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Leucine stimulates HGF production by hepatic stellate cells through mTOR pathway

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Abstract

Branched chain amino acids modulate various cellular functions in addition to providing substrates for the production of proteins. We examined the mechanism underlying the stimulation by leucine of hepatocyte growth factor (HGF) production by hepatic stellate cells. Both p70 S6 kinase activity and phosphorylation of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) were up-regulated rapidly after leucine treatment of a rat hepatic stellate cell clone. No such activation was observed following treatment with valine or isoleucine. Rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), suppressed leucine-induced activation of p70 S6 kinase and 4E-BP1 and negated the stimulatory effect of leucine on HGF production. An mTOR-dependent signaling pathway mediates the stimulatory effect of leucine on the production of HGF by hepatic stellate cells.

Keywords: Hepatocyte growth factor; Leucine; Hepatic stellate cells; Mammalian target of rapamycin; p70 S6 kinase; Eukaryotic initiation factor 4E-binding protein 1

Although hepatocyte growth factor (HGF) was identified initially as a mitogen for hepatocytes, further investigations revealed that HGF is a pleiotropic factor with mitogenic, motogenic, morphogenic, anti-apoptotic and tumor suppressive activities [1–3]. HGF is produced by cells in various organs, including hepatic stellate cells [1–3]. Exogenous administration of HGF has been shown to stimulate DNA synthesis in the liver, kidney and lung, facilitate liver function, reduce injuries of the liver, colon, kidney, lung, brain and heart, and suppress fibrogenesis of the liver and kidney in experimental models [1–8]. These observations indicate a potential application for HGF in the treatment of various diseases. In addition, up-regulation of HGF production may offer similar benefits. However, the mechanisms of regulation of HGF production are still under investigation.

In patients with chronic liver injury, it is known that serum levels of branched chain amino acids (BCAAs: valine, leucine and isoleucine) are reduced, depending on the degree of liver dysfunction [1,9]. Clinical trials of BCAA supplementation therapy have been performed to facilitate protein production, such as albumin by the cirrhotic liver [10–12]. In addition, administration of BCAAs has been reported to prevent or retard the progression of liver failure in cirrhotic patients [10–12]. However, the mechanisms through which the BCAAs generate the effects observed

Abbreviations: BCAAs, branched chain amino acids; cHSC, hepatic stellate cell clone; eIF4E, eukaryotic translation initiation factor 4E; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; HGF, hepatocyte growth factor; MEM, Eagle's minimal essential medium; mTOR, mammalian target of rapamycin; p70 S6, 70 kDa ribosomal protein S6.

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remain to be elucidated. In general, the most important role of amino acids has been considered to be providing the substrates for protein synthesis. Furthermore, recent studies show that amino acids, especially BCAAs, serve as regulatory molecules that modulate numerous cellular functions [1,13–17]. These observations imply that BCAAs can be physiological regulators of protein production, in addition to being essential amino acids for protein synthesis. Recently, we reported that HGF production by hepatic stellate cells is up-regulated by the addition of leucine to the culture medium [18]. In addition, we showed that leucine administration to rats increases circulating and hepatic HGF levels [19]. These results raise the possibility that leucine is a regulator of the production of HGF by hepatic stellate cells. Considering the above-mentioned effects of HGF on the liver, the clinical effect of BCAA administration in patients with liver cirrhosis might be attributable, at least in part, to the induction of HGF production by leucine.

In eukaryotic cells, one of the most important intracellular signaling transducers of amino acids is considered to be the rapamycin-sensitive pathway associated with the mammalian target of rapamycin (mTOR), which regulates protein production post-transcriptionally through activation of the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and the 70 kDa ribosomal protein S6 (p70 S6) kinase [20–23]. To evaluate the mechanism of regulation of HGF production by leucine, we studied the effect of leucine on an mTOR dependent signaling pathway in hepatic stellate cells in relation to HGF production.

