

# Association between vascular endothelial growth factor and metastasis after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma

Zheng-Ping Xiong, Shu-Ren Yang, Zhao-Yu Liang, En-Hua Xiao, Xiao-Ping Yu, Shen-Ke Zhou and Zi-Shu Zhang

Changsha, China

**BACKGROUND:** Hypoxia up-regulates vascular endothelial growth factor (VEGF) and stimulates the growth of hepatocellular carcinoma (HCC) cells. This study was designed to investigate the association between changes in plasma VEGF levels after transcatheter arterial chemoembolization (TACE) and HCC progression, especially in relation to metastasis.

**METHODS:** Plasma VEGF levels were measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA R&D system). Plasma VEGF levels were measured before, 3 days and 4 weeks after TACE in 30 patients with HCC. The development of metastasis was evaluated at the end of the third month after TACE.

**RESULTS:** The plasma VEGF levels of the 30 patients with HCC were  $154.47 \pm 90.17$  pg/ml. The total plasma VEGF levels after TACE increased compared with their basal levels ( $P < 0.05$ ), and the plasma VEGF levels had a tendency to increase in patients with heterogenous uptake of iodizdoil and portal vein thrombosis. Follow-up for six months showed metastatic foci in 20 patients (74%) with increased plasma VEGF, but none of the patients with decreased plasma VEGF developed metastasis.

**CONCLUSION:** Increased plasma VEGF expression is associated with the development of metastasis in HCC after TACE.

(*Hepatobiliary Pancreat Dis Int* 2004; 3: 386-390)

**KEY WORDS:** hepatocellular carcinoma; metastasis; vascular endothelial growth factor; transcatheter arterial chemoembolization

## Introduction

Transcatheter arterial chemoembolization (TACE) is one of the most useful palliative treatments for patients with inoperable hepatocellular carcinoma (HCC). Despite its effectiveness, however, intrahepatic or extrahepatic metastasis after TACE is one of the major factors limiting its overall therapeutic effect.

Vascular endothelial growth factor (VEGF) is believed to play an important role in fetal growth and development. In addition there is increasing evidence that the overexpression of VEGF and the activation of its signaling pathway play roles in oncogenesis and angiogenesis. In human HCC, the overexpression of VEGF and the reappearance of fetal VEGF transcripts also have been reported.<sup>[1]</sup>

TACE inevitably results in a hypoxic insult to HCC and the surrounding liver tissue. Hypoxia has been reported to up-regulate VEGF and thus stimulate the growth of HCC cells in vitro.<sup>[2,3]</sup> We prospectively evaluated changes in plasma VEGF levels after TACE and also determined the association between intrahepatic or extrahepatic metastasis in patients with HCC who underwent TACE.

## Methods

### Patients

Between February 2002 and August 2002, thirty consecutive patients were diagnosed with HCC. HCC was diagnosed either histologically (6 patients) or radiologically according to typical imaging findings of hypervascular liver masses (15) or atypical radiological findings with increased plasma  $\alpha$ -fetoprotein (AFP >400 ng/ml) (9).

**Author Affiliations:** Department of Radiology, Hunan Provincial Tumor Hospital, Changsha 410006, China (Xiong ZP, Liang ZY and Yu XP); Department of Radiology, Xiangya Second Hospital of Central South University, Changsha 410011, China (Yang SR, Xiao EH, Zhou SK and Zhang ZS)

**Corresponding Author:** Zheng-Ping Xiong, MD, Department of Radiology, Hunan Provincial Tumor Hospital, Changsha 410006, China (Tel: 86-731-8651678; Fax: 86-731-8651808; Email: xiongzhenping@163.net)

This study was supported by grant from the National Natural Science Foundation of China (30070235).

© 2004, Hepatobiliary Pancreat Dis Int. All rights reserved.

Of the 30 patients with HCC treated with TACE, 24 were found to be carriers of hepatitis B virus (HBV). The tumor size varied from 2.8 to 20.3 cm ( $10.71 \pm 4.82$  cm). Seven patients had single nodular tumors and the remaining 23 patients had multinodular tumors. Liver function showed Child-Pugh A in 17 patients and Child-Pugh B in 13.

