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Branched-chain amino acids, hepatocyte growth factor and protein production in the liver

Tomoaki Tomiya[a,*], Masao Omata[a], Kenji Fujiwara[b]

[a] *Department of Gastroenterology, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan*
[b] *Third Department of Internal Medicine, Saitama Medical School, Saitama, Japan*

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Abstract

Although the functions associated with differentiation are thought to be suppressed when cells proliferate, recent studies have shown that several mitogens can stimulate functions such as protein production under certain physiological conditions. Hepatocyte growth factor (HGF) is now considered to be a pluripotent factor and has been shown to stimulate the differentiated functions of hepatocytes, as well as their proliferation. The use of HGF for the treatment of liver disease, especially hepatic failure, has been suggested.

Because patients with decompensated liver cirrhosis have decreased plasma concentrations of branched-chain amino acids (BCAAs), many investigations in laboratory animals and patients have been designed to demonstrate the benefits of supplementation of BCAAs on the hepatic metabolism of proteins. However, the mechanisms involved in the specific actions of BCAAs remain to be elucidated. Amino acids are molecules that modulate numerous cellular functions. BCAAs are known to influence gene expression, cellular metabolism, amino acid transport, and protein turnover.

In this paper, we show the potential of BCAAs for stimulating HGF synthesis in the liver and discuss the possibility that BCAAs stimulate protein production by hepatocytes through the induction of HGF.

Keywords: HGF; BCAA; Leucine

Hepatocyte growth factor (HGF) was identified as a potent mitogen for hepatocytes [1–3]. However, recent studies have shown that HGF is pluripotent in its effects [1–7]. Furthermore, the administration of HGF in experimental models of various diseases has been shown to produce favorable effects [2–14]. These studies imply that HGF is a candidate therapeutic agent in many areas of disease. In addition, the up-regulation of the levels and/or activities of HGF in vivo may offer similar benefits.

In patients with liver cirrhosis, plasma concentrations of branched chain amino acids (BCAAs; valine, leucine and isoleucine) are usually reduced, depending on the stage of the disease [1,15]. Because a relationship between the occurrence of hepatic encephalopathy and a decrease of BCAAs, as well as an increase of aromatic amino acids, has been shown in the plasma of cirrhotic patients, BCAA-enriched formulas have been administered to such patients to prevent or treat hepatic encephalopathy [16]. Furthermore, the value of BCAA supplements as a nutritional support in cirrhosis, to prevent a decrease in protein production by hepatocytes, has been a topic of study for many years [17–22]. However, previous studies aimed at addressing this question in patients have generated conflicting results. Recently, a double-blind, randomized trial was performed, and some favorable effects on liver function were shown [23]. The potential benefits of supplementation in patients with liver cirrhosis with BCAAs were highlighted but the mechanisms of their action remain to be elucidated. BCAAs are known to be essential for protein

* Corresponding author. Tel.: +81 3 3815 5411x33017; fax: +81 3 5800 8806.
E-mail address: tomiya-1im@h.u-tokyo.ac.jp (T. Tomiya).

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nutrition. A very simple hypothesis is that supplementation of BCAAs provides the substrates for protein production, resulting in the increased production of proteins by hepatocytes. However, the overall capacity to produce proteins is known to be reduced in the cirrhotic liver, and controversy exists as to whether, and by what mechanism, the supplementation of substrates alone results in an increase in protein synthesis. Other mechanisms also should be considered.

Recent studies have shown that amino acids are molecules that modulate numerous cellular functions. Among them, the BCAAs have been reported to be able to regulate intracellular signaling pathways and gene expression in vitro [1,2,24–36]. The effect of BCAAs on HGF production should be examined as a possible mechanism of stimulation of protein production by hepatocytes, because HGF has the ability to enhance protein production.

