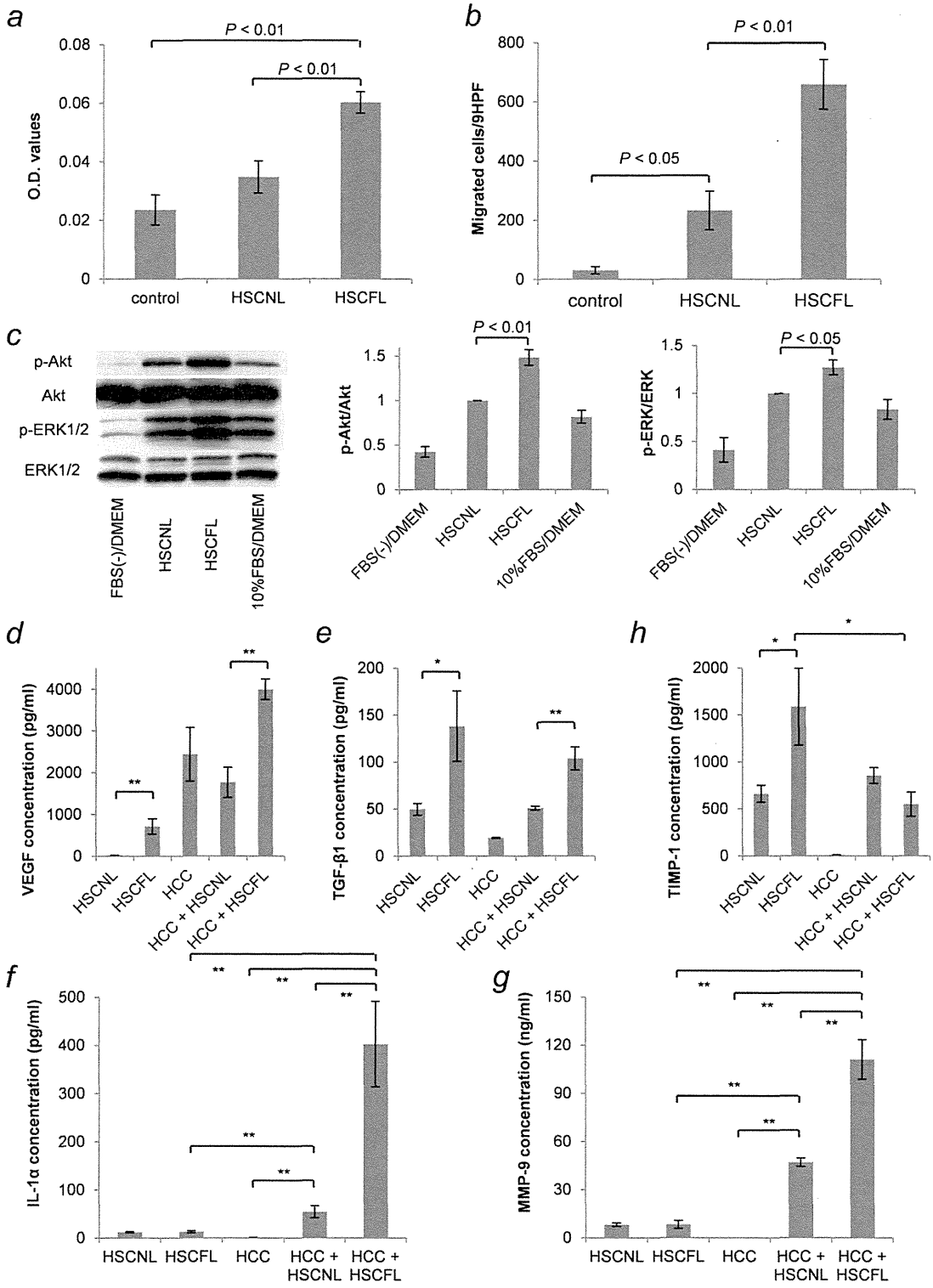

Figure 2. HSCFL promoted HCC progression *in vitro*. (*a* and *b*) HSC-FL promoted HCC proliferation and migration. (*c*) Protein lysates from McA-RH7777 cells treated with conditioned media from HSCNL or HSCFL were analyzed for Akt and ERK1/2 activation. The phosphorylation levels were normalized to total protein expression. (*d–h*) Cytokine secretion from McA-RH7777 cells and HSCs in monoculture and co-culture, using ELISA kits. Data shown are presented from more than 5 independent experiments. $*p < 0.05$, $**p < 0.01$.

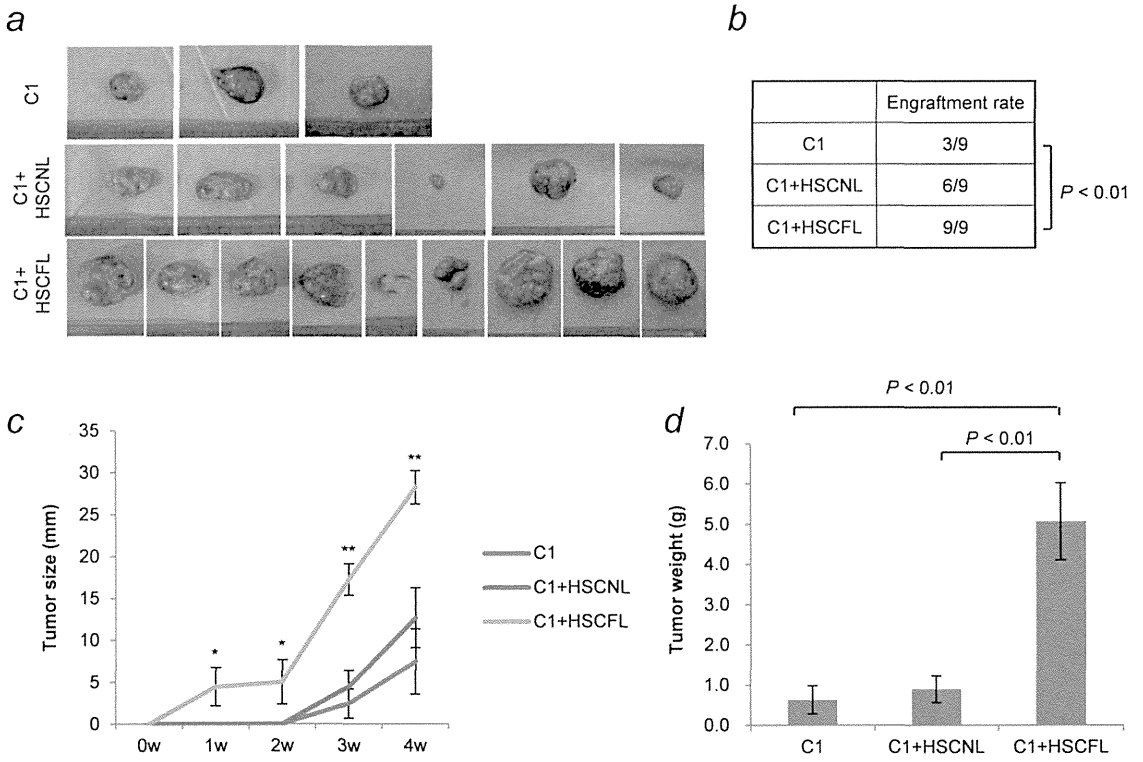

Figure 3. HSCFL promoted HCC progression *in vivo*. (*a*) Macrography of subcutaneous tumors in each group. (*b*) The table shows the engraftment rate in each group. (*c*) Tumor sizes were measured weekly. Data are presented as means (±SE). *$p < 0.05$, **$p < 0.01$ compared to the C1+HSCNL group (*d*) The graph shows the average tumor weight 4 weeks after co-implantation. Data are presented as means (±SE).

harvested from Y-27632-treated HSCFL and the levels of MMP-9 and TIMP-1 in CM harvested from a co-culture of HCC and HSCFL treated with Y-27632 were similar to those in CM harvested from untreated HSCFL and in CM harvested from a co-culture of HCC and untreated HSCFL, respectively (Supporting Information Figs. S5*a*–S5*d*). These results indicated that Rho-kinase inhibitor did not inhibit the overall features of HSC, but partially inhibited the activation of HSC.

High fat diet-induced FL also has a permissive microenvironment for HCC metastasis

We assessed the effect of HFD-induced FL (non-CDD-induced FL) on HCC metastasis. Rats fed on a HFD for 16 weeks developed microvesicular steatosis [approximately 20–30% steatosis, (Supporting Information Fig. S6*a*)], although the HFD-induced FL appeared less fibrotic compared to CDD-induced FL (Supporting Information Fig. S6*b*). Furthermore, the serum levels of AST were significantly lower in rats with HFD-induced FL than in those with CDD-induced FL (Supporting Information Fig. S6*c*). All the six rats fed on a HFD for 16 weeks developed several nodular tumors at 8 weeks after the inoculation of McA-RH7777 cells (5×10^5 cells/body). In contrast, one of the seven rats fed on a normal diet for 16 weeks developed several small tumors (Figs. 5*a* and 5*b*). Volumes of the HCC tumors were significantly greater in rats with HFD-induced FL than in rats with NL (Fig. 5*c*).

HSC derived from HFD-induced FL stimulate HCC cell proliferation and migration *in vitro*

We investigated whether HSCFL of rats fed on a HFD for 24 weeks (HSCHFD) could induce proliferation and migration of HCC cells. HSCHFD promoted HCC migration to a significantly greater extent than did HSC isolated from NL of rats fed on a normal diet for 24 weeks (Fig. 5*d*). In addition, HSCHFD promoted HCC proliferation to a significantly greater extent than did HSC isolated from NL of rats fed on a normal diet for 24 weeks (Fig. 5*e*).

Discussion

A rapidly growing literature indicates that NAFLD, including NASH, is associated with HCC.[1–3] However, whether NAFLD itself promotes the progression and metastasis of HCC is unclear. Therefore, we investigated whether FL either promotes or suppresses HCC progression. Our results have shown that both CDD- and HFD-induced FL have pro-metastatic microenvironments in the model of portal vein HCC cell inoculation. Furthermore, HSCs were activated in

