PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

*World J Gastroenterol* 2005;11(41):6521-6524
***World Journal of Gastroenterology*** ISSN 1007-9327


• *RAPID COMMUNICATION* •

# Significance and relationship between infiltrating inflammatory cell and tumor angiogenesis in hepatocellular carcinoma tissues

Shao-Hua Peng, Hong Deng, Jian-Feng Yang, Ping-Ping Xie, Cheng Li, Hao Li, De-Yun Feng

Shao-Hua Peng, Hong Deng, Jian-Feng Yang, Ping-Ping Xie, Cheng Li, Hao Li, Department of Pathology, Medical College, Hunan Normal University, Changsha 410006, Hunan Province, China
De-Yun Feng, Department of Pathology, Xiangya School of Medicine, Central South University, Changsha 410078, Hunan Province, China
Supported by the Hunan Provincial Natural Science Foundation of China, No. 03-JJY5031
Co-first authors: Shao-Hua Peng and Hong Deng
Co-correspondent: De-Yun Feng
Correspondence to: Shao-Hua Peng, Department of Pathology, Medical College, Hunan Normal University, Changsha 410006, Hunan Province, China. pengshaohua2004@sina.com
Telephone: +86-731-8630309
Received: 2004-09-13 Accepted: 2004-12-26

## Abstract

**AIM:** To investigate the relationship between infiltrating inflammatory cell and tumor angiogenesis in hepatocellular carcinoma (HCC) tissues and their clinicopathological features.

**METHODS:** The paraffin-embedded specimens from 70 cases with HCC were stained using EliVision immunohistochemistry with mAbs against CD68, tryptase, and CD34. The counts of tumor-associated macrophage (TAM), mast cell (MC) and tumor microvessel (MV) were performed in the tissue sections.

**RESULTS:** The mean counts of TAM, MC, and MV in HCC tissues were significantly higher than those in pericarcinomatous liver tissues (TAM: 69.31 ± 11.58 *vs* 40.23 ± 10.36; MC: 16.74 ± 5.67 *vs* 7.59 ± 4.18; MV: 70.11 ± 12.45 *vs* 38.52 ± 11.16, $P<0.01$). The MV count in the patients with metastasis was markedly higher than that with non-metastasis ($P<0.01$). In addition, the MC count in the patients with poorly differentiated HCC was obviously higher than that with well differentiated HCC ($P<0.01$). The correlation analysis showed that the TAM count was significantly correlated with the count of MV ($r=0.712$, $P<0.01$), and the MC count was obviously correlated with the MV count ($r=0.336$, $P<0.05$).

**CONCLUSION:** TAM and MC might be closely related to the enhancement of tumor angiogenesis. The MV count might be associated with tumor invasion and metastasis. Moreover, the MC count might be associated with tumor differentiation and prognosis of HCC.



**Key words:** Hepatocellular carcinoma; Mast cell; Microvessel; Macrophage

Peng SH, Deng H, Yang JF, Xie PP, Li C, Li H, Feng DY. Significance and relationship between infiltrating inflammatory cell and tumor angiogenesis in hepatocellular carcinoma tissues. *World J Gastroenterol* 2005; 11(41):6521-6524
http://www.wjgnet.com/1007-9327/11/6521.asp

## INTRODUCTION

Tumor angiogenesis is one of the fundamental requirements for tumor growth and proliferation, and the regulatory mechanisms of tumor angiogenesis are very complicated. The tumor neovascularization and its controlling factors have become investigative hot points in current tumor studies[1-5]. Tumor infiltrating inflammatory cells in the microenvironment plays very important functions in tumor angiogenesis[6-8]. Hepatocellular carcinoma (HCC) is a kind of a familiar malignant tumor in our country, though the treatment of HCC has obtained the substantial advance, the total treatment results are still unsatisfactory[9-12]. Until recently, there is some lack of knowledge in literature regarding the relationship among the count of tumor-associated macrophage (TAM), mast cell (MC), and microvessel (MV)_ in HCC tissues and their relation to the clinicopathological characteristics of HCC. In this study, we applied immunohistochemical method to detect the count of TAM, MC, and MV in HCC, and to investigate their relation to the clinicopathological characteristics of HCC, so that we could understand the regulating function of microenvironment in tumor angiogenesis. We believed that this study would offer some new clues for tumor angiogenesis research and biological therapeutic approach, such as suppressing tumor angiogenesis.