## Methods

### TACE procedure

At first, the superior mesenteric artery was catheterized to assess the blood flow of the portal vein. After confirming that it was adequate, the tumor feeding artery was visualized on a common hepatic arteriogram. Eighty mg of cisplatin, 8 mg of mitomycin (MMC), and 1250 mg of 5-Fu per  $m^2$  of body surface, dissolved in distilled water at a concentration of 0.5 mg/ml, were infused through the selected proper hepatic artery for 15 minutes. Approximately 10–30 ml of iodized oil mixed with 40 mg EADM also were infused slowly according to the size of the HCC. Subsequently, the selected feeding artery was embolized with gelatin sponge particles.

### Sample collection and plasma VEGF measurement

Blood samples were collected in unfertile tubes from all the patients 3–5 days before TACE, 3 days, and 4 weeks after TACE, respectively. The samples were centrifuged at 3000 r/min for 15 minutes at 4 °C, and the separated plasmas were stored at -20 °C until use for assay. The plasma VEGF levels were measured using an enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (Jinmei Company Systems). All the tests were duplicated and the VEGF level of each plasma sample was averaged for the results. The intra-assay and interassay coefficients of variation were less than 9.5% for all the measurements. The sensitivity was 15 pg/ml.

### Analysis of the changes in plasma VEGF levels

The changes in plasma VEGF levels in groups were classified according to sampling time. Group 1 was defined as having decreased post-TACE plasma VEGF levels compared with their baseline levels, and group 2 showed increased plasma VEGF levels. The relation was studied between clinical characteristics and changes of VEGF before and after TACE, then VEGF levels were also compared between the two groups.

### Statistical analysis

The differences between demographic, clinical, and biochemical characteristics were examined using Student's *t* test and the chi-square test with the statistical software SPSS 10.0 combined Fisher's exact test. Paired VEGF values were analyzed with Student's *t* test combined Fisher's exact test.

## Results

Comparing the plasma VEGF expression of 20 no-tumor healthy adults ( $22.82 \pm 10.19$  pg/ml) to the plasma VEGF levels of 30 patients with HCC ( $153.21 \pm 96.53$  pg/ml before TACE) showed that the plasma VEGF levels of HCC before TACE was higher than those of healthy adults ( $P < 0.05$ ).

### Relationship between baseline plasma VEGF levels and clinical characteristics

The baseline plasma VEGF level was higher in patients with the presence of portal vein thrombosis than in those without portal vein thrombosis ( $P < 0.05$ ).

The baseline plasma VEGF level tended to increase in patients with Child-Pugh class B, and/or in patients with tumor size  $> 3$  cm or AFP  $> 400$  ng/ml, and/or in patients with multiple nodules. But the difference was not statistically significant ( $P > 0.05$ ) (Table 1).

### Changes in plasma VEGF levels after TACE

In 11 of the 30 HCC patients, plasma VEGF levels decreased compared with their baseline levels 3 days after TACE (group 1); but the levels of the remaining 19 patients increased (group 2). The overall changes in the plasma VEGF levels were increased.

### Changes of plasma VEGF level 3 days after TACE in relation to the characteristics of hepatocellular carcinoma

The frequency of increased plasma VEGF levels in patients with heterogeneous uptake of iodized oil was higher than that in those with homogenous uptake of iodized oil (95% vs 20%;  $P = 0.0001$ ). The changes in the plasma VEGF levels, however, were not related to

**Table 1.** Baseline plasma levels of VEGF in relation to the characteristics of HCC

Variables	<i>n</i>	Plasma VEGF (pg/ml)
Plasma AFP level (ng/ml)		
<400	12	170.15 ± 70.59
≥400	18	158.06 ± 95.27 *
Child-Pugh class		
A	17	157.86 ± 103.01
B	13	169.40 ± 57.39 *
Tumor size (cm)		
≤3	2	107.10 ± 47.88
>3	28	166.84 ± 86.36 *
Tumor type		
Single nodule	7	156.41 ± 60.47
Multiple nodules	23	164.82 ± 92.44 *
Portal vein thrombosis		
Negative	19	139.79 ± 49.82
Positive	11	211.06 ± 112.11 **

Compared between the two groups, \* :  $P > 0.05$ ; \*\* :  $P < 0.05$ . (Student's *t* test).

