Psychopharmacology (Berl) 2004; **175**: 1-6

129 **Romero-Gómez M**, Diago M, Andrade RJ, Calleja JL, Salmerón J, Fernández-Rodríguez CM, Solà R, García-Samaniego J, Herrerías JM, De la Mata M, Moreno-Otero R, Nuñez O, Olveira A, Durán S, Planas R. Treatment of insulin resistance with metformin in naïve genotype 1 chronic hepatitis C patients receiving peginterferon alfa-2a plus ribavirin. *Hepatology* 2009; **50**: 1702-1708

130 **Khattab M**, Emad M, Abdelaleem A, Eslam M, Atef R, Shaker Y, Hamdy L. Pioglitazone improves virological response to peginterferon alpha-2b/ribavirin combination therapy in hepatitis C genotype 4 patients with insulin resistance. *Liver Int* 2009; Epub ahead of print

131 **Smith U**, Gogg S, Johansson A, Olausson T, Rotter V, Svalstedt B. Thiazolidinediones (PPARgamma agonists) but not PPARalpha agonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes. *FASEB J* 2001; **15**: 215-220

132 **Yuan L**, Ziegler R, Hamann A. Metformin modulates insulin post-receptor signaling transduction in chronically insulin-treated Hep G2 cells. *Acta Pharmacol Sin* 2003; **24**: 55-60

133 **Bailey CJ**, Turner RC. Metformin. *N Engl J Med* 1996; **334**: 574-579

134 **Shishido S**, Koga H, Harada M, Kumemura H, Hanada S, Taniguchi E, Kumashiro R, Ohira H, Sato Y, Namba M, Ueno T, Sata M. Hydrogen peroxide overproduction in megamitochondria of troglitazone-treated human hepatocytes. *Hepatology* 2003; **37**: 136-147

135 **Deacon CF**, Holst JJ. Dipeptidyl peptidase IV inhibitors: a promising new therapeutic approach for the management of type 2 diabetes. *Int J Biochem Cell Biol* 2006; **38**: 831-844

136 **DeFronzo RA**, Fleck PR, Wilson CA, Mekki Q. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor alogliptin in patients with type 2 diabetes and inadequate glycemic control: a randomized, double-blind, placebo-controlled study. *Diabetes Care* 2008; **31**: 2315-2317

137 **Suzuki K**, Suzuki K, Koizumi K, Ichimura H, Oka S, Takada H, Kuwayama H. Measurement of serum branched-chain amino acids to tyrosine ratio level is useful in a prediction of a change of serum albumin level in chronic liver disease. *Hepatol Res* 2008; **38**: 267-272

138 **Moriwaki H**, Miwa Y, Tajika M, Kato M, Fukushima H, Shiraki M. Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. *Biochem Biophys Res Commun* 2004; **313**: 405-409

139 **Nishitani S**, Takehana K, Fujitani S, Sonaka I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G1292-G1300

140 **Hinault C**, Mothe-Satney I, Gautier N, Lawrence JC Jr, Van Obberghen E. Amino acids and leucine allow insulin activation of the PKB/mTOR pathway in normal adipocytes treated with wortmannin and in adipocytes from db/db mice. *FASEB J* 2004; **18**: 1894-1896

141 **Broca C**, Breil V, Cruciani-Guglielmacci C, Manteghetti M, Rouault C, Derouet M, Rizkalla S, Pau B, Petit P, Ribes G, Ktorza A, Gross R, Reach G, Taouis M. Insulinotropic agent ID-1101 (4-hydroxyisoleucine) activates insulin signaling in rat. *Am J Physiol Endocrinol Metab* 2004; **287**: E463-E471

142 **She P**, Reid TM, Bronson SK, Vary TC, Hajnal A, Lynch CJ, Hutson SM. Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. *Cell Metab* 2007; **6**: 181-194

143 **Kawaguchi T**, Nagao Y, Matsuoka H, Ide T, Sata M. Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease. *Int J Mol Med* 2008; **22**: 105-112

144 **Kawaguchi T**, Taniguchi E, Itou M, Sumie S, Oriishi T, Matsuoka H, Nagao Y, Sata M. Branched-chain amino acids improve insulin resistance in patients with hepatitis C virus-related liver disease: report of two cases. *Liver Int* 2007; **27**: 1287-1292

145 **Muto Y**, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, Kato M, Nakamura T, Higuchi K, Nishiguchi S, Kumada H, Ohashi Y. 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**: 204-214

S- Editor Wang YR **L- Editor** Cant MR **E- Editor** Ma WH

The Pathogenesis, Complications and Therapeutic Strategy for Hepatitis C Virus-associated Insulin Resistance in the Era of Anti-viral Treatment

Takumi Kawaguchi[1,2,*], Eitaro Taniguchi[2], Minoru Itou[2], Shuji Sumie[2], Sho-ichi Yamagishi[3] and Michio Sata[1,2]

[1]*Department of Digestive Disease Information & Research,* [2]*Department of Medicine,* [3]*Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume 830-0011, Japan*

Abstract: Recent experimental and clinical studies have shown that chronic hepatitis C virus (HCV) infection causes insulin resistance. Since insulin resistance decreases response to antiviral treatments, promotes inflammatory and fibrogenic reactions and increases a risk of hepatocellular carcinoma (HCC), amelioration of insulin resistance may be a novel therapeutic target, which could improve the prognosis in patients with HCV-related chronic liver disease. Despite the increased awareness of health risk of insulin resistance, there is no common therapeutic strategy for HCV-associated insulin resistance. Indeed, treatments with exogenous insulin or sulfonylureas may be rather harmful because these treatments are associated with the development of HCC in patients with HCV infection. Meanwhile, we, along with others, have found distinctive treatments which improve HCV-associated insulin resistance. Administration of branched-chain amino acids (BCAA), especially as a late evening snack, improves glucose metabolism by improving insulin-signal cascades in insulin resistance patients with HCV infection. In this paper, we discuss the pathogenesis and complications for HCV-associated insulin resistance and further review a recent clinical therapeutic strategy using these agents for the treatment of this devastating disorder. We also discuss therapeutic potentialities of incretin-based therapies, new anti-diabetic agents for HCV-associated insulin resistance and the significance of insulin resistance in the era of new anti-viral treatments.

Keywords: Hepatitis C virus, insulin resistance, hepatocellular carcinoma, branched-chain amino acids, incretin.

INTRODUCTION

Since hepatitis C virus (HCV) was identified in 1989 [1, 2], underlying pathophysiology of chronic hepatitis C has been disclosed tremendously [3-7]. Individuals infected with HCV frequently develop chronic infection, which is associated with the development of liver cirrhosis and hepatocellular carcinoma (HCC) [8-10]. In addition, epidemiological data from East-West show an association between HCV infection and insulin resistance [11-21]. Recent basic and clinical researches have revealed the mechanisms of HCV-associated insulin resistance and insulin resistance is now recognized as a sequela of chronic HCV infection [14, 22-30].

Generally, insulin resistance is associated with the development of diabetes mellitus (DM), hypertension and cardiovascular diseases [31]. Besides these complications, insulin resistance is also involved in many events in patients with chronic hepatitis C, including antiviral treatment, fibrogenic reaction and the development of HCC [25, 28, 32-35].

It is now clear that insulin resistance is a critical factor for the progression of any stage of chronic hepatitis C [36]. Despite the accumulated evidences for the risk of insulin resistance, therapeutic strategy for HCV-associated insulin resistance has not been established yet [37]. In HCV-infected patients with diabetes, cardio-vascular disease occupies only less than 5% of cause of death and is not a significant prognostic factor [38]. Prognostic factors are HCC, liver failure, and esophageal varices even in HCV-infected patients with diabetes [38], and the therapeutic strategy should be considered based on mechanisms and life-threatening complications for HCV-associated insulin resistance.

Recently, we have found that an inactivation of incretin is a causative factor for HCV-associated insulin resistance [39]. Incretin mimetics and incretin enhancer are new anti-diabetic agents. Theoretically, it seems that replenishment and/or enhancement of incretin is proper therapeutic approach and these new anti-diabetic agents may ameliorate insulin resistance, prevent the development of HCC and improve the prognosis in patients with HCV infection.

In this review, we summarize the pathogenesis for HCV-associated insulin resistance and propose the clinical therapeutic strategy for the treatment of HCV-associated insulin resistance. In addition, therapeutic potentialities of incretin-based therapies and the significance of insulin resistance in the era of new anti-viral treatments are discussed.

MECHANISMS FOR HCV-ASSOCIATED INSULIN RESISTANCE

Indirect Effects of HCV

Various factors are reported to be associated with the development of HCV-associated insulin resistance (Table **1**). Similar to the life style-associated insulin resistance, obesity with decreased serum adiponectin levels [40-42], inflammatory cytokines [41, 43, 44], oxidative stress [45-47], hepatic steatosis [48], pancreatic beta-cell function [49], and serum

*Address correspondence to this author at the Department of Digestive Disease Information & Research, Kurume University School of Medicine. 67 Asahi-machi, Kurume 830-0011, Japan; Tel: +81-942-31-7902; Fax: +81-942-31-7820; E-mail: takumi@med.kurume-u.ac.jp

1574-8871/10 $55.00+.00

Table 1. Factors Associated with Insulin Resistance in Patients with HCV Infection

Factor	References
Obesity and decreased serum adiponectin levels	[40-42]
Inflammation and inflammatory cytokines	[41, 43, 44]
Oxidative stress	[45-47]
Decreased serum PEDF levels	[77, 187]
Portal-systemic shunt	[50]
Hepatic iron accumulation	[57, 58]
HCV core	[14, 26, 29]
Amino acid substitutions of the HCV core region (Gln70 (His70) and/or Met91)	[22]

pigment epithelium-derived factor (PEDF) levels are also involved in the development of HCV-associated insulin resistance. In addition to those common factors, liver specific factors are underlying in the development of HCV-associated insulin resistance.

Ferenci *et al.* reported that portal-systemic shunts caused not only hepatic encephalopathy, but also insulin resistance in patients with liver cirrhosis [50]. Reduced hepatic blood flow may lead liver dysfunction and subsequent insulin resistance. In fact, Tanabe *et al.* reported that occlusion of portal-systemic shunt improved glucose metabolism in patients with liver cirrhosis [51].

Hepatic iron accumulation is one of characters for HCV infection. Hepcidin is a key negative regulator of iron metabolism [52] and hepcidin levels are correlate with hepatic iron accumulation in HCV transgenic mice [53] and patients with chronic hepatitis C [54, 55]. Excessive iron induces reactive oxygen species mediated oxidative stress [56]. In patients with HCV infection, hepatic iron accumulation is associated with insulin resistance [57, 58]. Furthermore, iron depletion by phlebotomy reduces serum and hepatic levels of thioredoxin, a marker of oxidative stress, and homeostasis model assessment-insulin resistance, an index for insulin resistance in patients with chronic hepatitis C [46]. Thus, hepatic iron accumulation may cause insulin resistance through induction of inflammatory cytokines and oxidative stress.

Direct Effects of HCV on the Development of Insulin Resistance

Direct effects of HCV on the development of insulin resistance are still debatable. Tsochatzis E *et al.* reported that insulin resistance is not associated with viremia [59]. On the other hand, some previous studies reported that serum HCV RNA levels is associated with insulin resistance in a dose-dependent manner, independent of the visceral adipose tissue area [60-62] and HCV suppression by anti-viral treatment correlates with improvement in insulin resistance [63-65]. These findings suggest a possible role of HCV in the development of insulin resistance. Recently, HCV is now known to directly associate with insulin signaling molecules and cause insulin resistance. HCV core protein causes nuclear translocation of signal transducer and activation of transcription 3 and subsequent up-regulation of suppressor of cytokine signaling (SOCS) 3 proteins in various hepatoma cell lines [26]. SOCS3 is known to block insulin signaling cascade by ubiquitin-mediated degradation of insulin receptor substrate (IRS)1/2 [66]. Down-regulation of IRS1/2 are seen in livers from HCV-core transgenic mice and in livers of patients with HCV infection [26] and therefore, HCV core-induced SOCS3 up-regulation may promote proteasomal degradation of IRS1 and IRS2 through ubiquitination, leading to insulin resistance in patients with HCV infection [26, 67] (Fig. 1).

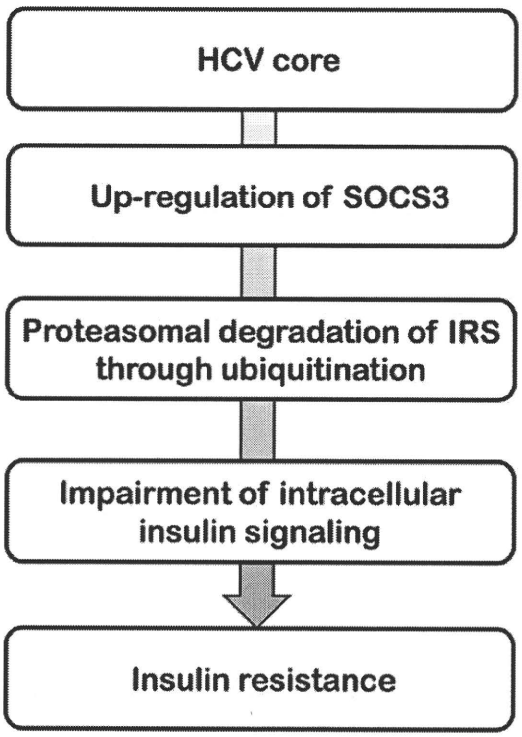

Fig. (1). Direct effects of HCV on the development of insulin resistance.
HCV core protein up-regulates suppressor of cytokine signaling (SOCS) 3 protein. SOCS3 ubiquitinates insulin receptor substrate (IRS) and causes proteasomal degradation of IRS. Since IRS is a central molecule of intarcellular insulin signaling, down-regulation of IRS blocks insulin signaling cascade, leading to insulin resistance in patients with HCV infection.

In addition to down-regulation of IRS1/2, HCV core protein also up-regulates serine phosphorylation of IRS1 or down-regulate tyrosine phosphorylation of IRS1 and subsequently impairs Akt signaling pathway, a downstream signaling of IRS1 *in vitro* [68] and in HCV-core transgenic mice [29]. Possible mechanism for changes in serine or tyrosine phosphorylation of IRS1 is due to increased activation of mammalian target of rapamycin [69] or Jun-N-terminal kinase [68]. Although changes in serine phosphorylation of IRS1 have not been validated in human liver tissue, down-regulation in tyrosine phosphorylation of IRS1 and subsequent Akt signaling pathway has been reported in human liver tissue [24].