## MATERIALS AND METHODS

### *Materials*

Fresh surgical specimens were obtained from 70 HCC

patients (all specimens contained pericarcinomatous liver tissues) who had undergone partial hepatectomy during the period from January 1996 to February 2003 in Xiangya Hospital. All specimens were fixed in 40 g/L formaldehyde, embedded in paraffin, sliced into consecutive sections, and pathologically diagnosed explicitly. Of 70 HCC patients, 53 were males and 17 were females with average age of 53.4 years (range, 27-68 years). The size of tumors was less than 5 cm in 25 cases, 5.1-10 cm in 29 cases and larger than 10 cm in 16 cases. Among all cases, 19 cases contained several tumor tubercles in liver tissues. None of the patients received any preoperative treatment, such as radiotherapy and chemotherapy. The HCC tissues were graded according to the Edmondson standard. Of them, 11 cases had grade I, 31 cases grade II, 23 cases grade III, and 5 cases grade IV tumors. All the pericarcinomatous liver tissues had liver fibrosis with cirrhosis of different degrees and 22 pericarcinomatous liver tissues had dysplasia. The antibodies against CD68, trypsin of MC and CD34, and "two step" EliVisions reagent box were all obtained from Maixin Biochemical Development Company of Fuzhou.

### Immunohistochemical staining

Briefly, after the sections were deparaffinized, the endogenous peroxidase was blocked by incubation with 30mL/L hydrogen peroxide for 30 min, followed by carrying out the two-step method of immunohistochemistry according to the manufacturer's instructions. The sections were incubated with the primary antibodies at room temperature overnight. For negative control, the primary antibodies were replaced with PBS.

Brown-yellow staining was observed in the cytoplasm of TAM, whereas brown-yellow grains were observed both in the cytoplasm and nucleus of MC. According to the count method of Molin *et al*[13], we chose five random microscopic fields in each section under high power, counting the number of TAM and MC in each visual field, respectively. In addition, in the same visual field, we counted the number of MC that had the phenomenon of taking-off grains. MV and its endothelial cells showed brown-yellow staining. Similarly, according to the count method of Weidner *et al*[14], we chose five random microscopic fields in each section under high power, counting the number of MV in each visual field.

### Statistical analysis

The data were analyzed using *t* test and relativity analyses in the statistical computer software SPSS10.0. A *P* value less than 0.05 was considered statistically significant.

## RESULTS

### Expressional characteristics of CD68, MC, CD34

The cytoplasm of CD68-positive cells was stained with brown-yellow color. We could observe in all the sections, that most of the CD68-positive cells had the characteristic of microphage in appearance and the TAM was primarily distributed in the perimeter of tumor cells. Moreover, brown-yellow grains were observed in the cytoplasm and nucleus of MC. In the relatively normal areas, MC was circular or oval with complete cell membrane, clear outline, abnormally dyed grains in cytoplasm and some of the MCs appearing to have the phenomenon of taking-off grains. But in the HCC tissue and its adjacent areas, the number of MC was obviously increased, the physical volume enlarged with a different appearance, and the majority of MC appeared to have the phenomenon of taking-off grains. The CD34 staining was located in the cytoplasm of vascular endothelial cells. We could observe a single endothelial cell or a cluster of cells in some areas containing partly irregular tube cavity and irregular MV distribution, which could mostly be seen in the perimeter areas of the tumor.

### Counts of TAM, MC, and MV

The counts of TAM, MC, and MV were all obviously higher in HCC tissues compared with the pericarcinomatous liver tissues ($P<0.01$, Table 1).

