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ORIGINAL ARTICLE

Basic Study

Hepatitis C virus NS5A promotes insulin resistance through IRS-1 serine phosphorylation and increased gluconeogenesis

Fahed Parvaiz, Sobia Manzoor, Jawed Iqbal, Mehuli Sarkar-Dutta, Muhammad Imran, Gulam Waris

Fahed Parvaiz, Sobia Manzoor, Muhammad Imran, Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad 44000, Pakistan

Jawed Iqbal, Mehuli Sarkar-Dutta, Gulam Waris, Department of Microbiology and Immunology, Rosalind Franklin University of Medicine and Science, Chicago, IL 60064, United States

Author contributions: Parvaiz F and Manzoor S designed the study and did the analysis; Parvaiz F performed most of the experiments and prepared the manuscript; Waris G provided technical support; Manzoor S and Waris G supervised the study and critically reviewed the manuscript; Iqbal J and Sarkar-Dutta M helped in performing Western blot and quantitative real-time polymerase chain reaction; Imran M helped in literature survey; all authors have read and approved the final version of the manuscript; Manzoor S is principal investigator and PhD supervisor of Parvaiz F.

Correspondence to: Sobia Manzoor, PhD, Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, H-12, Islamabad 44000, Pakistan. lcianunique@yahoo.com
Telephone: +92-51-90856147
Fax: +92-51-90856102

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Abstract

AIM: To investigate the mechanisms of insulin resistance in human hepatoma cells expressing hepatitis C virus (HCV) nonstructural protein 5A (NS5A).

METHODS: The human hepatoma cell lines, Huh7 and Huh7.5, were infected with HCV or transiently-transfected with a vector expressing HCV NS5A. The effect of HCV NS5A on the status of the critical players involved in insulin signaling was analyzed using real-time quantitative polymerase chain reaction and Western blot assays. Data were analyzed using Graph Pad Prism version 5.0.

RESULTS: To investigate the effect of insulin treatment on the players involved in insulin signaling pathway, we analyzed the status of insulin receptor substrate-1 (IRS-1) phosphorylation in HCV infected cells or Huh7.5 cells transfected with an HCV NS5A expression vector. Our results indicated that there was an increased phosphorylation of IRS-1 (Ser^{307}) in HCV infected or NS5A transfected Huh7.5 cells compared to their respective controls. Furthermore, an increased phosphorylation of Akt (Ser^{473}) was observed in HCV infected and NS5A transfected cells compared to their mock infected cells. In contrast, we observed decreased phosphorylation of Akt Thr308 phosphorylation in HCV NS5A transfected cells. These results suggest that Huh7.5 cells either infected with HCV or ectopically expressing HCV NS5A alone have the potential to induce insulin resistance by the phosphorylation of IRS-1 at serine residue (Ser^{307}) followed by decreased phosphorylation of Akt Thr^{308}, Fox01 Ser^{256} and GSK3β Ser^{9}, the downstream players of the insulin signaling

pathway. Furthermore, increased expression of PECK and glucose-6-phosphatase, the molecules involved in gluconeogenesis, in HCV NS5A transfected cells was observed.

CONCLUSION: Taken together, our results suggest the role of HCV NS5A in the induction of insulin resistance by modulating various cellular targets involved in the insulin signaling pathway.

Key words: Hepatitis C virus nonstructural protein 5A; Insulin resistance; Forkhead box protein 01; Glycogen synthase kinase-beta; Gluconeogenesis

Core tip: The underlying molecular mechanisms of insulin resistance in response to hepatitis C virus (HCV) infection are poorly understood. Previous studies have demonstrated the effect of HCV core and envelop proteins on insulin signaling in human hepatocytes. However, the role of HCV nonstructural protein 5A (NS5A) in insulin resistance is not known. Our data clearly indicate the role of NS5A in insulin resistance through increased phosphorylation of IRS-1Ser^{307} and decreased phosphorylation of AktThr^{308}, Fox01Ser^{256}, and GSK3βSer^{9}.

