FULL LENGTH ARTICLE

The potential value of CDV3 in the prognosis evaluation in Hepatocellular carcinoma

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Abstract CDV3 is correlated with tumorigenesis and may affect some biological process in cancer. In this study, we explore the role of CDV3 in HCC. According to the TCGA data base, CDV3 is over-expressed in HCC tissues. Up-regulation of CDV3 is correlated with lower over-all survival rate in HCC patients. In HCC samples from our hospital, CDV3 is also enriched in cancer tissues and CDV3 expression associated with HCC pathological T stage. What is more, higher CDV3 expression could forecast poor survival rate in HCC patients. In conclusion, CDV3 is a biomarker of HCC and could be a potential therapeutic target.
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Introduction

Hepatocellular carcinoma (HCC), a highly vascularized tumor, is the third leading cause of cancer death worldwide, and the second in China.[1,2] Due to existed therapies are insufficient for the high frequency of tumor recurrence after liver resection, the prognosis of HCC patients remains pessimistic. Therefore it is of great importance to establish the identity of new targets for therapeutic approach to improve the prognosis of HCC patients after surgical resection.

CDV3 (carnitine deficiency-associated gene expressed in ventricle 3), also known as H41, was documented as an unidentified gene in breast cancer in 1999.[3] CDV3 expression correlated to the expression of Her2 and the sensitivity

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of photon-irradiation and simultaneous PTX-treatment in breast cancer.[4] And CDV3 was up-regulated in colorectal adenocarcinoma and gastric cancer.[5−7] However, few is known about the role of CDV3 in HCC.

In our present research, we found that CDV3 was up-regulated in The Cancer Genome Atlas (TCGA) database. And we extensively evaluated the CDV3 expression pattern and confirmed its contribution to patients' survival rate after HCC surgical resection in our department. Results presented in this study suggest that CDV3 is significant overexpressed in HCC tissues, and its over-expression indicates poor prognosis. We propose that CDV3 is a novel powerful predictor for HCC prognosis and a new potential adjuvant treatment target for HCC after surgical resection.

Materials and methods

Study subjects

Samples from 50 patients with HCC receiving liver resection at our hospital (First Affiliated Hospital, Chongqing medical University, Chongqing, China) between 2006 and 2010 were collected in this study. Letter of consent was obtained from all patients, and the experimental protocols were approved by the local ethics committee. Patient charts were reviewed to obtain clinical data about age, gender, tumor size, TNM stage (AJCC), and death or time of last follow-up. Patient survival was calculated from the day of surgery until death, in month.

TCGA dataset and analysis of the differentially expressed mRNAs and the clinical significance

To validated the expression of mRNAs, 51 cases of normal liver tissues and 270 cases of HCC tissues were obtained from TCGA database (https://tcga-data.nci.nih.gov/tcga/). Furthermore, the Kaplan−Meier method was used to estimate the prognostic significance of the mRNAs for OS, and

survival curves were compared through the log-rank test. This study met the publication guidelines provided by TCGA.

RT-PCR and immunohistochemistry

The regents used and the detailed procedures of RT-PCR, western blot and IHC were performed as before.[8]

Statistical analysis

The analysis was performed using SPSS version 17.0. The chi-square test or Fisher's exact test was used to evaluate the clinicopathologic parameters. Over-all survival and tumor-free survival rates were calculated with the Kaplan−Meier method, and the statistical difference between survival curves was determined with the log-rank test. Statistical significance was accepted if $p < 0.05$.

Results

Validation of the CDV3 expression and impact of CDV3 expression on survival from the TCGA cohort

We analyzed CDV3 expression and impact of CDV3 expression on survival from the TCGA cohort. By compare 51 cases of normal liver tissues and 270 cases of HCC tissues in TCGA data base, CDV3 expression was significantly increased ($p < 0.001$) in HCC tissues (Fig. 1A). After analyzing 179 patients' survival rate in TCGA, CDV3 was found to be a bad prognostic value (HR = 1.71 and $p = 0.040$) for HCC after surgical resection (Fig. 1B).

