Introduction

Hepatocellular carcinoma (HCC), the sixth most prevalent cancer type, ranks the third leading cause of cancer-related mortality worldwide (Forner et al., 2012), especially in many countries of Asia (Fazeli et al., 2012). Although surgical resection is considered to be the most effective therapeutic method for treatment of patients with primary hepatic carcinoma, the high recurrence rate and early distant metastasis of HCC after surgery remains frustrating (Kobayashi et al., 2006; Tralhao et al., 2007). Identification of factors involved in oncogenesis of HCC may facilitate improvement of early diagnosis and therapeutic approaches (Ji et al., 2014).

DEP domain is a globular domain that consists of approximately 90 amino acids, which was first identified in three proteins: D. melanogaster Dishevelled, C. elegans EGL-10 and mammalian Pleckstrin (Ballon et al., 2006). These proteins are involved in Wnt signaling (Sokol, 2000), G-protein coupled receptor signaling , and signaling in platelets and neutrophils (Kharrat et al., 1998), respectively. DEP domain containing 1 (DEPDC1) is a highly conserved protein, which was reported in bladder cancer cells to act as transcriptional repressor through forming a complex with ZNF224 to suppress A20 transcription, leading to activation of anti-apoptotic pathway through NF-κB activation (Harada et al., 2010). A very recent report showed that, in HeLa and MCF-7 cells, DEPDC1 promotes JNK-dependent degradation of MCL1, an anti-apoptotic Bcl-2 family member, and therefore inhibits apoptosis (Sendoel et al., 2014). Several reports in bladder cancer, breast cancer and lung cancer demonstrated that DEPDC1 up-regulation might have important role in tumorigenesis (Kanehira et al., 2007; Harada et al., 2010; Kretschmer et al., 2011; Okayama et al., 2012; Kassambara et al., 2013). However, whether DEPDC1 also plays a pivotal role in hepatocellular carcinoma progression and what is its clinical significance in HCC patients are still unknown.

In this study, we detected DEPDC1 expression at mRNA level in HCC tissues, and analyzed the correlation with clinical parameters, as well as the diagnostic and prognostic value.

Materials and Methods

Patients and the source of specimens

A total of 205 cases of HCC samples and paired...
adjacent normal liver tissues were from patients who underwent curative surgical resection from 2001 and 2007 at the Affiliated Hospital of Guilin Medical University. All the cases met the “primary liver cancer clinical diagnosis and staging criteria” and none of them were from patients who received adjuvant chemotherapy or transhepatic arterial embolization. All of them were verified by clinical and pathological examination. The clinicopathological parameters were shown in Table 1. Fresh excised tumor and paired adjacent normal liver tissues for qRT-PCR and reverse-transcription PCR were immediately immersed in liquid nitrogen and stored at -80°C until use. The prognostic data were obtained via follow-up examination. Serum alpha-fetoprotein (AFP) test and ultrosonography (US) scan were conducted every 2 month during the first two years after surgical resection. If the AFP test or US results showed positive outcomes, catscan or magnetic resonance imaging (MRI) was applied to confirm the results. The follow-up process was ended in December 2012, with a median period of 36.0 months (median, 21.0 months, range, 20.0-84.0 months). Disease free survival (DFS) was defined as the period from the date of surgical resection to the date of recurrence, metastasis, death or last follow-up. Overall survival (OS) was defined as the period from the date of surgical resection to the date of death or last follow-up. The study protocol was approved by the Hospital Ethics Committee of Guilin Medical University. Written informed consent based on the 1964 Declaration of Helsinki and amendments was obtained from each patient.

### Table 1. Clinical and Biochemical Data of Examined Patients

| Parameter                | Mean±SD          |
|--------------------------|------------------|
| Age (years)              | 50.05±11.86      |
| Gender: female/male (n)  | 30/175           |
| Alcohol abuse: yes/no (n)| 107/98           |
| Cirrhosis: yes/no (n)    | 186/19           |
| HBsAg: negative/positive (n) | 37/168 |
| AFP (ng/ml)              | 3939.21±18817.12 |
| Platelets (10^9/L)       | 177.17±79.19     |
| Albumin (g/L)            | 40.98±4.68       |
| TBIL (μmol/L)            | 18.32±25.26      |
| ALT (U/L)                | 46.37±44.52      |
| AST (U/L)                | 56.58±79.65      |
| NLR                      | 2.55±1.86        |
| γGT (U/L)                | 109.62±97.83     |

*number of cases (n); hepatitis B surface antigen (HBsAg); alanine aminotransferase (ALT); aspartate aminotransferase (AST); γ-glutamyl transpeptidase (γGT) and total bilirubin (TBIL), Alpha Fetoprotein (AFP); neutrophil to lymphocyte ratio (NLR)