**Table 1** Counts of TAM, MC, and MV in HCC tissues and pericarcinomatous liver tissues (mean±SD)

| | TAM | MC | MV |
|---|---|---|---|
| HCC tissues | 69.31± 11.58 | 16.74±5.67 | 70.11±12.45 |
| Pericarcinomatous liver tissues | 40.23±10.36[b] | 7.59±4.18[b] | 38.52±11.16[b] |

[b]$P<0.01$ *vs* HCC tissues

### Relationship between the counts of TAM, MC, and MV and the clinicopathological characteristics of HCC

The counts of TAM, MC, and MV were significantly higher in metastatic HCC than that in non-metastatic HCC, and the count of MC in low grade HCC tissues was markedly higher compared to high grade HCC tissues ($P<0.05$). In addition, we also discovered that MC having the phenomenon of taking-off grains was more and more

**Table 2** Relationship of the counts of TAM, MC, and MV with the clinicopathological characteristics of HCC (mean±SD)

| Pathologic characteristics | *n* | TAM | MC | MV |
|---|---|---|---|---|
| Stage of HCC | | | | |
| I | 11 | 68.86±10.23 | 13.68±5.21 | 65.86±12.05 |
| II | 31 | 68.95±9.82 | 17.03±6.11 | 67.84±11.54 |
| III | 23 | 70.11±13.16 | 19.95±4.29[a] | 72.11±10.84 |
| IV | 5 | 69.84±12.57 | 23.56±3.86[a] | 68.70±9.53 |
| Tumor size | | | | |
| ≤5 cm | 25 | 69.32±10.17 | 16.57±5.56 | 66.56±11.84 |
| 5.1–10 cm | 29 | 71.35±9.54 | 16.98±4.81 | 65.84±10.53 |
| >10 cm | 16 | 70.83±12.11 | 17.02±6.12 | 73.13±11.30 |
| Metastasis | | | | |
| Yes | 29 | 68.36±12.17 | 16.99±6.49 | 73.49±12.32[c] |
| No | 41 | 69.14±11.69 | 16.76±6.88 | 64.39±13.17 |

[a]$P<0.05$ *vs* stage I; [c]$P<0.05$ *vs* non-transferred.

familiar in low grade HCC tissues. However, no obvious relationship was found between the counts of TAM, MC, and MV and the clinicopathological characteristics of HCC (Table 2).

### *Relationship of the counts of TAM and MC with the count of MV in HCC tissues*

Using the relativity analyses of the counts of TAM and MC with the count of MV in HCC tissues, we found that the TAM count and MV count in HCC tissues had a close and positive relationship of high degree ($r=0.712$, $P<0.01$), and the MC count and MV count in HCC tissues had a close and positive relationship ($r=0.336$, $P<0.05$).

## DISCUSSION

Tumor growth and proliferation are highly dependent on tumor neovascularization. Tumor angiogenesis is mediated by tumor-secreted angiogenic factors that interact with their surface receptors expressed on endothelial cells[15-19]. In fact, the mechanisms of regulation of tumor angiogenesis have become one of the investigative hot points in current tumor studies[1-5]. Our study showed that the MV count was significantly higher in HCC tissues as compared with the pericarcinomatous liver tissues, and also higher in the metastatic HCC compared with the metastatic HCC, suggesting that tumor angiogenesis has a crucial role in the development and metastasis of HCC.

Tumor angiogenesis is a complicated process, including many regulating factors[20-24]. The angiogenic response in the microvasculature is associated with the changes in cellular adhesive interactions between adjacent endothelial cells, pericytes and surrounding extracellular matrix. In addition, the infiltrating inflammatory cells in tumor are closely related with tumor angiogenesis. The infiltrating inflammatory cells in tumor, such as neutrophil, microphage, lymphocyte, eosinophil, MC, *etc*., can produce various cell factors and enzymes which participate in the growth and spread of tumor. Microphage is a main composition of the infiltrating inflammatory cells in the tumor and usually belongs to TAM in tumor tissues[23-28]. On one hand, it can produce several growth-stimulating and repressing factors, and albumen hydrolyze enzymes, such as fibroblast growth factor, vascular endothelial growth factor, etc. and thereby plays an important role in chain responding of tumor angiogenesis. On the other hand, tumor cells also can produce various chemoattractant factors, such as monocyte chemoattractant proteins, macrophage colony-stimulating factor etc, which have an important effect on the activation and migration of macrophage. These TAMs, being closely related to microvessel density, also have something to do with tumor angiogenesis, metastasis, and prognosis. In this study, we found that the count of TAM was obviously higher in HCC tissues as compared with the pericarcinomatous liver tissues, suggesting that TAM plays an important role in the development of HCC. In addition, the TAM count and the MV count in HCC tissues had a close and positive relationship, which suggested that TAM might promote tumor angiogenesis.