HCV Genotype and Insulin Resistance

Although associations between HCV genotypes and the development of insulin resistance are still controversial [26, 59, 70, 71], HCV genotype-specific interactions of insulin resistance are reported in patients with HCV genotype 1b, 3a, and 4 [61, 69].

HCV genotype 1b significantly suppresses IRS1 expression compared to HCV genotype 2 in HepG2 cell [67]. The suppression of IRS1 expression is due to two different pathways. HCV genotype 1b up-regulates SOCS3, which causes proteasomal degradation of IRS1 [26]. Alternatively, HCV genotype 1 activates mammalian target of rapamycin, which suppress IRS1 expression [69]. Recently, Akuta *et al.* found that amino acid substitutions in the HCV core region of genotype 1b were associated with severe insulin resistance in patients without cirrhosis [22]. Although the precise mechanisms for the development to insulin resistance by amino acid substitutions in the HCV core region are unclear, HCV core seems to impair intracellular insulin signaling through several pathways.

In *in vitro* experiments, HCV genotype 3a modulates SOCS7 expression and causes down-regulation of IRS1 [69, 72]. In fact, patients infected with HCV genotype 3 frequently associated with insulin resistance [73]. In patients infected with HCV genotype 3, a decrease in total and high molecular weight adiponectin is another causative factor for the development of insulin resistance [74].

High prevalence of insulin resistance is also seen in patients infected with HCV genotype 4 and insulin resistance is a major determinant of both rapid virologic response and sustained virologic response [61, 75]. Normal BMI and no significant fibrosis are characters for patients HCV genotype 4 infected with insulin resistance [61, 76]. Although molecular mechanisms of HCV genotype 4 associated insulin resistance is not unclear, these findings suggest the genotype specific interaction with intracellular insulin signaling.

COMPLICATIONS OF HCV-ASSOCIATED INSULIN RESISTANCE

Insulin regulates not only glucose metabolism, but also protein synthesis, lipid metabolism and cell proliferation through activation of various intracellular signaling molecules [77]. Therefore, insulin resistance is involved in not only the development of DM, but also non-response to antiviral treatment [25, 28] and hepatic fibrosis [32]. In addition, insulin resistance causes esophageal varices [78] and HCC [33-35], life-threatening complications. Recently, the development of lichen planus [79], multiple primary carcinomas [80], and other extrahepatic manifestations [81] are associated with insulin resistance. Thus, insulin resistance could play crucial roles in the development to variety of complications in patients with HCV infection. In this review, we focused on an association between insulin resistance and HCC, a major cause of death for patients with HCV infection.

DIABETES MELLITUS AND HCC

DM has been found as a potential risk factor for the development of HCC. Three large population based cohort studies, in Sweden [82], Denmark [83], and the United States [84], reported that the development of HCC was increased 2 to 4 fold in patients with diabetes. Moreover, in some case-control studies, the positive association between DM and HCC has been suggested [85-88]. However, in patient HCV infection, an association between DM and HCC remains unclear, because most of these study populations had not been routinely surveyed for serological marker of HCV or contained only small number patients with HCV infection.

Table **2** shows a summary of recent seven cohort studies that investigated an association between DM and the development of HCC. Three of 7 studies reported by Tazawa *et al.* [89], Chen *et al.* [90] and Wang *et al.* [91] found that DM was significantly associated with the development of HCC in patients with HCV infection, with relative risk ranging from 3.1 to 9.4. On the other hand, three studies reported by Ohota *et al.* [92], Lai *et al.* [93] and Henderson *et al.* [94] found that there was no significant association between DM and the development of HCC in patients with HCV infection. Although the reason for this discrepancy is unclear, one possible explanation is that DM is diagnosed based on fasting plasma glucose and hemoglobin A1c levels. Both plasma glucose and hemoglobin A1c levels are not adequate diagnostic markers for DM in patients with HCV infection because of depletion of hepatic glycogen content and increased turnover of erythrocytes [95]. Thus, underdiagnosis of DM is a possible reason for the discrepancy. Recently, measurement of serum insulin levels is reported as a relevant clinical marker for predicting the development of HCC [96].

INSULIN RESISTANCE/HYPERINSULINEMIA AND HCC

Insulin is known as one of the most important factors not only for a variety of metabolic pathways, but also for cell growth. Insulin stimulates, via phosphorylation of IRS, phosphatidylinositol 3-kinase and Akt cascade [97] as well as Ras and mitogen-activated protein kinase cascade [98]. Since these cascades regulate hepatocyte proliferation and apoptosis, hyperinsulinemia may stimulate growth of HCC. Saito *et al.* demonstrated that postprandial hyperinsulinemia accelerated tumor doubling time of HCC in patient with cirrhosis [99]. Moreover, several previous studies showed that insulin resistance and subsequent hyperinsulinemia contributed to progression of liver fibrosis in patients with HCV infection, regardless of the degree of steatosis [100, 101]. As

Table 2. A Summary of Seven Cohort Studies for an Association Between Diabetes Mellitus and the Development of HCC in the Population Based on HCV Infection

Year	Country	No. of Cases	No. of Cohort	No. of Cohort with HCV (%)	RR (95% CI)	Adjustment	References
2002	Japan	13	279	279 (100%)	9.4 (ND)*	age, sex, alcohol, blood transfusion, α-fetoprotein, biopsy, IFN, HCV genotype, viral road	[89]
2003	Japan	101	161	161 (100%)	1.58 (0.62-3.99)	age, sex, BMI, alcohol, ALT, triglyceride, cholesterol, IFN, HCV genotype, HCV core protein	[92]
2006	Taiwan	115	54979	2087 (3.8%)	0.62 (0.22-1.76)	age, sex, alcohol, cigarette	[93]
2008	Netherland Canada Germany Switzerland	38	541	541 (100%)	2.07 (0.95-4.47)	age, sex, BMI, bilirubin, albumin, platelet count, HCV genotype, HCV viral road, HBc-Ab	[188]
2008	Taiwan	291	23820	1095 (4.6%)	3.52 (1.29-9.24)*	age, sex, alcohol, cigarette	[90]
2009	Taiwan	111	5929	982 (16.6%)	3.1 (1.7-5.4)*	age, sex, BMI, alcohol, cigarette	[91]
2009	United State	262	32806	32806 (100%)	1.17 (0.90-1.52)	age, sex, race, duration on dialysis	[94]

Abbreviation: RR; relative risk, CI; confidence interval, ND; no description in the text, IFN; interferon, BMI; body mass index, ALT; alanine aminotransferase. * P value < 0.05

a pathogenesis of these positive associations, it has been demonstrated that hyperinsulinemia promotes fibrogenesis by stimulating the secretion of connective tissue growth factor from hepatic stellate cells [102]. Therefore, it is suggested that the insulin resistance/hyperinsulimemia causes progression of hepatic fibrosis, leading to development of HCC in patients with HCV infection.

THERAPEUTIC STRATEGY FOR HCV-ASSOCIATED INSULIN RESISTANCE

In this review, we propose the clinical therapeutic strategy for the treatment of HCV-associated insulin resistance, which can be determined by life-style and stage of the liver disease, but not by definition of metabolic syndrome (Fig. **2**).

The International Diabetes Federation definition of metabolic syndrome is central adiposity plus two or more of the following factors; 1) raised concentration of triglycerides, 2) reduced concentration of HDL cholesterol, 3) raised blood pressure, and 4) raised fasting plasma glucose concentration [103]. In general, overeating and less activity induce metabolic syndrome through changes in adipocytokines. Therefore, diet therapy and exercise are recommended to patients with central adiposity [104]. However, none of the adipocytokines is associated with insulin resistance in patients with HCV infection, suggesting life-style may not be a major cause of HCV-associated insulin resistance [105]. Excessive life style modification such as fasting and over exercise could worsen liver function in patients with chronic liver disease [106-108], diet therapy and exercise are recommended if patients are overeating or less active.

Nutritional Therapy

Treatment for the insulin resistance could be a therapeutic strategy to prevent the development of HCC and to improve the prognosis in patients with HCV infection. Nutritional therapy and exercise are fundamental for patients with metabolic syndrome, which is defined by as well as patients with HCV-associated insulin resistance. However, in patients with liver cirrhosis, glucose metabolism is characterized as postprandial hyperglycemia induced by decreased glucose uptake of the liver [109, 110] and as fasting hypoglycemia induced by decreased glycogen storage in the liver, accompanied with increased ratio of lipid combustion in fasting state [108, 111-114]. Therefore, in order to improve glucose metabolism, divided energy intake into a larger number of meals per day including a late evening snack has been recommended for cirrhotic patients [115].

Branched-chain Amino Acids (BCAA)

BCAA has been recently demonstrated to play a role in glucose metabolism [116-119], while it improves the complications and prognosis induced by liver cirrhosis, such as hyperammonemia, encephalopathy, or HCC [120-122]. Therefore, nutritional therapy containing BCAA is most essential when considering a nutritional therapy for liver cirrhosis as well as DM. The mechanism of BCAA action on glucose metabolism is suggested as follows. In a rat model

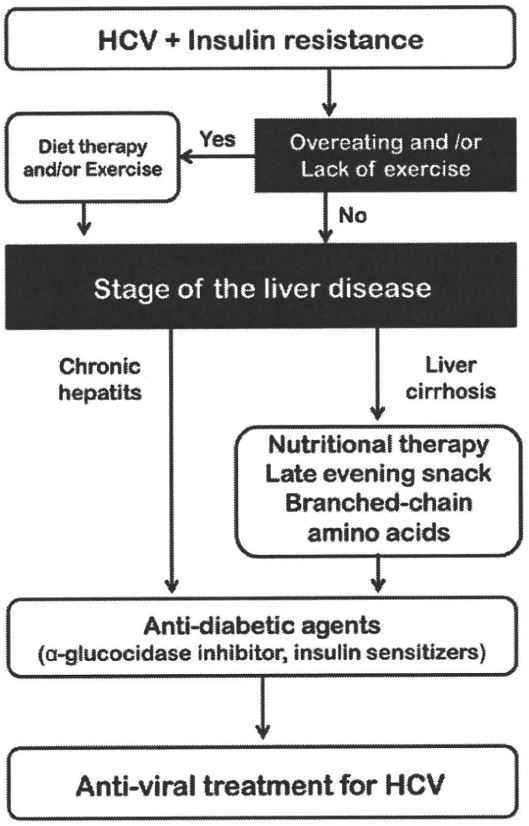

Fig. (2). Flow chart for the therapeutic strategy for HCV-associated insulin resistance. The therapeutic strategy for HCV-associated insulin resistance can be determined not only by life-style, but also by stage of the liver disease.

with liver cirrhosis, leucine and isolocine upregulate glucose uptake on skeletal muscle [123]. Similarly, a dipeptide constituted of leucine and isolocine also stimulates glucose uptake in myotube and skeletal muscle cells [124]. BCAA has potential to improve abnormal glucose metabolism such as insulin resistance through anti-obese effect in mice [125, 126]. In human, BCAA-enriched supplementation is also demonstrated to improve the insulin resistance or glucose tolerance of patients with chronic liver disease or liver cirrhosis [64, 127, 128]. Alternatively, intake of enteral nutrients for liver failure that are conditioned with enriched BCAA and high fisher's ratio as late evening snack would improve glucose metabolism [118].

It has been recently demonstrated that BCAA supplementation is potential to prevent the incidence of HCC [122]. Although the mechanism remains unclear, anti-diabetic and anti-obese effects might reduce the HCC incidence because both DM and obesity are shown to be risk factors for HCC. In addition, BCAA is shown to inhibit in vitro vascularization under the high concentration of glucose and/or insulin or in vivo vascularization of the liver in a diabetic rat model with liver injury [129]. Because HCC is a hypervascular tumor, the inhibitive effect of vascularization through the improvement of glucose metabolism by BCAA might reduce the incidence of HCC.

Anti-diabetic Agents

Anti-diabetic agents are generally used if nutritional and exercise therapies are not sufficient to improve hyperglycemia. When using anti-diabetic agents, the risk for adverse effects should be considered carefully because most of them are metabolized in liver. or α-glucocidase inhibitor [130] have been reported to have an adverse effect of severe liver injury that could be life-threatening complications for cirrhotic patients. In addition, use of biguanide in cirrhotic patients would be riskier for lactic acidosis than diabetic patients because lactic acid is also metabolized in liver. Thiazolidinedione is an insulin-sensitizing agent and improves virological response to peginterferon alpha-2b/ribavirin combination therapy in hepatitis C genotype 4 patients with insulin resistance [131]. However, thiazolidinedione causes overproduction of hydrogen peroxide, leading to severe hepatotoxicity [132-134]. Thus, these risks for adverse effects may limit the use of anti-diabetic agents and exogenous insulin tends to be administrated in patients with HCV infection.

An Association Between Anti-Diabetic Agents and Malignancies

Insulin therapy is generally selected for the treatment of cirrhotic patients with DM, however, insulin therapy has recently raised a question concerning to cancer incidence. In fact, use of anti-diabetic agents has been recently demonstrated to influence the tumor-free survival in diabetic patients. Currie CJ *et al.* demonstrated that overall tumor-free survival in diabetic patients receiving insulin-based therapy or sulfonylurea, but not metformin, was significantly worse than receiving no diabetes medications [135]. Although insulin or sulfonylurea does not seem to increase any tumor incidence, these agents could influence the incidence of digestive system cancers such as colorectal and pancreatic cancers. Similarly, Yang Y *et al.* demonstrated that the risk of colorectal cancer was increased by insulin therapy [136]. Li D *et al.* also demonstrated that the risk of pancreatic cancer was increased by insulin or sulfonylurea and reduced by metformin [137].