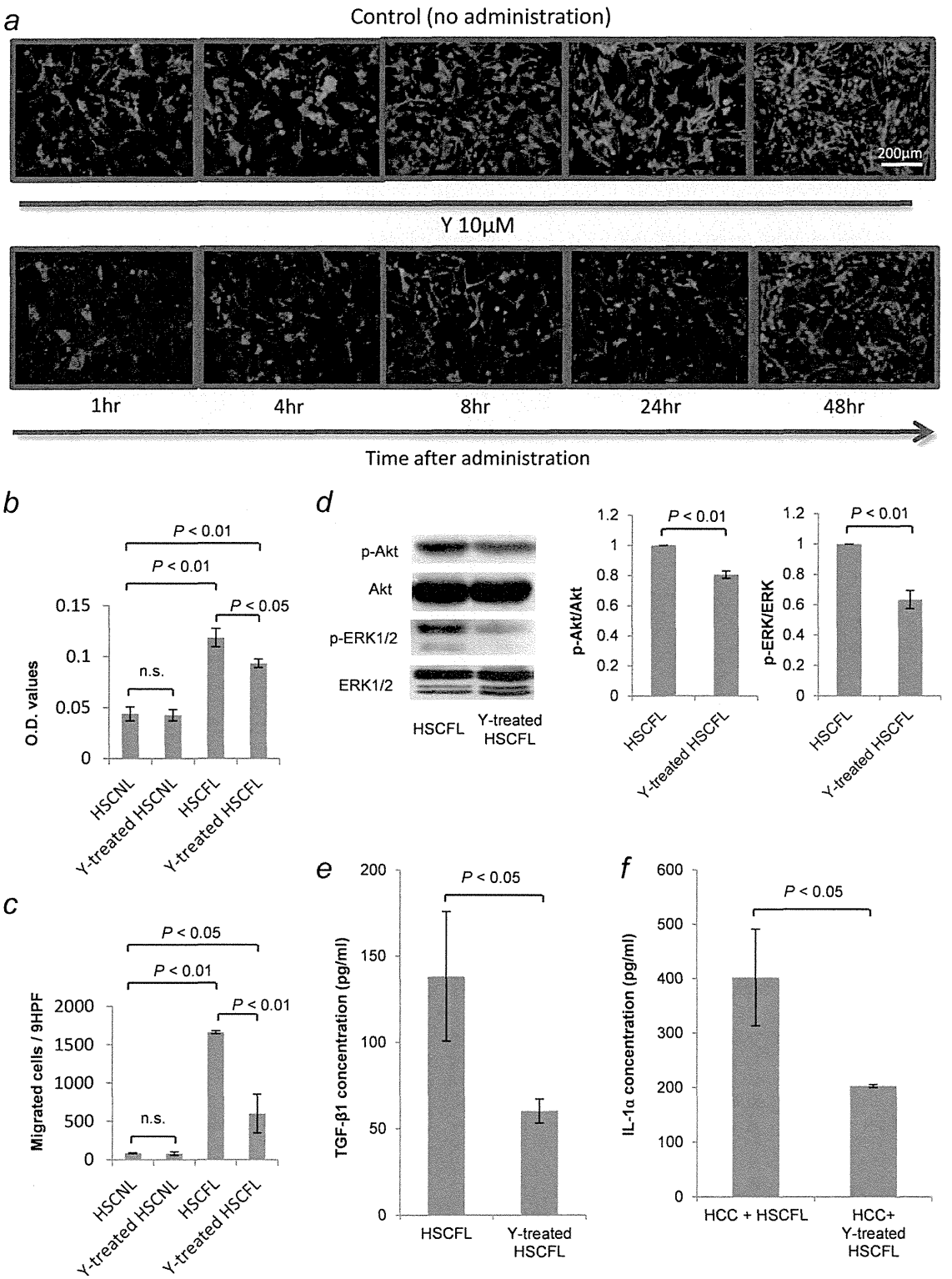

Figure 4. The Rho-kinase inhibitor, Y-27632, attenuated the HCC-promoting effect of HSCFL. (*a*) Stress fiber and F-actin expression in HSCFL and Y-27632-treated HSCFL (Y-treated HSCFL). (*b*) Conditioned media harvested from Y-treated HSCFL (Y-treated HSCFL-CM) significantly suppressed the proliferation of McA-RH7777 compared with that of untreated HSCFL-CM. (*c*) Migration of McA-RH7777 under the stimulation of Y-treated HSCFL was significantly suppressed compared with that of untreated HSCFL. (*d*) The phosphorylation levels of Akt and ERK were significantly suppressed in HCC cells treated with Y-treated HSCFL-CM, compared to those in HCC cells treated with untreated HSCFL-CM. (*e*) Y-27632 suppressed TGF-β1 secretion of HSCFL. (*f*) Y-27632 suppressed IL-1α secretion on co-culture of HCC and HSCFL.

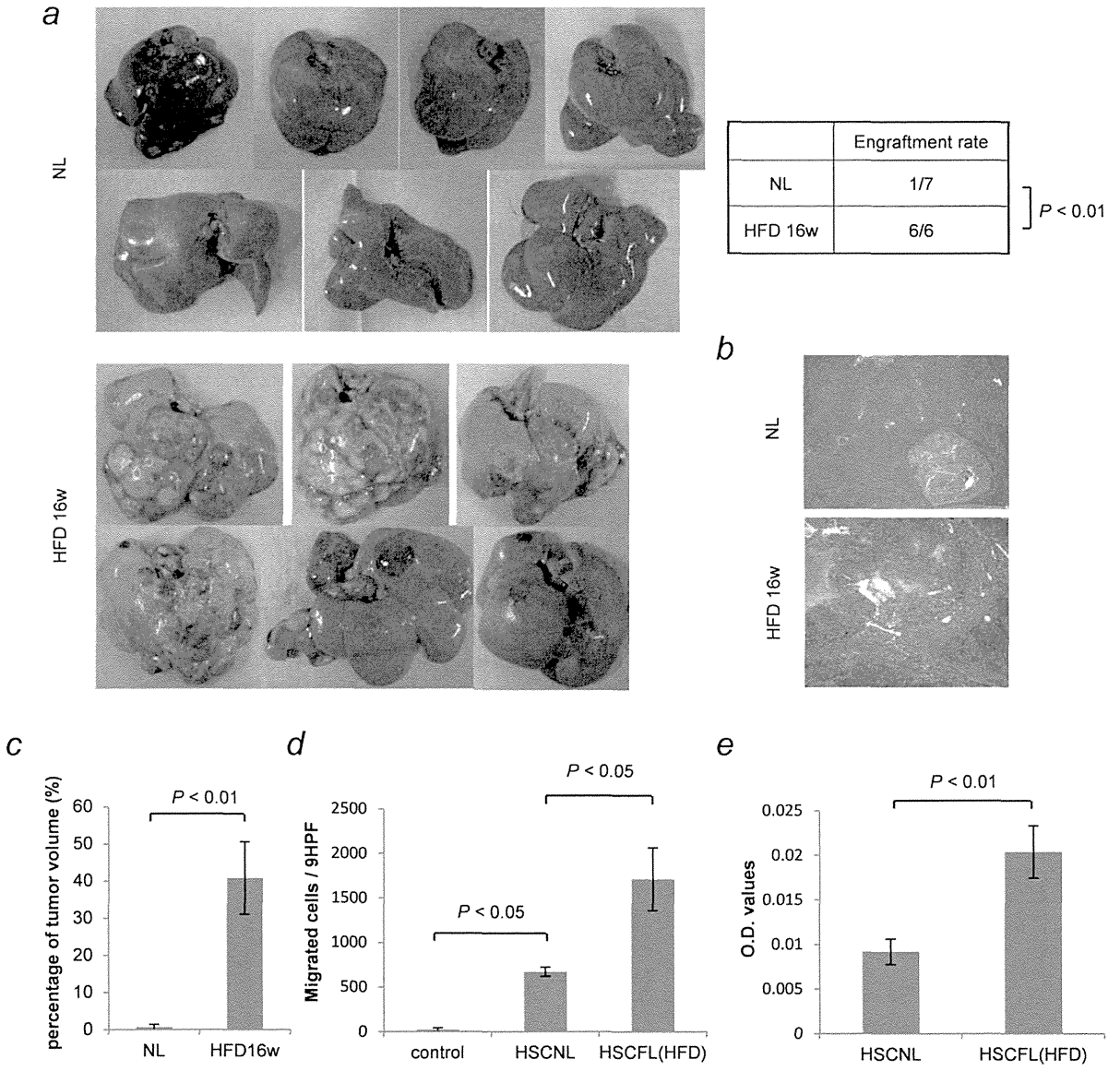

Figure 5. High fat diet-induced FL also provides a permissive microenvironment for HCC metastasis and progression. (*a*) Macrography of liver tumors in NL and high fat diet (HFD)-induced FL. Only one of seven rats with NL developed several nodular tumors after inoculation of HCC cells (McA-RH7777 cells, 5×10^5 cells/body), whereas all six rats with FL induced by feeding of a HFD for 16 weeks developed multiple nodular liver tumors. (*b*) Hematoxylin–eosin-stained images of liver tumors in NL and HFD-induced FL. (*c*) The graph shows the percentage of tumor volume in the liver. (*d* and *e*) HSCs derived from HFD-induced FL stimulated HCC migration and proliferation *in vitro*.

both CDD- and HFD-induced FL, and these activated HSCs enhanced the proliferation and migration of HCC cells. In addition, Y-27632, a Rho-kinase inhibitor, partially reduced the progression of HCC through deactivating activated HSCs. These results indicated that the steatotic liver microenvironment favors HCC progression and metastasis through the activation of HSCs.

In the current study, we have shown that CDD-induced FL activates HSCs to enhance the proliferation and migration of HCC in co-culture and co-implantation models through the secretion of paracrine signaling molecules such as VEGF. HSCs can transdifferentiate into highly proliferative and motile myofibroblasts during the activation process that follows liver injury.[8] In addition, we noted that the HSCs derived from HFD-induced FL, which appeared less fibrotic compared with CDD-induced FL, promoted the migration and proliferation of HCC cells, even though the HSCs may not be fully activated. Free fatty acids such as oleate and

palmitate reportedly stimulate the activation of fibrosis-related genes (*i.e.*, TGF-β, TIMP-1) in HSCs.[15] Activated HSCs produce growth factors and cytokines, such as TGF-β, hepatocyte growth factor (HGF), stromal-derived factor-1 (SDF-1), and IL-1, to stimulate the proliferation, adhesion, and migration of cancer cells.[16,17] It has been postulated that HSCs are a component of the prometastatic liver microenvironment.[10,11] Neaud *et al.*[18] showed that myofibroblasts increased the invasiveness of HCC cells by secreting HGF. Recently, Liu *et al.* demonstrated that the IQ motif containing GTPase-activating protein 1 (IQGAP1) binds to TGF-β receptor II (TGF-βRII) and suppresses TGF-βRII-mediated signaling in HSCs, thus preventing myofibroblastic differentiation. IQGAP1 deficiency in HSCs promoted myofibroblast activation, tumor implantation, and metastatic growth *via* upregulation of paracrine signaling molecules, including SDF-1/CXCL12 and HGF.[19] Our results are consistent with that report. Yoshimoto *et al.*[6] showed that senescence-associated secretory phenotype (SASP)[20] plays crucial roles in promoting obesity-associated HCC development in mice. In the current study, some of the SASP factors, such as IL-1α, CXCR2-binding chemokines, and TGF-β were increased in activated HSCs. These activated HSCs may be senescing, and this effect may be related to the promotion of metastatic growth of HCCs, but further study of this possibility is necessary. Sancho-Bru *et al.*[21] have examined the effect of hepatocarcinoma cells on HSCs in a co-culture system, and have reported the interaction of HSCs and HCC cells. In that study, co-culture of the cells reduced the expression of fibrogenic factors, such as procollagen-αI(I). Those results may be consistent with our results indicating the presence of decreased levels of TIMP-1 and increased levels of MMP-9 in CM harvested from co-culture of HCC cells and HSCs. Coulouarn *et al.* also showed that hepatocyte-HSC cross-talk generated a permissive proangiogenic microenvironment by inducing VEGF and MMP9 expression in HSCs.[22] Our results indicate that HCCs and HSCs have bidirectional cross-talk; that is, this interaction is proangiogenic and tumorigenic, but also antifibrogenic. These paradoxical results may have important implications for the progression of HCC. It is speculated that the remodeling of the extracellular matrix, along with the formation of new vessels, contributes to the invasiveness of HCC.

We have also shown that a ROCK inhibitor converted activated HSCs to inactivated HSCs, thereby suppressing the progression of HCC. The Rho signaling pathway and actomyosin system are reportedly involved in the motility and invasion of various cells, including cancer cells.[23–25] It is known that Rho signaling is involved in HSC activation, and a specific ROCK inhibitor, Y-27632, inhibited the activation of HSCs by regulating the formation of actin fibers and focal adhesion.[25–27] Our results are consistent with those reports. However, the production of TGF-β and IL-1α was suppressed by Y-27632, whereas HSCs reverted by treatment with Y-27632 still secreted several cytokines, including VEGF and MMP-9. These data indicated that HSCs did not fully revert to a quiescent state, but rather retained a preactivated intermediate state.[28] Several reports, including ours,[29] have also shown that treatment of tumor-bearing rats with a ROCK inhibitor suppressed peritoneal dissemination of cancer cells and intrahepatic metastasis of HCC cells.[30,31] This suggested that the ROCK inhibitor is implicated in suppressing HCC progression through not only a direct action on HCC (by inhibiting actomyosin contractility of HCC cells), but also through another indirect action within the cancer stroma, which includes HSCs.