Materials and methods

Cell culture

The hepatic stellate cell clone (cHSC), CFSC-8B, was kindly provided by Dr. Marcos Rojkind [24]. Cells were plated on plastic culture dishes at 7×10^4 cells/cm^2 in Eagle's minimal essential medium (MEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% (v/v) fetal calf serum (FCS, Gibco Laboratories, Life Technologies Inc., Grand Island, NY). After a 4 h attachment period, the culture medium was replaced with MEM supplemented with 0.5% FCS. After 20 h, cells were deprived of amino acids by incubation in Hanks' balanced salt solution (HBSS, Sigma Chemical Co., St. Louis, Missouri) supplemented with a vitamin mixture (Gibco Laboratories, Life Technologies Inc., Grand Island, NY). After 3 h, the cells were used for the following experiments.

Experiment I. The medium was changed to HBSS containing 10 mM leucine (Ajinomoto Co., Inc., Tokyo, Japan). Cells were frozen serially using liquid nitrogen and used for the assay of p70 S6 kinase activity and examination of the phosphorylation state of 4E-BP1.

Experiment II. The medium was changed to HBSS with 0.1–10 mM leucine or 10 mM valine or isoleucine (Ajinomoto Co., Inc., Tokyo, Japan). After a 40 min incubation period, cells were frozen quickly using liquid nitrogen and used for the assay of p70 S6 kinase activity and examination of the 4E-BP1 phosphorylation state.

Experiment III. The medium was changed to HBSS containing 0.1–2.5 nM of rapamycin. After a 30 min incubation period, 10 mM leucine was added to the medium. Forty minutes later, the cells were frozen quickly using liquid nitrogen and used for the assay of p70 S6 kinase activity and examination of the 4E-BP1 phosphorylation state.

Experiment IV. The cells were treated as in experiment III. HGF concentrations in the medium were determined 24 h after leucine addition.

Immunoprecipitation and p70 S6 kinase assays

The p70 S6 kinase activity was determined after immunoprecipitation using ribosomal subunits as substrate, as reported previously [15,25,26]. Briefly, cells were lysed in ice-cold buffer A (50 mM Tris–HCl pH 8.0, 1% Nonidet P-40, 120 mM NaCl, 20 mM NaF, 1 mM EDTA, 6 mM EGTA, 20 mM β-glycerophosphate, 0.5 mM dithiothreitol, 50 μM *p*-APMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) and incubated for 10 min. They were centrifuged at 10,000*g* for 30 min at 4 °C. An aliquot of the supernatants was incubated with anti-p70 S6 kinase antibody (SC-230, Santa Cruz Biotechnology, California) for 2 h at 4 °C and was absorbed to protein G–Sepharose. This was washed twice with buffer A containing 0.5 M NaCl and twice with the buffer containing 20 mM MOPS at pH 7.2, 10 mM β-glycerophosphate. The 40S S6 protein kinase reaction was performed at 30 °C by the addition of reaction mixture (50 mM MOPS pH 7.2, 12 mM $MgCl_2$, 2 mM EGTA, 1 mM dithiothreitol, 60 μM ATP, 10 mM β-glycerophosphate, 0.5 μM protein kinase inhibitor, 0.5 A_{260} subunits of 40S ribosomal subunits, and 5 μCi of [γ-^{32}P]ATP). After a 20 min incubation period, one half volume of SDS buffer (4% SDS, 20% glycerol, 100 mM Tris–HCl pH 6.8, 5% 2-mercaptoethanol) was added. The samples were separated by SDS–PAGE on 12% gels, transferred to nitrocellulose membrane and the radioactivity was visualized by autoradiography. The membrane also was used for the detection of p70 S6 kinase protein by Western blotting using anti-p70 S6 kinase antibody.

Examination of the 4E-BP1 phosphorylation state

An aliquot of the supernatant from the lysed cells, described above, was mixed with SDS buffer, separated by SDS–PAGE on a 12% gel, and transferred to a nitrocellulose membrane. An electrophoretic mobility was studied using anti-4E-BP1 antibody (sc-6024, Santa Cruz Biotechnology, California), as described previously [26,27].