Several studies have shown that MCs in the local milieu may have something to do with the growth, invasion, metastasis, and neovascularization of malignant tumor[29-30]. Our results showed that the MC count was obviously higher in HCC tissues as compared with the pericarcinomatous liver tissues, suggesting an important role of MC in the development of HCC. We also found that the MC count in the lower grade HCC tissues was higher than that in the higher grade HCC, which suggested that the high MC count might be related to the low grade and poor prognosis of HCC. In addition, we observed that MC showing the phenomenon of taking-off grains was more and more familiar in low grade HCC tissues; however, its concrete relationship still needs further research. The counts of MC and MV in HCC tissues had a close and positive relationship, which revealed that MC might promote HCC angiogenesis.

## REFERENCES


1 **Saaristo A,** Karpanen T, Alitalo K. Mechanisms of angiogenesis and their use in the inhibition of tumor growth and metastasis. *Oncogene* 2000; **19:** 6122-6129
2 **Lutsenko SV,** Kiselev SM, Severin SE. Molecular mechanisms of tumor angiogenesis. *Biochemistry(Mosc)* 2003; **68:** 286-300
3 **Boudreau N,** Myers C. Breast cancer-induced angiogenesis: multiple mechanisms and the role of the microenvironment. Breast *Cancer Res* 2003; **5:** 140-146
4 **Rofstad EK,** Halsør EF. Vascular endothelial growth factor, interleukin 8, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor promote angiogenesis and metastasis in human melanoma xenografts. *Cancer Res* 2000; **60:** 4932-4938
5 **Zheng S,** Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. *World J Gastroenterol* 2003; **9:** 1227-1230
6 **Kataki A,** Scheid P, Piet M, Marie B, Martinet N, Martinet Y, Vignaud JM. Tumor infiltrating lymphocytes and macrophages have a potential dual role in lung cancer by supporting both host-defense and tumor progression. *J Lab Clin Med* 2002; **140:** 320-328
7 **Yu JL,** Rak JW. Host microenvironment in breast cancer development: inflammatory and immune cells in tumour angiogenesis and arteriogenesis. Breast *Cancer Res* 2003; **5:** 83-88
8 **Elpek GO,** Gelen T, Aksoy NH, Erdoğan A, Dertsiz L, Demircan A, Keleş N. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus.*J Clin Pathol* 2001; **54:** 940-944
9 **Lu SY,** Sui YF, Li ZS, Ye J, Dong HL, Qu P, Zhang XM, Wang WY, Li YS. Superantigen-SEA gene modified tumor vaccine for hepatocellular carcinoma: an in vitro study. *World J Gastroenterol* 2004; **10:** 53-57
10 **Harris AL,** Reusch P, Barleon B, Hang C, Dobbs N, Marme D. Soluble Tie2 and Flt1 extracellular domains in serum of patients with renal cancer and response to antiangiogenic therapy. *Clin Cancer Res* 2001; **7:** 1992-1997
11 **Zhao WH,** Ma ZM, Zhou XR, Feng YZ, Fang BS. Prediction of recurrence and prognosis in patients with hepatocellular carcinoma after resection by use of CLIP score. *World J Gastroenterol* 2002; **8:** 237-242
12 **Qin LX,** Tang ZY. The prognostic molecular markers in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8:** 385-392
13 **Molin D,** Edström A, Glimelius I, Glimelius B, Nilsson G,

Sundström C, Enblad G. Mast cell infiltration correlates with poor prognosis in Hodgkin's lymphoma. *Br J Haematol* 2002; **119:** 122-124

14 **Weidner N,** Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* 1993; **143:** 401-409