1. Pharmacological actions of BCAAs

BCAAs are essential for protein nutrition and are abundant in mammalian proteins; their importance as substrates for protein production is well-established. In addition, leucine and isoleucine are known to be good sources of energy, producing 40 mol of ATP/mole [1,15]. Furthermore, as well as their nutritional aspects, BCAAs have pharmacological and biological activities. They are known to have specific effects in preserving body proteins by increasing the synthesis and decreasing the degradation of protein [1,30,33,36]. Recently, one of the BCAAs, leucine, was reported to activate the phosphorylation of two molecules involved in protein synthesis, p70 S6 kinase and eukaryotic initiation factor 4E binding protein 1 (4E-BP1) [1,25,33–36]. This activation seems to be mediated predominantly by the mammalian target of rapamycin. In theory, all mRNAs can be regulated by such mechanisms. However, some mRNAs are more sensitive to the changes than others, resulting in modulation of gene expression through altered patterns of translation of specific mRNAs [37]. In addition, BCAAs have been shown to have regulatory effects on cellular metabolism as well as amino acid transport. BCAAs can exert pharmacological effects on protein production through many mechanisms.

2. The multifaceted nature of HGF

Although HGF was originally isolated as a stimulator of DNA synthesis by hepatocytes in culture [1–3], it is now recognized as a pluripotent factor acting on various types of cells [1–7]. Epithelial cells initially were identified as the targets of HGF, although non-epithelial cells, such as hematopoietic, lymphoid, neural and muscle cells, also have been shown to respond to HGF. In the liver, HGF stimulates not only the proliferation of hepatocytes but also their differentiated functions, such as protein production, in vitro and in vivo [1–7,11–13]. In addition, HGF administration suppresses hepatic damage induced by treatment with alpha-naphthylisothiocyanate, alcohol, anti-Fas antibody, carbon tetrachloride, dimethylnitrosamine, lipopolysaccharide plus galactosamine, and warm ischemia/reperfusion and/or accelerates recovery from the hepatic injury in experimental models [4–7,11,14]. In such experiments, the contribution of mitogenic, anti-inflamatory, anti-apoptotic and anti-fibrogenetic activities of HGF have been postulated to be beneficial. Similar effects of HGF administration have been observed for other organs. HGF reduces injury to the kidney, lung, brain and heart, and suppresses fibrogenesis in the kidney [4,6,7]. In addition, HGF has been shown to induce angiogenesis and the development of neurons in experimental models [4–10]. HGF has been proposed as a therapeutic agent for liver disease, as well as a variety of disorders of other organs.

3. Stimulation of HGF synthesis

When HGF is administered intravenously or intraperitoneally, its half-life in the blood is quite short; approximately 5 min [3,12]. Thus, repeated injections of HGF would be necessary to maintain elevated levels. Other approaches are required, such as the use of the vectors expressing HGF or stimulation of intrinsic HGF production [14]. Previous reports have shown that many agents induce HGF expression in vitro. Cytokines (tumor necrosis factor α, interleukin 1α, 1β, and 6, and interferon α, β, and γ), growth factors (epidermal growth factor (EGF), fibroblast growth factor, and insulin-like growth factor families), cyclic AMP, phorbol-12-myristate-13-acetate, phorbol 12,13-dibutyrate, prostaglandin E_2, cholera toxin, norepinephrine, low density lipoprotein and injurin have stimulatory effects on HGF production by various cultured cells [38–45]. However, their efficacy in vivo and systemic reactions are still uncertain. The possibility of adverse effects following their administration in vivo should be considered.

