ANTICANCER RESEARCH *25*: 3335-3340 (2005)

Suppression of Renin-Angiotensin System Attenuates Hepatocarcinogenesis *Via* Angiogenesis Inhibition in Rats

HITOSHI YOSHIJI[1], RYUICHI NOGUCHI[1], SHIGEKI KURIYAMA[2], JUNICHI YOSHII[1], YASUHIDE IKENAKA[1], KOJI YANASE[1], TADASHI NAMISAKI[1], MITSUTERU KITADE[1], MASAHARU YAMAZAKI[1], MASAHITO UEMURA[1] and HIROSHI FUKUI[1]

[1]*Third Department of Internal Medicine, Nara Medical University, Nara;*
[2]*Third Department of Internal Medicine, Kagawa University School of Medicine, Kagawa, Japan*

Abstract. *Recent studies have shown that the renin-angiotensin system (RAS) as well as angiogenesis is involved in tumor development. The aim of the present study was to examine the interaction of RAS, angiogenesis and a potent angiogenic factor, namely the vascular endothelial growth factor (VEGF), in the hepatocarcinogenesis process. In a diethylnitrosamine-induced rat hepatocarcinogenesis model, a clinically used angiotensin-converting enzyme inhibitor, perindopril (PE), significantly suppressed glutathione S-transferase placental form (GST-P)-positive preneoplastic lesions along with inhibition of neovascularization in the liver. The hepatic expression of VEGF was also attenuated. The degree of angiogenesis correlated well with the development of preneoplastic lesions. Our in vitro study showed that PE significantly suppressed VEGF-induced tubular formation and the migration of endothelial cells (EC), whereas it did not affect the proliferation of EC. These results suggested that RAS plays an important role in hepatocarcinogenesis, at least partly through VEGF-mediated angiogenesis.*

Angiogenesis is a complex and critical process essential to support the growth of solid tumors (1, 2). Any tumor mass in excess of a few cubic millimeters totally depends on the formation of a vascular network that provides the growing tumor with oxygen and essential nutrients. Until recently, it was believed that angiogenesis starts at a relatively late stage when the tumor has attained a size of several hundred microns to 1 mm in diameter or when the tumor contains roughly 10^5-10^6 cells. Recently, it was demonstrated that angiogenesis begins at a very early stage, even when the tumor contains only 100-300 cells (3). The recent studies have revealed that angiogenesis can also be induced at the early stages of tumor formation, and clarified the carcinogenic procedures in several types of experimental models, such as the RIP1-Tag2 pancreatic β-cell islet carcinoma in transgenic mice (4, 5). In this model, treatment with an angiogenic inhibitor resulted in a significant reduction in the number of angiogenic islets and in a substantial reduction of tumor growth (6).

To date, many positive and negative angiogenic factors have been identified (2, 7, 8). Among the positive factors, the vascular endothelial growth factor (VEGF) is the most important factor regarding tumor angiogenesis. VEGF is a specific mitogen for endothelial cells (EC) *in vitro* and can be an angiogenic factor for neovascularization *in vivo* (9-11). It has been shown that it is secreted abundantly in several human tumors and animal experimental models. An increase of the VEGF expression in human surgical specimens has been shown to correlate with aggressive behavior and poor prognosis. In animal experimental models, overexpression of VEGF enhanced the tumor growth, angiogenesis and dissemination, whereas suppression of VEGF inhibited the tumor growth in many tumor cell types (9-11).

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world with an estimated incidence of more than one million new cases annually (12, 13). One of the notable features of HCC in clinical practice is hypervascularity. As such, several studies have shown that the VEGF expression was up-regulated in the tumor lesion of HCC more than in the non-cancerous lesion (14-19). We have reported that overexpression of VEGF significantly increased HCC development along with augmentation of neovascularization (20). A recent study on the EC markers in dysplastic lesions of the liver has suggested that alterations in the hepatic microcirculation occur at a very

Correspondence to: Hitoshi Yoshiji, MD, Ph.D., Third Department of Internal Medicine, Nara Medical University, Shijo-cho 840, Kashihara, Nara 634-8522, Japan. Tel: 81-744-22-3051 (ext 3415), Fax: 81-744-24-7122, e-mail: yoshijih@naramed-u.ac.jp