### Table 2. Correlation between DEPDC1 mRNA Expression and the Clinicopathologic Parameters in HCC

| Clinical character variable | No. of patients | DEPDC1 mRNA |
|----------------------------|-----------------|-------------|
| Age (years)                |                 |             |
| <55                        | 140             | 39 (27.9)   |
| ≥55                        | 65              | 22 (33.8)   |
| Gender                     |                 |             |
| Male                       | 175             | 53 (30.3)   |
| Female                     | 30              | 8 (26.7)    |
| Family history             |                 |             |
| No                         | 170             | 49 (28.8)   |
| Yes                        | 35              | 12 (34.3)   |
| HBsAg                      |                 |             |
| Negative                   | 37              | 11 (29.7)   |
| Positive                   | 168             | 50 (29.8)   |
| alpha-fetoprotein (ng/ml)  |                 |             |
| <100                       | 85              | 32 (37.6)   |
| ≥100                       | 120             | 29 (24.2)   |
| Median size (cm)           |                 |             |
| <4                         | 52              | 22 (42.3)   |
| ≥4                         | 153             | 39 (25.5)   |
| Cirrhosis                  |                 |             |
| No                         | 19              | 4 (21.1)    |
| Yes                        | 186             | 57 (30.6)   |
| Tumor number               |                 |             |
| Single                     | 140             | 45 (32.1)   |
| Multiple                   | 65              | 16 (24.6)   |
| BCLC stage                 |                 |             |
| 0-A                        | 100             | 38 (38)     |
| B-C                        | 105             | 23 (21.9)   |
| Portal vein tumor thrombus |                 |             |
| No                         | 164             | 50 (30.5)   |
| Yes                        | 41              | 11 (26.8)   |
| Recurrence                 |                 |             |
| No                         | 119             | 44 (37.0)   |
| Yes                        | 86              | 17 (19.8)   |

*HBsAg: hepatitis B surface antigen; BCLC: barcelona-clinic liver cancer
TGCAATGG-3’ (antisense). The PCR reaction was run for 95°C for 3 min; followed by 35 cycles (for DEPDC1) or 25 cycles (for β-actin) of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s; finally the reaction system was incubated at 70°C for 5 min and terminated at 4°C, products being visualized under UV light after ethidium bromide staining on 2% agarose gel.

The sequences of primers for qRT-PCR were as following: DEPDC1: 5’-ACCAAAATGGTGACACAGGCAGC-3’ (sense) and 5’-CAGCAAGCTTTTGTTGCCCAGTC-3’ (antisense); β-actin: 5’-GACAGGATGCAGAAGGAGATTACT-3’ (sense) and 5’-TGATCCACATGCCTGGAAGGT-3’ (antisense). qRT-PCR was carried out according to the manuscript of SYBR Green PCR Master Mix, Applied Biosystems was amplified using the ABI Prism 7500 Sequence Detector System Applied Biosystems (Foster City, CA, USA) with the following reaction conditions: incubation at 95°C for 10 min; then 40 cycles of denaturation at 95°C for 2 sec, annealing at 55°C for 30 s, and extension at 72°C for 30 s; finally the reaction system was incubated at 70°C for 5 min and terminated at 4°C, products being visualized under UV light after ethidium bromide staining on 2% agarose gel.

Statistical analysis

Results were analyzed using SPSS version 13.0, p < 0.05 was considered statistically significant. The Chi-square (χ²) test was used to compare the correlation between DEPDC1 expression and clinicopathological parameters, and the Students’ t test was used to analysis continuous variables. Kaplan-Meier method was used to plot survival curves, and the log-rank test was used to evaluate the differences in survival curves. Univariate and multivariate regression analysis were performed to identify prognostic factors.

### Results

**DEPDC1 expression in HCC**

To detect the expression of DEPDC1 in HCC patients, we analyzed DEPDC1 mRNA level in 20 cases of HCC tissues and adjacent normal liver tissues by reverse-transcription PCR. The results showed that DEPDC1 mRNA was significantly higher in 14 cases of HCC (Figure 1A). To further confirm RT-PCR results, DEPDC1 mRNA level in 205 cases of HCC was detected by quantitative real-time PCR. The results showed that DEPDC1 mRNA was up-regulated in 144 cases (70.24%), but down-regulated in 51 cases (29.76%) compared with adjacent normal liver tissues. The expression of DEPDC1 in HCC tissues was significantly higher than that in adjacent normal liver tissues (-9.96±0.2254 vs-14.50±0.2104, p<0.0001) (Figure 1B).

