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ORIGINAL PAPER

Prognostic value of serum vascular endothelial growth factor receptor 2 response in patients with hepatocellular carcinoma undergoing transarterial chemoembolization

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Abstract We aimed to elucidate whether serum VEGFR2 concentration before and after transarterial chemoembolization (TACE) can predict survival in patients with unresectable hepatocellular carcinoma (HCC). Serum VEGFR2 concentrations were serially measured in 169 patients with advanced HCC before and after TACE. We defined a decrease in the serum VEGFR2 level >10 % from the pretreatment level as response. Serum VEGFR2 concentrations decreased in 44 (26.0 %) patients at week 4. Patients who had a VEGFR2 response at week 4 had a longer median survival than those who did not have a VEGFR2 decrease (19.0 vs. 9.8 months, $p < 0.001$). Clinical variables associated with OS in addition to VEGFR2 response also included extrahepatic metastases ($p = 0.005$) and vascular invasion ($p = 0.035$). VEGFR2 decrease after TACE ($p = 0.012$) and presence of extrahepatic metastases ($p = 0.02$) were independently associated with OS by multivariate analysis. A serum VEGFR2 concentration decrease at 4 weeks after TACE may predict favorable overall survival in patients with advanced HCC.

Keywords Vascular endothelial growth factor receptor 2 · Hepatocellular carcinoma · Survival · Transarterial chemoembolization

Introduction

Hepatocellular carcinoma (HCC) accounts for 70–85 % of primary liver cancer. It is the sixth most common cancer in the world and the third most common cause of cancer-related death [1]. China is one of the areas with a high prevalence of HCC mainly because of chronic hepatitis B carriers accounting for >10 % of its population [2]. Most patients with HCC are diagnosed at advanced stages of disease, when curative treatments, such as hepatic resection and liver transplantation, are not feasible [3]. Transarterial chemoembolization (TACE) is the preferred first-line treatment for intermediate-stage HCC, and evidence obtained from randomized controlled trials has confirmed its beneficial effect in improving median survival [4]. However, TACE, as all currently available systemic options for HCC therapy, usually induce only short-termed disease stabilizations in majority of the patients [5], and early identification of potential treatment responders would be useful to both oncologists and patients.

Current determination of survival and prognosis in patients treated with TACE or TAE for unresectable HCC is mainly based on clinical assessment. Previous studies have illustrated the use of clinical variables, such as ascites, physical performance status, bilirubin, portal vein thrombosis, prothrombin time, a fetoprotein, and tumor size, as a means to predict survival in patients with HCC [6, 7].

HCC is a highly vascular tumor and the importance of the angiogenic process in hepatocellular carcinoma progression, and the correlation between tumor blood vessel

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density and clinical (or preclinical) outcome has already been described in the literature. Angiogenesis is an important step in development of cancer and is necessary for primary tumor growth, invasiveness, and metastasis. It is tightly regulated by a complex equilibrium among different pro- and antiangiogenic factors secreted both by tumor cells and by cells of the tumor microenvironment [8].

In the vascular endothelial growth factor (VEGF) family, three receptors have been identified, but earlier studies have shown that activation of VEGFR2 is sufficient to elicit all proangiogenic, proliferation, and survival effects associated with VEGF [9]. Previous study showed high expression of tissue VEGFR2 in HCC was related to large tumor diameter, poor differentiation, high serum α-fetoprotein, multifocal gross classification, and <5-years survival [10]. However, data on the relationship between TACE and serum VEGFR2 are little. In this respect, we decided to analyse the serum VEGFR2 levels in patients with HCC before and after TACE to determine the clinicopathological significance of VEGFR2, and to assess the clinical usefulness of serum VEGFR2 as a predictor of outcome in patients undergoing TACE therapy for HCC.

Materials and methods

Patients

Patients with unresectable HCC who underwent TACE as an initial treatment modality at the Department of Interventional Radiology in our hospital between 2009 and 2012 were selected from a prospectively maintained hepatobiliary tumor database. All patients satisfied the diagnostic criteria for HCC based on radiologic or histologic grounds according to the American Association for the Study of the Liver guidelines [11]. The study protocol was approved by the independent institutional review board of our institute.

Patients who met the following inclusion criteria were enrolled for this study: (1) age >18 years, (2) eastern cooperative oncology group performance status (ECOG PS) 0–1, and (3) Child–Pugh score <10. Patients were excluded from the study if they had one or more of the following: (1) severe underlying cardiac or renal diseases, (2) diffuse-type HCC, (3) evidence of hepatic decompensation, (4) complete occlusion of the entire portal venous system and without adequate collateral circulation around the occluded portal vein, and (5) clinical symptoms or signs of sepsis.