Key Words: Angiogenesis, ACE inhibitor, hepatocarcinogenesis, renin-angiotensin system.

early stage of liver carcinogenesis (21). Another clinical report showed that angiogenesis in the liver gradually increased from low-grade dysplastic nodules during hepatocarcinogenesis, before emergence of morphologically identifiable HCC (22). In an experimental study, a semi-synthetic compound of fumagilin, TNP-470, which possesses anti-angiogenic activity, suppressed the progression of HCC (23). In agreement with these studies, we found that neovascularization and VEGF expression increased stepwise during hepatocarcinogenesis (24).

The rennin-angiotensin system (RAS) normally regulates renal blood flow and fluid homeostasis, and plays an important role in the regulation of local hemodynamics in several organs (25). In the liver, it has been shown that RAS is frequently activated in patients with chronic liver diseases, such as cirrhosis (26). Angiotensin (AT)-II, which is produced by the proteolytic cleavage of its precursor AT-I *via* an angiotensin-converting enzyme (ACE), has been shown to induce neovascularization in experimental models both *in vitro* and *in vivo*, and it also increased the VEGF expression (26). We have previously reported that AT-II played an important role in HCC tumor development and hepatocarcinogenesis (27, 28). To date, however, studies on the correlation between RAS, VEGF and angiogenesis during hepatocarcinogenesis are very limited.

In the current study, we examined the effect of suppression of RAS using perindopril (PE), a clinically used ACE inhibitor (ACE-I), in conjunction with alteration of hepatic angiogenesis and VEGF. We also attempted to investigate the possible mechanisms involved *in vitro*.

Materials and Methods

Animals and reagents. Male Fisher 344 rats, aged 6 weeks, were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). They were housed in stainless-steel, mesh cages under controlled conditions of temperature (23±3˚C) and relative humidity (50±20%), with 10-15 air changes per hour and light illumination for 12 hours a day. The animals were allowed access to food and tap water *ad libitum* throughout the acclimatization and experimental periods. DEN (Nakarai, Kyoto, Japan) was diluted with 0.9 % sodium chloride at a concentration of 200 mg/ml. PE was supplied by Daiichi Pharmaceutical Co. (Tokyo, Japan).

Animal treatment. The experimental period was 12 weeks. In total, 30 rats were divided into 3 groups (n=10 each). After a week of acclimatization, the rats in groups 2 and 3 (G2 and G3, respectively) were given a single intraperitoneal (*i.p.*) dose of DEN (200 mg/kg body weight), were partially hepatectomized in week 3, given PE at doses of 0 and 2mg/kg/day by gavage from week 3 for 9 weeks, and then were killed at the end of week 12. In the negative control group (G1), phosphate-buffered saline (PBS) was injected instead of DEN and a partial hepatectomy was performed. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals.

Table I. *Effect of PE on general findings and GST-P-positive preneoplastic lesions in DEN-treated liver.*

Treatment	Final body weight (g)	Relative liver weight (g/100 g body wt.)	GST-P-positive lesions	
			No. ($/cm^2$)	Size ($mm^2/10^2$)
DEN	252±20[a]	2.84±0.22	28.8±3.9	4.9±1.2
DEN+PE	250±18	2.76±0.24	13.4±2.2[b]	1.6±0.4[b]
PBS	258±16	2.80±0.19	N.D.	N.D.

[a]Data represent mean±SD
[b]Statistically significant difference from DEN-treated group ($p<0.01$)

Immunohistochemistry. Five-millimeter-thick slices from the major liver lobes were fixed in ice-cold acetone and embedded in paraffin. Then, serial sections were prepared from each fixed liver. The first section was routinely stained with hematoxylin and eosin for histological examination. The other sections were immunohistochemically reacted for demonstration of anti-GST-P (Medical Biological Laboratories Co., Nagoya, Japan). The remaining portions of the liver specimen were snap-frozen and kept at –80˚C. Semi-quantitative analysis of the preneoplastic lesions was carried out with the Fuji-BAS 2000 image analyzing system (Fuji, Tokyo, Japan), as described previously (29).

mRNA expressions of CD31 and VEGF in the liver. The mRNA expressions of VEGF and CD31, which is used widely as a marker of neovascularization, were evaluated by real-time PCR as described previously (30). Real-time PCR was performed with the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA), according to the manufacturer's manual. Relative quantitation of the gene expression was performed as described in the manual by using glyceralaldehyde-3-phosphate dehydrogenase as an internal control.