CDV3 is up-regulated in HCC tissues

To validate the data of TCGA, we performed RT-PCR and Immunohistochemistry of CDV3 in HCC tissues. The expression of CDV3 in the samples from 50 patients with HCC receiving
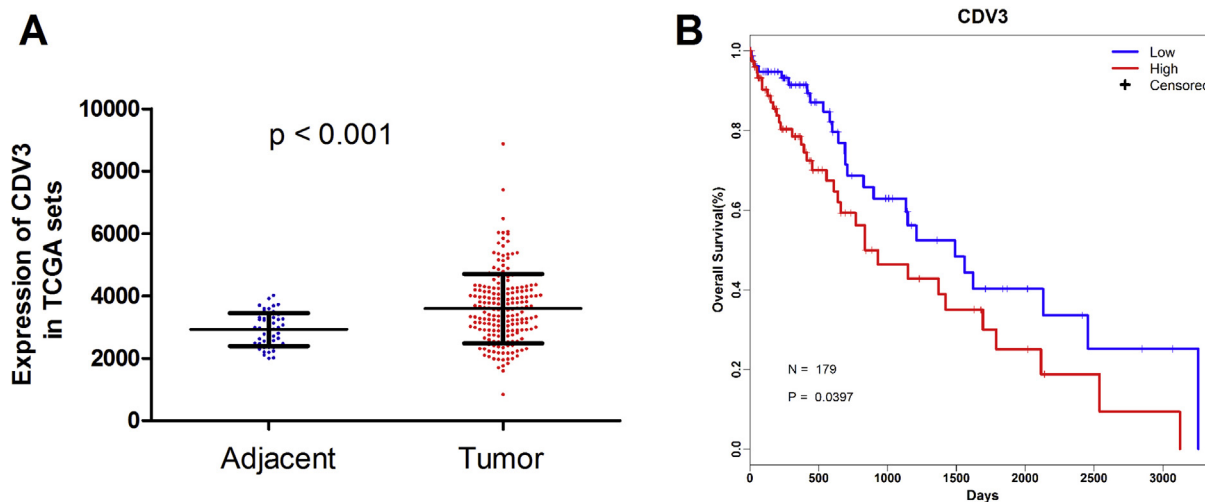

Figure 1 Validation of the CDV3mRNA expression and the impact of CDV3 expression on survival from the TCGA cohort. A. The expression of CDV3 mRNA for 51 cases of normal liver tissues and 270 cases of HCC tissues in TCGA data base. B. Kaplan−Meier survival analysis of overall survival (OS) for CDV3 mRNA.
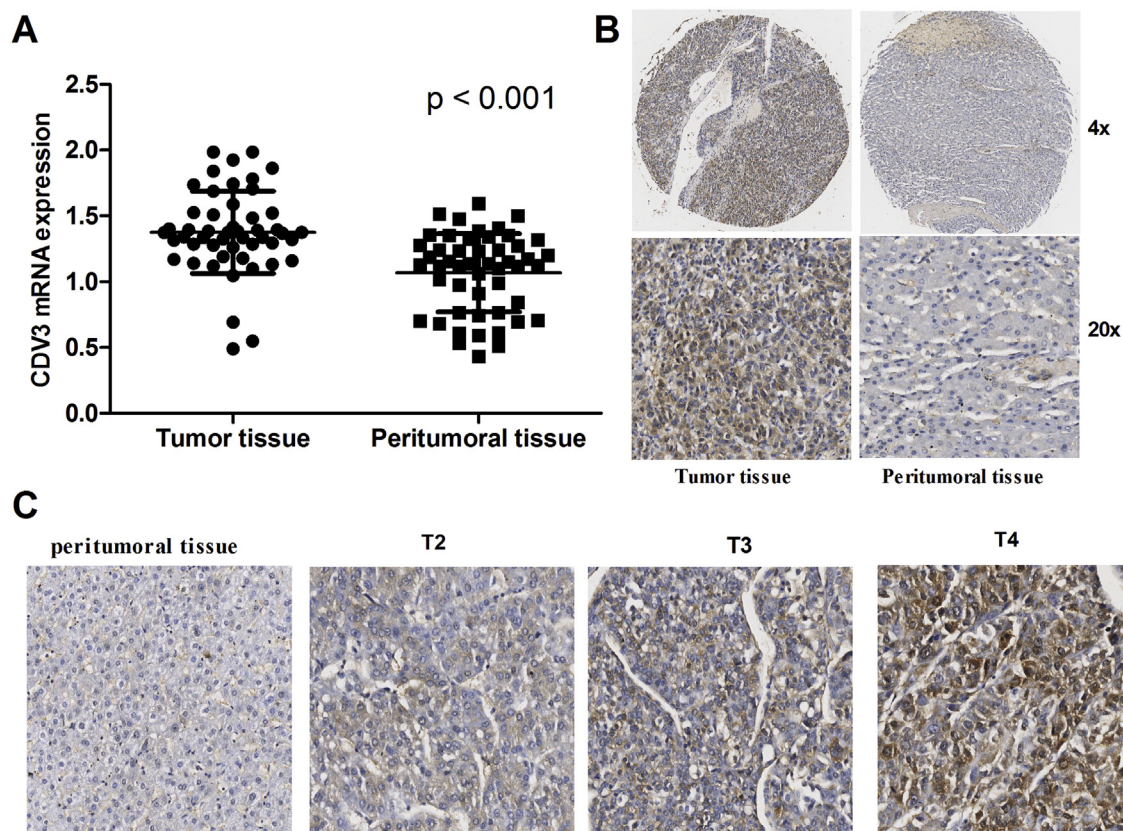
Figure 2 CDV3 is enriched in HCC tissues and is a significant prognosis factor after HCC surgical resection. A. Expression level of CDV3 was assayed by RT-PCR. Data were expressed as mean ± SD. B. HCC samples in a tissue microarray were immunostained with a monoclonal anti-TAZ antibody. C. CDV3 expression associated with HCC pathological T stage, normal liver tissue as control group.