To assess the diagnostic value of DEPDC1, we compared serum AFP level and DEPDC1 in each patient. Interestingly, we found that the increase of serum AFP level was not always accompanied by DEPDC1 mRNA up-regulation. As shown in Figure 1C, AFP alone was increased in 27 cases. DEPDC1 mRNA alone was increased in 35 cases.

| Clinical character | Category | No. of patients | Disease-free survival (months) | Overall survival (months) |
|-------------------|----------|----------------|-------------------------------|--------------------------|
|                   |          |                | Mean | 95% CI | p value | Mean | 95% CI | p value |
| **DEPDC1 expression** | Low | 61 | 55.32 | 46.88-63.76 | 0.002 | 60.24 | 52.74-67.74 | 0.004 |
|                   | High | 144 | 38.82 | 33.36-44.28 | 45.86 | 40.77-50.95 | |
| **Age (years)** | <55 | 140 | 42.90 | 37.18-48.62 | 0.453 | 48.71 | 43.41-54.01 | 0.393 |
|                   | ≥55 | 65 | 46.17 | 38.04-54.30 | 53.43 | 46.09-60.76 | |
| **Gender** | Female | 175 | 49.02 | 38.47-59.57 | 0.485 | 58.14 | 47.05-69.23 | 0.154 |
|                   | Male | 30 | 42.41 | 37.42-47.41 | 48.82 | 44.18-53.47 | |
| **Family history** | No | 170 | 41.84 | 36.77-46.92 | 0.053 | 48.35 | 43.66-53.04 | 0.055 |
|                   | Yes | 35 | 55.78 | 44.35-67.22 | 59.21 | 48.88-69.54 | |
| **HBsAg** | Positive | 37 | 40.98 | 29.65-52.30 | 0.621 | 49.32 | 39.07-59.56 | 0.832 |
|                   | Negative | 168 | 44.64 | 39.47-49.80 | 50.44 | 45.69-55.20 | |
| **AFP (ng/mL)** | <100 | 85 | 49.49 | 42.23-56.75 | 0.047 | 56.02 | 46.62-63.28 | 0.040 |
|                   | ≥100 | 120 | 40.28 | 34.19-46.37 | 46.13 | 40.42-51.84 | |
| **Tumor size (cm)** | <4 | 52 | 65.77 | 58.09-73.45 | p<0.001 | 70.18 | 63.88-76.47 | p<0.001 |
|                   | ≥4 | 153 | 36.41 | 31.19-41.64 | 43.52 | 38.59-48.45 | |
| **Cirrhosis** | No | 19 | 39.87 | 28.84-54.86 | 0.605 | 44.32 | 30.36-58.27 | 0.462 |
|                   | Yes | 186 | 44.48 | 39.53-49.43 | 50.79 | 46.27-55.31 | |
| **Tumor number** | Single | 140 | 49.24 | 43.44-55.05 | 0.002 | 54.93 | 47.94-60.12 | 0.001 |
|                   | Multiple | 65 | 32.48 | 25.70-39.27 | 40.05 | 32.90-47.20 | |
| **BCLC stage** | 0-A | 100 | 57.08 | 50.37-63.78 | p<0.001 | 62.62 | 56.99-68.26 | p<0.001 |
|                   | B-C | 105 | 32.22 | 28.66-36.26 | 38.13 | 32.56-43.71 | |
| **NLR** | <2.31 | 118 | 49.52 | 43.23-55.82 | 0.008 | 55.31 | 47.92-60.90 | 0.007 |
|                   | ≥2.31 | 87 | 35.41 | 29.10-41.72 | 43.26 | 36.77-49.74 | |
| **PVTT** | No | 164 | 49.36 | 44.04-54.69 | p<0.001 | 55.41 | 50.69-60.13 | p<0.001 |
|                   | Yes | 41 | 24.60 | 17.08-32.12 | 29.18 | 21.87-36.50 | |
| **Recurrence** | No | 186 | 44.48 | 39.53-49.43 | 50.79 | 46.27-55.31 | |
|                   | Yes | 41 | 24.60 | 17.08-32.12 | 29.18 | 21.87-36.50 | |

*CI, confidence interval; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; BCLC, Barcelona-clinic liver cancer; TNM, tumor-node-metastasis; PVTT, portal vein tumor thrombus; LNM, lymph node metastasis; NLR, neutrophil to lymphocyte ratio*
Increased in 39 cases. AFP and DEPDC1 mRNA both were increased in 48 cases. If combining serum AFP level and DEPDC1 mRNA in liver tissue, the diagnostic rate of HCC could reach more than 80% (Figure 1C).

**Correlation between DEPDC1 expression in HCC and clinicopathological parameters**

To explore whether increased DEPDC1 expression was relevant to clinicopathological parameters, we performed Chi-square test. As shown in Table 2, High DEPDC1 expression was significantly correlated with serum AFP level (≥100 µg/ml) ($\chi^2=4.326, p=0.038$), tumor size (≥ 4 cm) ($\chi^2=1.203, p=0.022$), B-C of BCLC stage ($\chi^2=6.348, p=0.012$) and recurrence ($\chi^2=7.072, p=0.008$). Nevertheless, no significant correlation was observed between high DEPDC1 expression and age, gender, family history, HBsAg, liver cirrhosis, and PVTT (all $p>0.05$).