Several limitations to our study should be considered. In the present study, we used hepatoma cells derived from rats. The animals develop large tumors within a few weeks of HCC inoculation, which diffusely infiltrate the liver. This may lead to the development of a different tumor microenvironment and different interactions between HCC and HSCs compared to those present in tumors that arise endogenously. Furthermore, we have shown that the progression of HCC in FL is associated with activated HSCs. However, the activation of HSCs is probably one of several mechanisms associated with the tumorigenic environment in FL. It has been shown that fatty change in hepatocytes induces hypoxic environments in the liver.[32] Indeed, fat droplet accumulation in the cytoplasm of hepatocytes is associated with an increase in cell volume, which may result in partial or complete obstruction of the hepatic sinusoidal space and reduction in sinusoidal blood flow. A state of chronic cellular hypoxia persists in FL, which induces hypoxia-inducible factor (HIF).[33] HIF can induce a vast array of gene products controlling energy metabolism, neovascularization, survival, and cell migration, and is recognized as a strong promoter of tumor growth.[34] The sinusoidal endothelial cells are injured in cases of FL.[32] The number of adherent leukocytes in the injured sinusoid cells induced by a methionine-and CDD was found to be significantly increased, compared with that in normal sinusoid cells. The injured sinusoid cells in the FL may promote tumor cell arrest and extravasation into the hepatic parenchyma. Additional studies of the other mechanisms by which a FL promotes tumor progression are needed. We have also shown that FL with approximately 10–20% steatosis, which was induced by a long-term feeding (16 weeks) of a HFD, promoted the progression of HCC. However, it remains unexplored whether FL with less than 10–20% steatosis promotes the progression of HCC.

In conclusion, our results indicate that the rat steatotic liver microenvironment favors HCC metastasis. This effect appears to be promoted through the activation of HSCs in the steatotic liver.

Acknowledgements

The authors like to thank K. Ogawa, National Institute of Health Sciences (Tokyo, Japan), for the generous gift of the HCC cell lines, as well as M. Kiyokawa and Y. Ishida for their excellent technical assistance.

References

1. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012;56:1384–91.
2. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010;51:1820–32.
3. Stickel F, Hellerbrand C. Non-alcoholic fatty liver disease as a risk factor for hepatocellular carcinoma: mechanisms and implications. *Gut* 2010;59:1303–7.
4. Ohki T, Tateishi R, Shiina S, et al. Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. *Gut* 2009;58:839–44.
5. Park EJ, Lee JH, Yu GY, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010;140:197–208.
6. Yoshimoto S, Loo TM, Atarashi K, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97–101.
7. Reynaert H, Thompson MG, Thomas T, et al. Hepatic stellate cells: role in microcirculation and pathophysiology of portal hypertension. *Gut* 2002;50:571–81.
8. Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008;134:1655–69.
9. Mederacke I, Hsu CC, Troeger JS, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nat Commun* 2013;4:2823.
10. Kang N, Gores GJ, Shah VH. Hepatic stellate cells: partners in crime for liver metastases? *Hepatology* 2011;54:707–13.
11. Hernandez-Gea V, Toffanin S, Friedman SL, et al. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology* 2013;144:512–27.
12. Washington K, Wright K, Shyr Y, et al. Hepatic stellate cell activation in nonalcoholic steatohepatitis and fatty liver. *Hum Pathol* 2000;31:822–8.
13. Reeves HL, Burt AD, Wood S, et al. Hepatic stellate cell activation occurs in the absence of hepatitis in alcoholic liver disease and correlates with the severity of steatosis. *J Hepatol* 1996;25:677–83.
14. Kuroda S, Tashiro H, Igarashi Y, et al. Rho inhibitor prevents ischemia-reperfusion injury in rat steatotic liver. *J Hepatol* 2012;56:146–52.
15. Bechmann LP, Zahn D, Gieseler RK, et al. Resveratrol amplifies profibrogenic effects of free fatty acids on human hepatic stellate cells. *Hepatol Res* 2009;39:601–8.
16. Holt AP, Haughton EL, Lalor PF, et al. Liver myofibroblasts regulate infiltration and positioning of lymphocytes in human liver. *Gastroenterology* 2009;136:705–14.
17. Amann T, Bataille F, Spruss T, et al. Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. *Cancer Sci* 2009;100:646–53.
18. Neaud V, Faouzi S, Guirouilh J, et al. Human hepatic myofibroblasts increase invasiveness of hepatocellular carcinoma cells: evidence for a role of hepatocyte growth factor. *Hepatology* 1997;26:1458–66.
19. Liu C, Billadeau DD, Abdelhakim H, et al. IQGAP1 suppresses TbetaRII-mediated myofibroblastic activation and metastatic growth in liver. *J Clin Invest* 2013;123:1138–56.
20. Kuilman T, Peeper DS. Senescence-messaging secretome: SMS-ing cellular stress. *Nat Rev Cancer* 2009;9:81–94.
21. Sancho-Bru P, Juez E, Moreno M, et al. Hepatocarcinoma cells stimulate the growth, migration and expression of pro-angiogenic genes in human hepatic stellate cells. *Liver Int* 2010;30:31–41.
22. Coulouarn C, Corlu A, Glaise D, et al. Hepatocyte-stellate cell cross-talk in the liver engenders a permissive inflammatory microenvironment that drives progression in hepatocellular carcinoma. *Cancer Res* 2012;72:2533–42.
23. Burridge K, Wennerberg K. Rho and Rac take center stage. *Cell* 2004;116:167–79.
24. Uehata M, Ishizaki T, Satoh H, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 1997;389:990–4.
25. Sanz-Moreno V, Gaggioli C, Yeo M, et al. ROCK and JAK1 signaling cooperate to control actomyosin contractility in tumor cells and stroma. *Cancer Cell* 2011;20:229–45.
26. Iwamoto H, Nakamuta M, Tada S, et al. A p160ROCK-specific inhibitor, Y-27632, attenuates rat hepatic stellate cell growth. *J Hepatol* 2000;32:762–70.
27. Murata T, Arii S, Nakamura T, et al. Inhibitory effect of Y-27632, a ROCK inhibitor, on progression of rat liver fibrosis in association with inactivation of hepatic stellate cells. *J Hepatol* 2001;35:474–81.
28. Troeger JS, Mederacke I, Gwak GY, et al. Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. *Gastroenterology* 2012;143:1073–83 e22.
29. Ogawa T, Tashiro H, Miyata Y, et al. Rho-associated kinase inhibitor reduces tumor recurrence after liver transplantation in a rat hepatoma model. *Am J Transplant* 2007;7:347–55.
30. Itoh K, Yoshioka K, Akedo H, et al. An essential part for Rho-associated kinase in the transcellular invasion of tumor cells. *Nat Med* 1999;5:221–5.
31. Takamura M, Sakamoto M, Genda T, et al. Inhibition of intrahepatic metastasis of human hepatocellular carcinoma by Rho-associated protein kinase inhibitor Y-27632. *Hepatology* 2001;33:577–81.
32. McCuskey RS, Ito Y, Robertson GR, et al. Hepatic microvascular dysfunction during evolution of dietary steatohepatitis in mice. *Hepatology* 2004;40:386–93.
33. Nath B, Levin I, Csak T, et al. Hepatocyte-specific hypoxia-inducible factor-1alpha is a determinant of lipid accumulation and liver injury in alcohol-induced steatosis in mice. *Hepatology* 2011;53:1526–37.
34. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 2006;441:437–43.

Hepatology Research

Hepatology Research 2014; 44: 149–158 doi: 10.1111/hepr.12194

Review Article

Clinical significance of therapy using branched-chain amino acid granules in patients with liver cirrhosis and hepatocellular carcinoma

Hiroki Nishikawa and Yukio Osaki

Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Osaka, Japan

The liver is the major organ for the metabolism of protein, fat and carbohydrate. A nutritional approach is required in the treatment of cirrhosis, which is frequently complicated with protein–energy malnutrition. Several advanced treatment approaches for hepatocellular carcinoma (HCC) have been established in the past decade. HCC is often complicated by cirrhosis, so treatment of the underlying liver diseases is also necessary to improve the prognosis. Branched-chain amino acid (BCAA) granules were developed originally for the treatment of hypoalbuminemia associated with decompensated cirrhosis. However, subsequent studies found various other pharmacological actions of this agent. We review the clinical significance of therapy using BCAA granules in patients receiving different treatment approaches for cirrhosis and HCC based on the published work as well as our own data.

Key words: branched-chain amino acid granules, hepatocellular carcinoma, liver cirrhosis, liver function, recurrence

INTRODUCTION

THE LIVER IS the major organ for the metabolism of protein, fat and carbohydrate.[1,2] Cirrhosis, which develops over a long period of time, is frequently complicated with protein–energy malnutrition (PEM).[1,2] Patients with cirrhosis-associated PEM starve even after a short period of fasting because of the increased energy consumption and decreased glycogen-storage capacity of the liver. The body consumes the endogenous fat as an energy substrate instead of carbohydrate. As a result, fasting hypoglycemia and postprandial hyperglycemia typically occur.[1–4] PEM affects the prognosis by increasing the risk of cirrhosis-associated events and deteriorating the patient's quality of life (QoL), so nutritional treatment is absolutely crucial.[1–3]

Correspondence: Dr Hiroki Nishikawa, Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, 5-30 Fudegasaki-cho, Tennoji-ku, Osaka 543-0027, Japan.
Email: h-nishikawa@osaka-med.jrc.or.jp
Conflict of interest: The authors have not received any financial support for this article and have no conflicts of interest to declare.
Received 11 April 2013; revision 24 June 2013; accepted 25 June 2013.

The treatment of hepatocellular carcinoma (HCC) has improved appreciably in the past 20–30 years. The current treatment for HCC with established efficacy is: (i) hepatectomy/liver transplantation; (ii) transcatheter arterial chemoembolization (TACE); (iii) percutaneous radiofrequency ablation (RFA); (iv) percutaneous ethanol injection; (v) percutaneous microwave coagulation therapy; and (vi) molecular-targeted therapy (e.g. sorafenib).[5–9] The most suitable treatment should be selected for individual patients based on thorough evaluation of HCC stage (tumor factor) and hepatic functional reserve.[5–10] In general, HCC develops after cirrhosis associated with viral hepatitis or alcoholic liver disease, so treatment of the underlying liver diseases is no less important than HCC treatment.[5–9,11] Preserving adequate hepatic reserves is necessary after HCC recurrence, which is quite frequent no matter how successful the initial radical treatment for HCC.[12–16]

Branched-chain amino acid (BCAA) granules (Livact; Ajinomoto Pharma, Tokyo, Japan) contain L-valine, L-leucine, and L-isoleucine at a ratio of 1.2:2:1. L-Leucine induces albumin synthesis in hepatic cells via transcription factors such as mammalian target of rapamycin.[1–3,17] BCAA granules were developed originally for the treatment of hypoalbuminemia associated

Table 1 Pharmacological effects of branched-chain amino acid granules

1. Improvement of hypoalbuminemia
2. Improvement of liver cirrhosis-related complications
3. Improvement of insulin resistance
4. Improvement of oxidative stress
5. Improvement of fatty acid metabolism
6. Activation of immune function
7. Suppression of angiogenesis
8. Suppression of liver carcinogenesis

with decompensated cirrhosis. However, subsequent studies found various other pharmacological actions of this drug. Therapy using BCAA granules improves hypoalbuminemia.[16–19] In addition, such therapy also inhibits cirrhosis-related complications such as esophageal varices and ascites,[17,18,20] reduces insulin resistance[17,21,22] and oxidative stress,[17,23] improves fatty-acid metabolism,[17,24] stimulates the immune system,[17,25,26] and inhibits angiogenesis.[17,21,27] The most noteworthy pharmacological action of BCAA granules, however, is the inhibition of hepatic carcinogenesis (Table 1).[17,19,20,22,27–29] Based on the significant inhibition of hepatic carcinogenesis observed after therapy using BCAA granules in patients with liver cirrhosis with a body mass index of 25 kg/m^2 or more shown in a multicenter, randomized, placebo-controlled study (the Lotus Study), the 2010 guidelines for comprehensive treatment of hepatitis virus-related cirrhosis in Japanese patients recommend the use of BCAA granules to preserve liver function and inhibit hepatic carcinogenesis.[16–19,28,30] Conversely, the American Society for Parental and Enteral Nutrition (ASPEN) and the European Society for Clinical Nutrition and Metabolism recommend that BCAA supplementation be carried out only in cirrhotic patients with chronic hepatic encephalopathy that is refractory to pharmacotherapy.[31,32]

Here, we review the clinical significance of therapy using BCAA granules in different treatment approaches for cirrhosis and HCC (i.e. hepatectomy, liver transplantation, RFA, TACE and molecular-targeted agents) mainly based on the published work as well as our own data published between 1997 and 2013. We searched the published work in the PubMed database, and the search strategy was based on the following terms: "branched-chain amino acid", "liver cirrhosis", "liver function", "complication", "clinical outcome", "carcinogenesis", "hepatocellular carcinoma", "recurrence", "hepatectomy", "liver transplantation", "RFA", "TACE" and "molecular-targeted therapy".