4. Stimulation of HGF production by BCAAs

We examined the effect of BCAAs on HGF secretion by hepatic stellate cells (HSCs) because they are a major source of HGF production in the liver [46,47]. Freshly isolated HSCs transform rapidly into myofibroblast-like cells during culture and lose the ability to produce HGF. Therefore, we used an HSC clone (cHSC) that maintains a consistent phenotype during culture [48,49]. The cells were cultured in a medium, deprived of amino acids for 3 h prior to their use in the following experiments. When the medium was changed to Hanks' balanced salt solution (HBSS), which does not contain amino acids, and supplemented with leucine up to 10 mM, the HGF levels in the culture medium increased in a dose-related manner. However, addition of 10 mM valine or isoleucine did not increase the levels of HGF. When cHSC was cultured

in HBSS supplemented with 10 mM leucine, HGF secretion into the medium was enhanced significantly at 18 h. The difference in the HGF levels detected in the medium of the leucine-treated and non-treated cultures were enhanced in a time-dependent manner up to day 3. Under these serum-free culture conditions, proliferation does not occur and the number of cHSC cells remains constant [48,49]. Thus, cHSC produces HGF constantly and leucine enhances this HGF production. The stimulatory effect of leucine on HGF production was observed even when the cells were cultured in Eagle's minimal essential medium, which contains amino acids including BCAAs, supplemented with 0.5 or 10% fetal calf serum, although the effect was less pronounced than that observed in amino acid- and serum-free culture conditions. The mechanisms of the stimulatory effect of leucine on HGF production remain to be elucidated. It is possible that the addition of leucine provides a substrate for HGF production and/or energy to the cells. However, the addition of valine or isoleucine had no significant effect on HGF levels in the medium. Furthermore, a dose-dependent response to leucine addition was observed even when the cells were cultured in medium containing various amino acids and serum, which provide sufficient energy and substrates for cellular functions. Thus, it is less likely that the stimulatory effect of leucine on HGF production depends on the substrate or energy supply provided by leucine. As mentioned above, leucine was reported to activate the phosphorylation of p70 S6 kinase and 4E-BP1 [1,25,33–36]. Leucine may stimulate HGF synthesis by HSCs pharmacologically by enhancing these pathways. In addition, HGF production may be enhanced by leucine in vivo, because it can stimulate HGF production even in the presence of various amino acids and serum in culture medium. BCAA treatment might be a safe and effective way of stimulation of HGF production.

5. A possible mechanism for selecting HGF activities

In clinical practice, only some of the many effects of HGF are desired in the target organs. In patients with liver cirrhosis, the aim of inducing the up-regulation of HGF production would be the stimulation of hepatocyte functions, including protein production. The cytoprotective and mitogenic activities of HGF also might be required in some cases of liver cirrhosis. One possible mechanism for the selection of HGF activities is their regulation downstream of the initial stimulus. To support this hypothesis, effector gene(s) or amplifier(s) could be induced differentially by the stimulation of HGF, depending on the situation or specific requirements. We examined this approach under experimental conditions that show an increase in HGF activity.

In our experiments in vitro, isolated rat hepatocytes were cultured at high and low densities. DNA synthesis increased in a dose-dependent manner in the hepatocytes cultured at low density following the addition of HGF, while the concentrations of albumin and fibrinogen in the medium were not affected. When the hepatocytes were cultured at high density, addition of HGF increased the concentration of albumin or fibrinogen in the medium in a dose-dependent manner but did not affect DNA synthesis. Only in hepatocytes cultured at low density, the activity of transforming growth factor α (TGFα), which is produced by hepatocytes in the liver and is known to be another mitogen for hepatocytes, contributed to the manifestation of the HGF activity, because total cellular TGFα increased in low-density cultured hepatocytes in a dose-related manner following the addition of HGF, and the addition of antisense TGFα mRNA oligonucleotides to the medium inhibited the increase in TGFα expression and DNA synthesis. The increase in DNA synthesis in the presence of HGF was also suppressed by the addition of anti-TGFα rabbit IgG, which does not recognize other ligands for the TGFα/EGF receptor, such as EGF and heparin-binding EGF-like growth factor [50]. Furthermore, we showed that HGF induced transcription factor p53 expression by the hepatocytes, and the suppression of p53 production or action resulted in reduced TGFα expression, followed by a decrease in DNA synthesis by the hepatocytes [51]. Therefore, the mitogenic activity of HGF in cultured hepatocytes seems to be linked to TGFα activity. Recently, similar effects have been reported by Russell and co-workers [52]. They showed that a tyrosine kinase inhibitor of EGF receptor (EGFR), a receptor for the EGF family including TGFα, blocked both basal and ligand-induced tyrosine phosphorylation of the EGFR, but not that of c-met, a receptor for HGF. Pharmacologic inhibition of the EGFR kinase abolished the proliferative actions of HGF. They concluded that the mitogenic effect of HGF might be secondary to increased synthesis or processing of EGFR ligands, such as TGFα. Furthermore, Kojima and co-workers showed that HGF induces branching tubulogenesis in MDCK cells by modulating the activin-follistatin system [53], suggesting the existence of regulatory mechanisms for HGF activity, other than its mitogenic action.