In vitro proliferation, angiogenesis and migration assay. Because PE is a prodrug, the active form, perindoprilat, was used for the *in vitro* studies. The *in vitro* proliferation, tubular formation and migration of EC in the presence or absence of perindoprilat (1 μM and 100 μM) were determined as described previously (n=6 per group) (28).

Statistical analysis. To assess the statistical significance of the inter-group differences in the quantitative data, Bonferroni's multiple comparison test was used after one-way analysis of variance (ANOVA). This was followed by Barlett's test to determine the homology of variance.

Results

General findings and GST-P-positive preneoplastic lesions. All rats survived throughout the experiment. Neither the body weight nor liver weights changed in any of the experimental groups (Table I). PE treatment did not cause alteration of

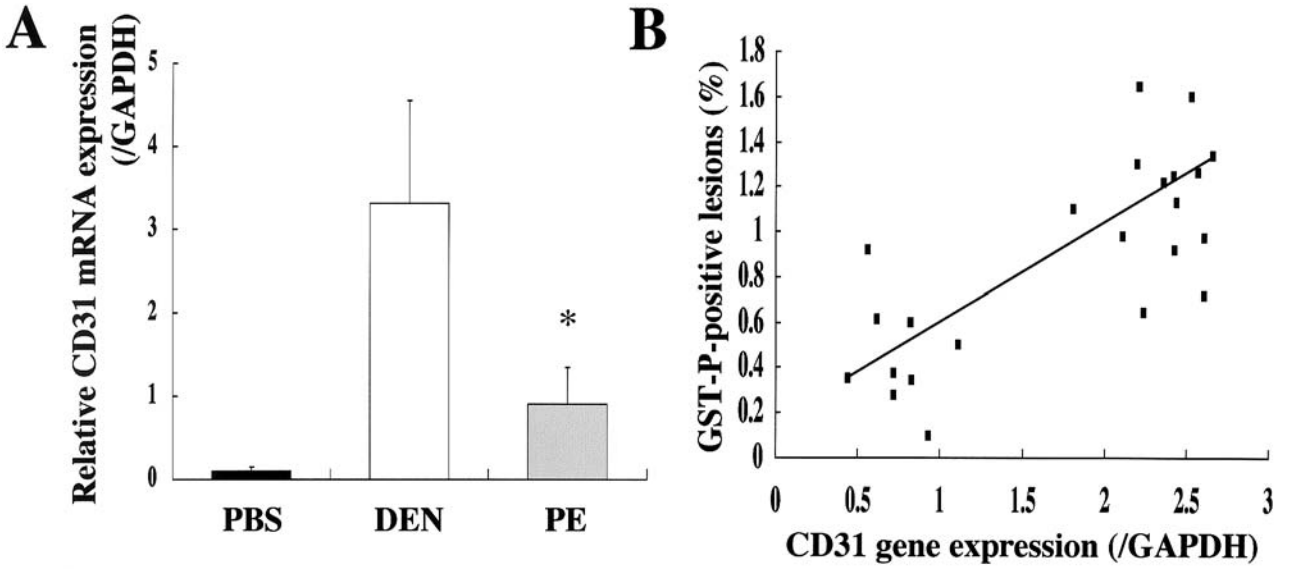

Figure 1. *(A): Effects of PE (an ACE-I) on the mRNA expressions of CD31 in the liver. The mRNA expressions were examined by real-time PCR as described in the "Materials and Methods" section. The mRNA expressions of CD31 significantly increased during hepatocarcinogenesis. PE significantly suppressed the CD31 gene expression as compared to the DEN-treated group. PBS: PBS-treated rats (G1); DEN: DEN-treated control rats (G2); PE: DEN+PE (2 mg/kg)-treated group (G3). The data represent means ± SD (n=10). *: Statistically significant differences as compared with the DEN-treated control group (p<0.01). (B): Relationship between the development of GST-P-positive preneoplastic lesions and the CD31 mRNA expression in the liver. The degree of angiogenesis correlated well with the development of preneoplastic lesions. The equation was y= 0.22+0.034x. The correlation efficient was 0.66.*