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INTRODUCTION

Hepatitis C virus (HCV) is a blood-borne pathogen, belonging to the family *Flaviviridae*. The HCV genome is a positive sense single stranded RNA molecule, which encodes a polyprotein that is cleaved by viral proteases and host cell signal peptidases into mature structural and non-structural proteins[1,2]. Chronic infection with HCV progresses into a number of pathological conditions including insulin resistance, fibrosis, steatosis, cirrhosis and ultimately hepatocellular carcinoma[3,4].

Insulin is the major anabolic hormone that has to utilize excessive glucose and maintain energy needs of the body. Insulin primarily performs this function by downregulating gluconeogenesis and glycogenolysis[4]. The primary targets of insulin action are skeletal muscles, cardiac muscles and the liver[5]. In certain pathological conditions, insulin is vulnerable to perform its function and results in abnormal metabolic condition known as insulin resistance that refers to the complex array of metabolic disorders involving lipid deposition, enhanced fatty acids release and unfolded protein response[6-11]. One of the key downstream insulin signaling molecules is Akt that has been implicated in insulin resistance with the accumulation of diacylglycerols and ceramides[12]. In addition to this, ceramides may acts through an atypical isoform of protein kinase C (PKC), *i.e.*, PKC-zeta that sequesters Akt and inhibits its function in normal insulin signaling[13,14].

In normal insulin signaling, insulin receptor substrate 1 (IRS-1) undergoes tyrosine phosphorylation and initiates a cascade of downstream signaling. However, there is impaired phosphorylation of IRS-1 in obese patients with type 2 diabetes mellitus[15]. Studies also reveal that pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 are involved in insulin resistance *via* serine phosphorylation of IRS-1[16]. During insulin signaling, the insulin receptor has to bind with the adaptar protein, IRS, and triggers the metabolic pathway effectively. In contrast, serine phosphorylation of IRS-1 leads to the degradation of IRS protein and hampers the insulin signaling pathway[4]. Akt is the downstream insulin signaling molecule which phosphorylates forkhead box protein 01 (Fox01) and glycogen synthase kinase-beta (GSK3β) and modulates insulin signaling[17,18]. Fox01 is the first direct downstream target of Akt and is involved in mediating hepatic glucose production *via* peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α)[19]. Fox01 regulates the expression of genes involved in glucose and lipid metabolism[20]. Fox01 undergoes posttranslational modifications including acetylation, ubiquitination and importantly phosphorylation to perform its stimulatory effect[18]. Fox01 has multiple phosphorylation sites but phosphorylation at serine 256 modulates its DNA binding activity and hampers normal metabolic pathways[21]. Downstream to the Akt/protein kinase B signaling pathway also lies another important protein GSK3β that gets phosphorylated and inactivated in response to insulin, and suppresses key gluconeogenic genes, *i.e.*, glucose-6-phosphatase (G6P) and phosphoenol pyruvate carboxykinase (PEPCK)[22,23]. Insulin regulates homeostasis by regulating two opposite pathways, *i.e.*, glycolysis and gluconeogenesis. Gluconeogenesis involves the conversion of pyruvate back to glucose with the aid of PEPCK, G6P and several other enzymes[22-28]. In the cyclic adenosine monophosphate (cAMP) axis, there is a cascade of genes that get activated and inactivated in response to the insulin mediated actions. Previous studies have shown that PGC-1α strongly upregulates gluconeogenic genes like cAMP response element binding protein (CREB), PEPCK and G6P[29-31]. Akt inactivates PGC-1α and inhibits gluconeogenesis under normal metabolic conditions[32].

During the course of chronic HCV infection, the insulin signaling pathway is altered and the glucose cannot be metabolized properly with the concomitant increased transcriptional and translational expression of gluconeogenic genes/proteins[33,34]. So far little is

Table 1 Primers used in the study

Gene	Forward primer	Reverse primer
CREB	GATCTTAGTGCCCAGCAACC	GACGGACCTCTCTCTTTCGT
PEPCK	GGCTACAACTTCGGCAAATACC	GGAAGATCTTGGGCAGTTTGG
G6P	CATTGACACCACACCCTTTGC	CCCTGTACATGCTGGAGTTGAG
TNF-α	AGGCGCTCCCCAAGAAGACA	TCCTTGGCAAAACTGCACCT

TNF-α: Tumor necrosis factor-alpha; PEPCK: Phosphoenol pyruvate carboxykinase; G6P: Glucose-6-phosphatase; CREB: CRE-binding protein.

known about the molecular mechanism behind the role of HCV in insulin resistance. Previously, HCV core protein has been shown to promote insulin resistance through serine phosphorylation of IRS-1 and modulating the Akt signaling pathway[33,35].