Concerning to HCC incidence, Donadon V *et al.* implied an association between HCC incidence and use of insulin or sulfonylurea [138]. Subsequently, they carried out a large-scale survey, demonstrating a direct association of HCC with use of insulin and sulfonylurea and an inverse relationship with metformin [139]. We also demonstrated that insulin or sulfonylurea was an independent risk factor for HCC incidence and hepatocarcinogenic effects of these anti-diabetic agents are evident in patients who were male or non-cirrhotic [140]. In addition, Komura T *et al.* described that insulin therapy was a significant factor contributing HCC recurrence after surgical treatment [141]. Thus, these results strongly suggest a potential risk factor of insulin for HCC incidence because either insulin administration or sulfonylurea intake increases insulin level in serum.

The mechanism between insulin and cancer incidence is little known. Since insulin has biological activities of cell proliferation, insulin may stimulate cancer cell proliferation and develop the cancer [142, 143]. In addition, it has been shown that the expression of phosphatase and tensin ho-

molog or SH2 domain-containing inositol phosphatase 2, suppressive molecules of insulin signaling in cells, is decreased in HCC tissues, indicating enhanced action of insulin in HCC [35, 144-146]. Thus, insulin therapy might worsen the prognosis of the patients with HCC because suppressors of intracellular insulin signaling are inactivated in HCC and therefore, insulin effects may be more evident in HCC than in hepatocytes.

Anti-viral Treatment for HCV

Since HCV itself plays crucial role in the development of insulin resistance, eradication of HCV by anti-viral treatment has a significant impact when patients meet the criteria. We along with others have shown that clearance of HCV improves insulin resistance, beta-cell function, and hepatic IRS1/2 expression [25, 28, 65]. Although clearance of HCV is a fundamental therapeutic strategy for patients with HCV infection, Tsochatzis *et al.* described that insulin resistance develops early in the course of the disease, and negatively affects treatment response and the development of liver cirrhosis and HCC, irrespective of genotype [147]. Thus, amelioration of insulin sensitivity may inhibit the progression of HCV-associated liver disease.

GUT HORMONES AND GLUCOSE METABOLISM

The gut has currently been recognized as an endocrine system that regulates glucose metabolism [148, 149]. Among several gut hormones called "incretin", glucagon-like peptide-1 (GLP-1) is well known to be involved in the glucose metabolism. GLP-1 is secreted from endocrine L-cells of the distal intestine and colon in response to enteric nutrient ingestion, such as carbohydrates, fatty acids, essential amino-acids and dietary fiber [150, 151]. GLP-1 exerts a direct insulinotoropic effect on the pancreatic β-cell [151, 152]. In addition, GLP-1 activates adenylate cyclase and subsequently enhances insulin secretion via GLP-1 receptor on the cell-membrane of pancreatic β-cell [153], and glucose disposal [154]. GLP-1 also inhibits glucagon secretion via GLP-1 receptor on pancreatic alpha-cells [155]. Thus, GLP-1 exerts carbohydrate assimilation and inhibits gluconeogenesis, consequently, GLP-1 is considered as a therapeutic target for DM as well as insulin resistance [150, 151, 155].

Active type of GLP-1 is rapidly inactivated by dipeptidyl peptidase-IV (DPP-4, enzyme code number 3.4.14.5) [151, 152, 156, 157]. DPP-4 is a membrane-associated peptidase and is widely distributed in numerous tissues, such as intestinal brush-border, endothelial cell and hepatocytes. DPP-4 inactivates GLP-1 within a few minutes. Therefore, DPP-4 inhibitor (incretin enhancer) may be a suitable agent for the treatment of insulin resistance.

GLP-1 AND GLUCOSE METABOLISM IN LIVER

GLP-1 reduces hepatic glucose production [158]. Although direct effect of GLP-1 on hepatocytes remains unclear, GLP-1 increases glycogen synthesis in hepatocytes by stimulating glycogen synthase alpha via GLP-1 receptor in rat hepatocytes [159, 160]. In addition, GLP-1 receptor agonist improve hepatic glucose homeostasis by promoting hepatic insulin signaling in diabetic rats [161]. In human study, GLP-1 receptor antagonist promotes hepatocyte proliferation via induced c-AMP [162] and GLP-1 inhibits glucose disposal rather than increasing glucose disposal [163]. These findings indicate that GLP-1 has a direct effect on hepatocytes in the regulation of glucose metabolism.

HCV-ASSOCIATED INSULIN RESISTANCE AND GLP-1

We have previously demonstrated that the up-regulation of DPP-4 causes a decrease in serum active GLP-1 levels, resulting in a decrease in hepatic glycogen contents and the development of insulin resistance in patients with HCV infection [39]. The mechanism of increased DPP-4 expression is unclear. However, a significant increase in DPP-4 expression is seen in a hepatoma cell line transfected with a HCV non-structural genome region [164]. In addition, eradication of HCV by treatment with interferon-alpha decreases serum DPP-4 activity [165]. These findings may indicate that HCV directly up-regulates DPP-4 expression. Although limited information is available for the effects of DPP-4 inhibitor in HCV-associated insulin resistance, this therapeutic agent could improve the initial step of the development of insulin resistance and is considered as a new therapeutic strategy for HCV-associated insulin resistance.

THE SIGNIFICANCE OF INSULIN RESISTANCE IN THE ERA OF NEW ANTI-VIRAL TREATMENTS

It is no doubt that these new anti-viral agents will markedly change the treatment for HCV infection in the near future. The most of new antiviral agents for HCV infection are currently in phase I-III [166-172] and the most studied agent is an inhibitor of the HCV non-structural 3 protease, telaprevir or boceprevir [173-183]. The addition of telaprevir or boceprevir to pegylated interferon-α and ribavirin combination therapy significantly enhance sustained virologic response rates even in HCV genotype 1 patients [168, 175, 180, 181, 184, 185]. However, the rates of sustained virologic response of triple therapy with telaprevir, pegylated interferon-α and ribavirin are still up to about 50% in patients who had previously treated by pegylated interferon-α and ribavirin [181]. In addition, the resistance profile of the HCV non-structural 3 protease inhibitor is elucidated. Thus, the triple therapy is not promising to cure all of patients with chronic HCV infection.

Recetnly, Akuta *et al.* examined the impact of substitution of amino acid in the core region of HCV genotype 1b in triple therapy with telaprevir, pegylated interferon-α and ribavirin and identified that substitutions of amino acid 70 and 91 as independent responsible factors associated with early virologic response [186]. Although the significance of insulin resistance in the triple therapy with telaprevir, pegylated interferon-α and ribavirin has never been investigated, insulin resistance may be a crucial factor even in the new era of anti-viral treatments because substitutions of amino acid 70 and 91 in the core region of HCV genotype 1b are closely associated with the development of insulin resistance [22].

CONCLUSION

In this review, we summarize the pathogenesis for HCV-associated insulin resistance. Similar to the life style-associated insulin resistance, obesity, inflammatory cytokines, oxidative stress, and serum PEDF levels are involved in the development of HCV-associated insulin resistance. Besides these factors, HCV itself also causes insulin resistance through down-regulation of hepatic IRS1/2. Insulin resistance is responsible for the development of cirrhotic complications including HCC, however, there is no common therapeutic strategy for HCV-associated insulin resistance.

Clearance of HCV by anti-viral treatment is a fundamental therapeutic strategy for patients with HCV infection. In addition, amelioration of insulin sensitivity may inhibit the progression of HCV-associated liver disease, and could improve the survival of these patients. Late evening snack and BCAA are nutritional therapies which could improve insulin resistance. However, use of anti-diabetic agents and exogenous insulin are not always recommended because of adverse effects and possible link to the development of HCC.

HCV also affects insulin resistance through activation of DPP-4 and subsequent inactivation of GLP-1, a key regulator of insulin secretion and hepatic glucose metabolism. Although availability of DPP-4 inhibitor in HCV-associated insulin resistance is yet unclear, this therapeutic agent could improve the early step of the development of insulin resistance and is expected to be a new therapeutic strategy for HCV-associated insulin resistance.

ACKNOWLEDGEMENTS

This study was supported, in part, by a Grant-in-Aid for Young Scientists (B) (No. 22790874 to T.K.) and a Grant-in-Aid for Scientific Research (C) (No. 21590865 to M.S.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by Health and Labour Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labour and Welfare of Japan, and by a Grant for Cancer Research from Fukuoka Cancer Society.

LIST OF ABBREVIATIONS

BCAA	=	branched-chain amino acids
DM	=	diabetes mellitus
DPP-4	=	dipeptidyl peptidase-IV
GLP-1	=	glucagon-like peptide-1
HCC	=	hepatocellular carcinoma
HCV	=	hepatitis C virus
IRS	=	insulin receptor substrate
PEDF	=	serum pigment epithelium-derived factor
SOCS	=	suppressor of cytokine signaling

REFERENCES

[1] Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244: 359-62.

[2] Houghton M. The long and winding road leading to the identification of the hepatitis C virus. J Hepatol 2009; 51: 939-48.

[3] Asselah T, Bieche I, Sabbagh A, *et al.* Gene expression and hepatitis C virus infection. Gut 2009; 58: 846-58.

[4] Maheshwari A, Ray S, Thuluvath P J. Acute hepatitis C. Lancet 2008; 372: 321-32.

[5] Seeff LB, Hoofnagle JH. Appendix: The national institutes of health consensus development conference management of hepatitis C 2002. Clin Liver Dis 2003; 7: 261-87.

[6] Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. Hepatology 2008; 48: 418-31.

[7] Thompson ND, Perz JF, Moorman AC, Holmberg SD. Nonhospital health care-associated hepatitis B and C virus transmission: United States, 1998-2008. Ann Intern Med 2009; 150: 33-9.

[8] Alter MJ, Margolis HS, Krawczynski K, *et al.* The natural history of community-acquired hepatitis C in the United States. The sentinel counties chronic non-a, non-b hepatitis study team. N Engl J Med 1992; 327: 1899-905.

[9] Marcellin P. Hepatitis B and hepatitis C in 2009. Liver Int 2009; 29 (Suppl 1): 1-8.

[10] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997; 349: 825-32.

[11] Ali SS, Ali I S, Aamir AH, Jadoon Z, Inayatullah S. Frequency of hepatitis C infection in diabetic patients. J Ayub Med Coll Abbottabad 2007; 19: 46-9.

[12] Bahtiyar G, Shin JJ, Aytaman A, Sowers JR, Mcfarlane SI. Association of diabetes and hepatitis C infection: epidemiologic evidence and pathophysiologic insights. Curr Diabetes Rep 2004; 4: 194-8.

[13] Gray H, Wreghitt T, Stratton IM, Alexander GJ, Turner RC, O'rahilly S. High prevalence of hepatitis C infection in Afro-Caribbean patients with type 2 diabetes and abnormal liver function tests. Diabet Med 1995; 12: 244-9.

[14] Kawaguchi T, Nagao Y, Tanaka K, *et al.* Causal relationship between hepatitis C virus core and the development of type 2 diabetes mellitus in a hepatitis C virus hyperendemic area: a pilot study. Int J Mol Med 2005; 16: 109-14.

[15] Mehta SH, Brancati FL, Strathdee SA, *et al.* Hepatitis C virus infection and incident type 2 diabetes. Hepatology 2003; 38: 50-6.

[16] Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med 2000; 133: 592-9.

[17] Nwokediuko SC, Oli JM. Hepatitis C virus infection in Nigerians with diabetes mellitus. Niger J Clin Pract 2008; 11: 94-9.

[18] Shaheen M, Echeverry D, Oblad MG, Montoya MI, Teklehaimanot S, Akhtar AJ. Hepatitis C, metabolic syndrome, and inflammatory markers: results from the Third National Health and Nutrition Examination Survey [NHANES III]. Diabetes Res Clin Pract 2007; 75: 320-6.

[19] Simo R, Hernandez C, Genesca J, Jardi R, Mesa J. High prevalence of hepatitis C virus infection in diabetic patients. Diabetes Care 1996; 19: 998-1000.

[20] Sporea I, Sirli R, Hogea C, Sink AA, Serban V. Diabetes mellitus and chronic HCV infection. Rom J Intern Med 2009; 47: 141-7.

[21] Wilson C. Hepatitis C infection and type 2 diabetes in American-Indian women. Diabetes Care 2004; 27: 2116-9.

[22] Akuta N, Suzuki F, Hirakawa M, *et al.* Amino acid substitutions in the hepatitis C virus core region of genotype 1b are the important predictor of severe insulin resistance in patients without cirrhosis and diabetes mellitus. J Med Virol 2009; 81: 1032-9.

[23] Allison ME, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. J Hepatol 1994; 21: 1135-9.

[24] Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology 2003; 38: 1384-92.

[25] Kawaguchi T, Ide T, Taniguchi E, *et al.* Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression

of insulin receptor substrate 1 and 2. Am J Gastroenterol 2007; 102: 570-6.

[26] Kawaguchi T, Yoshida T, Harada M, *et al.* Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 2004; 165: 1499-508.

[27] Negro F. Insulin resistance and HCV: will new knowledge modify clinical management? J Hepatol 2006; 45: 514-9.

[28] Romero-Gomez M. Insulin resistance and hepatitis C. World J Gastroenterol 2006; 12: 7075-80.

[29] Shintani Y, Fujie H, Miyoshi H, *et al.* Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 2004; 126: 840-8.

[30] Taura N, Ichikawa T, Hamasaki K, *et al.* Association between liver fibrosis and insulin sensitivity in chronic hepatitis C patients. Am J Gastroenterol 2006; 101: 2752-9.

[31] Alberti KG, Eckel RH, Grundy SM, *et al.* Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120: 1640-5.

[32] Balsano C, Alisi A, Nobili V. Liver fibrosis and therapeutic strategies: the goal for improving metabolism. Curr Drug Targets 2009; 10: 505-12.

[33] El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004; 127: S27-34.

[34] Negro F. Correction of insulin resistance in chronic hepatitis C patients not responding to the standard of care: more questions than answers. J Hepatol 2009; 50: 1271-2.

[35] Sumie S, Kawaguchi T, Komuta M, *et al.* Significance of glucose intolerance and SHIP2 expression in hepatocellular carcinoma patients with HCV infection. Oncol Rep 2007; 18: 545-52.

[36] Kawaguchi T, Sata M. Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. World J Gastroenterol 2010; 16: 1943-52.

[37] Kawaguchi T, Sata M. Importance of hepatitis C virus-associated insulin resistance-therapeutic strategies for insulin sensitization. World J Gastroenterol 2010; 16: 1943-52.

[38] Bianchi G, Marchesini G, Zoli M, Bugianesi E, Fabbri A, Pisi E. Prognostic significance of diabetes in patients with cirrhosis. Hepatology 1994; 20: 119-25.