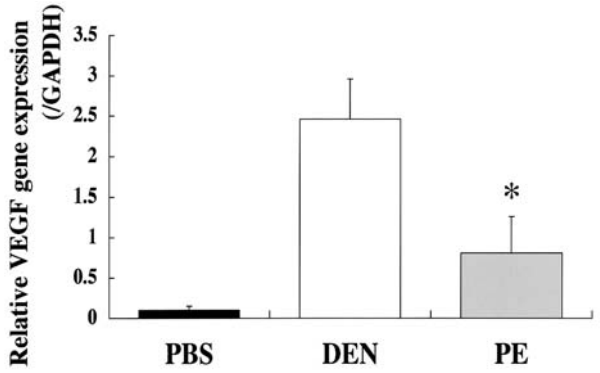

Figure 2. *Effects of PE on the mRNA expressions of VEGF in the liver. Similar to that of CD31 expression, the mRNA expression of VEGF significantly increased by treatment with DEN. PE significantly suppressed the VEGF gene expression as compared to the DEN-treated group. PBS: PBS-treated rats (G1); DEN: DEN-treated control rats (G2); PE: DEN+PE (2 mg/kg)-treated group (G3). The data represent means ± SD (n=10). *: Statistically significant differences as compared with the DEN-treated control group (p<0.01).*

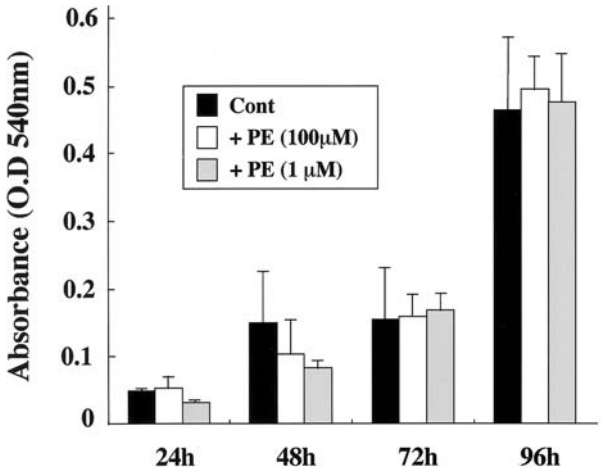

Figure 3. *Effect of PE on the VEGF-induced EC proliferation in vitro. Cell proliferation was measured by MTT assay after harvest from 24 h to 96 h as described previously (20). PE did not affect the proliferation of EC even at a high concentration (100 μM). The data represent means ± SD (n=6). Cont: VEGF (10 ng/ml)-treated control group; +PE (1) and (100): the active form of PE, perindoprilat,-treated group at 1 μM and 100 μM, respectively.*

the serum ALT levels. Neither ascites nor organ abnormalities were observed at the end of the experiment in any of the groups (data not shown). No histological changes indicating liver injury or liver fibrosis development were observed, except for the development of glutathione S-transferase placental form (GST-P)-positive preneoplastic lesions. As shown in Table I, the number and size of GST-P-positive preneoplastic lesions were significantly suppressed by treatment with PE. No GST-P-positive lesions developed in the PBS-treated group.