In this study, we investigated the mechanism by which NS5A modulates key insulin signaling molecules such as IRS-1, Akt, Fox01 and GSK3β at the posttranslational level and their target genes.

MATERIALS AND METHODS

Antibodies and reagents

Antibodies against IRS-1 (Ser^{307}) and phospho-Akt (Ser^{473}) were purchased from Calbiochem. Anti-phospho-Fox01 (Ser^{256}) and anti-phospho-GSK3β (α/β) were purchased from Cell Signaling. Anti-phospho Akt (Thr^{308}) and anti-actin were purchased from Santa Cruz Biotechnology and Sigma, respectively. Human recombinant insulin was purchased from Invitrogen. SYBR green master mix was purchased from Applied Biosystems.

Cell lines

Huh7 and Huh7.5 cell lines were grown in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum, 100 U of penicillin/mL and 100 μg/mL of streptomycin and cultured at 37 ℃ under 5% CO_2. Huh7.5 cell line was kindly provided by Dr. Charles Rice (Rockefeller University, NY) to Dr. Gulam Waris at Rosalind Franklin University of Medicine and Science (RFUMS), United States.

HCV cell culture infection system

HCV JFH-1 genomic RNA was *in vitro* transcribed and delivered into Huh7.5 cells by electroporation or liposome mediated transfection. These cells were then plated and passaged after 3 d. HCV infection in the cells and the corresponding cell culture supernatants was determined by quantitative real-time polymerase chain reaction (Applied Biosystems). The HCV cell culture supernatant was used to infect naive Huh7.5 cells at appropriate dilutions (*moi* of 1). Cells were then incubated at 37 ℃ for about 5-6 h in 5% CO_2 as previously described[36]. The infectious JFH-1 construct was provided by Dr. Takaji Wakita to Dr. Gulam Waris at RFUMS, United States.

Transient-transfection assays

Cells at about 70% confluence in 60 mm petri dishes were transfected with an HCV NS5A expressing plasmid using lipofectamine 2000 (Invitrogen, CA). The confluent cells were washed thrice with phosphate buffered saline to remove cell debris followed by treating with 100 nmol/L insulin for three hours before harvesting the transfected cells.

Reverse transcription and quantitative real-time polymerase chain reaction

Total cellular RNA was extracted from mock infected, HCV infected and HCV NS5A transfected Huh7.5 cells using Trizol (Invitrogen, CA). The extracted RNA was treated with DNase using RQ1 RNase-free DNase prior to cDNA synthesis. The cDNA was reverse-transcribed from 1 μg of total RNA using oligo(dT) primers according to the manufacturer's protocol (Applied Biosystems, CA). Quantitative RT-PCR was carried out using SYBR green master mix and specific primer sets in triplicate. The 18S ribosomal RNA (18S rRNA) was used as an internal control. Amplification reactions were performed in a 25 μL reaction mix using an real-time polymerase chain reaction (RT-PCR) reagent kit and the template RNA. Reactions were performed in a 96-well spectroflourometric thermal cycler under the following conditions: 2 min at 50 ℃, 10 min at 95 ℃, 40 cycles of 15 s at 95 ℃ and 1 min at 60 ℃. Relative transcript levels were calculated using ΔΔCt method as specified by the manufacturer. Primers were used as described previously (Table 1)[37,38].

Western blot analysis

Mock, HCV infected and NS5A transfected cells were harvested and cellular lysates were prepared by incubating the cells with RIPA buffer (50 mmol/L Tris Base pH 7.5, 150 mmol/L NaCl, 1% NP-40, 0.50% sodium deoxycholate, 0.10% SDS, 1 mmol/L orthovanadate, 1 mmol/L sodium formate and 10 μL/mL of protease inhibitor cocktail) on ice for 30 min. The lysates were subjected to SDS-PAGE followed by transfer to the nitrocellulose membrane in a transfer buffer (25 mmol/L Tris, 192 mmol/L glycine and 20% methanol). The membranes were then incubated for 1 h in a blocking buffer (20 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.5% Tween-20, and 5% non-fat dry milk). The membranes were then probed with respective