[39] Itou M, Kawaguchi T, Taniguchi E, *et al.* Altered expression of glucagon-like peptide-1 and dipeptidyl peptidase IV in patients with HCV-related glucose intolerance. J Gastroenterol Hepatol 2008; 23: 244-51.

[40] Eguchi Y, Mizuta T, Ishibashi E, *et al.* Hepatitis C virus infection enhances insulin resistance induced by visceral fat accumulation. Liver Int 2009; 29: 213-20.

[41] Hung CH, Lee CM, Chen CH, *et al.* Association of inflammatory and anti-inflammatory cytokines with insulin resistance in chronic hepatitis C. Liver Int 2009; 29: 1086-93.

[42] Lu JY, Chuang LM, Yang WS, *et al.* Adiponectin levels among patients with chronic hepatitis B and C infections and in response to IFN-alpha therapy. Liver Int 2005; 25: 752-9.

[43] Knobler H, Zhornicky T, Sandler A, Haran N, Ashur Y, Schattner A. Tumor necrosis factor-alpha-induced insulin resistance may mediate the hepatitis C virus-diabetes association. Am J Gastroenterol 2003; 98: 2751-6.

[44] Maeno T, Okumura A, Ishikawa T, *et al.* Mechanisms of increased insulin resistance in non-cirrhotic patients with chronic hepatitis C virus infection. J Gastroenterol Hepatol 2003; 18: 1358-63.

[45] Koike K, Miyoshi H. Oxidative stress and hepatitis C viral infection. Hepatol Res 2006; 34: 65-73.

[46] Mitsuyoshi H, Itoh Y, Sumida Y, *et al.* Evidence of oxidative stress as a cofactor in the development of insulin resistance in patients with chronic hepatitis C. Hepatol Res 2008; 38: 348-53.

[47] Vidali M, Tripodi MF, Ivaldi A, *et al.* Interplay between oxidative stress and hepatic steatosis in the progression of chronic hepatitis C. J Hepatol 2008; 48: 399-406.

[48] Narita R, Abe S, Tabaru A, Otsuki M. Impact of steatosis on insulin secretion in chronic hepatitis C patients. Am J Gastroenterol 2007; 102: 2173-80.

[49] Narita R, Abe S, Kihara Y, Akiyama T, Tabaru A, Otsuki M. Insulin resistance and insulin secretion in chronic hepatitis C virus infection. J Hepatol 2004; 41: 132-8.

[50] Ferenci P, Bratusch-Marrain P, Waldhausl WK, Nowotny P, Korn A. Impaired plasma amino-acid clearance in patients with cirrhosis of the liver and portocaval shunt--its relation to insulin resistance. Eur J Clin Invest 1984; 14: 255-61.

[51] Tanabe N, Ishii M, Sato Y, *et al.* Effects of collateral vessel occlusion on oral glucose tolerance test in liver cirrhosis. Dig Dis Sci 2000; 45: 581-6.

[52] Muckenthaler MU. Fine tuning of hepcidin expression by positive and negative regulators. Cell Metab 2008; 8: 1-3.

[53] Nishina S, Hino K, Korenaga M, *et al.* Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. Gastroenterology 2008; 134: 226-38.

[54] Fujita N, Sugimoto R, Takeo M, *et al.* Hepcidin expression in the liver: relatively low level in patients with chronic hepatitis C. Mol Med 2007; 13: 97-104.

[55] Nagashima M, Kudo M, Chung H, *et al.* Regulatory failure of serum prohepcidin levels in patients with hepatitis C. Hepatol Res 2006; 36: 288-93.

[56] Lecube A, Hernandez C, Simo R. Glucose abnormalities in non-alcoholic fatty liver disease and chronic hepatitis C virus infection: the role of iron overload. Diabetes Metab Res Rev 2009; 25: 403-10.

[57] Furutani M, Nakashima T, Sumida Y, *et al.* Insulin resistance/beta-cell function and serum ferritin level in non-diabetic patients with hepatitis C virus infection. Liver Int 2003; 23: 294-9.

[58] Sumida Y, Kanemasa K, Fukumoto K, Yoshida N, Sakai K. Hepatic iron accumulation may be associated with insulin resistance in patients with chronic hepatitis C. Hepatol Res 2007; 37: 932-40.

[59] Tsochatzis E, Manolakopoulos S, Papatheodoridis GV, *et al.* Serum HCV RNA levels and HCV genotype do not affect insulin resistance in nondiabetic patients with chronic hepatitis C: a multicentre study. Aliment Pharmacol Ther 2009; 30: 947-54.

[60] Hsu CS, Liu CJ, Liu CH, *et al.* High hepatitis C viral load is associated with insulin resistance in patients with chronic hepatitis C. Liver Int 2008; 28: 271-7.

[61] Moucari R, Asselah T, Cazals-Hatem D, *et al.* Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology 2008; 134: 416-23.

[62] Yoneda M, Saito S, Ikeda T, *et al.* Hepatitis C virus directly associates with insulin resistance independent of the visceral fat area in nonobese and nondiabetic patients. J Viral Hepatitis 2007; 14: 600-7.

[63] Delgado-Borrego A, Jordan SH, Negre B, *et al.* Reduction of insulin resistance with effective clearance of hepatitis C infection: results from the HALT-C trial. Clin Gastroenterol Hepatol 2010; 8(5): 458-62.

[64] Kawaguchi T, Taniguchi E, Itou M, *et al.* Branched-chain amino acids improve insulin resistance in patients with hepatitis C virus-related liver disease: report of two cases. Liver Int 2007; 27: 1287-92.

[65] Kawaguchi Y, Mizuta T, Oza N, *et al.* Eradication of hepatitis C virus by interferon improves whole-body insulin resistance and hyperinsulinaemia in patients with chronic hepatitis C. Liver Int 2009; 29: 871-7.

[66] Rui L, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem 2002; 277: 42394-8.

[67] Persico M, Russo R, Persico E, *et al.* SOCS3 and IRS-1 gene expression differs between genotype 1 and genotype 2 hepatitis C virus-infected HepG2 cells. Clin Chem Lab Med 2009; 47: 1217-25.

[68] Banerjee S, Saito K, Ait-Goughoulte M, Meyer K, Ray RB, Ray R. Hepatitis C virus core protein upregulates serine phosphorylation of insulin receptor substrate-1 and impairs the downstream akt/protein kinase B signaling pathway for insulin resistance. J Virol 2008; 82: 2606-12.

[69] Pazienza V, Clement S, Pugnale P, *et al.* The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. Hepatology 2007; 45: 1164-71.

[70] Cua IH, Hui JM, Kench JG, George J. Genotype-specific interac-

tions of insulin resistance, steatosis, and fibrosis in chronic hepatitis C. Hepatology 2008; 48: 723-31.

[71] Liu CJ, Jeng YM, Chen PJ, *et al.* Influence of metabolic syndrome, viral genotype and antiviral therapy on superimposed fatty liver disease in chronic hepatitis C. Antivir Ther 2005; 10: 405-15.

[72] Pazienza V, Vinciguerra M, Andriulli A, Mangia A. HCV core protein genotype 3a increases SOCS-7 expression through PPAR-{gamma} in Huh-7 cells. J Gen Virol 2010; 91(Pt 7): 1678-86.

[73] Duseja A, Dhiman RK, Chawla Y, *et al.* Insulin resistance is common in patients with predominantly genotype 3 chronic hepatitis C. Dig Dis Sci 2009; 54: 1778-82.

[74] Wang AY, Hickman IJ, Richards AA, Whitehead JP, Prins JB, Macdonald GA. High molecular weight adiponectin correlates with insulin sensitivity in patients with hepatitis C genotype 3, but not genotype 1 infection. Am J Gastroenterol 2005; 100: 2717-23.

[75] Khattab M, Eslam M, Sharwae MA, Shatat M, Ali A, Hamdy L. Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis C genotype 4 patients. Am J Gastroenterol 2010; [Epub ahead of print]

[76] Moucari R, Ripault MP, Martinot-Peignoux M, *et al.* Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-4. Gut 2009; 58: 1662-9.

[77] Kawaguchi T, Yamagishi S, Sata M. Branched-chain amino acids and pigment epithelium-derived factor: novel therapeutic agents for hepatitis C Virus-associated insulin resistance. Curr Med Chem 2009: 16(36): 4843-57.

[78] Camma C, Petta S, Di Marco V, *et al.* Insulin resistance is a risk factor for esophageal varices in hepatitis C virus cirrhosis. Hepatology 2009; 49: 195-203.

[79] Nagao Y, Kawasaki K, Sata M. Insulin resistance and lichen planus in patients with HCV-infectious liver diseases. J Gastroenterol Hepatol 2008; 23: 580-5.

[80] Nagao Y, Sata M. High incidence of multiple primary carcinomas in HCV-infected patients with oral squamous cell carcinoma. Med Sci Monit 2009; 15: CR453-9.

[81] Nagao Y, Kawaguchi T, Tanaka K, Kumashiro R, Sata M. Extrahepatic manifestations and insulin resistance in an HCV hyperendemic area. Int J Mol Med 2005; 16: 291-6.

[82] Adami HO, Chow WH, Nyren O, *et al.* Excess risk of primary liver cancer in patients with diabetes mellitus. J Natl Cancer Inst 1996; 88: 1472-7.

[83] Wideroff L, Gridley G, Mellemkjaer L, *et al.* Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst 1997; 89: 1360-5.

[84] El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004; 126: 460-8.

[85] Davila JA, Morgan RO, Shaib Y, Mcglynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 2005; 54: 533-9.

[86] El-Serag HB, Richardson PA, Everhart JE. The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. Am J Gastroenterol 2001; 96: 2462-7.

[87] La Vecchia C, Negri E, Decarli A, Franceschi S. Diabetes mellitus and the risk of primary liver cancer. Int J Cancer 1997; 73: 204-7.

[88] Lagiou P, Kuper H, Stuver SO, Tzonou A, Trichopoulos D, Adami HO. Role of diabetes mellitus in the etiology of hepatocellular carcinoma. J Natl Cancer Inst 2000; 92: 1096-9.

[89] Tazawa J, Maeda M, Nakagawa M, *et al.* Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. Dig Dis Sci 2002; 47: 710-5.

[90] Chen CL, Yang HI, Yang WS, *et al.* Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology 2008; 135: 111-21.

[91] Wang CS, Yao WJ, Chang TT, Wang ST, Chou P. The impact of type 2 diabetes on the development of hepatocellular carcinoma in different viral hepatitis statuses. Cancer Epidemiol Biomarkers Prev 2009; 18: 2054-60.

[92] Ohata K, Hamasaki K, Toriyama K, *et al.* Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Cancer 2003; 97: 3036-43.

[93] Lai MS, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: a cohort study in high prevalence area of hepatitis virus infection. Hepatology 2006; 43: 1295-302.

[94] Henderson WA, Shankar R, Gill JM, *et al.* Hepatitis C progressing to hepatocellular carcinoma: the HCV dialysis patient in dilemma. J Viral Hepatitis 2010; 17(1): 59-64.

[95] Koga M, Kasayama S, Kanehara H, Bando Y. CLD (chronic liver diseases)-HbA1C as a suitable indicator for estimation of mean plasma glucose in patients with chronic liver diseases. Diabetes Res Clin Pract 2008; 81: 258-62.

[96] Donadon V, Balbi M, Valentia G, *et al.* Association between type 2 diabetes mellitus and hepatocellular carcinoma: role of insulin resistance and hyperinsulinemia. Hepatology 2009; 50: 1081A.

[97] Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002; 296: 1655-7.

[98] Boulton TG, Nye SH, Robbins DJ, *et al.* ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 1991; 65: 663-75.

[99] Saito K, Inoue S, Saito T, *et al.* Augmentation effect of postprandial hyperinsulinaemia on growth of human hepatocellular carcinoma. Gut 2002; 51: 100-4.

[100] Bugianesi E, Marchesini G, Gentilcore E, *et al.* Fibrosis in genotype 3 chronic hepatitis C and nonalcoholic fatty liver disease: role of insulin resistance and hepatic steatosis. Hepatology 2006; 44: 1648-55.

[101] Fartoux L, Poujol-Robert A, Guechot J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut 2005; 54: 1003-8.

[102] Paradis V, Perlemuter G, Bonvoust F, *et al.* High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 2001; 34: 738-44.

[103] Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet 2005; 366: 1059-62.

[104] Kelesidis T, Kelesidis I, Chou S, Mantzoros CS. Narrative review: the role of leptin in human physiology: emerging clinical applications. Ann Intern Med 2010; 152: 93-100.

[105] Cua IH, Hui JM, Bandara P, *et al.* Insulin resistance and liver injury in hepatitis C is not associated with virus-specific changes in adipocytokines. Hepatology 2007; 46: 66-73.

[106] Degoricija V, Zjacic-Rotkvic V, Marout J, Sefer S, Troskot B. Clinical and neurohumoral response to posture, physical exercise, and ascites treatment in Child-Pugh C liver cirrhosis: randomized prospective trial. Croat Med J 2003; 44: 178-86.

[107] Kawaguchi T, Taniguchi E, Itou M, *et al.* Supplement improves nutrition and stresses caused by examination-associated fasting in patients with liver cirrhosis. Hepatol Res 2008; 38: 1178-85.

[108] Owen OE, Trapp VE, Reichard GA, Jr., *et al.* Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. J Clin Invest 1983; 72: 1821-32.

[109] Kalaitzakis E, Bosaeus I, Ohman L, Bjornsson E. Altered postprandial glucose, insulin, leptin, and ghrelin in liver cirrhosis: correlations with energy intake and resting energy expenditure. Am J Clin Nutr 2007; 85: 808-15.

[110] Nielsen MF, Caumo A, Aagaard NK, *et al.* Contribution of defects in glucose uptake to carbohydrate intolerance in liver cirrhosis: assessment during physiological glucose and insulin concentrations. Am J Physiol Gastrointest Liver Physiol 2005; 288: G1135-43.

[111] Miwa Y, Shiraki M, Kato M, *et al.* Improvement of fuel metabolism by nocturnal energy supplementation in patients with liver cirrhosis. Hepatol Res 2000; 18: 184-9.