Significance of cirrhosis treatment with BCAA granules

In cirrhotic patients, the plasma level of BCAA is positively correlated with the serum albumin level. Such a correlation is seen only in patients with chronic liver diseases such as cirrhosis. The albumin–BCAA correlation and the inability of cirrhotic patients to maintain an adequate plasma level of BCAA with diet alone serve as the theoretical rationale for the use of BCAA granules for the treatment of cirrhosis. In cirrhotic patients, BCAA uptake in skeletal muscle is increased for ammonia detoxification and energy production and, in turn, the plasma level of BCAA and albumin production decrease.[1–3]

Yatsuhashi *et al.* conducted a prospective multicenter study in 204 patients with decompensated cirrhosis and reported a mean increase in the serum albumin level of 0.2 g/dL after 6 months of treatment with BCAA granules as well as a significant increase in the serum albumin level in patients with intake of a poor diet (poor intake of energy).[33] Therapy using BCAA granules also significantly decreased the incidence of ascites even in patients with an unchanged serum albumin level because of qualitative improvement of the serum albumin level (specifically, an increase in the level of reduced albumin and decrease in the level of oxidized albumin).[33–35]

The importance of treatment compliance was suggested in a study conducted by Takaguchi *et al.*[36] That prospective, large-scale, multicenter, observational study in 2894 patients with decompensated cirrhosis reported that the incidence of cirrhosis-associated events was decreased significantly in patients with good adherence to BCAA treatment compared with those with poor adherence. The authors emphasized the importance of thorough instruction regarding medications to patients.[36]

The appropriate timing of the initiation of BCAA treatment is controversial. The approved indication of BCAA granules in Japan is for the treatment of decompensated cirrhosis in patients with a serum albumin level of 3.5 g/dL or less, and the Japanese Nutritional Study Group for Liver Cirrhosis has also recommended that BCAA granules should be administrated in cirrhotic patients with a serum albumin level of 3.5 g/dL or less, Fisher's ratio of 1.8 or less and/or BCAA : tyrosine ratio (BTR) of 3.5 or less.[37] Hence, therapy using BCAA granules is, in general, started when the serum

albumin level is 3.5 g/dL or less in clinical settings.[11,37] However, earlier initiation of BCAA treatment has been attempted in cirrhotic patients with a serum albumin level of 3.6 g/dL or more. Habu *et al.* classified their patients into four treatment arms based on their serum albumin level and the BTR.[38] The decrease in the serum albumin level was inhibited after therapy using BCAA granules even in patients with a serum albumin level of 3.6 g/dL or more if their BTR was 4 or less, so the authors highlighted the usefulness of early intervention with BCAA granules.[38,39] A prospective, multicenter study in Japanese patients with hepatitis C virus-related decompensated cirrhosis with a serum albumin level of 3.6 g/dL or more complicated with insulin resistance (BCAA Granules for patients with Hepatitis C virus-related Liver Cirrhosis and Insulin Resistance on the Effect of Reduction of Carcinogenic Risk in the Liver [BLOCK] study, Japan Liver Oncology Group [JLOG] 1004 Trial) is ongoing. If the superiority of therapy using BCAA granules is demonstrated in that study, BCAA granules will become available for a wider range of cirrhotic patients.

As mentioned above, BCAA granules can inhibit hepatic carcinogenesis.[17,19,20,22,27–29] Several reports have focused on the usefulness of BCAA granules for the inhibition of liver carcinogenesis through improvement of insulin resistance.[17,21,22] Insulin and insulin-like growth factor (IGF) can promote the growth of HCC.[40] Kawaguchi *et al.* reported that BCAA granules suppress liver carcinogenesis through amelioration of insulin resistance via: (i) BCAA activation of the insulin signaling cascade through upregulation of phosphatidylinositol 3-kinase with reduction of serum insulin levels; and (ii) inhibition of the IGF/IGF-1 receptor axis by suppressing the expressions of IGF-1, IGF-2 and IGF-1 receptor mRNA.[17,41] They also reported that the improvement of insulin resistance by BCAA granules may be related to the migration of HCC, suppression of angiogenesis and epithelial–mesenchymal transition of hepatocytes, and that BCAA granules may inhibit liver carcinogenesis (at least in part) by reduction of oxidative stress and strengthening of immune functions.[17]

There are several reports of the usefulness of BCAA supplementation on the QoL of patients with liver cirrhosis.[42,43] Kawamura *et al.* demonstrated that, in 453 patients with chronic liver disease, QoL decreased significantly according to the progression of disease as assessed by the scores from Short Form 36 ($P < 0.05$) and that the QoL of patients with chronic liver diseases was improved in the BCAA granules administration group ($n = 13$) compared with the control group ($n = 12$) after 6 months.[42] Hepatic encephalopathy (HE) is a major complication in patients with liver cirrhosis that is related to a poor prognosis and poor QoL.[44] Sleep disturbance may be associated with minimal HE.[45] Les *et al.* conducted a randomized study involving 116 patients who had experienced an episode of HE (58 patients in the BCAA group and 58 patients in the maltodextrin group) to examine the effect of BCAA: they reported that supplementation with BCAA improves minimal HE and muscle mass.[43] Tryptophan, which is a precursor of the neurotransmitter 5-hydroxytryptamine (which is related to sleep disturbance), may be regulated by BCAA supplementation.[46]

With the wide range of pharmacological actions, such as increasing the serum albumin level,[16–19] inhibiting cirrhosis complications/angiogenesis/hepatic carcinogenesis,[17–20,22,27–29] improving insulin resistance[17,21,22] and fatty-acid metabolism,[17,24] reducing oxidative stress,[17,23] and increasing stimulation of the immune system,[17,25,26] therapy using BCAA granules may be an indispensable treatment for cirrhosis.

Significance of BCAA granules in different approaches to HCC treatment

Hepatectomy

Along with liver transplantation, hepatectomy is a curative treatment approach for HCC.[6,8,9,47–49] According to guidelines set by the European Association for the Study of the Liver (EASL), hepatectomy is indicated in patients with a single tumor of 2 cm or less in diameter, performance status (PS) 0, Child–Pugh class A and no portal hypertension.[50] In Japan, however, hepatectomy is considered in patients with three or less tumors of less than 3 cm in diameter, no vascular invasion, Child–Pugh class A or B, and expected tolerance to surgery, or even in those with four or more tumors of more than 3 cm in diameter and vascular invasion if they are expected to tolerate surgery and the treatment may improve the prognosis.[51] Hepatectomy is considered the first-line initial treatment for resectable HCC because of generally good surgical outcomes and poor availability of brain-dead liver donors in Japan.[52,53]

In HCC patients in whom a large volume of liver has been removed and in those with concurrent cirrhosis, the hepatic functional reserve is expected to decrease after resection. In several studies, the serum albumin level has been identified as a contributing factor for the prolonged postoperative survival time in HCC patients.[13,54–57] Thus, nutritional treatment with BCAA granules would be an essential approach based on this

observation as well as the fact that BCAA therapy prevents perioperative complications.

Togo *et al.* reported, in their study in 43 HCC patients with advanced cirrhosis, that post-hepatectomy treatment with BCAA granules inhibited the progression of cirrhosis and improved the prognosis.[58] The usefulness of oral nutritional supplements to prevent post-hepatectomy hepatic failure[59] and the usefulness of BCAA granules to inhibit postoperative HCC recurrence[29] have also been reported. Ichikawa *et al.* reported, in their prospective study in 56 HCC patients aged 65 years or more, that post-hepatectomy HCC recurrence was suppressed significantly and that the postoperative clinical course was more favorable in the BCAA treatment group ($n = 26$) compared with the regular-diet group ($n = 30$).[29]

Treatment with BCAA granules has appreciable clinical significance in HCC patients (especially those with underlying advanced cirrhosis) in terms of preserving hepatic functional reserve, preventing perioperative complications and inhibiting postoperative recurrence.

Liver transplantation

As an important choice of HCC treatment in western countries,[8,60,61] liver transplantation is considered even in patients with decompensated cirrhosis of various causes.[62] Assuming that the Milan criteria are satisfied, living donor partial liver transplantation for the treatment of decompensated cirrhosis complicated by HCC has been covered by the national health insurance system in Japan since 2004.[63] As described above, living donor liver transplantation is the major choice of treatment because of the shortage of brain-dead donors in Japan.[8,60,61,63,64]

The usefulness of BCAA granules in patients who have undergone liver transplantation has been reported in two studies.[65,66] In a prospective randomized study in 56 Child–Pugh class A cirrhotic patients without major complications, Kawamura *et al.* reported that early intervention with BCAA granules significantly decreased cirrhosis-related complications and prolonged the time to liver transplantation.[65] In a retrospective study in 236 patients who underwent living donor liver transplantation, Shirabe *et al.* reported a significant decrease in post-transplantation septic complications in patients pretreated with BCAA granules.[66] Considering the global shortage of liver donors,[6–9] BCAA granules could be a promising treatment for subjects undergoing liver transplantation.