Transarterial chemoembolization

All of the percutaneous US-guided TACE procedures in our hospital were performed by the same team of surgeons. TACE was undertaken depending on the tumor burden and underlying liver function, and was repeated as needed until radiographic evidence was obtained of tumor necrosis, tumor progression, or decline in liver function or performance status. TACE was performed through the transfemoral route, and all feeding arteries of the tumor were identified by angiography. The vessels feeding the tumor were injected with an emulsion of lipiodol (5–10 mL), oxaliplatin (100–200 mg), and epirubicin (30–60 mg) followed by embolization with absorbable particles (gelatin foam). The doses of the chemotherapy drugs were adjusted by tumor size. After embolization, another angiography was performed to determine the extent of vascular occlusion and to assess blood flow in other arterial vessels. Patients were observed carefully after treatment, and analgesia was given when necessary.

Radiologic response evaluation

Radiologic images were reviewed in double-blind by two radiologists. Radiologic tumor response was evaluated using the modified response evaluation criteria in solid tumors (mRECIST) by CT or MRI at 1 month then every 3 months thereafter. In the mRECIST criteria, length of the major axis of a viable tumor was compared to baseline for calculating change in size. CR was taken as the absence of any enhancing tissue, PR as a 30 % decrease in enhancing tissue, and SD as a <20 % decrease [12].

Assay of serum VEGFR2 level

Peripheral venous blood samples were taken from the patients with HCC before initial treatment, 1 week and 4 weeks after first TACE treatment. Blood samples were drawn into serum separator tubes and centrifuged at 3,000 r/min for 5 min, then stored at −80 °C until analysis. The levels of serum VEGFR2 concentrations were measured quantitatively using an enzyme-linked immunosorbent assay kit (RAYBIO®; Human VEGFR2 ELISA Kit; RayBiotech, Inc., USA) according to the manufacturer's instructions. All samples were assayed in duplicate by an investigator blinded to the clinical data. We defined a decrease in the serum VEGFR2 level >10 % from the pretreatment level as a VEGFR2 response.

Follow-up

All patients treated in our center for HCC were required to be followed up according to our institutional protocol. Each follow-up session includes a detailed history and physical examination, ECOG PS classification, Child–Pugh score evaluation, serum AFP, and an abdominal enhanced CT/MRI scan every 4–6 weeks.

Table 1 Baseline demographics of patients

Characteristics	High VEGFR2	Low VEGFR2	*p*
Age (years)			0.45
≤60	76	45	
>60	29	19	
Gender			0.39
Male	82	48	
Female	23	16	
Cause of cirrhosis			0.076
HBV	61	45	
Other/unknown	44	19	
CP classification			0.42
A	75	44	
B	30	20	
Tumor number			0.712
1	64	39	
2	22	16	
>2	19	9	
Tumor size (cm)			0.358
>5	58	38	
≤5	47	26	
Major vascular invasion			0.105
Present	66	47	
Absent	39	17	
ECOG PS			0.447
0–1	97	58	
2–3	8	6	
AFP (ng/mL)			0.49
≤400	69	43	
>400	36	21	

VEGFR2 vascular endothelial growth factor receptor 2, *CP* Child–Pugh, *ECOG PS* eastern cooperative oncology group performance status, *AFP* α-fetoprotein

Statistical analyses

Data were evaluated using Statistical Package for Social Sciences, v.13.0. Continuous variables were expressed as mean ± SD and compared using t test. Categorical variables were expressed as frequency and compared using χ^2 test. The Kaplan–Meier method was used for survival analysis. A multivariate analysis was performed by Cox regression for significant variables on univariate analysis. All *p* values were based on two-sided testing and a *p* value <0.05 was considered as significant.