[112] Muller MJ, Lautz HU, Plogmann B, Burger M, Korber J, Schmidt FW. Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of cause, clinical staging and nutritional state. Hepatology 1992; 15: 782-94.

[113] Nakaya Y, Harada N, Kakui S, *et al.* Severe catabolic state after prolonged fasting in cirrhotic patients: effect of oral branched-chain amino-acid-enriched nutrient mixture. J Gastroenterol 2002; 37: 531-6.

[114] Tajika M, Kato M, Mohri H, *et al.* Prognostic value of energy metabolism in patients with viral liver cirrhosis. Nutrition 2002; 18: 229-34.

[115] 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:

1SA-138SA.

[116] Aoyama K, Tsuchiya M, Mori K, *et al.* Effect of a late evening snack on outpatients with liver cirrhosis. Hepatol Res 2007; 37: 608-14.

[117] Carty H. Scintigraphy in sports injuries: is it underutilized? Nucl Med Commun 1991; 12: 173-4.

[118] Tsuchiya M, Sakaida I, Okamoto M, Okita K. The effect of a late evening snack in patients with liver cirrhosis. Hepatol Res 2005; 31: 95-103.

[119] Utsugi T, Yoshida A, Kanda T, *et al.* Oral administration of branched chain amino acids improves virus-induced glucose intolerance in mice. Eur J Pharmacol 2000; 398: 409-14.

[120] Marchesini G, Bianchi G, Merli M, *et al.* Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. Gastroenterology 2003; 124: 1792-801.

[121] Muto Y, Sato S, Watanabe A, *et al.* Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol 2005; 3: 705-13.

[122] Muto Y, Sato S, Watanabe A, *et al.* 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.

[123] Nishitani S, Takehana K, Fujitani S, Sonaka I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. Am J Physiol Gastrointest Liver Physiol 2005; 288: G1292-300.

[124] Morifuji M, Koga J, Kawanaka K, Higuchi M. Branched-chain amino acid-containing dipeptides, identified from whey protein hydrolysates, stimulate glucose uptake rate in L6 myotubes and isolated skeletal muscles. J Nutr Sci Vitaminol (Tokyo) 2009; 55: 81-6.

[125] Ikehara O, Kawasaki N, Maezono K, Komatsu M, Konishi A. Acute and chronic treatment of L-isoleucine ameliorates glucose metabolism in glucose-intolerant and diabetic mice. Biol Pharm Bull 2008; 31: 469-72.

[126] She P, Reid TM, Bronson SK, *et al.* Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. Cell Metab 2007; 6: 181-94.

[127] Kawaguchi T, Nagao Y, Matsuoka H, Ide T, Sata M. Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease. Int J Mol Med 2008; 22: 105-12.

[128] Urata Y, Okita K, Korenaga K, Uchida K, Yamasaki T, Sakaida I. The effect of supplementation with branched-chain amino acids in patients with liver cirrhosis. Hepatol Res 2007; 37: 510-6.

[129] 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.

[130] Carrascosa M, Pascual F, Aresti S. Acarbose-induced acute severe hepatotoxicity. Lancet 1997; 349: 698-9.

[131] Khattab M, Emad M, Abdelaleem A, *et al.* Pioglitazone improves virological response to peginterferon alpha-2b/ribavirin combination therapy in hepatitis C genotype 4 patients with insulin resistance. Liver Int 2010; 30(3): 447-54.

[132] Al-Salman J, Arjomand H, Kemp DG, Mittal M. Hepatocellular injury in a patient receiving rosiglitazone. A case report. Ann Intern Med 2000; 132: 121-4.

[133] Gouda HE, Khan A, Schwartz J, Cohen RI. Liver failure in a patient treated with long-term rosiglitazone therapy. Am J Med 2001; 111: 584-5.

[134] Lenhard MJ, Funk WB. Failure to develop hepatic injury from rosiglitazone in a patient with a history of troglitazone-induced hepatitis. Diabetes Care 2001; 24: 168-9.

[135] Hemkens LG, Grouven U, Bender R, *et al.* Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study. Diabetologia 2009; 52: 1732-44.

[136] Yang YX, Hennessy S, Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. Gastroenterology 2004; 127: 1044-50.

[137] Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology 2009; 137: 482-8.

[138] Donadon V, Balbi M, Casarin P, Vario A, Alberti A. Association between hepatocellular carcinoma and type 2 diabetes mellitus in Italy: potential role of insulin. World J Gastroenterol 2008; 14: 5695-700.

[139] Donadon V, Balbi M, Ghersetti M, *et al.* Antidiabetic therapy and increased risk of hepatocellular carcinoma in chronic liver disease. World J Gastroenterol 2009; 15: 2506-11.

[140] Kawaguchi T, Taniguchi E, Morita Y, *et al.* Association of exogenous insulin or sulfonylurea treatment with an increased incidence of hepatoma in patients with HCV infection. Liver Int 2010; 30(3): 479-86.

[141] Komura T, Mizukoshi E, Kita Y, *et al.* Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment in patients with viral hepatitis. Am J Gastroenterol 2007; 102: 1939-46.

[142] Godsland IF. Insulin resistance and hyperinsulinaemia in the development and progression of cancer. Clin Sci (Lond) 2009; 118: 315-32.

[143] Sandow J. Growth effects of insulin and insulin analogues. Arch Physiol Biochem 2009; 115: 72-85.

[144] Dong-Dong L, Xi-Ran Z, Xiang-Rong C. Expression and significance of new tumor suppressor gene PTEN in primary liver cancer. J Cell Mol Med 2003; 7: 67-71.

[145] Wan XW, Jiang M, Cao HF, *et al.* The alteration of PTEN tumor suppressor expression and its association with the histopathological features of human primary hepatocellular carcinoma. J Cancer Res Clin Oncol 2003; 129: 100-6.

[146] Wu SK, Wang BJ, Yang Y, Feng XH, Zhao XP, Yang DL. Expression of PTEN, PPM1A and P-Smad2 in hepatocellular carcinomas and adjacent liver tissues. World J Gastroenterol 2007; 13: 4554-9.

[147] Tsochatzis EA, Manolakopoulos S, Papatheodoridis GV, Archimandritis AJ. Insulin resistance and metabolic syndrome in chronic liver diseases: old entities with new implications. Scand J Gastroenterol 2009; 44: 6-14.

[148] Creutzfeldt W. The [pre-] history of the incretin concept. Regul Pept 2005; 128: 87-91.

[149] Seltzer HS, Allen EW, Herron AL, Jr., Brennan MT. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 1967; 46: 323-35.

[150] Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007; 132: 2131-57.

[151] Drucker DJ. The biology of incretin hormones. Cell Metab 2006; 3: 153-65.

[152] Holst JJ. Glucagon-like peptide-1: from extract to agent. The Claude Bernard Lecture, 2005. Diabetologia 2006; 49: 253-60.

[153] Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci USA 1992; 89: 8641-5.

[154] Vilsboll T, Krarup T, Madsbad S, Holst JJ. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul Pept 2003; 114: 115-21.

[155] Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993; 36: 741-4.

[156] Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ. Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. Am J Physiol 1996; 271: E458-64.

[157] Hansen L, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 1999; 140: 5356-63.

[158] Larsson H, Holst JJ, Ahren B. Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. Acta Physiol Scand 1997; 160: 413-22.

[159] Mommsen TP, Andrews PC, Plisetskaya EM. Glucagon-like peptides activate hepatic gluconeogenesis. FEBS Lett 1987; 219: 227-32.

[160] Redondo A, Trigo MV, Acitores A, Valverde I, Villanueva-Penacarrillo ML. Cell signalling of the GLP-1 action in rat liver. Mol Cell Endocrinol 2003; 204: 43-50.

[161] Park S, Hong SM, Ahn IS. Exendin-4 and exercise improve hepatic glucose homeostasis by promoting insulin signaling in diabetic rats.

Metabolism 2010; 59: 123-33.

[162] Aviv V, Meivar-Levy I, Rachmut IH, Rubinek T, Mor E, Ferber S. Exendin-4 promotes liver cell proliferation and enhances the PDX-1-induced liver to pancreas transdifferentiation process. J Biol Chem 2009; 284: 33509-20.

[163] Prigeon RL, Quddusi S, Paty B, D'alessio DA. Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. Am J Physiol Endocrinol Metab 2003; 285: E701-7.

[164] Harada T, Kim DW, Sagawa K, *et al.* Characterization of an established human hepatoma cell line constitutively expressing nonstructural proteins of hepatitis C virus by transfection of viral cDNA. J Gen Virol 1995; 76 (Pt 5): 1215-21.

[165] Maes M, Bonaccorso S, Marino V, *et al.* Treatment with interferon-alpha (IFN alpha) of hepatitis C patients induces lower serum dipeptidyl peptidase IV activity, which is related to IFN alpha-induced depressive and anxiety symptoms and immune activation. Mol Psychiatry 2001; 6: 475-80.

[166] Asselah T, Benhamou Y, Marcellin P. Protease and polymerase inhibitors for the treatment of hepatitis C. Liver Int 2009; 29 (Suppl 1): 57-67.

[167] Harrison SA. Small molecule and novel treatments for chronic hepatitis C virus infection. Am J Gastroenterol 2007; 102: 2332-8.

[168] Lange CM, Sarrazin C, Zeuzem S. Review article: HCV - STAT-C era of therapy. Aliment Pharmacol Ther 2010; [Epub ahead of print]

[169] Moucari R, Marcellin P. Hepatitis C: The role of new interferons in the era of STAT-C. Nat Rev Gastroenterol Hepatol 2009; 6: 509-11.

[170] Parfieniuk A, Jaroszewicz J, Flisiak R. Specifically targeted antiviral therapy for hepatitis C virus. World J Gastroenterol 2007; 13: 5673-81.

[171] Pereira AA, Jacobson IM. New and experimental therapies for HCV. Nat Rev Gastroenterol Hepatol 2009; 6: 403-11.

[172] Schinazi RF, Bassit L, Gavegnano C. HCV drug discovery aimed at viral eradication. J Viral Hepatitis 2010; 17: 77-90.

[173] Berman K, Kwo PY. Boceprevir, an NS3 protease inhibitor of HCV. Clin Liver Dis 2009; 13: 429-39.

[174] Flint M, Mullen S, Deatly AM, *et al.* Selection and characterization of hepatitis C virus replicons dually resistant to the polymerase and protease inhibitors HCV-796 and boceprevir (SCH 503034). Antimicrob Agents Chemother 2009; 53: 401-11.

[175] Hezode C, Forestier N, Dusheiko G, *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009; 360: 1839-50.

[176] Hoofnagle JH. A step forward in therapy for hepatitis C. N Engl J Med 2009; 360: 1899-901.

[177] Kao JH. Telaprevir for chronic HCV infection. N Engl J Med 2009; 361: 534-5.

[178] Kronenberger B, Zeuzem S. Current and future treatment options for HCV. Ann Hepatol 2009; 8: 103-12.

[179] Liu-Young G, Kozal MJ. Hepatitis C protease and polymerase inhibitors in development. AIDS Patient Care STDS 2008; 22: 449-57.

[180] Mchutchison JG, Everson GT, Gordon SC, *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009; 360: 1827-38.

[181] Mchutchison JG, Manns MP, Muir AJ, *et al.* Telaprevir for previously treated chronic HCV infection. N Engl J Med 2010; 362: 1292-303.

[182] Njoroge FG, Chen KX, Shih NY, Piwinski JJ. Challenges in modern drug discovery: a case study of boceprevir, an HCV protease inhibitor for the treatment of hepatitis C virus infection. Acc Chem Res 2008; 41: 50-9.

[183] Van der Meer AJ, De Knegt RJ. Telaprevir for chronic HCV infection. N Engl J Med 2009; 361: 533-4; author reply 34-5.

[184] Gentile I, Viola C, Borgia F, Castaldo G, Borgia G. Telaprevir: a promising protease inhibitor for the treatment of hepatitis C virus infection. Curr Med Chem 2009; 16: 1115-21.

[185] Suzuki F, Akuta N, Suzuki Y, *et al.* Rapid loss of hepatitis C virus genotype 1b from serum in patients receiving a triple treatment with telaprevir (MP-424), pegylated interferon and ribavirin for 12 weeks. Hepatol Res 2009; 39: 1056-63.

[186] Akuta N, Suzuki F, Hirakawa M, *et al.* Amino acid substitutions in the hepatitis C virus core region of genotype 1b affect very early viral dynamics during treatment with telaprevir, peginterferon, and ribavirin. J Med Virol 2010; 82: 575-82.

[187] Kawaguchi T, Yamagishi SI, Itou M, *et al.* Pigment epithelium-derived factor inhibits lysosomal degradation of Bcl-xL and apoptosis in HepG2 cells. Am J Pathol 2010; 176(1): 168-76.

[188] Veldt BJ, Chen W, Heathcote EJ, *et al.* Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatology 2008; 47: 1856-62.

Received: December 25, 2009 Revised: May 06, 2010 Accepted: May 12, 2010

Structure-Function Relationships of PEDF

T. Kawaguchi*[,1], S.-I. Yamagishi[2] and M. Sata[1]

[1]*Department of Digestive Disease Information & Research and Department of Medicine,* [2]*Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume 830-0011, Japan*

Abstract: Pigment epithelial-derived factor (PEDF) is a 50-kDa secreted glycoprotein that belongs to the non-inhibitory serpin. It has an α/β core serine-protease inhibitor domain, 3 major β-sheets, and 10 α-helices. Although PEDF does not inhibit either serine or cysteine proteinases, PEDF exerts diverse physiological activities including anti-angiogenesis, anti-vasopermeability, anti-tumor, and neurotrophic activities. Recent studies have shown that a variety of peptides derived from PEDF possess activities similar to those of the parent molecule through interactions with the extracellular matrix, binding to PEDF receptors, nuclear localization and phosphorylation. Thus, peptides derived from PEDF have therapeutic potential for various diseases and therefore, it is important to clarify the structure-function relationship of PEDF. In this review, we summarize structural features of PEDF that could affect various target organs such as blood vessels, tumors, and the central nervous system. In addition, since PEDF is recently identified as a regulator for glucose and lipid metabolism, we also discuss PEDF structures specially related to insulin-sensitizing and triglyceride-reducing properties.

Keywords: Pigment epithelial-derived factor, functional domain, anti-angiogenic activity, anti-vasopermeability activity, anti-tumor activity, neurotrophic activity, glucose metabolism, lipid metabolism.