Assays for HGF concentrations in the culture medium

HGF concentrations in the culture medium were determined using a rat HGF enzyme-linked immunosorbent assay kit (Institute of Immunology, Tokyo, Japan) [18,28]. The lower limit of detection of the assay was 0.1 ng/ml.

Results

Effect of leucine on p70 S6 kinase activity and phosphorylation of 4E-BP1 in cHSC

p70 S6 kinase activity increased rapidly and peaked 40 min after treatment with 10 mM leucine, although total p70 S6 kinase protein levels did not change (Fig. 1A). As for 4E-BP1, cHSC cells exhibited a predominance of faster-migrating unphosphorylated forms of 4E-BP1 (α and β bands) before the addition of leucine (Fig. 1B). The proportion of slower-migrating highly phosphorylated form of 4E-BP1 (γ band) increased 10 min after leucine addition, peaked at 20 min, and remained at plateau levels thereafter (Fig. 1B).

Effect of BCAAs on p70 S6 kinase activity and phosphorylation of 4E-BP1 in cHSC

As shown in Fig. 2A, the addition of leucine increased p70 S6 kinase activity in a dose-dependent manner. How-

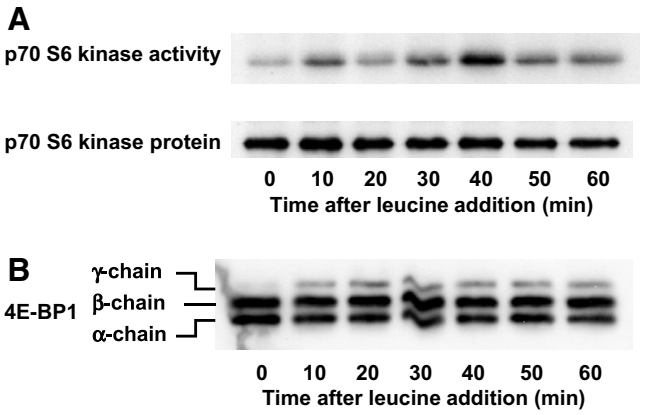

Fig. 1. p70 S6 kinase activity and phosphorylation of 4E-BP1 in cHSC cells cultured in medium supplemented with leucine. (A) The p70 S6 kinase activity was determined in the immunoprecipitates using ribosomal subunits as substrate. The samples were separated by SDS–PAGE, transferred to a nitrocellulose membrane and ^{32}P radioactivity was visualized by autoradiography (upper panel). The membrane was also used for the detection of p70 S6 kinase protein by western blotting using anti-p70 S6 kinase antibody (lower panel). (B) An electrophoretic mobility was studied using anti-4E-BP1 antibody. Faster-migrating bands (α and β bands) of 4E-BP1 indicate unphosphorylated or lower phosphorylated forms of 4E-BP1, while the slower-migrating band (γ band) indicates the highly phosphorylated form.

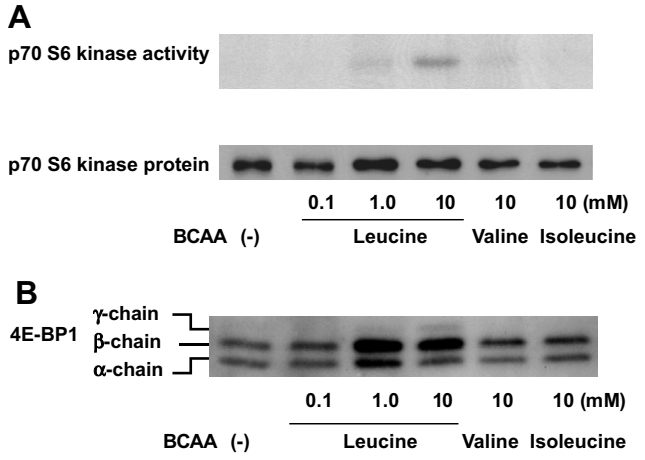

Fig. 2. p70 S6 kinase activity and phosphorylation of 4E-BP1 in cHSC cells cultured in medium supplemented with BCAAs. (A) The p70 S6 kinase activity is shown in the upper panel and the detection of p70 S6 kinase protein is shown in the lower panel. (B) Unphosphorylated or lower phosphorylated, faster-migrating bands of 4E-BP1 (α and β bands) and the highly phosphorylated slower-migrating band (γ band) were revealed by electrophoretic mobility using anti-4E-BP1 antibody.