We determined whether similar mechanisms could be observed in vivo. Partial hepatectomy induced an increase in hepatic and circulating levels of HGF, followed by an increase in the production of TGFα and of hepatocyte proliferation in rats [54]. When an anti-TGFα antibody was administered to partially hepatectomized rats, hepatocyte proliferation was reduced, irrespective of any increase in HGF levels [50]. In rats, after sham-operations, hepatic and circulating levels of HGF increased moderately. However, no increases in the TGFα levels and hepatocyte proliferation were observed in these rats [54]. These results suggest that TGFα activity is related to the mitogenic effect of HGF during liver regeneration in rats. In addition, the observations in clinical settings seem to be comparable with these results in rats [55–59]. In patients who received partial hepatectomy, serum HGF levels were increased, followed by an increase in serum levels of TGFα, which reached a maximum later than the serum HGF levels. The maximum levels that were achieved in each case correlated significantly with the resected volume of the liver and the increased volume of the remaining liver. In contrast,

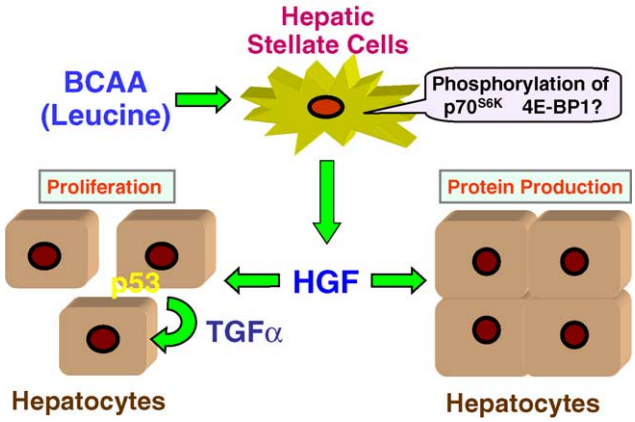

Fig. 1. Schematic potential pathways of BCAA, HGF and protein production, in addition to proliferation of hepatocytes.

TGFα levels did not increase in patients who underwent operations other than partial hepatectomy, while HGF levels did increase moderately. Also, in patients suffering from acute hepatitis, serum HGF levels reached a maximum earlier than the TGFα levels. In these patients, the maximum serum HGF levels correlated positively with the maximum serum TGFα levels, and the TGFα levels correlated with the maximum serum alanine aminotransferase levels observed in each case. Because livers in acute hepatitis may regenerate depending on the degree of liver damage, the TGFα levels should correlate with the degree of liver regeneration in patients with acute hepatitis. Together, these results suggest that serum TGFα levels increase in patients in relation to the extent of liver regeneration after an increase of serum HGF levels.

6. Conclusions and future prospects

BCAAs seem to have significant effects, not only as a nutrient, but also as a drug. BCAAs, especially leucine, stimulate HGF synthesis by hepatic stellate cells. Increased HGF production induced by BCAAs can stimulate protein production by hepatocytes (Fig. 1). Further investigations will be required to elucidate the regulatory mechanisms of HGF induction by BCAAs and the selection of HGF activities, especially in vivo.

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