Pigment epithelial-derived factor (PEDF) is widely expressed throughout the human body and has multiple biological activities. A variety of peptides derived from PEDF exerts diverse physiological activities including anti-angiogenesis, anti-vasopermeability, anti-tumor, and neurotrophic activities as shown in Table **1**. In this review, we summarize structure-function relationships of PEDF.

REGULATION OF SECRETION OF PEDF

C-terminal amino-acid residues play an important role in the secretion of various proteins [1-5]. The insertion of a reactive center loop (RCL) into the β-sheet, which is called "loop-sheet polymerization" is involved in impaired secretion of various types of proteins [6, 7]. PEDF is a secretory protein, and the C-terminal of PEDF contains highly exposed typical RCL [8-10]. Truncation of the C-terminal tail of PEDF (Pro415–Pro418) inhibits the secretion of PEDF by Chinese hamster ovary cells [11]. Since Pro415 is mostly buried and interacts with Phe231 and Lue223, truncation of PEDF at Pro415 causes disruption of the hydrophobic interactions imposed by Pro415 and exposure of Asp414 to the negatively charged C-terminus, resulting in inefficient secretion of PEDF [11]. In addition, not only deletion of Pro373-Ala380, but also alanine substitution at Gly376 and Leu377 inhibits the secretion of PEDF. Gly376 and Leu377 are located within the highly exposed segment of the RCL. Therefore, these two residues are indispensable for (i) interactions of PEDF with components of the quality control system in the endoplasmic reticulum and (ii) subsequent efficient secretion of PEDF [11].

INTERACTIONS OF PEDF WITH THE EXTRA-CELLULAR MATRIX

PEDF accumulates in the extracellular matrix [12]. The extracellular matrix is a complex of proteins, proteoglycans, and glycosaminoglycans, and plays a crucial role in the mechanical strength of cells and the regulation of cell proliferation and differentiation [13, 14]. It has been speculated, therefore, that PEDF exerts its diverse biological activities by interacting with different components of the extracellular matrix [15].

The crystal structure of human PEDF shows an asymmetrical charge distribution, which is one of the structural characters of PEDF [8]. A high density of basic residues exists at the center of β sheet A-strand 2 and 3, and helix F. This region is densely populated with lysines exposed to the surface (aa134, aa137, aa189, aa191, aa212, and aa124), which interact with various glycosaminoglycans [16-18].

The heparin-binding motif is XBBXBX (where B represents basic amino acids: X represents residues excluding acidic amino acids) [19] and is localized at the basic surface of PEDF (aa145-148), which is in the loop region between sheet 2A and helix E [8, 16]. Studies using site-directed mutagenesis showed that three clustered basic amino acid residues, Arg145, Lys146, and Arg148, are necessary for heparin binding [18]. Binding with heparin increases the proteolytic susceptibility of PEDF by trypsin and induces a conformational change in the vicinity of Lys178 of PEDF [20]. Heparin mediates the binding of PEDF to a receptor on the cell surface of Y-79 retinoblastoma

*Address correspondence to this author at the Department of Digestive Disease Information & Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan; Tel: +81-942-31-7902; Fax: +81-942-31-7820;
E-mail: takumi@med.kurume-u.ac.jp

1566-5240/10 $55.00+.00

Table 1. Functional Sites of PEDF

Amino Acids	Function	References
16-26	Anti-angiogenic activity	[28]
24	Protein kinase CK2 phosphorylation site Neutrophic activity Anti-angiogenic activity	[59, 137]
24-57	PEDF receptor binding site Anti-angiogenic activity Apoptotic activity in endothelial cells	[28]
32-380	Neurotrophic activity	[43]
39-57	Chemotaxis activity Apoptotic activity in endothelial cells	[51]
40-64	Anti-tumor activity Osteogenic differentiation activity Collagen I-binding site	[55]
41-44	Collagen I-binding site	[22]
44-77	PEDF receptor (Laminin receptor) binding site	[31]
44-121	Neurotrophic activity	[43]
46-70	PEDF receptor (Laminin receptor) binding site Apoptotic activity in endothelial cells Anti-migration activity in endothelial cells Anti-tube-like formation activity in endothelial cells Anti-angiogenic activity	[31]
58-101	Neuroendocrine differentiation activity	[28]
64	Collagen I-binding site	[22]
78-94	PEDF receptor binding site Neuroendocrine differentiation activity Neurotrophic activity	[28]
78-102	Anti-proliferation activity Collagen I-binding site	[55]
78-121	PEDF receptor (PNPLA2) binding site Neuroendocrine differentiation activity Lipase activity Anti-vasopermeability activity	[24, 29, 73, 91]
82-121	Neutrophic activity	[93]
90-114	Collagen I-binding site Anti-VEGF expression activity	[55]
101, 103, 112	Anti-vasopermeability activity	[73]
114	Protein kinase CK2 phosphorylation site Neutrophic activity Anti-angiogenic activity	[59, 137]
115	Anti-vasopermeability activity	[73]
127, 128, 130	Heparin binding site	[20]
134, 137	Glycosaminoglycans binding site	[16]
139-147	Cytotoxic T-lymphocyte activity	[138]

(Table 1). Contd.....

Amino Acids	Function	References
141-149	HA-binding site	[23]
145-148	Heparin binding site	[18]
146-149	Nuclear localization signal motif	[35]
149, 166, 167	Heparin binding site	[139]
189, 191	Glycosaminoglycans/polyamions	[16]
189-194	HA-binding site	[23]
212, 214	Glycosaminoglycans binding site	[16]
227	Protein kinase A phosphorylation site Anti-angiogenic activity Neutrophic activity	[59, 137]
255-258, 290, 291, 296, 299, 300	Collagen-I binding site	[17, 18, 22]
285	Secretion activity	[140]
272-279	Cytotoxic T-lymphocyte activity	[138]
354-359	Cell differentiation activity Neuroprotective activity	[94, 141, 142]
373-380, 376, 377	Secretion activity	[11]
387-411	Anti-invation activity Anti-VEGF expression activity	[55]
389-397	Cytotoxic T-lymphocyte activity	[138]
415-418	Secretion activity	[11]

cells, and a structural change in PEDF is thought to be a mechanism for enhanced receptor binding [12].

PEDF binds to collagen type I and type III, but not collagen type II and type IV [21]. An increase in ionic strength, lower pH, or modifications of carboxylic groups of PEDF decrease the affinity of PEDF for collagen I, suggesting that acidic and /or negatively charged sites of PEDF (Glu41, Glu42, Glu43, Asp44, Asp64, Asp256, Asp258, Glu290, Glu291, Glu296, Asp300, and Glu304) are possible collagen I-binding sites [22]. Collagen I-binding sites are also localized to the side opposite the heparin-binding site. At this site, the acidic amino acid residues Asp255, Asp257, and Asp299 are critical to collagen I-binding [18]. Mutation of the collagen I-binding site (aa299) of PEDF abolishes anti-tumor activity through anti-angiogenic activity [17].

PEDF is found within hyaluronan (HA) rich tissues and contains amino acids sequence for putative HA-binding motifs, BXBX2BX2B and BX3AB2XB motifs (B represents basic amino acids: X represents residues excluding acidic amino acids: A represents negatively charged amino acids). Becerra *et al.* examined the HA-binding site of PEDF by site-directed mutagenesis and identified two HA-binding motifs (aa141-149 and aa189-194) in PEDF [23]. The BXBX2BX2B motif (aa189-194) was located between α-helix F and β-strand s3A and the BX3AB2XB motif (aa141-149) was localized between β-strand s2A and α-helix F. Although PEDF is a member of the serine-protease inhibitor (serpin) superfamily, none of the other serpins have these HA-binding sites [23].

PEDF RECEPTOR-BINDING SITES

PEDF is a secreted protein with various biological effects and deposits in the cell membrane. In addition, effects of PEDF are blocked by antibodies which are cell surface-binding antagonists [24-27]. These findings suggest an interaction between PEDF and its receptor(s) [12]. Radioligand-binding assays and crystallization analysis demonstrated that cleaved PEDF (aa24-57, aa32-380, aa78-94, and aa44–121) and N-terminal regions of PEDF are possible regions that bind to PEDF receptors [8, 24, 28].

The 44-mer peptide (aa78-121) binds to the cell surface 80-kDa protein in retinoblastoma cells and some neuronal cells, and the 44-mer peptide competes with 121I-PEDF binding in retinoblastoma Y-79 cells [24]. Notari *et al.* identified an 80-kDa phospholipase A2/nutrin/patarin-like phospholipase domain-containing 2 (PNPLA2) as a putative receptor for PEDF in retinal epithelial cells and, using a cell-free system, and showed that the interaction was involved in lipase activity of PEDF [29].

We found that a protein of molecular mass of ca. 60-kDa could be one of the candidates for PEDF receptor on endothelial cells [30]; later, 67-kDa laminin receptor (67LR) was shown to be a putative receptor

for PEDF in endothelial cells [31]. The 67LR consists of two 37-kDa laminin receptor precursor (37LRP) polypeptide chains, and both yeast two hybrid and immunoprecipitation methods revealed the interaction between PEDF and 37LPR/67LR. Further, the 25-mer peptide (aa46-70) derived from a helix-loop-helix structure of PEDF co-localized with the LR on plasma membranes and caused apoptosis of endothelial cells, inhibition of endothelial cell migration, and angiogenesis *in vitro* and *ex vivo* [31].

NUCLEAR LOCALIZATION OF PEDF

Although PEDF is a secreted protein, it is localized in the nucleus of mammalian cells [32-34]. The nuclear localization signal motif, KKRK, is located in aa146-149 domain of PEDF [35]. PEDF regulates cell cycle [36] and interacts with p53 [37-40]. Moreover, a response element specific for nuclear molecules (p63 and p73) is found in the PEDF promoter region, suggesting that PEDF is a direct target of nuclear molecules [41]. Thus, PEDF may play a crucial role in cell cycle in the nucleus.

NON-INHIBITORY SERINE PROTEASE ACTIVITY OF PEDF

PEDF is a member of the serpin superfamily. Serpins are a group of proteins with the same overall tertiary structure [42]. The C-terminal region of all serpins has an RCL that is susceptible to proteolysis [43]. The serpin active site (P1) binds to the primary specificity pocket of the target protease, leading to a change in serpin conformation from the stressed form to the relaxed form by incorporation of the serpin-exposed loop into the β-sheet. This conformational change increases stability and protects against denaturation, and the protease-serpin complex inhibits proteolytic activity [43].

Inhibitory activity against serpin proteases is not found for all serpins [44]. Although PEDF has a Leu residue at P1, which is known to be specific for inhibition of chymotrypsin and chymotrypsin-like activities, PEDF does not have typical inhibitory activities of a serpins [45]. A possible explanation for this discrepancy is that the N-terminal residues of the P1 of PEDF are unfavorable for the insertion of the serpins loop into the β-sheet of the folded serpin protein. Alternatively, alanine residues between P12 and P9 of the RCL are also known to be linked to the inhibitory property of serpins [46]. Although PEDF has an RCL like other serpins, its RCL lacks the tetrad of alanine residues between P12 and P9 [47]. In addition, the three proline residues are found in the RCL of PEDF, which could block the interaction of PEDF with target proteases [48]. Thus, changes in the RCL are likely to be responsible for non-inhibitory property of serine protease in PEDF.

ANTI-ANGIOGENIC ACTIVITY OF PEDF

PEDF exerts anti-angiogenic effects through several different mechanisms; induction of apoptosis in endothelial cells [39, 49-51], inhibition of capillary tube formation [17, 18], migration of endothelial cells [52-54], reduction of vascular endothelial growth factor (VEGF) expression [25, 55, 56] and translocation or phosphorylation of VEGF receptor 1 [57]. Although the precise underlying mechanisms for its anti-angiogenic activity are unknown, several structures in PEDF are reported to be involved in its anti-angiogenic activity. A summary of our current understanding of the structure and anti-angiogenic activity of PEDF is shown in Fig. (**1**).

Collagen type I is an angiogenic scaffold and promotes capillary tube formation through endothelial integrin engagement of collagen type 1 [58]. Collagen type 1-binding sites of PEDF, that is, the interaction of collagen type I and PEDF, play an important role in anti-angiogenic property of PEDF [17, 18, 22, 23]. In fact, mutation of the collagen type I-binding site of PEDF causes tumor progression with neovascularization in tumor xenograft study, while wild type PEDF and mutation of the heparin binding site suppresses both tumor progression and neovascularization [17].

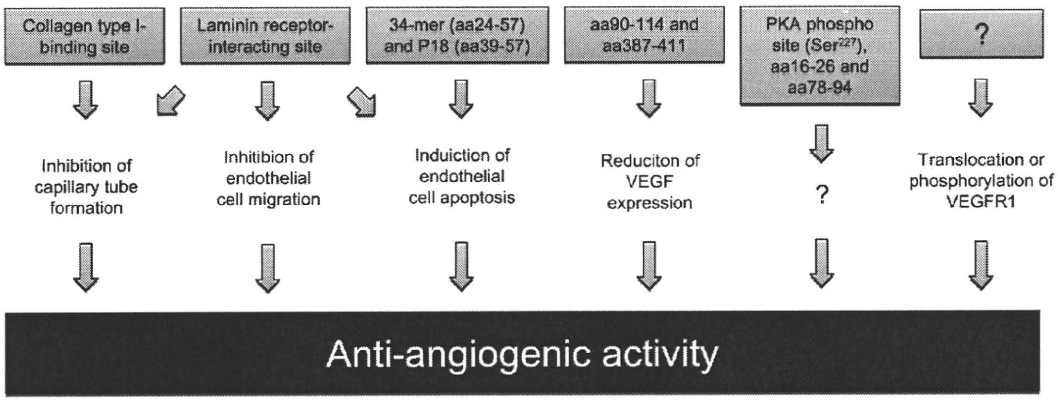

Fig. (1). Relationship between structure and anti-angiogenic activity of PEDF. VEGF, endothelial growth factor; VEGFR1, vascular endothelial growth factor receptor-1.