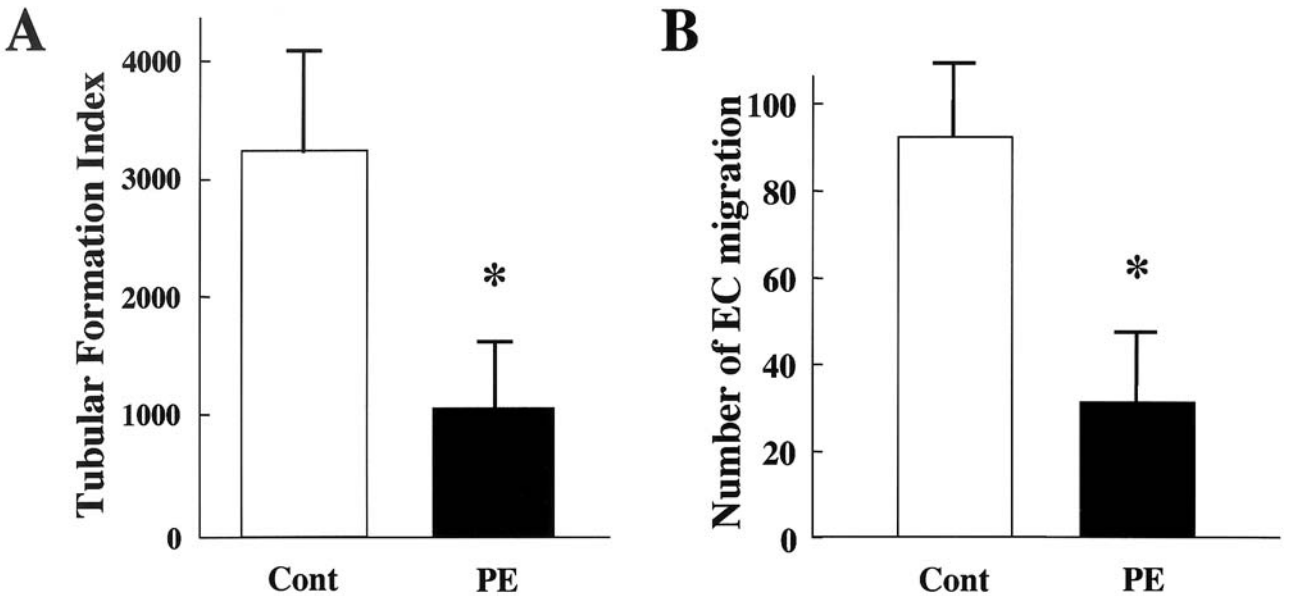

Figure 4. *Effect of PE on the VEGF-induced EC tubular formation and migration in vitro. PE significantly suppressed the EC tubular formation (A) and migration (B) even at a low concentration (1 μM). The microvessel index was measured by an image analyzer system, and the double chamber system was employed for the migration assay, as described previously (20). The data represent means±SD (n=6). Cont: VEGF (10 ng/ml)-treated control group; PE: the active form of PE, perindoprilat,-treated group at 1 μM.*

Relationship between neovascularization and the GST-P-positive preneoplastic lesions. The effect of PE on neovascularization in the liver was examined to elucidate whether or not the inhibitory effect of RAS inhibition by PE on the GST-P-positive lesions is associated with any alteration of angiogenesis. As shown in Figure 1A, CD31 mRNA expression was significantly increased in the DEN-treated control group, and the expression of CD31 in the PE-treated group was markedly suppressed as compared to the control group. Next, the possible relationship between neovascularization and the GST-P-positive preneoplastic lesions was examined. The development of GST-P-positive lesions strongly correlated with CD31 gene expression (r=0.66: y=0.22+0.034x) (Figure 1B).

Effect of RAS inhibition on VEGF expression. Since it has been reported that RAS inhibition suppressed VEGF expression in HCC (28), we examined the effect of PE on the VEGF expression during hepatocarcinogenesis. Almost parallel to the results of CD31 expression, VEGF expression was significantly increased during hepatocarcinogenesis. Moreover, the treatment with PE markedly attenuated the VEGF expression in the DEN-treated liver (Figure 2).

In vitro proliferation, angiogenesis and migration assays. To examine the involvement of RAS in angiogenesis *in vitro*, we elucidated the direct effect of PE on EC from several aspects. As shown in Figure 3, PE did not affect the proliferation of EC even at a high concentration (100 μM). The VEGF-induced EC tubule formation in the presence or absence of PE was also investigated. Contrary to the effect on proliferation, PE significantly suppressed EC tubule formation even at a low dose (1 μM). Our semi-quantitative analysis showed that the total length of tubules formed in the PE-treated group was significantly less than that in the untreated control group ($p<0.01$) (Figure 4A). Figure 4B shows the results of the VEGF-induced EC migration assay. Similar to the EC tubular formation, EC migration was significantly suppressed as compared to the control group by treatment with PE ($p<0.01$).

