insulin signaling, phosphatase and tensin homologue[69], and SH2 domain-containing inositol phosphatase- 2[20] occur in HCC. Thus, HCC may be sensitive to insulin stimulation.

Extrahepatic manifestations

HCV causes extrahepatic manifestations including mixed cryoglobulinemia, Sjögren's syndrome, and non-Hodgkin lymphoma, oral lichen planus, oral squamous cell carcinoma, and malignancies other than HCC[22-24,70-73]. In patients with extrahepatic manifestations of HCV, fasting insulin levels and homeostasis model assessment for insulin resistance are significantly higher than for patients without extrahepatic manifestations[22]. Among various extrahepatic manifestations, insulin resistance is associated with oral lichen planus[23], oral squamous cell carcinoma[24], and multiple primary cancers including gastric cancer[24]. Although reasons for this association remain unclear, a high prevalence of precancerous lesions and cancers are seen in patients with type 2 diabetes mellitus[74,75], suggesting that insulin resistance or hyperinsulinemia may enhance carcinogenic activities.

DISTINCTIVE THERAPEUTIC STRATEGY FOR HCV-ASSOCIATED INSULIN RESISTANCE

Despite awareness of the increased risk of insulin resistance, therapeutic guidelines to inhibit distinctive complications of HCV-associated insulin resistance have not yet been established. HCV itself has a significant impact on the development of insulin resistance, and eradication of HCV improves insulin resistance[44,46,76]. Thus, anti-viral therapy is a fundamental therapeutic strategy for patients with HCV infection. In addition, amelioration of insulin resistance is considered to inhibit complications and improve prognosis. Here, we summarize treatments which could improve HCV-associated insulin resistance as therapeutic strategies (Figure 1).

Late evening snack

Proper diet and exercise are fundamental for patients with lifestyle-associated insulin resistance as well as patients with HCV-associated insulin resistance[77-80]. As a nutritional treatment for liver cirrhosis, divided energy intake (4 to 6 meals/d) has been recommended[77,79]. As postprandial hyperglycemia is characteristic of HCV-associated insulin resistance[77-80], a decrease in energy intake per meal reduces postprandial hyperglycemia and hyperinsulinemia. In particular, a late evening snack is reported not only to improve glucose intolerance[81-84], but also to suppress hepatocarcinogenesis in cirrhotic patients[85].

Coffee consumption

Coffee consumption reduces the risk of elevated serum alanine aminotransferase activity[86], hepatic fibrosis[87], and disease progression in chronic hepatitis C[88]. Coffee consumption also reduces the risk of HCC independent of HCC etiology[89]. Caffeine is metabolized by hepatic cytochrome P450 1A2 into 3 metabolites, the dimethylxanthines paraxanthine, theobromine, and theophylline. Of these metabolites, theophylline inhibits transforming growth factor-β-stimulated CTGF expression through PPARγ and Smad 2/3-dependent pathways. Since CTGF and transforming growth factor-β are important factors associated with progression of hepatic fibrosis and hepatocarcinogenesis, a metabolite of caffeine, theophylline, may have an inhibitory effect on the development of complications associated with HCV infection. In addition, coffee has significant effects on glucose metabolism[90]. In an animal experiment, the insulin-sensitizing effects of coffee have been demonstrated[91]. Similarly, in a human study, coffee consumption reduced fasting glucose and insulin levels[90,92]. Although the mechanisms for the coffee-induced insulin-sensitizing effect remain unclear, some possibilities exist. Chlorogenic acids, a constituent of coffee, inhibits hepatic glucose-6-phosphate translocation[90,93], limits glucose absorption from the gut by inhibiting Na^+-dependent transport[94], and increases the secretion of glucose regulating hormone, glucagon-like peptide (GLP)-1, from the gut[90,95,96]. These findings suggest that a constituent of coffee, chlorogenic acid, directly ameliorates HCV-associated insulin resistance. Furthermore, coffee modulates lipid metabolism[97,98] and lowers body weight[90], indicating that coffee may suppress the lipid-induced increase in oxidative stress and ameliorates HCV-associated insulin resistance.

Phlebotomy

Hepatic iron overload produces oxidative stress and is a factor responsible for the development of HCV-associated insulin resistance[4,99-101]. Although the pathogenesis of hepatic iron overload remains unclear, recent studies showed that iron-regulating molecules are modulated by HCV infection. Hepcidin is a negative regulator of duodenal iron absorption and macrophage iron release[100] and decreased hepatic expression of hepcidin is seen in both HCV polyprotein transgenic mice[102] and patients with HCV infection[103-105]. In addition, upregulation of hepatic expression of transferrin receptor 2, a mediator of iron uptake, is responsible for hepatic iron overload[106].

In order to reduce hepatic iron deposition, dietary iron restriction and phlebotomy are effective. Dietary iron restriction (less than 7 mg/d) decreases serum alanine aminotransferase levels in patients with HCV infection[107]. Phlebotomy reduces oxidative stress as well as insulin resistance in patients with HCV infection[101,108,109]. A long-term combination treatment with phlebotomy and dietary iron restriction reduces the risk of development of HCC in patients with HCV infection[110].