Percutaneous treatment

Since its introduction in Japan in 1999, RFA has rapidly gained popularity because of its excellent antitumor effect and low extent of invasiveness. Percutaneous RFA is the first-line percutaneous treatment for HCC.[5–9,11,14,67–72] EASL guidelines recommend percutaneous RFA for HCC of PS 0–2, Child–Pugh class A or B, and three or less unresectable tumors of 3 cm or less in diameter. In Japan, percutaneous RFA is, in general, indicated for patients of Child–Pugh class A or B and three or less unresectable tumors of 3 cm or less in diameter. Even in patients with unresectable tumors of 3 cm or more in diameter, percutaneous RFA in combination with TACE is recommended to expand the ablated area.[50,51,73]

Percutaneous RFA is less invasive than hepatectomy, but hepatic functional reserve may decrease after RFA in some patients.[74–76] The possible causes of a postoperative decrease in the serum albumin level include: (i) decreased albumin synthesis secondary to hepatocyte decrease; (ii) inhibition of albumin synthesis by inflammatory cytokines; and (iii) loss of protein due to inflammation at the ablation site.[74–76] We reported the association between the serum albumin level and survival of HCC patients treated with percutaneous RFA, so therapy using BCAA granules may be a useful treatment for RFA-treated HCC frequently complicated by cirrhosis.[11,67]

One of the disadvantages of percutaneous RFA is the high prevalence of recurrence of HCC.[6,8,9,15,48,67] We found the prevalence of HCC 5 years after RFA to be approximately 80% even in patients with a single HCC.[67] The regimen to prevent HCC after RFA includes antiviral therapy (interferon therapy for hepatitis C and nucleoside analog therapy for hepatitis B) and liver-support therapy to keep the hepatic enzymes at a low level.[67,77–83] BCAA granules with potential anticarcinogenic effects may also be useful for preventing HCC recurrence post-RFA.[11,27]

Yoshiji *et al.* focused on the inhibitory action of BCAA granules and an angiotensin-converting enzyme inhibitor (ACE-I) against angiogenesis, and evaluated the effect of these agents in preventing post-RFA recurrence of HCC in a prospective randomized study.[27] The post-RFA prevalence of HCC and levels of vascular endothelial growth factor were decreased significantly in the combined BCAA granules and ACE-I treatment group compared with the control group, suggesting a possible synergistic effect of the two drugs to inhibit HCC recurrence after RFA.[27] Our retrospective controlled study in 256 HCC patients with a serum albumin level of

3.5 g/dL or less treated with percutaneous RFA showed significantly higher overall and recurrence-free survival in patients treated with BCAA granules ($n = 115$) compared with those receiving a regular diet ($n = 141$).[11] The use of BCAA granules was identified as a contributing factor to prolonged survival in a multivariate analysis.[11] The mechanism of the inhibitory effect of BCAA granules against HCC recurrence after RFA needs to be verified in a large-scale prospective study. BCAA granules may inhibit HCC recurrence in patients who have undergone percutaneous RFA as well as in those who have undergone hepatectomy.[11,29]

TACE

Transcatheter arterial chemoembolization is a combination of local chemotherapy through feeding blood vessels and the use of embolizing material.[16,84–87] TACE is most frequently used for the treatment of HCC in Japan, where it was originally developed.[84,87–90] EASL guidelines recommend TACE for unresectable, Child–Pugh class A or B multiple HCC with no vascular invasion, whereas in Japan the therapy is recommended even for HCC with vascular invasion if it is Vp1 or Vp2.[50,51]

The factors affecting the survival of HCC patients treated with TACE are: (i) tumor stage; (ii) tumor markers; and (iii) hepatic functional reserve.[84] Preserving hepatic functional reserve is a critical issue in HCC patients who, in general, are treated repeatedly with TACE.[16,88–92] However, in some patients, hepatic functional reserve decreases after TACE because of complications such as post-TACE syndrome.[93]

The usefulness of BCAA granules or BCAA-enriched "snacks" for patients with unresectable HCC treated with TACE has been suggested in several studies.[16,91,92] In a randomized controlled trial (RCT) in 56 HCC patients treated with TACE, Takeshita *et al.* found that the post-TACE decrease in liver function was suppressed significantly in patients who received an enteral nutritional formula for hepatic failure given as a late-evening snack (LES) compared with the control group.[91,94] Our retrospective controlled study in 99 HCC patients treated with TACE showed that therapy using BCAA granules significantly inhibited the decrease in hepatic functional reserve at 3 months and 6 months compared with the regular diet group.[16] According to EASL guidelines, if HCC with Child–Pugh class B treated with TACE recurs as Child–Pugh class C, TACE is not indicated for the recurred HCC. The significance of therapy using BCAA granules is considerable in terms of permitting repeated TACE.

Molecular-targeted drugs (sorafenib)

There had long been a lack of evidence to support systemic chemotherapy for unresectable advanced HCC.[95] However, after the efficacy of a molecular-targeted drug, sorafenib, for unresectable advanced HCC was demonstrated in two RCT (SHARP trial and Asia–Pacific trial), the drug was approved for the treatment of unresectable advanced HCC in Japan in 2009.[96,97]

The action of sorafenib against tumor growth and angiogenesis is based on the inhibition of the activities of intracellular kinase and receptor tyrosine kinase.[96–106] The new era of systemic chemotherapy for unresectable advanced HCC was started with the introduction of sorafenib.[96–103,106] EASL guidelines recommend sorafenib for unresectable, advanced, Child–Pugh class A or B HCC with PS 0–2 and vascular invasion or distant metastasis.[50] According to Japanese guidelines, sorafenib is recommended for unresectable, advanced, Child–Pugh class A HCC with vascular invasion or distant metastasis as well as for patients intolerant to TACE or in whom the procedure is anatomically unsuitable.[51,104,105]

Several cases of adverse events associated with the use of sorafenib have been reported.[96–106] Patients should be monitored carefully for hepatic dysfunction during sorafenib therapy because decreased hepatic reserve caused by sorafenib may result in irreversible hepatic failure.[102] Even if hepatic failure is avoided, sorafenib treatment may have to be discontinued or the dose reduced.[102] Many HCC patients treated with sorafenib have concurrent cirrhosis.[96–106] Hence, intervention with BCAA granules has appreciable importance in terms of preserving hepatic functional reserve and ensuring continued sorafenib treatment.[107] Our previous study revealed that therapy using BCAA granules significantly inhibited the decrease in serum albumin level and prolonged the duration of sorafenib treatment and survival in patients with a serum albumin level of 3.5 g/dL or less compared with the regular diet group.[107] The synergistic effect of sorafenib and therapy using BCAA granules to inhibit angiogenesis may have contributed to the better prognosis.

There remains a lack of evidence to support the effect of nutritional intervention in patients with unresectable advanced HCC treated with sorafenib. However, therapy using BCAA granules should be considered as a treatment option.

CONCLUSION

WE DISCUSSED THE significance of the use of BCAA granules in the treatment of cirrhosis and

Table 2 Summary of current knowledge of branched-chain amino acid granules for hepatocellular carcinoma (HCC) therapy

1. Prolongation of survival due to the improvement of hypoalbuminemia after HCC therapy
2. Improvement of liver cirrhosis-related complications after HCC therapy
3. Suppression of septic complications due to the activation of immune function after HCC therapy
4. Possibility of suppression of HCC recurrence after HCC therapy

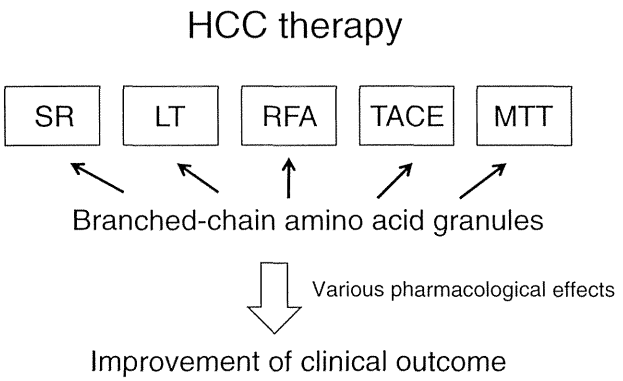

Figure 1 Schematic presentation of the effect of branched-chain amino acid granules for HCC therapy. HCC, hepatocellular carcinoma; LT, liver transplantation; MTT, molecular-targeted therapy; RFA, radiofrequency ablation; SR, surgical resection; TACE, transcatheter arterial chemoembolization.

HCC based on a review of the published work as well as our own data. With a variety of pharmacological actions, BCAA granules are a promising treatment for HCC. (Fig. 1) Summary of current knowledge of BCAA granules for HCC therapy is shown in Table 2.

REFERENCES

1 Moriwaki H, Miwa Y, Tajika M *et al.* Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. *Biochem Biophys Res Commun* 2004; **313**: 405–9.
2 Charlton MR. Branched-chain amino acid enriched supplements as therapy for liver disease. *J Nutr* 2006; **136** (1 Suppl): 295S–8S.
3 Tajika M, Kato M, Mohri H *et al.* Prognostic value of energy metabolism in patients with viral liver cirrhosis. *Nutrition* 2002; **18**: 229–34.
4 Greco AV, Mingrone G, Benedetti G *et al.* Daily energy and substrate metabolism in patients with cirrhosis. *Hepatology* 1998; **27**: 346–50.
5 Kudo M. Radiofrequency ablation for hepatocellular carcinoma: updated review in 2010. *Oncology* 2010; **78**: 113–24.
6 Livraghi T, Mäkisalo H, Line PD. Treatment options in hepatocellular carcinoma today. *Scand J Surg* 2011; **100**: 22–9.
7 El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264–73.
8 de Lope CR, Tremosini S, Forner A *et al.* Management of HCC. *J Hepatol* 2012; **56** (Suppl 1): S75–S87.
9 El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118–27.
10 Mazzaferro V, Regalia E, Doci R *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693–9.
11 Nishikawa H, Osaki Y, Iguchi E *et al.* The effect of long-term supplementation with branched-chain amino acid granules in patients with hepatitis C virus-related hepatocellular carcinoma after radiofrequency thermal ablation. *J Clin Gastroenterol* 2013; **47**: 359–66.
12 Zhou WP, Lai EC, Li AJ *et al.* A prospective, randomized, controlled trial of preoperative transarterial chemoembolization for resectable large hepatocellular carcinoma. *Ann Surg* 2009; **249**: 195–202.
13 Nishikawa H, Arimoto A, Wakasa T *et al.* Effect of transcatheter arterial chemoembolization prior to surgical resection for hepatocellular carcinoma. *Int J Oncol* 2013; **42**: 151–60.
14 Nishikawa H, Osaki Y, Iguchi E *et al.* Percutaneous radiofrequency ablation therapy for recurrent hepatocellular carcinoma. *Anticancer Res* 2012; **32**: 5059–65.
15 Nishikawa H, Osaki Y, Kita R *et al.* Transcatheter arterial infusion chemotherapy prior to radiofrequency thermal ablation for single hepatocellular carcinoma reduces the risk of intrahepatic distant recurrence. *Int J Oncol* 2012; **41**: 903–9.
16 Nishikawa H, Osaki Y, Inuzuka T *et al.* Branched-chain amino acid treatment before transcatheter arterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 2012; **18**: 1379–84.
17 Kawaguchi T, Izumi N, Charlton MR *et al.* Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology* 2011; **54**: 1063–70.
18 Muto Y, Sato S, Watanabe A, Moriwaki H *et al.* Long-Term Survival Study Group. Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. *Clin Gastroenterol Hepatol* 2005; **3**: 705–13.
19 Moriwaki H, Shiraki M, Fukushima H *et al.* Long-term outcome of branched-chain amino acid treatment in patients with liver cirrhosis. *Hepatol Res* 2008; **38**: S102–S106.