ever, the addition of isoleucine or valine did not stimulate activation of p70 S6 kinase. Addition of leucine also increased the slower migrating γ band, indicating a higher state of phosphorylation of 4E-BP1, in a dose-dependent manner, while the addition of isoleucine or valine did not affect the state of phosphorylation of 4E-BP1 (Fig. 2B).

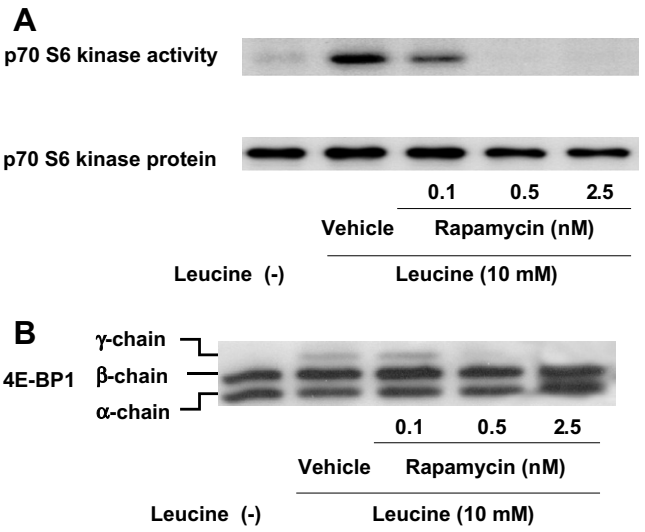

Fig. 3. Effect of rapamycin on p70 S6 kinase activity and phosphorylation of 4E-BP1 in cHSC cells cultured in the medium supplemented with leucine. (A) The p70 S6 kinase activity is shown in the upper panel and the detection of p70 S6 kinase protein is shown in the lower panel. (B) Unphosphorylated or lower phosphorylated faster-migrating bands of 4E-BP1 (α and β bands) and the highly phosphorylated slower-migrating band (γ band) were revealed by electrophoretic mobility using anti-4E-BP1 antibody.

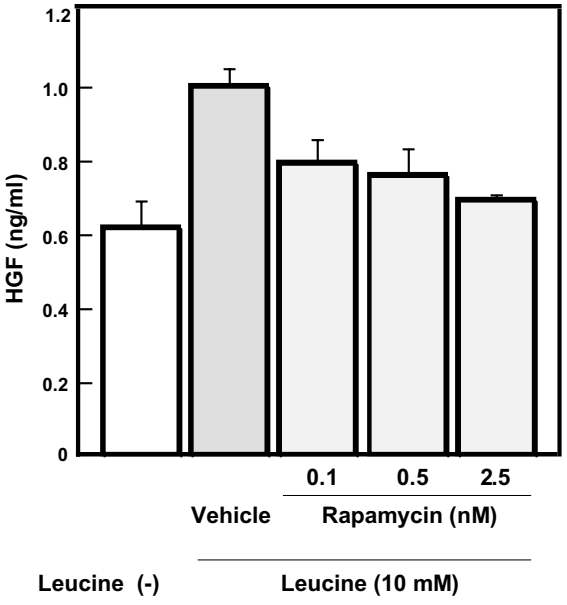

Fig. 4. Effect of rapamycin on HGF concentrations in the medium of cHSC cells cultured in HBSS supplemented with leucine. Data are means ± SEM of 5 dishes.