Supplementation of zinc

Zinc plays a crucial role in the metabolism of protein, carbohydrate, lipid, nucleic acid, and ammonia[111-113]. In fact, zinc supplementation improves glucose disposal

in patients with cirrhosis[114]. Zinc also inhibits hepatic inflammation[115] and hepatic fibrosis[116]. More recently, zinc supplementation was shown to lower the cumulative incidence of HCC in patients with HCV infection[117]. It is unclear whether these inhibitory effects of zinc on progression of liver disease are mediated by amelioration of insulin resistance. However, zinc participates in the synthesis, storage and secretion of insulin[118] and regulates the binding ability of insulin to bind to its receptor[113]. As the serum zinc level is decreased in patients with HCV infection[115,117], supplementation of zinc could be a therapeutic option.

Anti-diabetic agents

Exogenous insulin and sulfonylurea agents: Anti-diabetic agents are effective for decreasing plasma glucose and HbA1c levels, leading to prevention of diabetes mellitus-associated complications including cardiovascular diseases[119,120]. However, it has never been determined whether anti-diabetic agents prevent complications or improve prognosis in patients with HCV infection. Use of exogenous insulin or sulfonylurea agents may worsen hyperinsulinemia. In fact, we, along with others, recently reported an association between exogenous insulin or sulphonylurea treatment and the development of HCC in patients with HCV infection[29,30,121]. Use of exogenous insulin is also reported to be associated with the development of colon cancer[122] and other malignancies[123]. Although a causal relationship between exogenous insulin and the development of HCC remains controversial[124], the reduction of serum insulin levels is a first line therapeutic strategy for insulin resistance[125-128].

Insulin-sensitizing agents: Insulin resistance is associated with a poor response to anti-viral treatment in patients with HCV infection[10,43-46]. Amelioration of insulin resistance may improve the response to anti-viral treatment. However, the impact of insulin-sensitizing agents, biguanides and thiazolidinediones, on sustained virologic response (SVR) rates has not yet been established. Recently, metformin, a biguanide agent, has been reported to ameliorate HCV-associated insulin resistance, and increase the SVR rate in HCV genotype 1 infected patients with normalization of homeostasis model assessment for insulin resistance at week 24 of therapy[129]. Pioglitazone, a thiazolidinedione agent, has also been reported to ameliorate insulin resistance and increase SVR rates in patients with HCV genotype 4 infection[130]. Although the insulin-sensitizing mechanisms of metformin and of pioglitazone are different, both agents are known to up-regulate IRS[131,132], which is the molecule responsible for HCV-associated insulin resistance[3,6,50], and to improve HCV-associated insulin resistance. Because both agents have severe adverse effects, neither is recommended for patients with liver cirrhosis. Biguanides predispose cirrhotic patients to lactic acidosis[133]. Thiazolidinediones cause overproduction of hydrogen peroxide leading to severe hepatotoxicity[134]. Thus, further validation for safety is required.

Dipeptidyl peptidase IV (DPPIV) inhibitor is a new therapeutic agent[135] and its clinical efficacy in type 2 diabetes has been shown[136]. Although no study has examined the effect of DPPIV inhibitor on HCV-associated insulin resistance, we found that activation of DPPIV is a factor responsible for HCV-associated insulin resistance[27]. Thus, a DPPIV inhibitor may be suited for ameliorating HCV-associated insulin resistance.

BCAA supplementation, a possible insulin-sensitizing agent

BCAA are constituents of proteins and are required for protein synthesis[19,78,137,138]. In addition, BCAA are reported to modulate glucose metabolism. Leucine and isoleucine induce glucose transporter 1 and 4 translocation to the plasma membrane of muscle cells and improve glucose metabolism in a carbon tetrachloride-induced cirrhotic rat model[139]. In addition, leucine enhances the insulin-induced activation of the Akt/mammalian target of the rapamycin pathway in adipocytes of db/db mice[140]. Moreover, isoleucine increases hepatic phosphatidylinositol 3-kinase activity and improves insulin resistance in Zucker fa/fa rats, a model of severe insulin resistance[141]. Recently, knockout of the mitochondrial BCAA aminotransferase gene in mice, in which results in elevated plasma BCAA levels, was found to ameliorate insulin resistance[142]. Thus, BCAA improve insulin signaling in various animal models *via* several pathways. In good agreement with these results in animals, in human studies, we have recently shown that BCAA-enriched supplementation reduces insulin resistance in patients with HCV infection[143,144]. In a multicenter, randomized, controlled trial, BCAA supplementation led to a reduction in the risk of HCC in cirrhotic patients[145]. This suppressive effect on hepatocarcinogenesis was more evident in obese patients with HCV infection[145]. Both obesity and HCV induce the development of insulin resistance. Thus, BCAA may improve insulin resistance and subsequently inhibit insulin resistance-induced hepatocarcinogenesis[19,145].