20 Hayaishi S, Chung H, Kudo M *et al.* Oral branched-chain amino acid granules reduce the incidence of hepatocellular carcinoma and improve event-free survival in patients with liver cirrhosis. *Dig Dis* 2011; **29**: 326–32.
21 Yoshiji H, Noguchi R, Kaji K *et al.* Attenuation of insulin-resistance-based hepatocarcinogenesis and angiogenesis by combined treatment with branched-chain amino acids and angiotensin-converting enzyme inhibitor in obese diabetic rats. *J Gastroenterol* 2010; **45**: 443–50.
22 Yoshiji H, Noguchi R, Kitade M *et al.* Branched-chain amino acids suppress insulin-resistance-based hepatocarcinogenesis in obese diabetic rats. *J Gastroenterol* 2009; **44**: 483–91.
23 Ohno T, Tanaka Y, Sugauchi F *et al.* Suppressive effect of oral administration of branched-chain amino acid granules on oxidative stress and inflammation in HCV-positive patients with liver cirrhosis. *Hepatol Res* 2008; **38**: 683–8.
24 Nishimura J, Masaki T, Arakawa M *et al.* Isoleucine prevents the accumulation of tissue triglycerides and upregulates the expression of PPARalpha and uncoupling protein in diet-induced obese mice. *J Nutr* 2010; **140**: 496–500.
25 Kakazu E, Ueno Y, Kondo Y *et al.* Branched chain amino acids enhance the maturation and function of myeloid dendritic cells ex vivo in patients with advanced cirrhosis. *Hepatology* 2009; **50**: 1936–45.
26 Nakamura I, Ochiai K, Imai Y *et al.* Restoration of innate host defense responses by oral supplementation of branched-chain amino acids in decompensated cirrhotic patients. *Hepatol Res* 2007; **37**: 1062–7.
27 Yoshiji H, Noguchi R, Ikenaka Y *et al.* Combination of branched-chain amino acids and angiotensin-converting enzyme inhibitor suppresses the cumulative recurrence of hepatocellular carcinoma: a randomized control trial. *Oncol Rep* 2011; **26**: 1547–53.
28 Muto Y, Sato S, Watanabe A *et al.* for the Long-Term Survival Study (LOTUS) Group. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* 2006; **35**: 204–14.
29 Ichikawa K, Okabayashi T, Maeda H *et al.* Oral supplementation of branched-chain amino acids reduces early recurrence after hepatic resection in patients with hepatocellular carcinoma: a prospective study. *Surg Today* 2013; **43**: 720–6.
30 Kumada H, Okanoue T, Onji M *et al.* Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, Ministry of Health, Labour and Welfare of Japan. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40** (1): 8–13.
31 ASPEN Board of Directors and the Clinical Guidelines Task Force. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. *JPEN J Parenter Enteral Nutr* 2002; **26** (1 Suppl): 1SA–138SA.
32 Plauth M, Cabre E, Riggio O *et al.* ESPEN Guidelines on Enteral Nutrition: liver disease. *Clin Nutr* 2006; **25**: 285–94.
33 Yatsuhashi H, Ohnishi Y, Nakayama S *et al.* Anti-hypoalbuminemic effect of branched-chain amino acid granules in patients with liver cirrhosis is independent of dietary energy and protein intake. *Hepatol Res* 2011; **41**: 1027–35.
34 Fukushima H, Miwa Y, Shiraki M *et al.* Oral branched-chain amino acid supplementation improves the oxidized/reduced albumin ratio in patients with liver cirrhosis. *Hepatol Res* 2007; **37**: 765–70.
35 Jalan R, Schnurr K, Mookerjee RP *et al.* Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality. *Hepatology* 2009; **50**: 555–64.
36 Takaguchi K, Moriwaki H, Doyama H *et al.* Effects of branched-chain amino acid granules on serum albumin level and prognosis are dependent on treatment adherence in patients with liver cirrhosis. *Hepatol Res* 2013; **43**: 459–66.
37 Suzuki K, Endo R, Kohgo Y *et al.* for the Japanese Nutritional Study Group for Liver Cirrhosis 2008. Guidelines on nutritional management in Japanese patients with liver cirrhosis from the perspective of preventing hepatocellular carcinoma. *Hepatol Res* 2012; **42**: 621–6.
38 Habu D, Nishiguchi S, Nakatani S *et al.* Comparison of the effect of BCAA granules on between decompensated and compensated cirrhosis. *Hepatogastroenterology* 2009; **56** (96): 1719–23.
39 Habu D, Nishiguchi S, Nakatani S *et al.* Relationship between branched-chain amino acid to tyrosine ratio (BTR) and porto-systemic shunt in the Child-Pugh grade A cirrhosis determined by per-rectal portal scintigraphy. *Hepatol Res* 2003; **27**: 57–61.
40 Shimizu M, Kubota M, Tanaka T *et al.* Nutraceutical approach for preventing obesity-related colorectal and liver carcinogenesis. *Int J Mol Sci* 2012; **13**: 579–95.
41 Kawaguchi T, Nagao Y, Matsuoka H *et al.* Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease. *Int J Mol Med* 2008; **22**: 105–12.
42 Kawamura N, Nakajima H, Takashi SI. Administration of granulated BCAA and quality of life. *Hepatol Res* 2004; **30S**: 42–5.
43 Les I, Doval E, García-Martínez R *et al.* Effects of branched-chain amino acids supplementation in patients with cirrhosis and a previous episode of hepatic encephalopathy: a randomized study. *Am J Gastroenterol* 2011; **106**: 1081–8.

44 Marchesini G, Bianchi G, Amodio P *et al.* Italian Study Group for quality of life in cirrhosis. Factors associated with poor health-related quality of life of patients with cirrhosis. *Gastroenterology* 2001; **120**: 170–8.
45 Bajaj JS, Saeian K, Schubert CM, Franco R, Franco J, Heuman DM. Disruption of sleep architecture in minimal hepatic encephalopathy and ghrelin secretion. *Aliment Pharmacol Ther* 2011; **34**: 103–5.
46 Saleem DM, Haider S, Khan MM, Shamsi T, Haleem DJ. Role of tryptophan in the pathogenesis of hepatic encephalopathy. *J Pak Med Assoc* 2008; **58**: 68–70.
47 Makuuchi M, Sano K. The surgical approach to HCC: our progress and results in Japan. *Liver Transpl* 2004; **10** (2 Suppl 1): S46–52.
48 Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362** (9399): 1907–17.
49 Rahbari NN, Mehrabi A, Mollberg NM *et al.* Hepatocellular carcinoma: current management and perspectives for the future. *Ann Surg* 2011; **253**: 453–69.
50 European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908–43.
51 Kudo M, Izumi N, Kokudo N *et al.* HCC Expert Panel of Japan Society of Hepatology. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis* 2011; **29**: 339–64.
52 Makuuchi M, Donadon M, Torzilli G. Hepatic resection for hepatocellular carcinoma in cirrhosis. *Ann Ital Chir* 2008; **79**: 111–15.
53 Ikai I, Arii S, Okazaki M *et al.* Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. *Hepatol Res* 2007; **37**: 676–91.
54 Horino K, Beppu T, Kuroki H *et al.* Glasgow Prognostic Score as a useful prognostic factor after hepatectomy for hepatocellular carcinoma. *Int J Clin Oncol* 2012 Jul 21. [Epub ahead of print].
55 Takuma Y, Nouso K, Makino Y *et al.* Outcomes after curative treatment for cryptogenic cirrhosis-associated hepatocellular carcinoma satisfying the Milan criteria. *J Gastroenterol Hepatol* 2011; **26**: 1417–24.
56 Ikai I, Arii S, Kojiro M *et al.* Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. *Cancer* 2004; **101**: 796–802.
57 Chang WT, Kao WY, Chau GY *et al.* Hepatic resection can provide long-term survival of patients with non-early-stage hepatocellular carcinoma: extending the indication for resection? *Surgery* 2012; **152**: 809–20.
58 Togo S, Tanaka K, Morioka D *et al.* Usefulness of granular BCAA after hepatectomy for liver cancer complicated with liver cirrhosis. *Nutrition* 2005; **21**: 480–6.
59 [No authors listed] Long-term oral administration of branched chain amino acids after curative resection of hepatocellular carcinoma: a prospective randomized trial. The San-in Group of Liver Surgery. Long-term oral administration of branched chain amino acids after curative resection of hepatocellular carcinoma: a prospective randomized trial. The San-in Group of Liver Surgery. *Br J Surg* 1997; **84**: 1525–31.
60 Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis* 2010; **30**: 61–74.
61 Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A, OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13** (1): e11–22.
62 Tsochatzis EA, Bosch J, Burroughs AK. New therapeutic paradigm for patients with cirrhosis. *Hepatology* 2012; **56**: 1983–92.
63 Sugawara Y, Makuuchi M. Living donor liver transplantation: present status and recent advances. *Br Med Bull* 2006; **75-76**: 15–28.
64 Sugawara Y, Makuuchi M. Advances in adult living donor liver transplantation: a review based on reports from the 10th anniversary of the adult-to-adult living donor liver transplantation meeting in Tokyo. *Liver Transpl* 2004; **10**: 715–20.
65 Kawamura E, Habu D, Morikawa H *et al.* A randomized pilot trial of oral branched-chain amino acids in early cirrhosis: validation using prognostic markers for pre-liver transplant status. *Liver Transpl* 2009; **15**: 790–7.
66 Shirabe K, Yoshimatsu M, Motomura T *et al.* Beneficial effects of supplementation with branched-chain amino acids on postoperative bacteremia in living donor liver transplant recipients. *Liver Transpl* 2011; **17**: 1073–80.
67 Nishikawa H, Osaki Y, Iguchi E *et al.* Radiofrequency ablation for hepatocellular carcinoma: the relationship between a new grading system for the ablative margin and clinical outcomes. *J Gastroenterol* 2012 Oct 12. [Epub ahead of print].
68 Nishikawa H, Inuzuka T, Takeda H *et al.* Percutaneous radiofrequency ablation therapy for hepatocellular carcinoma: a proposed new grading system for the ablative margin and prediction of local tumor progression and its validation. *J Gastroenterol* 2011; **46**: 1418–26.
69 Shiina S, Tateishi R, Arano T *et al.* Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569–77.
70 Tateishi R, Shiina S, Teratani T *et al.* Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005; **103**: 1201–9.
71 Nishikawa H, Osaki Y, Iguchi E *et al.* Percutaneous radiofrequency ablation for hepatocellular carcinoma: clinical outcome and safety in elderly patients. *J Gastrointestin Liver Dis* 2012; **21**: 397–405.