Effect of rapamycin on p70 S6 kinase activity and phosphorylation of 4E-BP1 in cHSC stimulated by leucine

The addition of rapamycin attenuated the increase of p70 S6 kinase activity and the phosphorylation state of 4E-BP1 in cHSC cells stimulated by leucine (Fig. 3).

Effect of rapamycin on HGF production in cHSC stimulated by leucine

HGF levels in the medium of cHSC increased significantly following leucine treatment. The increase was suppressed in a dose-dependent manner by the addition of rapamycin ($F = 5.61$, $p < 0.01$, tested by one-way analysis of variance) (Fig. 4).

Discussion

We showed that leucine induces HGF production by cHSC through a rapamycin-sensitive pathway which activates p70 S6 kinase and 4E-BP1.

Hepatic stellate cells are one of the major sources of HGF production in the liver. However, freshly isolated hepatic stellate cells become transformed into myofibroblast-like cells during culture, resulting in diminution of HGF production with time [29]. Thus, we utilized a cHSC which maintains a stable phenotype during culture [24] and produces HGF continuously [18].

BCAAs are essential in protein nutrition. In addition, both leucine and isoleucine are known to be good sources of energy, producing 40 mol of ATP/mole [1,9]. One possible hypothesis is that the addition of leucine provides a substrate for HGF synthesis and/or energy to the cells, which results in the increased production of HGF. However, neither isoleucine nor valine stimulated HGF production by cHSC [18]. Therefore, we studied the mechanism of the stimulatory effect of leucine on HGF production, other than by the supply of a substrate or energy for HGF production.

It has been shown that activation of mTOR leads to the phosphorylation of both p70 S6 kinase and 4E-BP1 [20,21]. p70S6 kinase and 4E-BP1 control the step in initiation of translation involving the binding of mRNA to the 40S ribosomal subunit [20,21]. 4E-BP1 regulates protein synthesis through its association with eukaryotic translation initiation factor 4E (eIF4E). Though, theoretically, the translation of all mRNAs can be regulated equally through mTOR, it is known that some mRNAs are more susceptible to the changes in eIF4E than others, resulting in modulation of gene expression through altered patterns of translation of specific mRNAs [20,21]. Our results showed that addition of leucine to the medium rapidly increased both p70 S6 kinase activity and phosphorylation of 4E-BP1 in hepatic stellate cells. Furthermore, the addition of rapamycin, a specific inhibitor of mTOR [22,23,30], prevented the up-regulation of p70 S6 kinase and 4E-BP1 activity by leucine. Treatment with rapamycin also attenuated the leucine-induced increase of HGF production. In contrast, the other BCAAs, valine and isoleucine, did not increase the activities of p70 S6 kinase and 4E-BP1. Our previous report showed that neither valine nor isoleucine stimulated HGF production in hepatic stellate cells [18]. These observations indicate that up-regulation of HGF production by leucine is dependent on the activation of an mTOR dependent signaling pathway.

Regulation of protein production by amino acids has been a subject of study for many years. mTOR is known as an evolutionarily conserved nutrient sensor that directs the cellular response to nutrient status, especially the availability of amino acids [31,32]. Leucine is one of the most potent amino acids in facilitating protein synthesis through an mTOR signaling pathway [15,33]. Administration of leucine has been reported to activate this pathway in various kinds of cells, such as adipocytes and hepatocytes *in vitro* and muscle, liver, and adipose tissue *in vivo* [26,34–37]. Through this pathway, it has been shown that leucine treatment stimulates the production of specific proteins, such as leptin by adipocytes and albumin by hepatocytes [26,34]. Our observations may support the possible role of leucine as a physiological or pharmacological regulator of the production of HGF.