Discussion

HCC is one of the common malignancies with poor prognosis in the world (12). Since most cases of HCC develop in patients with chronic liver diseases, such as liver cirrhosis, only a few patients can undergo a radical operation due to their limited hepatic reserves. Consequently, several alternative therapies have been employed, such as a trans-arterial embolization and percutaneous intratumoral ethanol injection. However, to date there is still no satisfactory improvement in prognosis of HCC. One of the reasons for the poor prognosis of HCC is the high rate of recurrence. It has been shown that this high recurrence rate, even after curative therapy, is due to intrahepatic metastasis or multicentric development of each

respective neoplasm clone (13). Since the high-risk group of HCC development seems to be clearer than in other types of tumors, it is likely that a primary or secondary chemopreventive agent would be beneficial in improving the prognosis of HCC. Several agents, such as interferon and acyclic retinoid, have been shown to prevent secondary HCC recurrence. However, there are still problems in their clinical application concerning the cost and long-term toxicity, respectively (31). A retrospective cohort study of 5207 patients receiving ACE-I or other hypertensive drugs, with a 10-year follow-up, demonstrated that ACE-I treatment may decrease the incidence of adult cancer and fetal cancer (32). The other hypertensive drugs, *e.g.*, calcium channels blockers, diuretics and β-blockers, have no apparent effects on the risk of cancer development.

In the current study, it was found that PE significantly inhibited the hepatic preneoplastic foci at a clinically comparable low dose, along with suppression of neovascularization and VEGF expression. The degree of development of the preneoplastic lesions correlated with the induction of neovascularization in the liver. An *in vitro* study revealed that PE did not inhibit the proliferation of EC. We previously reported that PE did not affect the proliferation of HCC cells *in vitro* either (33), suggesting that the inhibitory effect of PE was not related to cytotoxicity. We also reported that VEGF expression increased stepwise during hepatocarcinogenesis and that suppression of VEGF signaling markedly attenuated HCC development (24). It has been shown that AT-II induced VEGF in several types of cells, including tumor cells in a dose-dependent fashion. AT-II also induces the proliferation of EC (34). PE significantly suppressed the VEGF mRNA expression in HCC cells and EC (35). In the current study, we observed that PE markedly attenuated VEGF-induced EC tubular formation and migration *in vitro*. PE also markedly suppressed the VEGF-induced augmentation of tumor growth (36). Taken together, it is likely that PE suppressed VEGF expression, which increased during hepatocarcinogenesis, and this suppression consequently attenuated the neovascularization in the liver.

Recently, the VEGF-neutralizing monoclonal antibody (Bevacizumab) has been approved for treatment of metastatic colon cancer in the U.S.A. (37). However, it is likely that a considerable time will be required before this agent is applied for patients with HCC. The treatment with Bevacizumab is an injection therapy, which may not always be convenient for all patients since long-term administration is commonly required for chemoprevention against HCC. ACE-I is an orally available agent, and it is already widely used without serious side-effects such as myelosuppression. PE is used in more than 100 countries, and the safety of its administration to patients with liver cirrhosis has been reported (38). A noteworthy finding in this study was that a significant inhibitory effect on hepatocarcinogenesis along with suppression of neovascularization by PE could be observed at a clinically comparable low dose, as described previously (28).

In summary, we have shown that RAS plays an important role in hepatocarcinogenesis, at least partly through VEGF-mediated angiogenesis, and that treatment with ACE-I significantly inhibits hepatocarcinogenesis along with suppression of neovascularization and VEGF expression in the liver. Since ACE-I is widely used in clinical practice, suppression of RAS by ACE-I may represent a potential new strategy for chemoprevention against HCC in the future.