hepatic resection at our hospital was found higher in HCC tissues than the peritumoral tissues (Fig. 2A), and staining reaction of CDV3 in patients with HCC is shown in Fig. 2B.

Relationship between CDV3 expression and clinicopathologic characteristics of patients with HCC

The study included HCC tissue from 50 patients (37 males and 13 females). Patients' characteristics are shown in Table 1. CDV3 staining was significantly associated with pathological T factor and prognosis (Fig. 2C) ($p < 0.001$ and $p = 0.016$), but not with tumor size ($p = 0.553$). High expression of CDV3 in HCC patients assessed was found as follows: CDV3 in 30 (60.0%) cases.

Association between CDV3 status and clinical outcome of the patients

As shown in Fig. 3A, CDV3 status was significantly associated with an increased incidence of death in HCC patients ($p = 0.016$). The 5-year over-all survival rate for patients with low expression of CDV3 and for patients with high expression of CDV3 was 60.0% and 22.0% ($p = 0.043$), respectively. In addition, significant associations between CDV3 status and Over-all survival rate were also detected in pT3-4 cases ($p = 0.028$) (Fig. 3B). As shown in Table 2,

univariate analyses for HCC survival revealed CDV3 as significant prognostic variables in these patients (HR = 2.576 (1.148−5.781), $p = 0.022$).

Discussion

Hepatocellular carcinoma is of poor prognosis and always short of effective adjuvant medicine. In this study, we uncover that CDV3 was up-regulated in HCC, and high CDV3 expression could predict unfavorable overall survival in HCC patients. Thus, we provide clinic evidence that CDV3 expression analysis can be an addition for risk assessment and therapeutic decision making for HCC patients.

CDV3 (carnitine deficiency-associated gene expressed in ventricle 3), also known as H41, is located on the chromosomal regions 3q22. This region is known to regulate a large number of tumors, such as non-small cell lung cancer, prostate cancer, ovarian cancer, bladder cancer, HCC and many others.[4,9−12] In HCC, chromosomal 3q22 aberrations were found to be associated with tumor recurrence and poor overall patient survival rate and correlated with the advanced stage tumors and the progression of hepatitis B virus-related HCC.[12,13] However, there was no definite mechanism about CDV3 in HCC. We could just propose hypothesis that CDV3 is of great importance in HCC development and progression from CDV3's chromosome location.

Some reports showed that CDV3 might have an positive effect on colorectal cancer risk and be involved in cell

Table 1 Relationship between CDV3 expression and clinicopathologic features.

Variable	n	CDV3 expression (n%)		p value
		Low	High	
Adjacent tissue	50	41 (82.0)	9 (18.0)	<**0.001**
Tumor tissue	50	20 (40.0)	30 (60.0)	
Age (year)				
<50	29	13 (44.8)	16 (55.2)	0.56
≥50	21	7 (33.3)	14 (66.7)	
Sex				
Male	37	16 (43.2)	21 (56.8)	0.522
Female	13	4 (30.8)	9 (69.2)	
Tumor size (cm)				
<5	19	9 (47.4)	10 (52.6)	0.553
≥5	31	11 (35.5)	20 (64.5)	
HBsAg				
Negative	6	2 (33.3)	4 (66.7)	0.722
Positive	44	18 (40.9)	26 (59.1)	
Liver cirrhosis				
No	27	13 (48.1)	14 (51.9)	0.254
Yes	23	7 (30.4)	16 (69.6)	
AFP (ng/ml)				
≤400	21	9 (42.9)	12 (57.1)	0.57
>400	29	10 (34.5)	19 (65.5)	
≤200	12	4 (33.3)	8 (66.7)	0.556
>200	38	14 (36.8)	24 (63.2)	
Pathological T factor				
T1-T2	13	11 (84.6)	2 (15.4)	<**0.001**
T3-T4	37	9 (24.3)	28 (75.7)	
Prognosis				
survival	19	12 (63.2)	7 (16.8)	**0.016**
dead	31	8 (25.8)	23 (74.2)	

Statistical significance was accepted if $p<0.05$.