References

[1] A. Ichihara, BCA, HGF, and proteasomes, Biochem. Biophys. Res. Commun. 266 (1999) 647–651.
[2] R. Zarnegar, G.K. Michalopoulos, The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis, J. Cell Biol. 129 (1995) 1177–1180.
[3] K. Matsumoto, T. Nakamura, Hepatocyte growth factor (HGF) as a tissue organizer for organogenesis and regeneration, Biochem. Biophys. Res. Commun. 239 (1997) 639–644.
[4] H. Funakoshi, T. Nakamura, Hepatocyte growth factor: from diagnosis to clinical applications, Clin. Chim. Acta. 327 (2003) 1–23.
[5] L.B. Ware, M.A. Matthay, Keratinocyte and hepatocyte growth factors in the lung: roles in lung development, inflammation, and repair, Am. J. Physiol. 282 (2002) L924–L940.
[6] Y. Ishiki, H. Ohnishi, Y. Muto, K. Matsumoto, T. Nakamura, Direct evidence that hepatocyte growth factor is a hepatotrophic factor for liver regeneration and has a potent antihepatitis effect in vivo, Hepatology 16 (1992) 1227–1235.
[7] K. Fujiwara, S. Nagoshi, A. Ohno, K. Hirata, Y. Ohta, S. Mochida, T. Tomiya, K. Higashio, K. Kurokawa, Stimulation of liver growth by exogenous human hepatocyte growth factor in normal and partially hepatectomized rats, Hepatology 18 (1993) 1443–1449.
[8] M. Yamaoka, K. Hirata, I. Ogata, T. Tomiya, S. Nagoshi, S. Mochida, K. Fujiwara, Enhancement of albumin production by hepatocyte growth factor in rat hepatocytes: distinction in mode of action from stimulation of DNA synthesis, Liver 18 (1998) 52–59.
[9] A.J.L. Cooper, Role of the liver in amino acid metabolism, in: T. Zakim, T.D. Boyer (Eds.), Hepatology: A Text Book of Liver Disease, W. B. Saunders company, Philadelphia, 1996, pp. 563–601.
[10] G. Marchesini, G. Bianchi, B. Rossi, M. Brizi, N. Melchionda, Nutritional treatment with branched-chain amino acids in advanced liver cirrhosis, J. Gastroenterol. 35 (2000) 7–12.
[11] G. Marchesini, G. Bianchi, M. Merli, P. Amodio, C. Panella, C. Loguercio, F.R. Fanelli, R. Abbiati, Italian BCAA Study Group, Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial, Gastroenterology 124 (2003) 1792–1801.
[12] Y. Muto, S. Sato, A. Watanabe, H. Moriwaki, K. Suzuki, A. Kato, M. Kato, T. Nakamura, K. Higuchi, S. Nishiguchi, H. Kumada, Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis, Clin. Gastroenterol. Hepatol. 3 (2005) 705–713.