CONCLUSION

In this review, we summarize the distinctive complications of, and therapeutic strategies for, HCV-associated insulin resistance. Although cardiovascular diseases, renal failure, and infections are well-known complications of lifestyle-associated insulin resistance, these complications are not major causes of death in cirrhotic patients with insulin resistance. HCV-associated insulin resistance rather causes (1) hepatic steatosis, (2) resistance to anti-viral treatment, (3) hepatic fibrosis and esophageal varices, (4) hepatocarcinogenesis and proliferation of HCC, and (5) extrahepatic manifestations. These complications are life-threatening, and therapeutic strategies for HCV-associated insulin resistance have to be considered on the basis of its pathogenic mechanisms.

Pathogenic mechanisms for HCV-associated insulin resistance differ from those for lifestyle-associated insulin resistance. Postprandial hyperglycemia, lipid-induced oxi-

dative stress, hepatic iron overload, and depletion of zinc are responsible for the development of HCV-associated insulin resistance. Therefore, a late evening snack, coffee consumption, dietary iron restriction, phlebotomy, and supplementation of zinc are recommended therapeutic strategies. No clinical guidelines for the use of anti-diabetic agents are available for patients with HCV-associated insulin resistance. However, use of exogenous insulin or sulphonylurea may increase the risk for HCC. On the other hand, insulin-sensitizing agents may improve the SVR rate of anti-viral treatment. In addition, BCAA supplementation has an insulin-sensitizing effect as well as a suppressive effect on hepatocarcinogenesis. Thus, in order to ameliorate HCV-associated insulin resistance, various therapeutic approaches are required.

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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

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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 cardio-vascular 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

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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**).

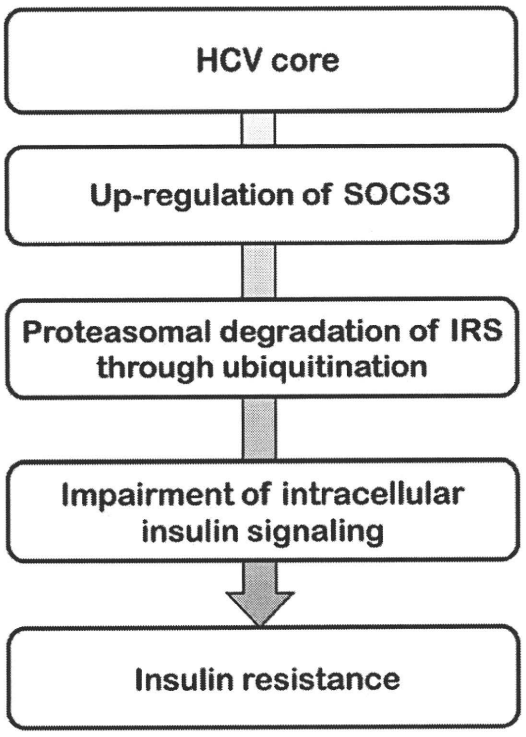

Fig. (1). Direct effects of HCV on the development of insulin resistance.
HCV core protein up-regulates suppressor of cytokine signaling (SOCS) 3 protein. SOCS3 ubiquitinates insulin receptor substrate (IRS) and causes proteasomal degradation of IRS. Since IRS is a central molecule of intarcellular insulin 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

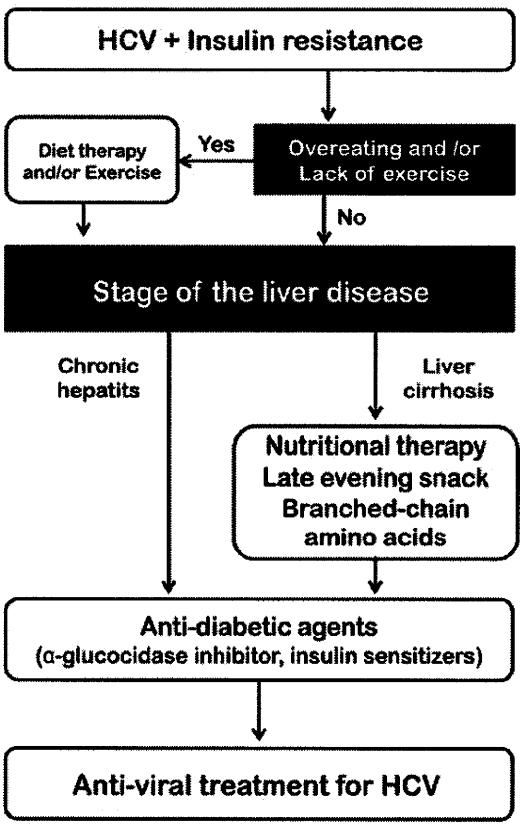

Fig. (2). Flow chart for the therapeutic strategy for HCV-associated insulin resistance. The therapeutic strategy for HCV-associated insulin resistance can be determined not only by life-style, but also by stage of the liver disease.

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

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

Structure-Function Relationships of PEDF

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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 EXTRACELLULAR 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

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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]
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78-94	PEDF receptor binding site Neuroendocrine differentiation activity Neurotrophic activity	[28]
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