Table 2 Unvariate analyses of HCC survival in 50 HCC patients examined.

Variable	HR (95% CI)	df	p value
Sex (female)	0.807 (0.348–1.874)	1	0.618
age	1.035 (0.999–1.072)	1	0.054
TNM stage (III-IV)	1.009 (0.464–2.193)	1	0.982
Tumor size ≥5 cm	0.859 (0.420–1.757)	1	0.678
CDV3 high expression	2.576 (1.148–5.781)	1	**0.022**

Statistical significance was accepted if $p<0.05$.

So, we could put forward the hypothesis that the up-regulated CDV3 expression might be related with over-expressing c-erbB-2 HCC and promote the development of hepatitis C virus-related HCC. In human chondrosarcoma cells, it reported that CDV3 level had elevated during the long-term hypoxia.[17] As an important tumor microenvironment, hypoxia regulates proliferation, apoptosis, metastasis, inflammation, and angiogenesis in HCC.[18,19] The hypoxia condition was also observed to be associated with the development and prognosis of HCC in our previous study.[20]

Therefore, we thought that CDV3 might participate in the progress of HCC and its increased malignancy under hypoxia condition, and play a important role in the development of virus-induced HCC and involved in the angiogenesis that could promote invasion and metastasis of HCC. These lines of evidence demonstrated that CDV3 might be the predisposing gene of HCC.

At present, many biomarkers have applicated in prognosis prediction in HCC, such as mRNA, micro RNA, Long none-coding RNA, circulating RNA, circular RNA et al. However, early diagnostic markers and novel therapeutic targets which were transformed into effective targeted drugs were extremely limited. The most successful example of targeted drugs is sorafenib, however, it could only prolong life for up to 2.8 months.[21,22]

In summary, we have analyzed the clinical significance of human CDV3. This is the first report shows that CDV3 is an oncogene in HCC. The biological function and the mechanism of the CDV3 gene on HCC progress remains unclear and awaits for further study.
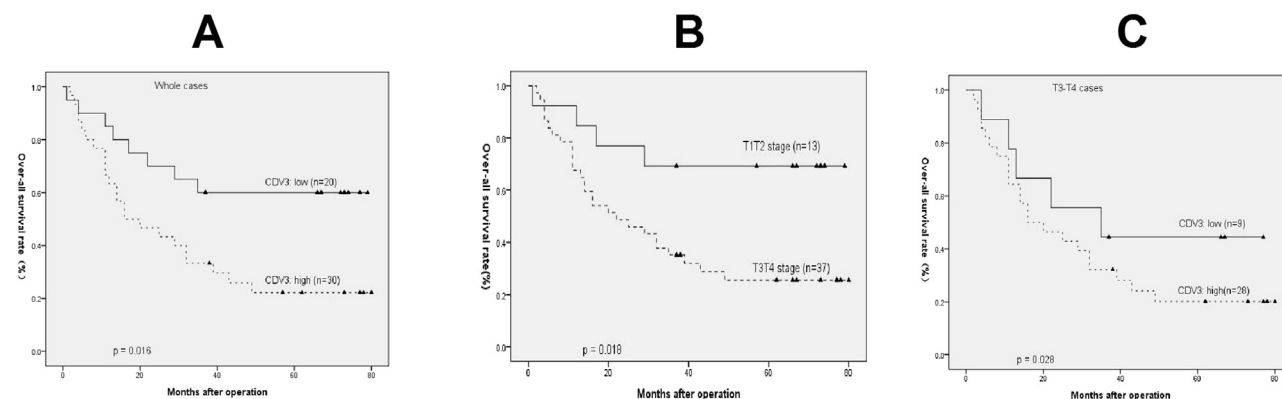
proliferation in gastric cancer.[6,7] CDV3 has been reported to be up-regulated in breast cancer cells overexpressing cellular-erythroblastosis B-2 (c-erbB-2, a kind of tyrosine kinase).[14] Meanwhile, many studies have reported that c-erbB-2 expression was an early event in the pathogenesis of HCC and associated with hepatitis C virus-related HCC.[15,16]

Figure 3 CDV3 has prognostic value for HCC after HCC surgical resection. A. The over-all survival rates of all 50 patients with HCC after hepatic resection were compared between the low-CDV3 and high-CDV3 groups. B. The over-all survival rates of all 50 patients with HCC after hepatic resection were compared between T1T2 stage and T3T4 stage. C. The over-all survival rates of pT3-4 patients after hepatic resection were compared between the low-CDV3 and high-CDV3 groups.

Conflicts of interest

None.

Acknowledgment

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