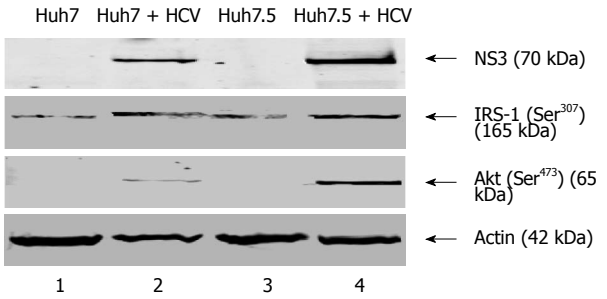

Figure 1 Hepatitis C virus infection modulates insulin signaling in hepatitis C virus infected human hepatoma cells. Cellular lysates were made from mock and hepatitis C virus (HCV) infected Huh7 and Huh7.5 cells. An equal amount of cellular lysates were subjected to Western blot assay using p-IRS-1 $Ser^{307}$and p-Akt Ser^{473}. HCV NS3 protein expression represents the level of HCV infection. Cellular actin was used as an internal control to verify protein loading in each lane. IRS: Insulin receptor substrate; NS: Nonstructural protein.

primary antibody for 1 h at room temperature followed by washing thrice with blocking buffer without milk and then probed with respective secondary antibody for 1 h at room temperature. After doing an additional washing step, the membranes were visualized using the Odyssey Infrared imaging system (Li-Cor Biosciences, Lincoln, NE). The expression of actin protein was used to determine protein loading control in each lane.

Statistical analysis

Data were analyzed using Graph Pad Prism version 5.0 and 2-tail error bars represent standard error of mean ± SE of the data from three individual trials. A *P* value < 0.05 was regarded as statistically significant.

RESULTS

Modulation of insulin signaling pathway in HCV infected hepatoma cells

To investigate the effect of HCV upon the insulin signaling pathway, we infected human hepatoma cells (Huh7 and Huh7.5) with HCV (JFH1) and confirmed the HCV infection by Western blot analysis of HCV NS3 as shown in Figure 1. Our results also showed that Huh7.5 cells were more susceptible to JFH-1 HCV infection than Huh7 cells. To examine the effect of HCV infection on the status of IRS-1 and Akt, the above cellular lysates were subjected to Western blot analysis. The results showed an increased phosphorylation of IRS-1 (Ser^{307}) and Akt (Ser^{473}) in HCV infected Huh7 and Huh7.5 cells compared to their respective controls (Figure 1). Furthermore, the phosphorylation of IRS-1 and Akt was more in HCV infected Huh7.5 cells compared to HCV infected Huh7 cells (Figure 1, lane 4).

HCV infected Huh7.5 cells show resistance towards insulin signaling

Since we observed a pronounced effect in Huh7.5 cells compared to Huh7 cells, we selected Huh7.5 cells for further experiments. To examine the effect of HCV infection on the insulin signaling pathway in HCV infected Huh7.5 cells, uninfected and HCV infected Huh7.5 cells were incubated with insulin. The results showed increased phosphorylation of IRS-1 (Ser^{307}) in HCV infected cells which was reduced in HCV infected cells treated with insulin (Figure 2, lane 4). Furthermore, the phosphorylation of Akt (Ser^{473}) was slightly enhanced in HCV infected cells treated with insulin. Collectively, these results suggest that HCV impairs insulin signaling *via* phosphorylation of IRS-1 (Ser^{307}).

HCV-NS5A alters phosphorylation level of IRS-1 (Ser^{307})

To investigate the role of HCV NS5A in the induction of insulin resistance, Huh7.5 cells were transfected with an HCV NS5A expression plasmid and the status of various cellular proteins involved in insulin signaling was examined. We observed an increased phosphorylation of IRS-1 (Ser^{307}) in NS5A transfected cells but not in cells treated with insulin (Figure 3). In addition, NS5A transfected cells showed an increased phosphorylation of Akt Ser^{473}, compared to untransfected cells (Figure 3). In contrast, we observed a decreased phosphorylation of AktThr^{308} in HCV NS5A transfected cells and it was not further affected upon insulin treatment (Figure 3). These results indicate the potential role of HCV NS5A in the modulation of the insulin signaling pathway by the increased serine phosphorylation of IRS-1 and decreased phosphorylation of Akt Thr^{308}.