72 Tiong L, Maddern GJ. Systematic review and meta-analysis of survival and disease recurrence after radiofrequency ablation for hepatocellular carcinoma. *Br J Surg* 2011; **98**: 1210–24.
73 Morimoto M, Numata K, Kondou M, Nozaki A, Morita S, Tanaka K. Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: a randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer* 2010; **116**: 5452–60.
74 Li JX, Wu H, Huang JW, Zeng Y. The influence on liver function after transcatheter arterial chemoembolization combined with percutaneous radiofrequency ablation in patients with hepatocellular carcinoma. *J Formos Med Assoc* 2012; **111**: 510–15.
75 Kuroda H, Ushio A, Miyamoto Y *et al.* Effects of branched-chain amino acid-enriched nutrient for patients with hepatocellular carcinoma following radiofrequency ablation: a one-year prospective trial. *J Gastroenterol Hepatol* 2010; **25**: 1550–5.
76 Morihara D, Iwata K, Hanano T *et al.* Late-evening snack with branched-chain amino acids improves liver function after radiofrequency ablation for hepatocellular carcinoma. *Hepatol Res* 2012; **42**: 658–67.
77 Shimomura S, Ikeda N, Saito M *et al.* Long-term interferon therapy after radiofrequency ablation is effective in treating patients with HCV-associated hepatocellular carcinoma. *Hepatol Int* 2010; **5**: 559–66.
78 Kudo M, Sakaguchi Y, Chung H *et al.* Long-term interferon maintenance therapy improves survival in patients with HCV-related hepatocellular carcinoma after curative radiofrequency ablation. A matched case-control study. *Oncology* 2007; **72** (Suppl 1): 132–8.
79 Xia F, Lai EC, Lau WY *et al.* High serum hyaluronic acid and HBV viral load are main prognostic factors of local recurrence after complete radiofrequency ablation of hepatitis B-related small hepatocellular carcinoma. *Ann Surg Oncol* 2012; **19**: 1284–91.
80 Goto T, Yoshida H, Tateishi R *et al.* Influence of serum HBV DNA load on recurrence of hepatocellular carcinoma after treatment with percutaneous radiofrequency ablation. *Hepatol Int* 2011; **5**: 767–73.
81 Oyama K, Shiota G, Ito H, Murawaki Y, Kawasaki H. Reduction of hepatocarcinogenesis by ursodeoxycholic acid in rats. *Carcinogenesis* 2002; **23**: 885–92.
82 Chung GE, Yoon JH, Lee JH *et al.* Ursodeoxycholic acid-induced inhibition of DLC1 protein degradation leads to suppression of hepatocellular carcinoma cell growth. *Oncol Rep* 2011; **25**: 1739–46.
83 Chen CJ, Yang HI, Su J *et al.* REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65–73.
84 Takayasu K, Arii S, Ikai I *et al.* Liver Cancer Study Group of Japan. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; **131**: 461–9.
85 Matsui O. Current status of hepatocellular carcinoma treatment in Japan: transarterial chemoembolization. *Clin Drug Investig* 2012; **32** (Suppl 2): 3–13.
86 Matsui O, Miyayama S, Sanada J *et al.* Interventional oncology: new options for interstitial treatments and intravascular approaches: superselective TACE using iodized oil for HCC: rationale, technique and outcome. *J Hepatobiliary Pancreat Sci* 2010; **17**: 407–9.
87 Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S. Hepatic artery embolization in 120 patients with unresectable hepatoma. *Radiology* 1983; **148**: 397–401.
88 Takayasu K. Transarterial chemoembolization for hepatocellular carcinoma over three decades: current progress and perspective. *Jpn J Clin Oncol* 2012; **42**: 247–55.
89 Takayasu K, Arii S, Kudo M *et al.* Superselective transarterial chemoembolization for hepatocellular carcinoma. Validation of treatment algorithm proposed by Japanese guidelines. *J Hepatol* 2012; **56**: 886–92.
90 Ikeda K, Kumada H, Saitoh S, Arase Y, Chayama K. 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**: 2150–4.
91 Takeshita S, Ichikawa T, Nakao K *et al.* A snack with oral branched-chain amino acids prevents a fall in albumin in patients with liver cirrhosis undergoing chemoembolization for hepatocellular carcinoma. *Nutr Res* 2009; **29**: 89–93.
92 Poon RT, Yu WC, Fan ST, Wong J. Long-term oral branched chain amino acids in patients undergoing chemoembolization for hepatocellular carcinoma: a randomized trial. *Aliment Pharmacol Ther* 2004; **19**: 779–88.
93 Pomoni M, Malagari K, Moschouris H *et al.* Post embolization syndrome in doxorubicin eluting chemoembolization with DC bead. *Hepatogastroenterology* 2012; **59** (115): 820–5.
94 Koreeda C, Seki T, Okazaki K, Ha-Kawa SK, Sawada S. Effects of late evening snack including branched-chain amino acid on the function of hepatic parenchymal cells in patients with liver cirrhosis. *Hepatol Res* 2011; **41**: 417–22.
95 Nishikawa H, Osaki Y, Kita R, Kimura T. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma in Japan. *Cancers* 2012; **4**: 165–83.
96 Llovet JM, Ricci S, Mazzaferro V *et al.* SHARP Investigators Study Group: sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378–90.
97 Cheng AL, Kang YK, Chen Z *et al.* Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III

randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25–34.
98 Abou-Alfa GK, Schwartz L, Ricci S *et al.* Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 4293–300.
99 Baek KK, Kim JH, Uhm JE *et al.* Prognostic factors in patients with advanced hepatocellular carcinoma treated with sorafenib: a retrospective comparison with previously known prognostic models. *Oncology* 2011; **80**: 167–74.
100 Llovet JM, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J, on behalf of the SHARP Investigators Study Group. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2290–300.
101 Takeda H, Nishikawa H, Iguchi E *et al.* Sorafenib-induced acute interstitial pneumonia in patients with advanced hepatocellular carcinoma: report of three cases. *Clin J Gastroenterol* 2012; **5**: 407–12.
102 Takeda H, Nishikawa H, Iguchi E *et al.* Impact of pretreatment serum cholinesterase level in unresectable advanced hepatocellular carcinoma patients treated with sorafenib. *Mol Clin Oncol* 2013; **1**: 241–48.
103 Nishikawa H, Osaki Y, Iguchi E *et al.* Comparison of the efficacy of transcatheter arterialchemoembolization and sorafenib for advanced hepatocellular carcinoma. *Exp Ther Med* 2012; **4**: 381–6.
104 Kudo M, Tateishi R, Yamashita T *et al.* Current status of hepatocellular carcinoma treatment in Japan: case study and discussion-voting system. *Clin Drug Investig* 2012; **32** (Suppl 2): 37–51.
105 Kudo M, Ueshima K, Arizumi T. Real-life clinical practice with sorafenib in advanced hepatocellular carcinoma: a single-center experience. *Dig Dis* 2012; **30**: 609–16.
106 Inuzuka T, Nishikawa H, Sekikawa A *et al.* Complete response of advanced hepatocellular carcinoma with multiple lung metastases treated with sorafenib: a case report. *Oncology* 2011; **81** (Suppl 1): 152–7.
107 Takeda H, Nishikawa H, Iguchi E *et al.* Effect of treatment with branched-chain amino acids during sorafenib therapy for unresectable hepatocellular carcinoma. *Hepatol Res* 2013. doi: 10.1111/hepr.12125

Accuracy of Spleen Stiffness Measurement in Detection of Esophageal Varices in Patients With Chronic Liver Disease: Systematic Review and Meta-analysis

Siddharth Singh,* John E. Eaton,* Mohammad H. Murad,‡ Hironori Tanaka,§ Hiroko Iijima,§ and Jayant A. Talwalkar*

*Division of Gastroenterology and Hepatology, Department of Internal Medicine, and ‡Knowledge and Evaluation Research Unit, Mayo Clinic, Rochester, Minnesota; and §Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

BACKGROUND & AIMS: **Spleen stiffness measurement (SSM) is a promising noninvasive alternative to esophagogastroduodenoscopy (EGD) that could be used in the diagnosis of esophageal varices (EV) in patients with cirrhosis. However, its overall diagnostic accuracy in various clinical settings is unknown. We conducted a systematic review and meta-analysis of studies that compared the accuracy of SSM with that of EGD in detecting EV in patients with chronic liver disease.**

METHODS: **Through a systematic search of bibliographic databases and conference proceedings, and contact with authors, we identified 12 studies that reported the accuracy of SSM, compared with EGD, in the diagnosis of any and/or clinically significant EV in adults with chronic liver disease. In a meta-analysis, we combined measures of test performance of individual studies.**

RESULTS: **Based on pooled estimates, SSM detected the presence of any EV with 78% sensitivity (95% confidence interval [CI], 75%–81%), 76% specificity (95% CI, 72%–79%), a positive likelihood ratio (LR) of 3.4 (95% CI, 2.3–4.9), a negative LR of 0.2 (95% CI, 0.1–0.4), and a diagnostic odds ratio of 19.3 (95% CI, 7.5–49.8). In a meta-analysis of 9 studies, SSM detected the presence of clinically significant EV with 81% sensitivity (95% CI, 76%–86%), 66% specificity (95% CI, 61%–69%), a positive LR of 2.5 (95% CI, 1.7–3.9), a negative LR of 0.2 (95% CI, 0.1–0.5), and a diagnostic odds ratio of 12.6 (95% CI, 5.5–28.7). There was significant heterogeneity among studies owing to differences in elastography techniques and study locations. The included studies that were at risk for spectrum bias, review bias, and disease progression bias.**

CONCLUSIONS: **Based on a meta-analysis, current techniques for measuring spleen stiffness are limited in their accuracy of EV diagnosis; these limitations preclude widespread use in clinical practice at this time.**

Keywords: Elastography; Portal Hypertension; Accuracy; Cirrhosis.

Esophageal varices (EV) are present in 50% of patients with cirrhosis, and bleeding from EV is associated with high mortality.[1,2] Endoscopic screening for EV is recommended for all patients at the time of cirrhosis diagnosis, followed by surveillance at frequent intervals depending on the size and treatment of varices.[1] However, because the point prevalence of medium/large varices, which are at highest risk of bleeding and that benefit from prophylactic therapy with β-blockers, is only 15% to 25%, the majority of cirrhotic patients who undergo screening esophagogastroduodenoscopy (EGD) either do not have varices or have small EV that do not warrant prophylactic therapy.[1] This invasive test is potentially associated with complications related to sedation and the procedure itself, and also increased costs of medical care.[3] Hence, there is great interest in developing noninvasive techniques to detect EV.[4]

Recent studies have shown that spleen stiffness correlates with hepatic fibrosis and portal hypertension in

Abbreviations used in this paper: ARFI, acoustic radiation force impulse imaging; AUROC, area under receiver operating curve; BMI, body mass index; CI, confidence interval; EGD, esophagogastroduodenoscopy; EV, esophageal varices; LR, likelihood ratio; OR, odds ratio; ROC, receiver operating curve; RTE, real-time tissue elastography; SSM, spleen stiffness measurement; TE, transient elastography; VTTQ, virtual touch tissue quantification.

1542-3565/$36.00
http://dx.doi.org/10.1016/j.cgh.2013.09.013

patients with chronic liver disease.[5,6] Subsequent published studies have suggested that spleen stiffness measurement (SSM) can be used to predict the presence and size of EV in patients with chronic liver disease with high diagnostic accuracy,[5,7] although these results have not been replicated universally.[8] In addition, there is considerable variability across different techniques of measuring spleen stiffness, including transient elastography (TE) and acoustic radiation force impulse imaging (ARFI), as well as the performance of SSM across different stages and etiologies of chronic liver disease.[9]

Hence, we sought to conduct a systematic review and meta-analysis to characterize the diagnostic performance of SSM as compared with EGD as the reference standard, for predicting the presence and size of EV, in patients with chronic liver diseases.

Methods

This systematic review was conducted following guidance provided by the Cochrane handbook for systematic reviews of diagnostic test accuracy,[10] and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[11] The process followed an a priori–established protocol.

Search Strategy

With the assistance of an expert librarian, we first performed a systematic search of PubMed, EMBASE, Web of Science, and Cochrane Library from database inception through March 31, 2013, for all relevant articles on the assessment of spleen stiffness for diagnosis of EV. Medical subject heading terms used in the search included "stiff*," "elast*," AND "spleen." The title and abstract of studies identified in the search were reviewed by 2 authors independently (S.S. and J.E.E.) to exclude studies that did not answer the research question of interest (details of selection criteria are described later). The full text of the remaining articles was examined to determine whether it contained relevant information. Next, the bibliographies of the selected articles, as well as review articles on the topics, were searched manually for additional articles. Third, a manual search of abstracts from major gastroenterology and hepatology conferences between 2008 and 2012 (American Association for the Study of the Liver annual meeting, European Association for the Study of the Liver annual meeting, and Digestive Diseases Week) was performed for additional studies on the topic. In case of missing or incomplete data, the primary authors' of the studies were contacted for additional information.

Selection Criteria

Studies included in this meta-analysis were observational studies that met the following inclusion criteria: (1) performed in patients with intrinsic chronic liver diseases, due to any etiology with or without evidence of portal hypertension or cirrhosis, (2) provided adequate description of SSM using either ultrasound-based or magnetic resonance–based elastography, as well as (3) assessment of EV based on upper endoscopy (EGD) as the gold standard, and (4) provided sufficient data (either in the primary article or after contact with study authors) to allow estimation of test performance (sensitivity, specificity, prevalence of EV in the study population). Inclusion was not otherwise restricted by study size, language, or publication type. When there were multiple publications from the same cohort, data from the most recent comprehensive report were included. Discrepancies in article selection were resolved by joint re-evaluation of the article and through consensus with a senior reviewer (J.A.T.).

