

In conclusion, we demonstrate that the upregulation of CIP2A in nontumoral liver tissues indicates aggressive tumor behavior and predicts a worse clinical outcome. Therefore, CIP2A may be a useful biomarker to identify patients who are at high risk of death and postoperative recurrence.

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Declaration of interest

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