A ligand-receptor interaction is also important for elicitation of divergent PEDF signals. We found that PEDF exerted anti-inflammatory properties in endothelial cells *via* the interaction with a putative PEDF receptor at a molecular mass of about 60-kDa [30]. Bernard *et al.* identified a 67LR as a PEDF receptor [31], and the 25-mer peptide (aa46-70) of PEDF bound to the LR on plasma membranes and subsequently caused anti-angiogenic reactions both *in vitro* and *ex vivo* [31].

The 34-mer peptide (aa24-57) of PEDF is also reported to act on endothelial cells and cause c-jun-NH2 kinase (JNK)-dependent endothelial cell apoptosis. This effect appears to be mediated by the inhibition of nuclear factor of activated T cells c2 (NFAT), which is regulated by JNK. A NFAT target, caspase-8 inhibitor cellular Fas-associated death domain-like interleukin 1beta-converting enzyme inhibitory protein (c-FLIP) is blocked by PEDF, which was involved in the anti-angiogenic properties of PEDF [28]. Recently, Mirochnik *et al.* analyzed the function of the 34-mer of PEDF and designed 3 peptides that covered its COOH terminus: P14 (aa43-57), P18 (aa39-57), and P23 (aa34-57) [51]. Only P18, but not P14 or P23, was found to induce apoptosis in basic fibroblast growth factor-treated or VEGF-treated endothelial cells, similar to that of the parental 34-mer peptide [51].

PEDF could exert anti-angiogenic activity by reducing VEGF levels [25, 55, 56]. Ek *et al.* generated 25-mer peptides (aa90-114 and aa387-411) and found that the peptides reduced VEGF expression in human osteosarcoma cells [55]. Although the underlying mechanisms are unclear, the protein kinase A (PKA) phosphorylation site of PEDF, Ser227 [59] and peptides aa16-26 and aa78-94 could also have anti-angiogenic activity [28].

ANTI-VASOPERMEABILITY OF PEDF

Increased vascular permeability has a pathophysiologic impact on non-proliferative diabetic retinopathy [60-63], nephritic syndrome [64-66], and hypotension [67]. Moreover, increased vascular permeability accelerates cancer invasion [68, 69]. VEGF disrupts the vascular barrier by uncoupling endothelial cell-cell junctions [70].

PEDF behaves as a functional antagonist of VEGF [71, 72]. Liu *et al.* found that the 44-mer peptide of PEDF (aa78-121) counteracted the VEGF-induced increases in vascular permeability in mouse eyes [73]. The 44-mer peptide contains the exposed elements of hC, one turn of hD, and the connecting loops [8] and a study using chimeric peptides showed that Glu101, Iso103, Leu112, and Ser115 were the amino acids responsible for the anti-vasopermeability effect of PEDF [73]. Thus, the 44-mer peptide or chimeric peptides have therapeutic potential for diseases resulting from excessive vascular permeability.

ANTI-TUMOR ACTIVITY OF PEDF

Besides its anti-angiogenic effects, PEDF also has direct anti-tumor activity by inducing tumor apoptosis and by inhibiting tumor growth and invasion [47, 74-78]. A summary of our current understanding of the structure and anti-tumor activity of PEDF is shown in Fig. (**2A** and **2B**).

Members of the family of HA-binding proteins are known to be associated with apoptosis [79-81]. PEDF contains HA-binding sites that activate caspase-8, caspase-3, and poly (ADP-ribose) polymerase, leading to apoptosis of cancer cells [2].

PEDF also acts as a tumor differentiator [9, 28, 55, 76-78, 82-87]. Alberdi *et al.* found that the 44-mer peptide (aa78-121) bound to a cell surface 80-kDa protein and induced neuronal differentiation in retinoblastoma Y-79 cells [24]. Filleur *et al.* also discovered that the 44-mer peptide (aa58-101) and its fragment (aa78-94) caused a decrease in cytokeratin K8 expression and an increase in mRNA levels of gastrin-releasing peptide/bombesin, thus suggesting the neuroendocrine differentiation of prostate adenocarcinoma cells by PEDF peptides [28]. In addition, the 25-mer peptide (aa78-102) of PEDF was also shown to suppress proliferation of osteosarcoma cells [55]. The 25-mer peptide of PEDF had similar differentiation-promoting activity in neuroblastoma [82, 86] and osteosarcoma cells [55, 83].

The extracellular matrix is deeply involved in tumor migration and invasion [88, 89]. PEDF contains an HA-binding motif and formation of PEDF-HA complexes exerts indirect anti-tumor effects by the blocking biological effects of HA, including loosening the matrix for migration and invasion [23]. Twenty five-mer peptides (aa40-64, aa78-102, and aa90-114) increased cellular adhesion to collagen type-1 [55]. Moreover, the 25-mer peptide (aa387-411) inhibits Matrigel invasion of osteosarcoma [55].

Although the precise structure has not been identified, PEDF exerts anti-tumor effects through activation of a Fas/Fas ligand pathway [25, 49] and induction of cell cycle arrest at G1 phase [87].

NEUROTROPHIC ACTIVITY OF PEDF

PEDF has neurotrophic and neuroprotective activities in the central nervous system [26, 47, 90]. The crystal structure of PEDF indicates that its neurotrophic activity is located at the exposed parts of helices C and D and at loop 90 [8]. Becerra *et al.* examined neurotrophic activity of PEDF using cleaved PEDF. Cleaved PEDF peptides (aa32-380 and aa44–121) can induce morphological differentiation and neurite outgrowth in human Y-79 retinoblastoma cells [43]. These findings suggest that the N-terminal region of PEDF is a neurotrophically active site [43]. In fact, Alberdi *et al.* generated synthetic peptides and found that the 44-mer peptide (aa78–121) derived from the N-terminal edge of PEDF had neurotrophic activity in

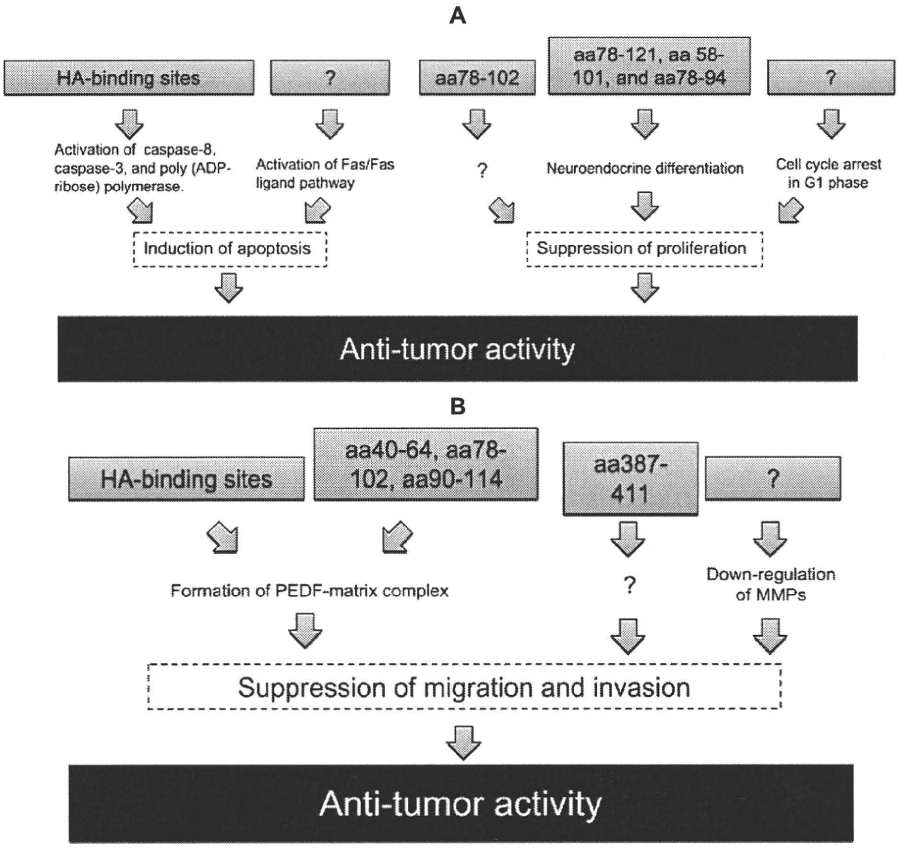

Fig. (2). Relationship between structure and anti-tumor activity of PEDF. (**A**) Apoptosis and growth suppression. (**B**) Inhibition of migration and invasion. HA, hyaluronan; MMP, matrix metalloproteinase.

retinoblastoma cells and cerebellar granuleneurons [24]. The 44-mer peptide also has a neurotrophic function in motor neurons [91] and a fragment (aa78-94) within the 44-mer can induce neuroendocrine differentiation in prostate cancer cells [28].

PEDF prevents damage to retinal ganglion cells after transient ischemia reperfusion in an ischemic rat model [92]. Li *et al*. demonstrated that the 40-mer peptide (aa82-121) protectd the retina from ischemic injury [93]. The thinning of the inner plexiform layer was also protected by the 40-mer peptide (aa82-121) of PEDF [93].

The crystal structure revealed the existence of protein kinase CK2 and PKA phosphorylation sites (Ser24 and Ser114, and Ser227) in PEDF [8]. Mutagenesis studies disclosed that protein kinase CK2 phosphorylated mutants (Ser24Glu and Ser114Glu) reduced the neurotrophic effect of PEDF, but enhanced its anti-angiogenic activity, while the PKA phosphorylation site mutant (Ser227Glu) reduced anti-angiogenic activity of PEDF [59]. These observations suggest that extracellular phosphorylation could completely change the nature of PEDF from a neutrophic to an anti-angiogenic factor.

Besides the N-terminal region of PEDF, the C-terminal site also has neuroprotective properties. A fragment from the C-terminal region (aa354-359) induces both cell differentiation and neuroprotective properties in human promyelocytic leukemia cells through inhibition of phosphatidylinositol-specific phospholipase C [94]. In addition, a fragment from the C-terminal region (aa354-359) was shown to possess neuroprotective activity and counteracts the toxic effects of beta-amyloid peptides in a rat model of Alzheimer's disease [95].

EFFECTS OF PEDF ON GLUCOSE METABOLISM

PEDF is highly expressed in the liver [96], a major organ for glucose metabolism [97-104], and PEDF has a significant role in the development of insulin resistance and the pathogenesis of diabetic complications [105-120]. Although the protein structure of PEDF that is responsible for glucose metabolism has never been determined, analysis of the genomic structure of PEDF indicates an association between PEDF and glucose metabolism.

Hepatocyte nuclear factor-4 (HNF-4), CCAAT/enhancer-binding protein homologous protein

(CHOP), and upstream stimulatory factor (USF) bind to the DNA-binding sites of the PEDF gene, which are located 200 bp upstream of the transcription start site [121]. HNF-4 enhances insulin sensitivity through activation of a phosphatidylinositol 3-kinase/Akt pathway in hepatocytes [122, 123]. CHOP induces insulin stimulation and up-regulates mammalian tribbles homologs, which is associated with insulin resistance and metabolic syndromes [124]. USF also enhances hepatic insulin signaling through up-regulation of glucokinase [125]. Moreover, HNF-4, CHOP, and USF genes are responsible for the development of diabetes mellitus [126-128]. Thus, the genomic structure of PEDF suggests an association between PEDF and glucose metabolism.

EFFECTS OF PEDF ON LIPID METABOLISM

Studies have shown the existence of high affinity PEDF-binding sites and about 80-kDa proteins in plasma membranes of various cells [24, 27, 28, 91, 129, 130]. Alberdi *et al.* found that a 44-mer peptide (aa78-121) bound to a cell surface 80-kDa protein in retinoblastoma cells and some neuronal cells [24]. Using a yeast two-hybrid screening method, Notari *et al.* found that the PEDF fragments (aa35–418, aa35–266, aa35–229, and aa35–119 of the human PEDF) bound to an 80 kDa lipase-linked membrane protein, adipose triglyceride lipase (ATGL) [29]. ATGL is a member of the newly identified calcium-independent PNPLA2 family, which possesses triglyceride lipase and acylglycerol transacylase activities [131]. In fact, PEDF is highly expressed in the liver [96], a major organ for lipid metabolism [100, 101, 132-134] and reduces hepatocyte triglyceride contents *in vitro* [135]. In a mouse model of ethanol-induced hepatic steatosis, absence of PEDF is associated with triglyceride accumulation in hepatocytes, which can be reversed by administration of exogenous PEDF [136]. Thus, PEDF modulates hepatic lipid metabolism through ATGL activation and the 44-mer peptide (aa78-121) of PEDF is a potential drug target for fatty liver diseases.

CONCLUSION

PEDF is widely expressed throughout the human body and has multiple biological activities. Administration of recombinant PEDF causes anti-tumor activity, neurotrophic activity, and ameliorates glucose and lipid metabolism, thus suggesting that PEDF is a potential therapeutic target for patients with various disorders, including cancer, neurological disorders, and the metabolic syndrome. Shorter peptides have more advantages in terms of side effects and drug delivery than full-length PEDF. PEDF consists of 418 amino acids, and it has been demonstrated that peptides derived from PEDF also possess biological activities. In this review, we summarized the known structure-function relationship of PEDF. Further study of structure-function relationship of PEDF may help us to develop peptides that can serve as new therapeutics for a broad spectrum of diseases.

ACKNOWLEDGEMENTS

This study was supported, in part, by a Grant-in-Aid for Young Scientists (B) (No. 19790643 to T.K.) and a Grant-in-Aid for Scientific Research (C) (No. 21590865 to M.S.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by Health and Labour Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labour and Welfare of Japan, and by a Grant for Cancer Research from Fukuoka Cancer Society.