HCV-NS5A decreases the phosphorylation levels of Fox01 (Ser^{256}) and GSK-3β (Ser^{9})

Fox01 is an important downstream target of the insulin signaling pathway. The two important residues of Fox01, *i.e.*, Ser^{256} and Ser^{319}, are known to be involved in nuclear exclusion of Fox01 and the regulation of the normal insulin mediated signaling[33]. We observed that HCV NS5A expressing cells showed a decreased phosphorylation of Fox01Ser^{256}, and insulin treatment did not change the phosphorylation of Fox01, indicating that Fox01 may be involved in the modulation of subsequent downstream targets. In previous studies, insulin resistance has been linked with GSK3β signaling[17]. Similar to the Fox01Ser^{256}, our results also showed a decreased phosphorylation of GSK3βSer^{9} in NS5A expressing cells, indicating that the active form of GSK3β favors gluconeogenesis in NS5A expressing cells (Figure 4).

HCV infection promotes hepatic gluconeogenesis

Downstream to the GSK3β are several gluconeogenic genes and transcription factors that are involved in gluconeogenesis. The rate limiting step of gluconeogenesis is controlled by *PEPCK* gene. Therefore, we first examined the transcriptional level of PEPCK in HCV infected cells. The results showed sign-

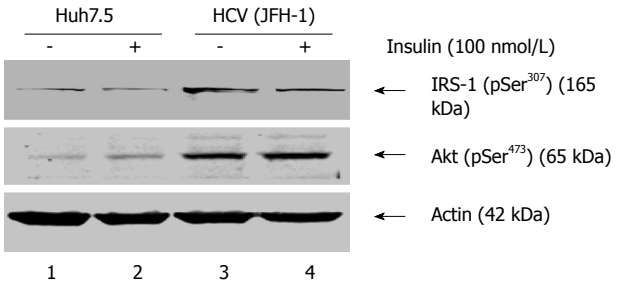

Figure 2 Status of p-Ser^{307} insulin receptor substrate-1 and p-Ser^{473} Akt phosphorylation in hepatitis C virus infected hepatoma cells upon insulin treatment. Total cellular lysates were prepared from hepatitis C virus (HCV) infected and mock infected Huh7.5 cells that were treated or untreated with insulin (100 nmol/L). An equal amount of cellular lysates were subjected to Western blot assay using anti-p-IRS-1 Ser^{307} and anti-p-Akt Ser^{473}. Cellular actin was used as a protein loading control in each lane. IRS: Insulin receptor substrate.

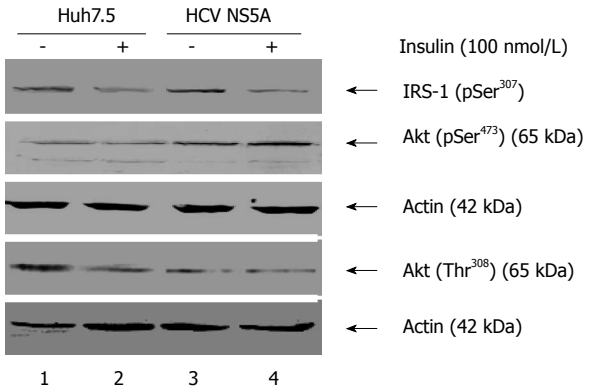

Figure 3 Hepatitis C virus nonstructural protein 5A modulates phosphorylation levels of key insulin signaling molecules. Untransfected and nonstructural protein 5A (NS5A) transfected cells were incubated with insulin (100 nmol/L) for 3 h. An equal amount of cellular lysates were subjected to Western blot assay using anti-p-Akt Ser^{473}, anti-p-Akt Thr^{308} and anti-p-IRS-1 Ser^{307}. Cellular actin was used as an internal control to verify protein loading in each lane. IRS: Insulin receptor substrate.

ificantly increased expression of PEPCK, indicating that HCV infected cells have potentially enhanced gluconeogenesis (Figure 5A). In addition, CREB is another important transcription factor that regulates the transcriptional activity of PEPCK. Our results showed significantly increased expression of CREB in the HCV infected cells (Figure 5A). Furthermore, TNF-α has also been linked to various HCV induced metabolic disorders. This prompted us to examine the transcriptional level of TNF-α in HCV infected cells. The results showed a significantly increased expression of TNF-α (Figure 5A), indicating the fact that this proinflammatory cytokine might have an important role in the insulin resistance mechanism.