References

1 Carmeliet P and Jain RK: Angiogenesis in cancer and other diseases. Nature *407*: 249-257, 2000.
2 Carmeliet P: Angiogenesis in health and disease. Nat Med *9*: 653-660, 2003.
3 Li CY, Shan S, Huang Q, Braun RD, Lanzen J, Hu K, Lin P and Dewhirst MW: Initial stages of tumor cell-induced angiogenesis: evaluation *via* skin window chambers in rodent models. J Natl Cancer Inst *92*: 143-147, 2000.
4 Bergers G and Benjamin LE: Tumorigenesis and the angiogenic switch. Nat Rev Cancer *3*: 401-410, 2003.
5 Bergers G, Javaherian K, Lo KM, Folkman J and Hanahan D: Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. Science *284*: 808-812, 1999.
6 Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z and Hanahan D: Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol *2*: 737-744, 2000.
7 Harris SR and Thorgeirsson UP: Tumor angiogenesis: biology and therapeutic prospects. In Vivo *12*: 563-570, 1998.
8 Fox SB, Gatter KC and Harris AL: Tumour angiogenesis. J Pathol *179*: 232-237, 1996.
9 Ferrara N, Gerber HP and LeCouter J: The biology of VEGF and its receptors. Nat Med *9*: 669-676, 2003.
10 Shibuya M: Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. Cell Struct Funct *26*: 25-35, 2001.
11 Bruns CJ, Liu W, Davis DW, Shaheen RM, McConkey DJ, Wilson MR, Bucana CD, Hicklin DJ and Ellis LM: Vascular endothelial growth factor is an *in vivo* survival factor for tumor endothelium in a murine model of colorectal carcinoma liver metastases. Cancer *89*: 488-499, 2000.
12 Befeler AS and Di Bisceglie AM: Hepatocellular carcinoma: diagnosis and treatment. Gastroenterology *122*: 1609-1619, 2002.
13 Schafer DF and Sorrell MF: Hepatocellular carcinoma. Lancet *353*: 1253-1257, 1999.
14 Chow N H, Hsu PI, Lin XZ, Yang HB, Chan SH, Cheng KS, Huang SM and Su IJ: Expression of vascular endothelial growth factor in normal liver and hepatocellular carcinoma: an immunohistochemical study. Hum Pathol *28*: 698-703, 1997.
15 Mise M, Arii S, Higashituji H, Furutani M, Niwano M, Harada T, Ishigami S, Toda Y, Nakayama H, Fukumoto M, Fujita J and Imamura M: Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. Hepatology *23*: 455-464, 1996.