**Table 2.** Changes of plasma VEGF level 3 days after TACE in relation to the characteristics of HCC

Clinical characteristics	n	Group 1 (%)	Group 2 (%)
Plasma AFP (ng/ml)			
<400	12	5(41.7)	7(58.3)
≥400	18	6(33.3)	12(66.7) *
Child-Pugh class			
A	17	6(35.3)	11(64.7)
B	13	5(38.5)	8(61.5) *
Tumor size (cm)			
≤3	2	0	2(100)
>3	28	11(39.3)	17(60.7) *
Tumor type			
Single nodule	7	3(42.9)	4(57.1)
Multiple nodules	23	8(34.8)	15(65.2) *
Portal vein thrombosis			
Negative	19	5(26.3)	14(73.7)
Positive	11	6(54.5)	5(45.5) *
Uptake of iodized oil			
Homogeneous	10	8(80.0)	2(20.0)
Heterogeneous	20	1(5.0)	19(95.0) **
Response to TACE			
Partial	13	5(38.5)	8(61.5)
Complete	6	4(66.7)	2(33.3)
Progressive	11	2(18.2)	9(81.8) *

Compared between the two groups. \* :  $P > 0.05$  ; \*\* :  $P < 0.05$ . (Fisher's exact test combined with the chi-square test). Group 1 was defined as the group in which the plasma vascular endothelial growth factor-2 (VEGF) levels decreased compared with their basal levels; the levels in group 2 increased compared with their basal levels. The numbers in parentheses indicate percentages, which may be not total 100 because of rounding.

Child-Pugh class, tumor type, portal vein thrombosis, tumor size, AFP level and response to TACE. Moreover, the plasma VEGF levels increased in 61.5% of the patients who achieved a partial response (Table 2). The difference was not statistically significant ( $P > 0.05$ ).

#### Occurrence of intrahepatic or extrahepatic metastasis 4 weeks after TACE in relation to changes in the plasma VEGF levels

Metastasis of HCC was found in 20 patients within 3 months after TACE (lung metastasis in 8 patients, intra-abdominal lymph node metastasis in 4, bone metastasis in 3, and intra-hepatic metastasis with main portal vein thrombosis in 5). Univariate analysis showed that these metastases appeared to be common in patients with large-sized, multi-nodular HCC. The poor response to TACE Plasma VEGF also tended to be associated with frequent metastasis after treatment ( $P < 0.05$ , Table 3).

Using risk factors for metastasis that were determined by univariate-analysis, plasma VEGF level, tumor size, tumor-type and response to TACE were found to be independent risk factors for the occurrence of metastasis HCC after TACE ( $P < 0.05$ ).

**Table 3.** Risk factors for intrahepatic or extrahepatic metastasis on univariate analysis

Clinical variables	n	No. of metastasis (%)
Plasma AFP (ng/ml)		
<400	12	8(66.7)
≥400	18	12(66.7) *
Child-Pugh class		
A	17	12(70.6)
B	13	8(61.5) *
Tumor size (cm)		
≤3	2	0
>3	28	20(71.4) **
Tumor type		
Single nodule	7	1(14.3)
Multiple nodules	23	19(82.6) **
Portal vein thrombosis		
Negative	19	12(63.2)
Positive	11	8(72.7) *
Uptake of iodized oil		
Homogeneous	10	7(70.0)
Heterogeneous	20	13(65.0) *
Response to TACE		
Partial	13	11(84.6)
Complete	6	1(16.7)
Progressive	11	8(72.7) **
Changes in plasma VEGF levels		
Group 1	3	0
Group 2	27	20(74.1) **

Compared between the two groups. \* :  $P > 0.05$  ; \*\* :  $P < 0.05$ . (Fisher's exact test). Group 1 was defined as the group in which the plasma vascular endothelial growth factor (VEGF) levels decreased compared with their basal levels and the levels in group 2 increased compared with their basal levels. The numbers in parentheses indicate percentages, which were rounded up.