NS5A promotes gluconeogenesis through transcriptional upregulation of PEPCK and G6P

In order to investigate the role of HCV-NS5A in the upregulation of gluconeogenesis, we transfected

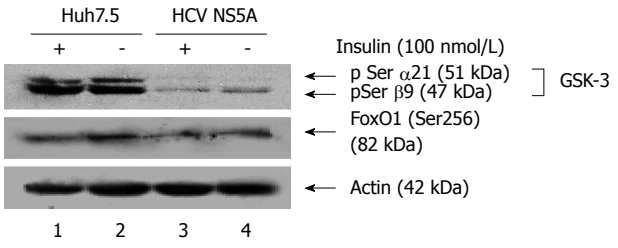

Figure 4 Effect of hepatitis C virus nonstructural protein 5A on the phosphorylation levels of Fox01 Ser^{256} and GSK-3β Ser^{9}. Using the cellular lysates from nonstructural protein 5A (NS5A) transfected cell line and the controlled treated hepatoma cell line, Western blot assay was performed using anti-p-GSK3 Ser (α^{21}/β^{9}) and anti-p-Fox01 Ser^{256}. HCV: Hepatitis C virus.

Huh7.5 cells with NS5A and observed that there was significantly increased expression of PEPCK. The ultimate downstream step of gluconeogenesis, *i.e.*, conversion of glucose-6-phosphate to glucose, is governed by G6P. Similarly, the results indicated significantly increased transcriptional expression of G6P in HCV-NS5A transfected cells (as shown in Figure 5B). These results suggest that the gluconeogenic pathway is increased in NS5A expressing cells.

Model of HCV NS5A induced insulin resistance

The model of HCV-NS5A induced insulin resistance is shown in Figure 6.

DISCUSSION

Insulin resistance is a multifaceted disorder that involves modulation of various genes at transcriptional and translational levels. Hepatic gluconeogenesis is the core phenomenon that provokes insulin resistance. Our data suggest that HCV infection or ectopic expression of HCV NS5A increases hepatic gluconeogenesis as well as modulates phosphorylation status of various cellular proteins required for the induction of insulin resistance. Normal insulin signaling involves the binding of the insulin to insulin receptor and promotes tyrosine phosphorylation of IRS. Downstream to the IRS-1 are the Akt, Fox01 and GSK3β that get differentially phosphorylated, metabolize glucose and favor homeostasis[5,17,18]. Our results suggest that HCV infection in the hepatoma cell lines favors serine phosphorylation of IRS-1 (Ser^{307}) that is required for the insulin resistance. Furthermore, we observed an increased phosphorylation of Akt Ser^{473} and a decreased phosphorylation of Akt Thr^{308} in HCV NS5A transfected cells. These results are consistent with the previous studies where Akt Thr^{308} but not Akt Ser^{473} phosphorylation plays an important role in insulin resistance process[33,35,39].

Fox01 is an important insulin signaling molecule downstream of Akt and has been implicated in the modulation of transcriptional regulation of various genes (including PEPCK, G6P, *etc.*) involved in gluco-