16 Miura H, Miyazaki T, Kuroda M, Oka T, Machinami R, Kodama T, Shibuya M, Makuuchi M, Yazaki Y and Ohnishi S: Increased expression of vascular endothelial growth factor in human hepatocellular carcinoma. J Hepatol *27*: 854-861, 1997.
17 Suzuki K, Hayashi N, Miyamoto Y, Yamamoto M, Ohkawa K, Ito Y, Sasaki Y, Yamaguchi Y, Nakase H, Noda K, Enomoto N, Arai K, Yamada Y, Yoshihara H, Tujimura T, Kawano K, Yoshikawa K and Kamada T: Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res *56*: 3004-3009, 1996.
18 Yamaguchi R, Yano H, Nakashima Y, Ogasawara S, Higaki K, Akiba J, Hicklin DJ and Kojiro M: Expression and localization of vascular endothelial growth factor receptors in human hepatocellular carcinoma and non-HCC tissues. Oncol Rep *7*: 725-729, 2000.
19 Yamaguchi R, Yano H, Iemura A, Ogasawara S, Haramaki M and Kojiro M: Expression of vascular endothelial growth factor in human hepatocellular carcinoma. Hepatology *28*: 68-77, 1998.
20 Yoshiji H, Kuriyama S, Yoshii J, Yamazaki M, Kikukawa M, Tsujinoue H, Nakatani T and Fukui H: Vascular endothelial growth factor tightly regulates *in vivo* development of murine hepatocellular carcinoma cells. Hepatology *28*: 1489-1496, 1998.
21 Park YN, Kim YB, Yang KM and Park C: Increased expression of vascular endothelial growth factor and angiogenesis in the early stage of multistep hepatocarcinogenesis. Arch Pathol Lab Med *124*: 1061-1065, 2000.
22 Frachon S, Gouysse G, Dumorti J, Couvelard A, Nejjari M, Mion F, Berger F, Paliard P, Boillot O and Scoazec JY: Endothelial cell marker expression in dysplastic lesions of the liver: an immunohistochemical study. J Hepatol *34*: 850-857, 2001.
23 Kin M, Torimura T, Ueno T, Nakamura T, Ogata R, Sakamoto M, Tamaki S and Sata M: Angiogenesis inhibitor TNP-470 suppresses the progression of experimentally-induced hepatocellular carcinoma in rats. Int J Oncol *16*: 375-382, 2000.
24 Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, Wu Y, Yanase K, Namisaki T, Kitade M, Yamazaki M, Tsujinoue H, Masaki T and Fukui H: Halting the interaction between vascular endothelial growth factor and its receptors attenuates liver carcinogenesis in mice. Hepatology *39*: 1517-1524, 2004.
25 Matsusaka T and Ichikawa I: Biological functions of angiotensin and its receptors. Annu Rev Physiol *59*: 395-412, 1997.
26 Yoshiji H, Kuriyama S and Fukui H: Angiotensin-I-converting enzyme inhibitors may be an alternative anti-angiogenic strategy in the treatment of liver fibrosis and hepatocellular carcinoma. Possible role of vascular endothelial growth factor. Tumour Biol *23*: 348-356, 2002.
27 Yoshiji H, Yoshii J, Ikenaka Y, Noguchi R, Tsujinoue H, Nakatani T, Imazu H, Yanase K, Kuriyama S and Fukui H: Inhibition of renin-angiotensin system attenuates liver enzyme-altered preneoplastic lesions and fibrosis development in rats. J Hepatol *37*: 22-30, 2002.
28 Yoshiji H, Kuriyama S, Kawata M, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H and Fukui H: The angiotensin-I-converting enzyme inhibitor perindopril suppresses tumor growth and angiogenesis: possible role of the vascular endothelial growth factor. Clin Cancer Res *7*: 1073-1078, 2001.
29 Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H and Fukui H: Angiotensin-II type 1 receptor interaction is a major regulator for liver fibrosis development in rats. Hepatology *34*: 745-750, 2001.
30 Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, Huber J, Nakatani T, Tsujinoue H, Yanase K, Imazu H and Fukui H: Synergistic effect of basic fibroblast growth factor and vascular endothelial growth factor in murine hepatocellular carcinoma. Hepatology *35*: 834-842, 2002.
31 Okita K, Sakaida I and Hino K: Current strategies for chemoprevention of hepatocellular carcinoma. Oncology *62(Suppl 1)*: 24-28, 2002.
32 Lever AF, Hole DJ, Gillis CR, McCallum IR, McInnes GT, MacKinnon PL, Meredith PA, Murray LS, Reid JL and Robertson JW: Do inhibitors of angiotensin-I-converting enzyme protect against risk of cancer? Lancet *352*: 179-184, 1998.
33 Noguchi R, Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Yanase K, Namisaki T, Kitade M, Yamazaki M, Mitoro A, Tsujinoue H, Imazu H, Masaki T and Fukui H: Combination of interferon-beta and the angiotensin-converting enzyme inhibitor, perindopril, attenuates murine hepatocellular carcinoma development and angiogenesis. Clin Cancer Res *9*: 6038-6045, 2003.
34 Monton M, Castilla MA, Alvarez Arroyo MV, Tan D, Gonzalez-Pacheco FR, Lopez Farre A, Casado S and Caramelo C: Effects of angiotensin II on endothelial cell growth: role of AT-1 and AT-2 receptors. J Am Soc Nephrol *9*: 969-974, 1998.
35 Yoshiji H, Kuriyama S and Fukui H: Perindopril: possible use in cancer therapy. Anticancer Drugs *13*: 221-228, 2002.
36 Yoshiji H, Yoshii J, Ikenaka Y, Noguchi R, Yanase K, Tsujinoue H, Imazu H and Fukui H: Suppression of the renin-angiotensin system attenuates vascular endothelial growth factor-mediated tumor development and angiogenesis in murine hepatocellular carcinoma cells. Int J Oncol *20*: 1227-1231, 2002.
37 Gerber HP and Ferrara N: Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res *65*: 671-680, 2005.
38 Thiollet M, Funck-Brentano C, Grange JD, Midavaine M, Resplandy G and Jaillon P: The pharmacokinetics of perindopril in patients with liver cirrhosis. Br J Clin Pharmacol *33*: 326-328, 1992.

Received March 22, 2005
Accepted May 23, 2005