Predictive value of tumor markers in patients with recurrent hepatocellular carcinoma in different vascular invasion pattern

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BACKGROUND: Four tumor markers for hepatocellular carcinoma (HCC), alpha-fetoprotein (AFP), glypican-3 (GPC3), vascular endothelial growth factor (VEGF) and des-gamma-carboxy prothrombin (DCP), are closely associated with tumor invasion and patient's survival. This study estimated the predictability of preoperative tumor marker levels along with pathological parameters on HCC recurrence after hepatectomy.

METHODS: A total of 140 patients with HCC who underwent hepatectomy between January 2012 and August 2012 were enrolled. The demographics, clinical and follow-up data were collected and analyzed. The patients were divided into two groups: patients with macroscopic vascular invasion (MaVI+) and those without MaVI (MaVI-). The predictive value of tumor markers and clinical parameters were evaluated by univariate and multivariate analysis.

RESULTS: In all patients, tumor size (>8 cm) and MaVI were closely related to HCC recurrence after hepatectomy. For MaVI+ patients, VEGF (>900 pg/mL) was a significant predictor for recurrence (RR=2.421; 95% CI: 1.272-4.606; *P*=0.007). The 1- and 2-year tumor-free survival rates for MaVI+ patients with VEGF ≤900 pg/mL versus for those with VEGF >900 pg/mL were 51.5% and 17.6% versus 19.0% and 4.8% (*P*<0.001). For MaVI- patients, DCP >445 mAu/mL and tumor size >8 cm were two independent risk factors for tumor recurrence (RR=2.307, 95% CI: 1.132-4.703, *P*=0.021; RR=3.150, 95% CI: 1.392-7.127, *P*=0.006; respectively). The 1- and 2-year tumor-free survival rates for the patients with DCP ≤445 mAu/mL and those with DCP >445 mAu/mL were 90.4% and 70.7% versus 73.2% and 50.5% respectively (*P*=0.048). The 1- and 2-year tumor-free survival rates for the patients with tumor size ≤8 cm and >8 cm were 83.2% and 62.1% versus 50.0% and 30.0%, respectively (*P*=0.003).

CONCLUSIONS: The MaVI+ patients with VEGF ≤900 pg/mL had a relatively high tumor-free survival than those with VEGF >900 pg/mL. In the MaVI- patients, DCP >445 mAu/mL and tumor size >8 cm were predictive factors for postoperative recurrence.

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KEY WORDS: tumor markers; hepatocellular carcinoma; recurrence; vascular invasion; prediction

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most prevalent malignancy and the third most frequent cause of tumor-related death throughout the world.[1] Though current options for treatment of HCC consist of surgical resection, liver transplantation, transcatheter arterial embolization and radiofrequency

ablation, hepatectomy is still the first choice.[2-4] However, the high incidence of HCC recurrence following resection reduces the survival rate.

Many studies[5-8] have developed various serum and tissue tumor markers to predict the prognosis of patients with HCC. Alpha-fetoprotein (AFP) is the first introduced and most extensively utilized marker for diagnosis, prognosis and monitoring of HCC. Glypican-3 (GPC3), vascular endothelial growth factor (VEGF) and des-gamma-carboxy prothrombin (DCP) were found to be associated with carcinogenesis and vascular invasion that enabled them to be potential and reliable biomarkers for predicting tumor recurrence and survival in HCC patients after resection.

Nevertheless, the studies investigating predictive ability focus on the universality of multiple tumor markers in spite of varied tumor features, such as tumor size, number and vascular invasion. Macroscopic vascular invasion (MaVI) is usually evaluated before treatment and regarded as a critical feature reflecting biological behavior of tumor and influencing outcome of patients.[9, 10]

Therefore, the aim of the present study was to estimate the prognostic role of preoperative levels of serum tumor markers and pathological parameters in the recurrence of HCC in different vascular invasion patterns after hepatectomy. Early prediction of tumor recurrence after treatments is essential for precise selection of personalized therapeutic strategies, thus improving the survival of HCC patients.

Methods

Patients

A cohort of 140 adult patients who had been diagnosed with HCC and received liver resection at the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China from January 2012 to August 2012 were enrolled while excluding those who had received preoperative adjuvant therapy or died of noncancerous causes or took oral warfarin. Demographics and clinical information were analyzed from original medical records after approval from the institutional review board. Tumor invasiveness was divided into 3 types: type 1, single nodular type with a clear demarcation; type 2, single nodular type with extranodular growth; type 3, contiguous multi-nodular type formed by a cluster of small and contiguous nodules.[11, 12] And tumor differentiation was determined by biopsy. Serum samples were taken from each patient for the measurement of the levels of four tumor markers within 3 days before operation. The diagnosis of HCC was confirmed by pathologic examination of resected specimens. MaVI was defined as portal vein invasion, and venous invasion was evaluated by imagings including computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography-computed tomography (PET-CT).

The patients were followed up with contrast enhanced CT scan of the chest and abdomen or contrast enhanced MRI of the abdomen and non-contrast CT of the chest every 3 months for the first year, and subsequently every 6 months. The end of follow-up was as follows: the last follow-up (May 2015), tumor recurrence or death.

Assays

A 5-mL blood sample was drawn into a serum separator tube and centrifuged at 1500 ×g for 10 minutes to obtain serum that was subsequently stored at -80 ℃. The serum was diluted to fit the standard curve of each measurement. Serum concentrations of VEGF were measured in duplicate with an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN, USA), by an investigator who was blinded to the clinical information of the patients. AFP, GPC3, and DCP were measured with an ELISA kit (Uscn Life Science Inc., Wuhan, China). The detection limits and the quantitation limits are 0.27 ng/mL and 0.78 ng/mL for AFP, 57 ng/L and 156 ng/L for GPC3, 9.00 pg/mL and 31.20 pg/mL for VEGF, and 5 mAu/mL and 100 mAu/mL for DCP.

Statistical analysis

Statistical analysis was performed with the software SPSS, version 16.0 (Chicago, IL, USA). Variables were expressed as means and standard deviations or as medians and interquartile ranges. Pearson's correlation test, the Chi-square test, the Mann-Whitney *U* test or Student's *t* test was used appropriately to calculate the statistical significance of variables. Tumor-free survival rates were calculated using the Kaplan-Meier method. Univariate analysis was performed to identify the risk factors for tumor recurrence. After the univariate analysis, only variables with P value <0.1 were used in the multivariate analysis, which used the Cox proportional hazard regression analysis to identify independent survival predictors. Pearson's correlation test was used to assess the correlation between serum levels of tumor markers and pathological features of the resected HCC. A P value <0.05 was considered statistically significant.

Results

Table 1 presents baseline demographics and clinical parameters. The study population is composed of 124