High DEPDC1 expression is an independent predictor for DFS and OS

Kaplan-Meier survival analysis revealed that high DEPDC1 expression is related to poor OS and DFS.
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Figure 2. Correlation between DEPDC1 expression and disease free survival (DFS) or overall survival (OS). Patients with high DEPDC1 expression had a shorter DFS A) and OS B). The solid line represents the patient with low DEPDC1 expression, and the dashed line represents the patient with high DEPDC1 expression.

Discussion

In the current study, we detected that DEPDC1 is up-regulated in over 70% HCC tissues compared with adjacent normal liver tissues, found that high DEPDC1 expression is correlated with these clinicopathologic parameters: AFP ≥100ng/ml, tumor size ≥4cm, B-C of BCLC stage and recurrence, revealed that high DEPDC1 expression, together with AFP ≥100, tumor size ≥4cm, multiple tumor number, B-C of BCLC stage, PVTT and NLR ≥2.31 were relevant to poor DFS and OS, and that high DEPDC1 expression might be an independent predictor for DFS and OS.

Previous reports have demonstrated that DEPDC1 is up-regulated in bladder cancer (Kanehira et al., 2007), breast cancer (Kretschmer et al., 2011) and lung cancer (Okayama et al., 2012). To our knowledge, our report is the first one showed DEPDC1 up-regulation in HCC. AFP is a broadly used serum HCC marker. But its levels remain normal in up to 40% HCC patients. Thus, it is urgent to identify novel biomarkers to improve diagnostic rate of HCC. Our data demonstrated that HCC positive rate could reach over 80% if serum AFP level and tissue DEPDC1 mRNA are both used as the diagnostic markers. Therefore, DEPDC1 might be a novel diagnostic biomarker of HCC that is capable of compensating the shortcoming of AFP to improve HCC diagnostic rate significantly.

Our study revealed that high DEPDC1 expression, tumor size ≥4cm, PVTT, and B-C of BCLC stage are independent prognostic indicators for DFS and OS. High AFP as an indicator of poor survival was reported previously (Peng et al., 2004; Cheng et al., 2011). A systematic review of 72 studies revealed that PVTT, large tumor size, tumor number are robust indicators of poor prognosis (Tandon and Garcia-Tsao, 2009). Larger size tumor and multifocal tumors tend to invade portal veins, and lead to intrahepatic tumor recurrence and PVTT. Therefore, microvascular portal vein thrombosis is associated with disease free survival and recurrence rates (Ercolani et al., 2003). In addition, cells derived from PVTT showed a typical migratory tendency and aggressive phenotype (Wang et al., 2010). As our data demonstrated that high DEPDC1 expression is associated with these aggressive features, it will be very interesting to elucidate the mechanisms by which DEPDC1 expression is connected to these factors in the future.

High DEPDC1 expression was identified as an independent prognostic indicator for DFS and OS in our study, indicating DEPDC1 may play an important role in tumor development. Given that DEPDC1 is also up-regulated in bladder cancer (Kanehira et al., 2007), breast cancer (Kretschmer et al., 2011) and lung cancer (Okayama et al., 2012), it is crucial to unveil how DEPDC1 is regulated in future study. Researchers found DEPDC1 was up-regulated in bladder cancer tissues, but undetectable in 24 normal human tissues except in testis (Kanehira et al., 2007), and developed peptide vaccine based on this cancer/testis antigen, which effectively
induced peptide-specific cytotoxic T lymphocyte in vivo (Obara et al., 2012). Another cell permeable peptide capable of disrupting interaction between DEPDC1 and zinc finger transcription factor ZNF224 showed promising results in bladder cancer cells (Harada et al., 2010). These two reports demonstrated that DEPDC1 is a therapeutic target, at least in bladder cancer. Based on our findings that DEPDC1 is up-regulated in HCC, it might be very interesting to explore the possibility of utilizing DEPDC1 as a therapeutic target in HCC.

In conclusion, DEPDC1 is up-regulated in HCC, and might be a novel HCC diagnostic marker, prognosis predictor, as well as a therapeutic target for HCC patients. Further studies on its regulation and function will ultimately benefit patients with HCC or other related cancers.

List of abbreviations: HCC, hepatocellular carcinoma; DFS, disease-free survival; OS, overall survival; AFP, alpha-fetoprotein; PVTT, portal vein tumor thrombus; NLR, neutrophil to lymphocyte ratio; BCLC, Barcelona-clinic liver cancer.

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