Results

Patient characteristics

There were 169 patients who underwent initial treatment with TACE for HCC. Baseline clinical characteristics of the patients are shown in Table 1. The median age was 60.5 (range 32.5–83.6) years, and the majority of patients were men ($n = 130$, 77 %). All in all, 103 patients had one nodule, 38 patients had two nodules, and 28 patients had three nodules or more. The majority of patients were classified with intermediate HCC according to the BCLC staging algorithm (70 %), with compensated liver function (Child–Pugh class A, 70 %). With aspect to etiologies, 106 patients were positive for hepatitis B virus surface antigen (HBsAg), 14 patients were positive for anti-hepatitis C virus (HCV) antibody, 8 patients belonged to alcoholic cirrhosis, and 41 patients had unknown etiologies. Complete radiographic response was noted in 35 patients (20.1 %), partial responses were observed in 58 (34.3 %), stable disease in 48 (28.4 %) and progressive disease in 28 (16.7 %). The most common post-embolization complications were transient fever, abdominal pain, and increased alanine aminotransferase levels, which were controlled with symptomatic treatments. No chemoembolization-related deaths were recorded.

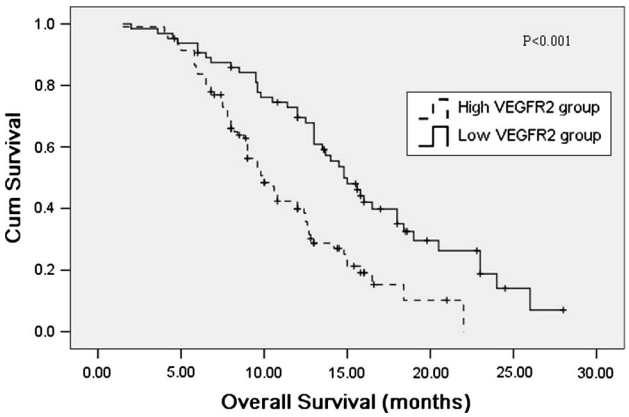

Fig. 1 Correlation of pre-TACE serum vascular endothelial growth factor receptor 2 (VEGFR2) and overall survival in patients with HCC

Pretreatment serum VEGFR2 concentration and prognosis

The baseline mean serum VEGFR2 was 8.7 ± 2.5 μg/L and the median TTP and OS of all patients were 5.5 (95 % CI 3.4–6.8) and 12.5 months (95 % CI 11.2–13.8), respectively. Patients with high VEGFR2 (dichotomized by median split) had significant worse outcome. The median survival of patients with a high VEGFR2 was 10 months (95 % CI 8.6–11.4) compared with 14.8 months (95 % CI 12.8–16.8) for patients with a low VEGFR2, $p < 0.001$ (Fig. 1). The only clinical factor associated with TTP was the radiographic response to initial TACE. No difference was observed in the demographic and clinicopathologic variables of HCC at baseline between patients with higher

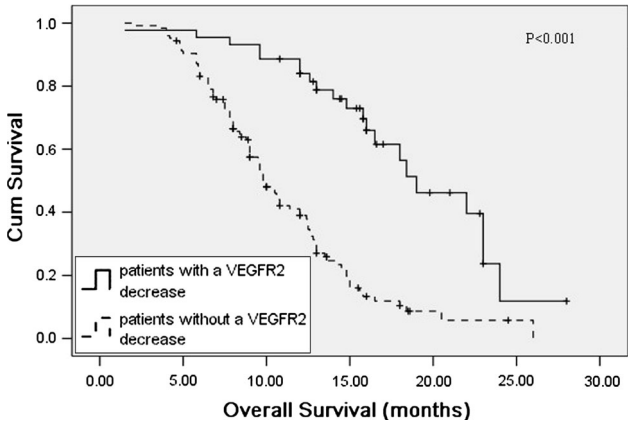

Fig. 2 This Kaplan–Meier plot illustrates overall survival according to changes in vascular endothelial growth factor receptor 2 (VEGFR2) concentrations

versus lower pretreatment serum VEGFR2 concentrations. No correlation was found between serum VEGFR2 and AFP ($r = 0.45$, $p = 0.56$).

Changes in serum VEGF concentrations and overall survival

Serum VEGFR2 concentrations at 1 week and 4 weeks after the initial TACE treatment were 11.8 ± 3.1 and 10.5 ± 3.3 μg/L, higher than baseline concentration ($p = 0.014$ and $p = 0.04$, respectively). Serum VEGFR2 concentrations decreased in 35 (20.7 %) patients at first week. No significant difference in OS between changes in serum VEGFR2 at first week was identified (14.5 vs. 12.8 months, $p = 0.51$). Serum VEGFR2 concentrations decreased in 44 (26.0 %) patients at week 4. Patients who had a VEGFR2 decrease at week 4 had a longer median survival than those who did not have a VEGFR2 decrease (19.0 vs. 9.8 months, $p < 0.001$), suggesting that a decrease in VEGFR2 concentration 4 weeks after TACE treatment is closely associated with a favorable prognosis (Fig. 2). Among 44 patients with decreased VEGFR2, their best radiologic response were 7 CR, 17 PR, 10 SD, and 10 PD. VEGFR2 trend after TACE was not associated with radiographic response to TACE ($p = 0.89$).