## Discussion

Metastasis is a major characteristic of malignant tumor. The process is ignited by the changing gene activity in the early stage of the primary tumor. The malignant tumor cell can obtain the ability of invasiveness. Hematogenous seeding of tumor cells usually precedes intra-hepatic and extra-hepatic metastases in patients with HCC. The tumor cells need to escape the immune surveillance of the host for their continuous clone growth. The process of angiogenesis is also essential to produce a visible mass at the metastatic site.<sup>[3-5]</sup> Therefore, the invasiveness of the tumor, host immunity, and tumor factors influencing the growth of various cells within tumors may contribute to the likelihood of metastasis in patients with HCC. The tumor cell is dependent on oxygen and other substances for their growth. During the growth of a tumor, cells obtain oxygen by perfusion, but node diameter will be less than 2-3 mm and cell number can not exceed  $10^7$  without angiogenesis. The tumor cell will be in a stable state.<sup>[6-10]</sup>

In the present study, metastases were more frequent

in patients with large-sized tumors and multi-nodular HCC, and high plasma VEGF levels. Response to TACE also tended to be associated with metastases after treatment. Usually, the response to TACE is likely to be incomplete in patients with large-sized HCC, high level of plasma AFP, or heterogeneous uptake of iodized oil after TACE.<sup>[11]</sup> In addition, resected HCC from these patients after TACE remains viable with incomplete necrosis.<sup>[12]</sup> Therefore, it is not surprising to observe an increased risk of metastasis in these patients. Moreover, it is also possible that a certain type (multiple nodules) of HCC unresponsive to therapy and spreading easily may have an increased tendency to metastasize after TACE.<sup>[13]</sup>

In this study, the increased expression of VEGF after TACE was associated with intra-hepatic and extra-hepatic metastases. Several possibilities could explain the association between the increased post-TACE plasma VEGF levels and the occurrence of HCC metastasis after TACE. In group 1 VEGF was decreased because HCC cell almost completely necrosed and in group 2 VEGF expression was increased only because hypoxia stimulated tumor cell. VEGF and its signaling pathway have been known to initiate tyrosyl phosphorylation of the vascular endothelial growth factor receptor-1 and thus activate multiple signaling pathways that are essential to liver cell growth. Host capillaries become hyper-permeability in response to an angiogenesis stimulator, and breakdown of the tumor capillary basement membrane. Therefore, increased VEGF expression may increase the risk of HCC metastasis by enhancing the growth and invasiveness of HCC cells. Also it may enhance the hematogenous spread of tumor cells and promote the growth of pre-existing HCC cells to form a visible mass at the metastatic sites by enhancing neovascularization through its direct angiogenic activity or by inducing a potent angiogenic factor, such as IGF-2, PlGF, FGF, and HIF-1.

It is not uncommon to observe the early spread of HCC cells after TACE or surgical resection, which results in multiple intra-hepatic or extra-hepatic metastasis in patients who have no evidence of angio-invasion. In contrast, some large-sized tumors with evident portal vein thrombosis tend to be confined within the liver for a long period. These findings suggest that certain tumor factors may be induced by TACE or surgery and may promote the spread or clone growth of HCC cells.<sup>[13]</sup> It was reported that TACE might change the nature of tumor cells from so-called "adherent subline" to "floating subline" and result in the residual viable tumor cells lacking mutual contacts. The results of the current study together with previous reports could be interpreted to indicate that ischemic insults caused by TACE up-regulate VEGF expression and thereby promote the spread of tumor cells into the peripheral circulation, which might facilitate the occurrence of metastasis.

In spite of increased plasma VEGF level after TACE, TACE indeed played a very important role. Almost

influent-oxygen tumor cells died, but their changes from hypoxia state need time. Studies have pointed out the importance of anti-angiogenesis therapy.<sup>[14,15]</sup>

Our data indicated that plasma VEGF levels increase in patients with large-sized tumors and heterogeneous uptake of iodized oil after TACE. We speculate that the plasma VEGF induced by ischemia after TACE is likely to originate from remnant viable tumors. Furthermore, per-HCC liver tissue also over-expresses VEGF compared with liver tissue without HCC.<sup>[16]</sup> VEGF originates from malignant cell and is transferred by blood cells including platelets and leukocytes.<sup>[17]</sup> Therefore, increased plasma VEGF level induced by ischemia after TACE is likely to exert autocrine, paracrine, or endocrine effects on the metastasis of HCC.<sup>[18]</sup>

Our findings demonstrate that increased VEGF plasma levels after TACE is associated with the metastasis of HCC. But it is possible to decrease the metastasis of HCC after TACE by anti-angiogenesis therapy.<sup>[19-22]</sup>

## Acknowledgment

We are grateful to all nurses of the 32nd ward and 35th ward for collecting blood samples, and to Deng JW for providing us with central experimental room (CSU).

## Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

## References

- 1 Eriksson A, Cao R, Pawliuk R. Placenta growth factor-1 antagonizes VEGF-induced angiogenesis and tumor growth by the formation of functionally inactive PlGF-1/VEGF heterodimers. *Cancer Cell* 2002;1:99-108.
- 2 Mao H, Yuan AL, Zhao MF. Metastasis of hepatocellular carcinoma induced by vascular endothelial growth factor. *Chin J Hepatobiliary Surg* 2001;7:34-36.
- 3 Passe TJ, Bluemke DA, Siegelman SS. Tumor angiogenesis: tutorial on implications for imaging. *Radiology* 1997;203:593-600.
- 4 Fidler I. The biology of human cancer metastasis. *Acta Oncology* 1991;30:669-675.
- 5 Folkman J. Clinical application of research on angiogenesis. *N Engl J Med* 1995;28:1757-1763.
- 6 Laughner E, Taghavi P, Chiles K. HER2 (neu) signaling increases the rate of hypoxia-inducible factor-1a (HIF-1a) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Molecular Cellular Biology* 2001; 21:3995-4004.
- 7 L'Allemain G. The hypoxia-inducible factor HIF as a new target in cancer research. *Bull Cancer* 2002;89:257-260.
- 8 Folkman J, D'Amore PA. Blood vessel formation: what is its molecular basis. *Cell* 1996;87:1153-1155.

- 9 Dhar DK, Naora H, Yamanoi A. Requisite role of VEGF receptors in angiogenesis of hepatocellular carcinoma: a comparison with angiopoietin/Tie pathway. *Anticancer Res* 2002;22:379-386.
- 10 Ruan G, Liu Y, Chen S. Inhibition of K562 cell growth and tumor angiogenesis in nude mice by antisense VEGF(121) cDNA transfection. *Zhonghua Xue Ye Xue Za Zhi* 2002;23:179-182.
- 11 Ikeda K, Kumada H, Saitoh S. Effect of repeated transcatheter arterial embolization on the survival time in patients with hepatocellular carcinoma. An analysis by the cox proportional hazard model. *Cancer* 1991;68:2150-2154.
- 12 Hsu HC, Wei TC, Tsang YM. Histologic assessment of resected hepatocellular carcinoma after transcatheter hepatic arterial embolization. *Cancer* 1986;57:1184-1191.
- 13 Byung C, Song YH, Chung J. Association between vascular endothelial growth factor-2 and metastases after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. *Cancer* 2001;91:2386-2393.
- 14 Baker CH, Solorzano CC, Fidler IJ. Blockade of vascular endothelial growth factor receptor and epidermal growth factor receptor signaling for therapy of metastatic human pancreatic cancer. *Cancer Res* 2002;62:1996-2003.
- 15 Wang F, Tian YH, Li L. XF inhibition of tumor angiogenesis, growth and metastasis by blocking VEGF paracrine pathway. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao* (Shanghai) 2002;34:165-170.
- 16 Li J, Wang WL, Liu B. Angiogenesis and apoptosis in human hepatocellular carcinoma. *WCJD* 1998;6:1057-1060.
- 17 Kenji JN, Masahito T, Ichinosuke H. Circulating vascular endothelial growth factor (vegf) is a possible tumor marker for metastasis in human hepatocellular carcinoma. *J Gastroenterol* 1998;33:376-382.
- 18 Rosen LS. Clinical experience with angiogenesis signaling inhibitors: focus on vascular endothelial growth factor (VEGF) blockers. *Cancer Control* 2002;9:36-44.
- 19 Kane M, Furu T, Takena K. A 5-year experience of lipiodolization: selective regional chemotherapy for 200 patients with hepatocellular carcinoma. *Hepatology* 1989;10:98-102.
- 20 Xiong ZP, Yang SR, Xiao EH. Relation between vascular endothelial growth factor and metastases after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. *Chin J Oncol* 2003;25:262-265.
- 21 Margolin K. Inhibition of vascular endothelial growth factor in the treatment of solid tumors. *Curr Oncol Rep* 2002;4:20-28.
- 22 Yoshiji H, Yoshii J, Ikenaka Y. Suppression of the renin-angiotensin system attenuates vascular endothelial growth factor-mediated tumor development and angiogenesis in murine hepatocellular carcinoma cells. *Int J Oncol* 2002;20:1227-1231.

*Received January 28, 2004*

*Accepted after revision June 4, 2004*