REFERENCES

[1] Brodbeck, R. M. and Brown, J. L. (1994) *J. Biol. Chem.*, **269**, 17252-17256.
[2] Domowicz, M.S., Pirok, E.W., 3rd, Novak, T.E. and Schwartz, N.B. (2000) *J. Biol. Chem.*, **275**, 35098-35105.
[3] Haneishi, A., Terasawa, F., Fujihara, N., Yamauchi, K., Okumura, N. and Katsuyama, T. (2009) *Thromb. Res.*, **124**, 368-372.
[4] Massoulie, J. and Bon, S. (2006) *J. Mol. Neurosci.*, **30**, 233-236.
[5] Vitikainen, M., Lappalainen, I., Seppala, R., Antelmann, H., Boer, H., Taira, S., Savilahti, H., Hecker, M., Vihinen, M., Sarvas, M. and Kontinen, V.P. (2004) *J. Biol. Chem.*, **279**, 19302-19314.
[6] Lomas, D.A., Elliott, P.R., Sidhar, S.K., Foreman, R.C., Finch, J.T., Cox, D. W., Whisstock, J.C. and Carrell, R.W. (1995) *J. Biol. Chem.*, **270**, 16864-16870.
[7] Sidhar, S.K., Lomas, D.A., Carrell, R.W. and Foreman, R.C. (1995) *J. Biol. Chem.*, **270**, 8393-8396.
[8] Simonovic, M., Gettins, P.G. and Volz, K. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 11131-11135.
[9] Tombran-Tink, J., Chader, G.G. and Johnson, L.V. (1991) *Exp. Eye Res.*, **53**, 411-414.
[10] Tombran-Tink, J. and Johnson, L.V. (1989) *Invest. Ophthalmol. Vis. Sci.*, **30**, 1700-1707.
[11] Shao, H., Schvartz, I. and Shaltiel, S. (2003) *Eur. J. Biochem.*, **270**, 822-831.
[12] Alberdi, E.M., Weldon, J.E. and Becerra, S.P. (2003) *BMC Biochem.*, **4**, 1.
[13] Taipale, J. and Keski-Oja, J. (1997) *FASEB J.*, **11**, 51-59.
[14] Tanaka, Y., Kimata, K., Adams, D.H. and Eto, S. (1998) *Proc. Assoc. Am. Phys.*, **110**, 118-125.
[15] Chader, G.J. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 2122-2124.
[16] Alberdi, E., Hyde, C.C. and Becerra, S.P. (1998) *Biochemistry*, **37**, 10643-10652.
[17] Hosomichi, J., Yasui, N., Koide, T., Soma, K. and Morita, I. (2005) *Biochem. Biophys. Res. Commun.*, **335**, 756-761.
[18] Yasui, N., Mori, T., Morito, D., Matsushita, O., Kourai, H., Nagata, K. and Koide, T. (2003) *Biochemistry*, **42**, 3160-3167.
[19] Cardin, A.D. and Weintraub, H.J. (1989) *Arteriosclerosis*, **9**, 21-32.
[20] Valnickova, Z., Petersen, S.V., Nielsen, S.B., Otzen, D.E. and Enghild, J.J. (2007) *J. Biol. Chem.*, **282**, 6661-6667.
[21] Kozaki, K., Miyaishi, O., Koiwai, O., Yasui, Y., Kashiwai, A., Nishikawa, Y., Shimizu, S. and Saga, S. (1998) *J. Biol. Chem.*, **273**, 15125-15130.
[22] Meyer, C., Notari, L. and Becerra, S.P. (2002) *J. Biol. Chem.*, **277**, 45400-45407.
[23] Becerra, S.P., Perez-Mediavilla, L.A., Weldon, J.E., Locatelli-Hoops, S., Senanayake, P., Notari, L., Notario, V. and Hollyfield, J.G. (2008) *J. Biol. Chem.*, **283**, 33310-33320.

[24] Alberdi, E., Aymerich, M.S. and Becerra, S.P. (1999) *J. Biol. Chem.*, **274**, 31605-31612.

[25] Takenaka, K., Yamagishi, S., Jinnouchi, Y., Nakamura, K., Matsui, T. and Imaizumi, T. (2005) *Life Sci.*, **77**, 3231-3241.

[26] Taniwaki, T., Hirashima, N., Becerra, S.P., Chader, G.J., Etcheberrigaray, R. and Schwartz, J. P. (1997) *J. Neurochem.*, **68**, 26-32.

[27] Wu, Y.Q., Notario, V., Chader, G.J. and Becerra, S.P. (1995) *Protein Expr. Purif.*, **6**, 447-456.

[28] Filleur, S., Volz, K., Nelius, T., Mirochnik, Y., Huang, H., Zaichuk, T.A., Aymerich, M.S., Becerra, S.P., Yap, R., Veliceasa, D., Shroff, E.H. and Volpert, O.V. (2005) *Cancer Res.*, **65**, 5144-5152.

[29] Notari, L., Baladron, V., Aroca-Aguilar, J.D., Balko, N., Heredia, R., Meyer, C., Notario, P.M., Saravanamuthu, S., Nueda, M.L., Sanchez-Sanchez, F., Escribano, J., Laborda, J. and Becerra, S.P. (2006) *J. Biol. Chem.*, **281**, 38022-38037.

[30] Yamagishi, S., Inagaki, Y., Nakamura, K., Abe, R., Shimizu, T., Yoshimura, A. and Imaizumi, T. (2004) *J. Mol. Cell. Cardiol.*, **37**, 497-506.

[31] Bernard, A., Gao-Li, J., Franco, C.A., Bouceba, T., Huet, A. and Li, Z. (2009) *J. Biol. Chem.*, **284**, 10480-10490.

[32] Kawaguchi, T., Yamagishi, S., Itou, M., Okuda, K., Sumie, S., Kuromatsu, R., Taniguchi, E., Koga, H., Harada, M., Ueno, T. and Sata, M. (2009) *Hepatology*, 60th Annual Meeting of the AASLD. Abstract #1175.

[33] Tombran-Tink, J., Lara, N., Apricio, S.E., Potluri, P., Gee, S., Ma, J.X., Chader, G. and Barnstable, C.J. (2004) *Exp. Eye Res.*, **78**, 945-955.

[34] Tombran-Tink, J., Shivaram, S.M., Chader, G.J., Johnson, L.V. and Bok, D. (1995) *J. Neurosci.*, **15**, 4992-5003.

[35] Tombran-Tink, J., Aparicio, S., Xu, X., Tink, A.R., Lara, N., Sawant, S., Barnstable, C.J. and Zhang, S.S. (2005) *J. Struct. Biol.*, **151**, 130-150.

[36] Pignolo, R.J., Francis, M.K., Rotenberg, M.O. and Cristofalo, V.J. (2003) *J. Cell Physiol.*, **195**, 12-20.

[37] Gaetano, C., Colussi, C. and Capogrossi, M.C. (2007) *Cardiovasc. Res.*, **76**, 195-196.

[38] Ho, T.C., Chen, S.L., Yang, Y.C., Liao, C.L., Cheng, H.C. and Tsao, Y.P. (2007) *Cardiovasc. Res.*, **76**, 213-223.

[39] Ho, T.C., Chen, S.L., Yang, Y.C., Lo, T.H., Hsieh, J.W., Cheng, H.C. and Tsao, Y.P. (2009) *Am. J. Physiol. Cell. Physiol.*, **296**, C273-284.

[40] Ho, T.C., Yang, Y.C., Chen, S.L., Kuo, P.C., Sytwu, H.K., Cheng, H.C. and Tsao, Y.P. (2008) *Mol. Immunol.*, **45**, 898-909.

[41] Sasaki, Y., Naishiro, Y., Oshima, Y., Imai, K., Nakamura, Y. and Tokino, T. (2005) *Oncogene*, **24**, 5131-5136.

[42] Huber, R. and Carrell, R.W. (1989) *Biochemistry*, **28**, 8951-8966.

[43] Becerra, S.P., Sagasti, A., Spinella, P. and Notario, V. (1995) *J. Biol. Chem.*, **270**, 25992-25999.

[44] Hunt, L.T. and Dayhoff, M.O. (1980) *Biochem. Biophys. Res. Commun.*, **95**, 864-871.

[45] Becerra, S.P., Palmer, I., Kumar, A., Steele, F., Shiloach, J., Notario, V. and Chader, G.J. (1993) *J. Biol. Chem.*, **268**, 23148-23156.

[46] Patston, P.A. and Gettins, P.G. (1996) *FEBS Lett.*, **383**, 87-92.

[47] Tombran-Tink, J. (2005) *Front. Biosci.*, **10**, 2131-2149.

[48] Whisstock, J.C., Pike, R.N., Jin, L., Skinner, R., Pei, X.Y., Carrell, R.W. and Lesk, A.M. (2000) *J. Mol. Biol.*, **301**, 1287-1305.

[49] Abe, R., Shimizu, T., Yamagishi, S., Shibaki, A., Amano, S., Inagaki, Y., Watanabe, H., Sugawara, H., Nakamura, H., Takeuchi, M., Imaizumi, T. and Shimizu, H. (2004) *Am. J. Pathol.*, **164**, 1225-1232.

[50] Chen, L., Zhang, S.S., Barnstable, C.J. and Tombran-Tink, J. (2006) *Biochem. Biophys. Res. Commun.*, **348**, 1288-1295.

[51] Mirochnik, Y., Aurora, A., Schulze-Hoepfner, F.T., Deabes, A., Shifrin, V., Beckmann, R., Polsky, C. and Volpert, O. V. (2009) *Clin. Cancer Res.*, **15**, 1655-1663.

[52] Chung, C., Doll, J.A., Stellmach, V.M., Gonzales, J., Surapureddi, S., Cornwell, M., Reddy, J.K. and Crawford, S.E. (2008) *Adv. Exp. Med. Biol.*, **617**, 591-597.

[53] Elayappan, B., Ravinarayannan, H., Sardar Pasha, S.P., Lee, K.J. and Gurunathan, S. (2009) *Angiogenesis*, **12**, 313-324.

[54] Nakamura, K., Yamagishi, S., Matsui, T., Yoshida, T., Takenaka, K., Jinnouchi, Y., Yoshida, Y., Ueda, S., Adachi, H. and Imaizumi, T. (2007) *Am. J. Pathol.*, **170**, 2159-2170.

[55] Ek, E.T., Dass, C.R., Contreras, K.G. and Choong, P.F. (2007) *J. Orthop. Res.*, **25**, 1671-1680.

[56] Tsuchiya, T., Nakahama, K., Asakawa, Y., Maemura, T., Tanaka, M., Takeda, S., Morita, M. and Morita, I. (2009) *Gynecol. Endocrinol.*, **25**, 104-109.

[57] Cai, J., Jiang, W.G., Grant, M.B. and Boulton, M. (2006) *J. Biol. Chem.*, **281**, 3604-3613.

[58] Sweeney, S.M., DiLullo, G., Slater, S.J., Martinez, J., Iozzo, R.V., Lauer-Fields, J.L., Fields, G.B. and San Antonio, J.D. (2003) *J. Biol. Chem.*, **278**, 30516-30524.

[59] Maik-Rachline, G., Shaltiel, S. and Seger, R. (2005) *Blood*, **105**, 670-678.

[60] Antonetti, D.A., Barber, A.J., Hollinger, L.A., Wolpert, E.B. and Gardner, T.W. (1999) *J. Biol. Chem.*, **274**, 23463-23467.

[61] Antonetti, D.A., Lieth, E., Barber, A.J. and Gardner, T.W. (1999) *Semin. Ophthalmol.*, **14**, 240-248.

[62] Ishibashi, T., Tanaka, K. and Taniguchi, Y. (1979) *Nippon Ganka Gakkai Zasshi*, **83**, 783-794.

[63] Mathews, M.K., Merges, C., McLeod, D.S. and Lutty, G.A. (1997) *Invest. Ophthalmol. Vis. Sci.*, **38**, 2729-2741.

[64] Brenchley, P.E. (2003) *Nephrol. Dial. Transplant.*, **18**, vi21-25.

[65] Lagrue, G., Branellec, A., Blanc, C., Xheneumont, S., Beaudoux, F., Sobel, A. and Weil, B. (1975) *Biomedicine*, **23**, 73-75.

[66] Tomizawa, S., Nagasawa, N., Maruyama, K., Shimabukuro, N., Arai, H. and Kuroume, T. (1990) *Nephron*, **56**, 341-342.

[67] Horowitz, J.R., Rivard, A., van der Zee, R., Hariawala, M., Sheriff, D.D., Esakof, D.D., Chaudhry, G.M., Symes, J.F. and Isner, J.M. (1997) *Arterioscler. Thromb. Vasc. Biol.*, **17**, 2793-2800.

[68] Harmey, J.H., Bucana, C.D., Lu, W., Byrne, A.M., McDonnell, S., Lynch, C., Bouchier-Hayes, D. and Dong, Z. (2002) *Int. J. Cancer*, **101**, 415-422.

[69] Yano, S., Shinohara, H., Herbst, R.S., Kuniyasu, H., Bucana, C.D., Ellis, L.M. and Fidler, I.J. (2000) *Am. J. Pathol.*, **157**, 1893-1903.

[70] Weis, S.M. and Cheresh, D.A. (2005) *Nature*, **437**, 497-504.

[71] Dieudonne, S.C., La Heij, E.C., Diederen, R.M., Kessels, A.G., Liem, A.T., Kijlstra, A. and Hendrikse, F. (2007) *Ophthalmic. Res.*, **39**, 148-154.

[72] Pollina, E.A., Legesse-Miller, A., Haley, E.M., Goodpaster, T., Randolph-Habecker, J. and Coller, H.A. (2008) *Cell Cycle*, **7**, 2056-2070.

[73] Liu, H., Ren, J.G., Cooper, W.L., Hawkins, C.E., Cowan, M.R. and Tong, P.Y. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 6605-6610.

[74] Abe, R., Fujita, Y., Yamagishi, S. and Shimizu, H. (2008) *Curr. Pharm. Des.*, **14**, 3802-3809.

[75] Dass, C.R., Ek, E.T. and Choong, P.F. (2008) *Curr. Cancer Drug Targets*, **8**, 683-690.

[76] Ek, E.T., Dass, C.R. and Choong, P.F. (2006) *Trends Mol. Med.*, **12**, 497-502.

[77] Ek, E.T., Dass, C.R. and Choong, P.F. (2006) *Mol. Cancer The.r*, **5**, 1641-1646.

[78] Fernandez-Garcia, N.I., Volpert, O.V. and Jimenez, B. (2007) *J. Mol. Med.*, **85**, 15-22.

[79] Bartolazzi, A., Peach, R., Aruffo, A. and Stamenkovic, I. (1994) *J. Exp. Med.*, **180**, 53-66.

[80] Charrad, R.S., Li, Y., Delpech, B., Balitrand, N., Clay, D., Jasmin, C., Chomienne, C. and Smadja-Joffe, F. (1999) *Nat. Med.*, **5**, 669-676.

[81] Yu, Q., Toole, B.P. and Stamenkovic, I. (1997) *J. Exp. Med.*, **186**, 1985-1996.