Clinical variables associated with OS in addition to VEGFR2 included extrahepatic metastases ($p = 0.005$) and vascular invasion ($p = 0.035$). VEGFR2 decrease after TACE ($p = 0.012$) and presence of extrahepatic metastases ($p = 0.02$) were independently associated with OS by multivariate analysis (Table 2).

Table 2 Univariate and multivariate analysis of risk factors affecting overall survival

Characteristic	Univariate (*p*)	Multivariate (*p*)	OR, 95 % CI
Age (>60 years)	0.37		
Gender (male)	0.76		
Tumor size (>5 cm)	0.28		
ECOG PS (>1)	0.41		
CP class (B)	0.22		
Extrahepatic metastases	0.005	0.02	1.81–4.22
Vascular invasion	0.035	0.32	0.71–5.12
Baseline AFP (>400)	0.53		
Baseline VEGFR2	0.023	0.28	0.28–1.93
VEGFR2 decrease	0.003	0.012	0.23–0.87

ECOG PS eastern cooperative oncology group performance status, *CP* Child–Pugh, *AFP* α-fetoprotein, *VEGFR2* vascular endothelial growth factor receptor 2

Discussion

In the current study, we demonstrated that surum VEGFR2 concentrations change dynamically after TACE procedure, and changes in VEGFR2 concentration are closely associated with OS in patients who underwent TACE. The VEGF family members are secreted, dimeric glycoproteins of B40 kDa, consisting of five members, VEGF A, B, C, D and placental growth factor (PLGF), and binding to specific receptors (VEGFR). VEGF and their receptors are believed to be important for the process of regulation of angiogenesis [9]. VEGFR2 is the principal receptor that promotes the proangiogenic action of VEGFA and has been the principal target of antiangiogenic therapies [13]. Huang et al. [10] revealed that high expression of tissue VEGFR2 in HCC was related to large tumor diameter, poor differentiation, high serum α-fetoprotein, multifocal gross classification, and <5-years survival. However, few studies focused on the relationship between TACE and serum VEGFR2 level.

The objective of our study was to elucidate whether an on-treatment change in VEGFR2 is a potentially useful biomarker for predicting prognosis in patients who underwent TACE, because such an on-treatment predictor among patients has not yet been elucidated. We found that the mean level of serum VEGFR2 increased after TACE procedure. These may be due to the aggravated tumor anoxic microenvironment after TACE. This hypothesis is in accordance with the demonstration that plasma VEGF concentrations increased shortly after treatment with TACE [14, 15]. Tumor microenvironment hypoxia resulted in up-regulated levels of hypoxia inducible factor-1 (HIF-1), which in turn up-regulates VEGF and VEGFR and increases tumor angiogenesis [16]. Hypoxia induced by local treatment and associated production of VEGF were considered to be a reason for disease progression and metastasis after local therapy [17].

Although the mean level of VEGFR2 increased after TACE, our study found that decreases in serum VEGFR2 were observed in 35 and 44 patients at one and 4 weeks after TACE, and patients with a decrease at 4 weeks had better OS. One possible reason for this association may be that the decrease in VEGFR2 concentrations reflects a decrease in the number of tumor cells secreting VEGFR2. An association between changes in VEGF concentrations and overall survival was observed in a previous study of a multikinase inhibitor, sorafenib, in patients with advanced HCC. Tsuchiya et al. [18] reported a plasma VEGF concentration decrease at 8 weeks after starting sorafenib treatment may predict favorable overall survival in patients with advanced HCC. Although the precise mechanism underlying the association between serial changes in VEGF or VEGFR2 and disease progression is unclear, the findings of the current study are extremely valuable for clinical practice in predicting the prognosis of patients who underwent TACE.

There are some limitations in correlating serum VEGFR2 with our clinical findings. The relative small size and the nature of the retrospective design are main limitations. Although the current study suggests that serum VEGFR2 level may be a useful prognostic biomarker for TACE therapy in HCC, further studies with larger patient populations are needed to validate its prognostic value and determine the optimum cut-off value. Furthermore, the biologic significance of circulating VEGFR2 in cancer patients remains to be clarified.

Conflict of interest None.

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