15 **Strohmeyer D,** Rössing C, Bauerfeind A, Kaufmann O, Schlechte H, Bartsch G, Loening S. Vascular endothelial growth factor and its correlation with angiogenesis and p53 expression in prostate cancer. *Prostate* 2000; **45:** 216-224

16 **Guang-Wu H,** Sunagawa M, Jie-En L, Shimada S, Gang Z, Tokeshi Y, Kosugi T. The relationship between microvessel density, the expression of vascular endothelial growth factor (VEGF), and the extension of nasopharyngeal carcinoma. *Laryngoscope* 2000; **110:** 2066-2069

17 **Fidler IJ.** Angiogenesis and cancer metastasis. *Cancer* J 2000; **6 Suppl 2:** S134-S141

18 **Streit M,** Detmar M. Angiogenesis, lymphangiogenesis, and melanoma metastasis. *Oncogene* 2003; **22:** 3172-3179

19 **Yamaguchi R,** Yano H, Nakashima Y, Ogasawara S, Higaki K, Akiba J, Hicklin DJ, Kojiro M. Expression and localization of vascular endothelial growth factor receptors in human hepatocellular carcinoma and non-HCC tissues. *Oncol Rep* 2000; **7:** 725-729

20 **Etoh T,** Inoue H, Tanaka S, Barnard GF, Kitano S, Mori M. Angiopoietin-2 is related to tumor angiogenesis in gastric carcinoma: possible in vivo regulation via induction of proteases. *Cancer Res* 2001; **61:** 2145-2153

21 **Yudoh K,** Kanamori M, Ohmori K, Yasuda T, Aoki M, Kimura T. Concentration of vascular endothelial growth factor in the tumour tissue as a prognostic factor of soft tissue sarcomas.*Br J Cancer* 2001; **84:** 1610-1615

22 **Mitsuhashi N,**Shimizu H, Ohtsuka M, Wakabayashi Y, Ito H, Kimura F, Yoshidome H, Kato A, Nukui Y, Miyazaki M. Angiopoietins and Tie-2 expression in angiogenesis and proliferation of human hepatocellular carcinoma. *Hepatology* 2003; **37:** 1105-1113

23 **Feoktistov I,** Ryzhov S, Goldstein AE, Biaggioni I. Mast cell mediated stimulation of angiogenesis: cooperative interaction between A2B and A3 adenosine receptors. *Circ Res* 2003; **92:** 485-492

24 **Aoki M,** Pawankar R, Niimi Y, Kawana S. Mast cells in basal cell carcinoma express VEGF, IL-8 and RANTES. *Int Arch Allergy Immunol* 2003; **130:** 216-223

25 **Torisu H,** Ono M, Kiryu H, Furue M, Ohmoto Y, Nakayama J, Nishioka Y, Sone S, Kuwano M. Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma: possible involvement of TNFalpha and IL-1alpha. *Int J Cancer* 2000; **85:** 182-188

26 **Ishigami S,** Natsugoe S, Tokuda K, Nakajo A, Okumura H, Matsumoto M, Miyazono F, Hokita S, Aikou T. Tumor-associated macrophage (TAM) infiltration in gastric cancer. *Anticancer Res* 2003; **23:** 4079-4083

27 **Li C,** Shintani S, Terakado N, Nakashiro K, Hamakawa H. Infiltration of tumor-associated macrophages in human oral squamous cell carcinoma. *Oncol Rep* 2002; **9:** 1219-1223

28 **Ono M,** Torisu H, Fukushi J, Nishie A, Kuwano M. Biological implications of macrophage infiltration in human tumor angiogenesis. *Cancer Chemother Pharmacol* 1999; **43:** S69-71

29 **Hiromatsu Y,** Toda S. Mast cells and angiogenesis. *Microsc Res Tech* 2003; **60:** 64-69

30 **Ribatti D,** Ennas MG, Vacca A, Ferreli F, Nico B, Orru S, Sirigu P. Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. *Eur J Clin Invest* 2003; **33:** 420-425

**Science Editor** Guo SY **Language Editor** Elsevier HK