Data Abstraction and Quality Assessment

The following data from each study were abstracted: (1) study characteristics: primary author; time period of study/year of publication; and country of study; (2) patient characteristics: age, sex, body mass index (BMI), underlying etiology of the chronic liver disease (viral vs nonviral), stage of liver disease (noncirrhotic, compensated cirrhosis, decompensated cirrhosis), and Child–Pugh class; (3) spleen stiffness assessment: technique (TE, ARFI, magnetic resonance elastography, real-time tissue elastography [RTE], virtual touch tissue quantification [VTTQ]), diagnostic threshold (or cut-off) corresponding to maximum sensitivity and specificity values from the receiver operator curve (ROC); (4) outcomes reported: presence or absence of EV, assessment and definition of clinically significant EV; (5) test performance of SSM: sensitivity, specificity, prevalence of outcome of interest in study (to impute numbers of true-positive, true-negative, false-positive, and false-negative results), and area under ROC (AUROC).

The quality assessment of included studies was performed by 2 investigators independently (S.S. and J.E.E.) using the quality assessment of diagnostic accuracy studies (QUADAS) questionnaire, which was designed to assess the internal and external validity of diagnostic accuracy studies included in systematic reviews.[12] This tool is a 14-item instrument that allows for the identification of important design elements in diagnostic accuracy studies such as patient spectrum, the presence or absence of observer blinding and verification bias, handling of indeterminate results, and reporting of patient loss to follow-up evaluation. Each item was scored as "yes" if reported (1 point) or as "no" if not reported, or as "unclear" if there is no adequate information in the article to make an accurate judgment (0 points). A score of 10 or higher was considered suggestive of a high-quality study.

Outcomes Assessed

The primary outcome for analysis was the diagnostic performance of SSM for the detection of EV in patients with chronic liver disease compared with the reference standard of EGD. Assessing the diagnostic accuracy of SSM for detecting clinically significant EV was analyzed as a secondary outcome. A priori hypotheses to explain potential heterogeneity included technique of SSM (TE vs ARFI vs RTE/VTTQ), location of study (Asian population vs Western population), and etiology of chronic liver disease (viral vs nonviral).

Statistical Analysis

Because of a priori assumptions about the likelihood for heterogeneity between primary studies, the random-effects model of DerSimonian and Laird[13] was used for meta-analysis. Analyses were performed using the statistical software Meta-DiSc (version 1.1.1; Ramón y Cajal Hospital, Madrid, Spain).

Diagnostic test characteristics. Pooled summary statistics (and 95% confidence intervals [CIs]) for sensitivity, specificity, positive likelihood ratio [LR], negative LR, and diagnostic odds ratios (ORs) for the test performance of SSM for the diagnosis of EV and clinically significant EV was calculated. A positive LR is the probability of a person who has the disease (ie, EV), testing positive (ie, positive SSM) divided by the probability of a person who does not have the disease testing positive (ie, positive LR = sensitivity/[1-specificity]); negative LR is the probability of a person who has the disease, testing negative divided by the probability of a person who does not have the disease testing negative (ie, negative LR = [1-sensitivity]/specificity). A positive LR higher than 5 and a negative LR less than 0.2 provide strong diagnostic evidence.[14] From the positive and negative LRs, the diagnostic OR was estimated, which represents the odds of having a positive SSM in patients with EV compared with the odds of a positive SSM in patients without EV, as a single indicator of test accuracy that comprises a combination of sensitivity and specificity information.[15] Based on hypothetical situations of low (25%), ambiguous (50%), and high (75%) pretest probabilities of the presence of EV in patients with chronic liver disease, we estimated the post-test probabilities of EV with a positive or negative SSM test result using the Bayes' theorem and represented it using the Fagan nomogram.[16] We also used the derived estimates of sensitivity, specificity, and respective variances to construct a summary ROC curve using a weighted linear model according to Littenberg and Moses,[17] and the AUROC was used as an alternative global measure of test performance.

Besides conventional pooling of sensitivity and specificity and reporting a summary ROC curve, we also performed a sensitivity analysis by using the fitted bivariate regression model, as suggested by Reitsma et al,[18] and modified by Chu and Cole.[19] This test assumes the presence of an implicit threshold effect and that sensitivity and specificity likely are dependent and correlated variables in estimating pooled sensitivity and specificity.

Evaluation of heterogeneity. The between-study heterogeneity of all diagnostic test parameters was evaluated initially by graphic examination of Forest plots for sensitivity and specificity. Statistical assessment then was performed using 2 methods: first, the chi-square test of homogeneity, which tests the null hypothesis that all studies in a meta-analysis have the same underlying magnitude of effect, was measured.[20] Because this test is underpowered to detect moderate degrees of heterogeneity, a P value of less than .10 was considered suggestive of significant heterogeneity.[20] Second, the inconsistency index (I^2 statistic), which estimates what proportion of total variation across studies was caused by heterogeneity rather than chance, was calculated.[20] In this, I^2 value of greater than 50% was suggestive of considerable heterogeneity.[21] Sources of heterogeneity were investigated using subgroup analyses by stratifying original estimates according to study characteristics as described earlier.

Publication bias. We tested for the presence of publication bias by using a regression of the diagnostic log OR against $1/(\text{effective sample size})^{1/2}$ and weighting according to the effective sample size, with a P value less than .10 indicating significant asymmetry, and suggestive of a significant publication bias.[22]

Results

Of 120 unique full-text articles identified using the search strategy, 8 studies met the inclusion criteria (7 studies on the presence or absence of EV, 1 study reporting only on the presence of clinically significant EV with insufficient data on diagnostic accuracy of SSM for the presence or absence of EV).[5–8,23–26] The coefficient of agreement between the 2 reviewers for study identification ($\kappa = 0.88$; 95% CI, 0.72–1.00) was very good. One study reported the diagnostic performance in 2 separate populations, one as a pilot cohort and another as a validation cohort, and this was treated as 2 separate studies.[6] In addition, 4 studies presented at conferences with relevant data were included.[27–30] Of the 11 investigators contacted for additional data, 5 responded and graciously provided additional data for relevant analysis.[5,6,24,28,30] Two studies were excluded because they reported the diagnostic accuracy of SSM in patients with idiopathic portal hypertension[31] or extrahepatic portal venous obstruction.[32] Hence, a total of 12 studies on 1497 patients with chronic liver disease reporting diagnostic performance of SSM for the presence or absence of EV (735 patients with any grade of EV) were included.[5–8,23–25,27–30] Figure 1 shows the flow diagram summarizing study identification and selection.

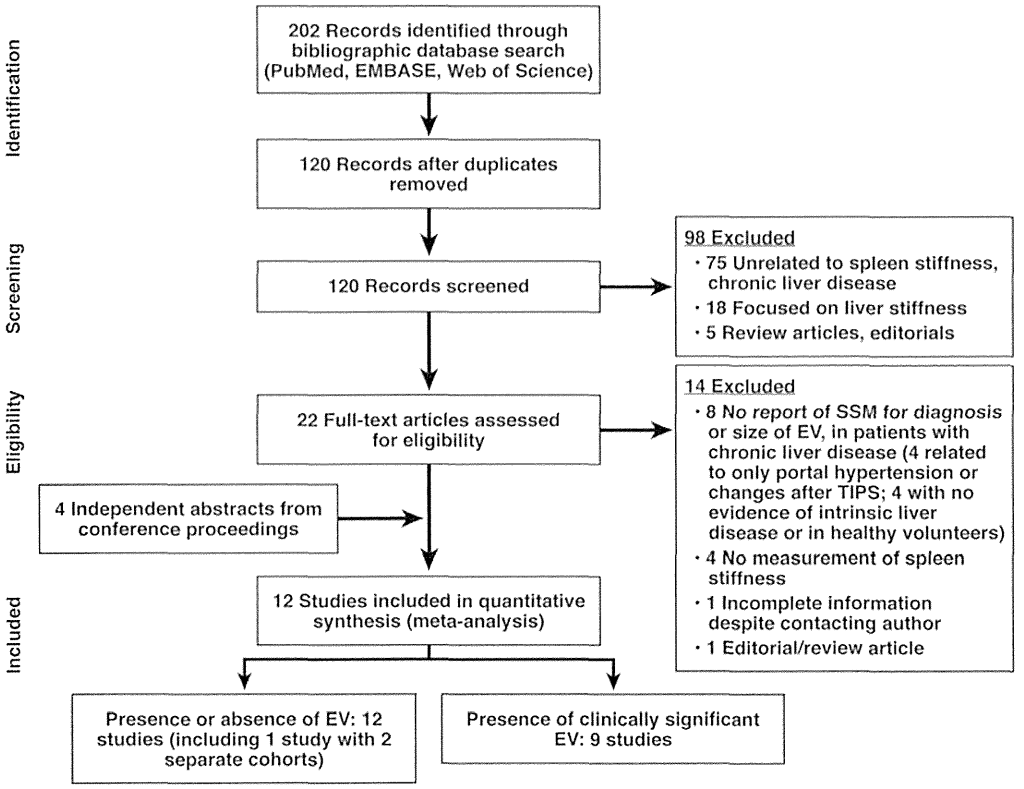

Figure 1. Flow diagram summarizing study identification and selection.

Nine studies (5 full-text studies,[7,8,24–26] 4 abstracts or author contact[6,28–30]) provided sufficient data to estimate the diagnostic performance of SSM for assessing the presence of clinically significant EV.

Characteristics and Quality of Included Studies

The characteristics of the included studies are shown in Tables 1 and 2. Seven studies were performed in Western populations (all in Europe)[5,24–29] and 6 studies were performed in Asian populations.[6–8,23,30] The first study recruited patients in 2008. The weighted mean age of the participants in the studies was 59.3 years (mean age range in individual studies, 39.3–68 y); 884 patients were males (59%; range in individual studies, 50%–78%). Viral hepatitis was the leading etiology of chronic liver disease in the included studies, with 5 studies performed only in patients with hepatitis B or C.[5,8,23,27,29] Ten studies focused primarily on patients with cirrhosis,[5,7,8,23–29] with at least 3 of them performed only in patients with compensated Child–Pugh A cirrhosis.[5,28,29] SSM was performed using TE in 5 studies (range of diagnostic threshold in individual studies, 46.0–50.4 kPa),[5,23,25,27,29] ARFI in 5 studies (range, 3.08–3.48 m/s),[7,8,24,26,28] RTE in 2 studies (diagnostic threshold expressed as elastic ratio, 8.24),[6] and VTTQ in 1 study (diagnostic threshold, 2.73 m/s).[30]

The overall quality of evidence was moderate: 8 of the included studies had a QUADAS score of 10 or greater (including 2 separate cohorts from 1 study) (Supplementary Table 1). Nine studies provided insufficient information whether the results of the elastography were interpreted while blinded to EGD results, or vice versa, putting them at risk for review bias.[6–8,23,25–27,29,30] The time period between performance of EGD and SSM was too long or not clearly stated in 7 studies,[8,23,25–28,30] putting them at risk for disease progression bias. The failure rate of SSM was not reported in 6 studies, and it was unclear how patients with failed SSM examinations were handled.[7,8,23,27,28,30]

Spleen Stiffness for Detection of Esophageal Varices

The pooled sensitivity of 12 studies was 78% (95% CI, 75%–81%), whereas the pooled specificity was 76% (95% CI, 72%–79%) (Figure 2*A* and *B*). The positive and negative LR was 3.4 (95% CI, 2.3–4.9) and 0.2 (95% CI, 0.1–0.4), respectively; the diagnostic OR was 19.3 (95% CI, 7.5–49.8). The AUROC was 0.86 (95% CI, 0.79–0.93) (Supplementary Figure 1). Because of significant differences in the SSM technique, a single threshold corresponding to the summary ROC could not be inferred. In patients with chronic liver disease with a low pretest probability of EV (ie, 25% probability of having EV as is the case in patients with compensated cirrhosis), a negative SSM was able to decrease the post-probability to 6% (Supplementary Figure 2). In patients with pretest ambiguity for the presence of EV (ie, 50% probability of having EV as is the case in patients with decompensated cirrhosis), a negative SSM result decreased the post-test probability to 16% while a positive SSM increased the probability of EV to 78%.