[13] S.M. Hutson, R.A. Harris, Symposium: leucine as a nutritional signal: introduction, J. Nutr. 131 (2001) 839–840.
[14] T.A. Gautsch, J.C. Anthony, S.R. Kimball, G.L. Paul, D.K. Layman, L.S. Jefferson, Availability of eIF4E regulates skeletal muscle protein synthesis during recovery from exercise, Am. J. Physiol. 274 (1998) C406–C414.
[15] K. Shigemitsu, Y. Tsujishita, H. Miyake, S. Hidayat, N. Tanaka, K. Hara, K. Yonezawa, Structural requirement of leucine for activation of p70 S6 kinase, FEBS Lett. 447 (1999) 303–306.
[16] T. Tomiya, M. Omata, K. Fujiwara, Significance of branched chain amino acids as possible stimulators of hepatocyte growth factor, Biochem. Biophys. Res. Commun. 313 (2004) 411–416.
[17] T. Tomiya, M. Omata, K. Fujiwara, Branched-chain amino acids, hepatocyte growth factor and protein production in the liver, Hepatol. Res. 30S (2004) 14–18.
[18] T. Tomiya, Y. Inoue, M. Yanase, M. Arai, H. Ikeda, K. Tejima, K. Nagashima, T. Nishikawa, K. Fujiwara, Leucine stimulates the secretion of hepatocyte growth factor by hepatic stellate cells, Biochem. Biophys. Res. Commun. 297 (2002) 1108–1111.
[19] T. Tomiya, Y. Inoue, M. Yanase, M. Arai, H. Ikeda, K. Tejima, K. Nagashima, T. Nishikawa, N. Watanabe, M. Omata, K. Fujiwara, Treatment with leucine stimulates the production of hepatocyte growth factor in vivo, Biochem. Biophys. Res. Commun. 322 (2004) 772–777.
[20] C.G. Proud, Regulation of mammalian translation factors by nutrients, Eur. J. Biochem. 269 (2002) 5338–5349.
[21] S.R. Kimball, L.S. Jefferson, Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids, Biochem. Biophys. Res. Commun. 313 (2004) 423–427.
[22] D.C. Fingar, J. Blenis, Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression, Oncogene 23 (2004) 3151–3171.
[23] E.K. Rowinsky, Targeting the molecular target of rapamycin (mTOR), Curr. Opin. Oncol. 16 (2004) 564–575.
[24] P. Greenwel, J. Rubin, M. Schwartz, E.L. Hertzberg, M. Rojkind, Liver fat-storing cell clones obtained from a CCl4-cirrhotic rat are heterogeneous with regard to proliferation, expression of extracellular matrix components, interleukin-6, and connexin 43, Lab. Invest. 69 (1993) 210–216.
[25] K. Hara, K. Yonezawa, Q.P. Weng, M.T. Kozlowski, C. Belham, J. Avruch, Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism, J. Biol. Chem. 273 (1998) 14484–14494.
[26] C. Ijichi, T. Matsumura, T. Tsuji, Y. Eto, Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system, Biochem. Biophys. Res. Commun. 303 (2003) 59–64.
[27] K. Shigemitsu, Y. Tsujishita, K. Hara, M. Nanahoshi, J. Avruch, K. Yonezawa, Regulation of translational effectors by amino acid and mammalian target of rapamycin signaling pathways. Possible involvement of autophagy in cultured hepatoma cells, J. Biol. Chem. 274 (1999) 1058–1065.
[28] A. Yamada, K. Matsumoto, H. Iwanari, K. Sekiguchi, S. Kawata, Y. Matsuzawa, T. Nakamura, Rapid and sensitive enzyme-linked immunosorbent assay for measurement of HGF in rat and human tissues, Biomed. Res. 16 (1995) 105–114.
[29] P. Schirmacher, A. Geerts, A. Pietrangelo, H.P. Dienes, C.E. Rogler, Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells, Hepatology 15 (1992) 5–11.
[30] J. Chung, C.J. Kuo, G.R. Crabtree, J. Blenis, Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases, Cell 69 (1992) 1227–1236.
[31] T. Schmelzle, M.N. Hall, TOR, a central controller of cell growth, Cell 103 (2000) 253–262.
[32] J. Rohde, J. Heitman, M.E. Cardenas, The TOR kinases link nutrient sensing to cell growth, J. Biol. Chem. 276 (2001) 9583–9586.
[33] C.J. Lynch, Role of leucine in the regulation of mTOR by amino acids: revelations from structure-activity studies, J. Nutr. 131 (2001) 861S–865S.
[34] C. Roh, J. Han, A. Tzatsos, K.V. Kandror, Nutrient-sensing mTOR-mediated pathway regulates leptin production in isolated rat adipocytes, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E322–E330.
[35] J.C. Anthony, T.G. Anthony, S.R. Kimball, L.S. Jefferson, Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine, J. Nutr. 131 (2001) 856S–860S.
[36] A.K. Reiter, T.G. Anthony, J.C. Anthony, L.S. Jefferson, S.R. Kimball, The mTOR signaling pathway mediates control of ribosomal protein mRNA translation in rat liver, Int. J. Biochem. Cell Biol. 36 (2004) 2169–2179.
[37] C.J. Lynch, B.J. Patson, J. Anthony, A. Vaval, L.S. Jefferson, T.C. Vary, Leucine is a direct-acting nutrient signal that regulates protein synthesis in adipose tissue, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E503–E513.