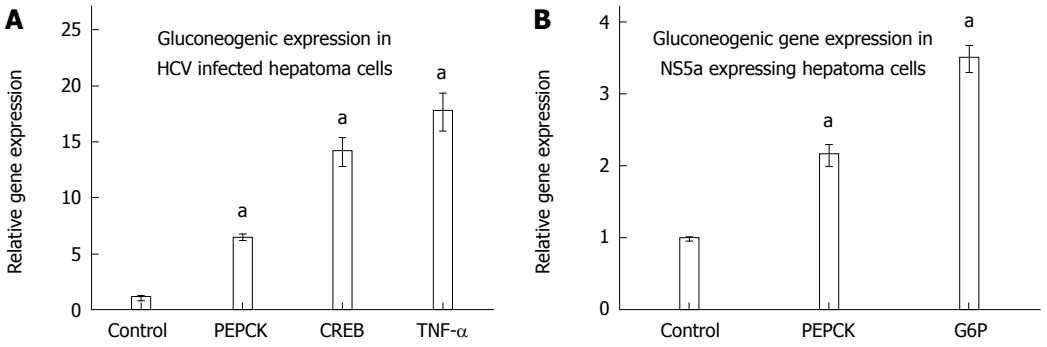

Figure 5 Hepatitis C virus nonstructural protein 5A favors gluconeogenic gene expression. Total cellular RNA was extracted from hepatitis C virus (HCV, A) nonstructural protein 5A (NS5A, B) transfected cells and control cells. The quantitative real-time polymerase chain reaction was performed for the targeted genes as described in Materials and Methods. 18S rRNA was used as a housekeeping gene. Data represent mean of three independent experiments. $^{a}P < 0.05$ *vs* control group. Data were analyzed with Graph Pad Prism, and 2-tail error bars represent SE of the data. TNF-α: Tumor necrosis factor-alpha; PEPCK: Phosphoenol pyruvate carboxykinase; G6P: Glucose-6-phosphatase; CREB: CRE-binding protein.

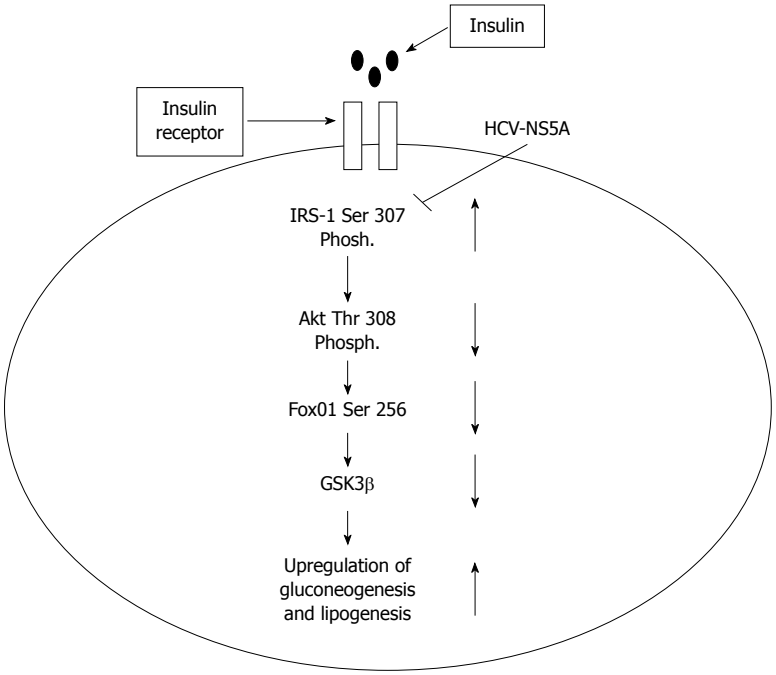

Figure 6 Schematic representation of hepatitis C virus nonstructural protein 5A induced insulin resistance. Based on our findings, a model has been proposed that depicts various check points in the insulin signaling pathway that gets modulated by nonstructural protein 5A (NS5A) protein. The up and down arrows represent upregulation and downregulation of proteins involved in insulin signaling cascades. The blunt headed line represents the check point that gets blocked by NS5A as this protein favors serine phosphorylation of insulin receptor substrate-1 (IRS-1) while under normal conditions tyrosine phosphorylation is known to take place. HCV: Hepatitis C virus.

neogenesis[40]. Fox01 Ser^{256} is an important phosphorylation site that modulates its DNA binding ability, inhibits Fox01 nuclear translocation to the cytoplasm and modulates the metabolic gene expression[33]. Furthermore, GSK3β is another important downstream insulin signaling molecule that gets hypophosphorylated and becomes activated which, in turns, selectively upregulates the gluconeogenic gene expression, and inhibitors of GSK3β can selectively downregulate the transcriptional expression of these gluconeogenic genes[22,41]. Previous studies have shown that decreased phosphorylation of GSK3β leads to its activation and favors gluconeogenesis[18,39]. To further discern the effect of HCV-NS5A on downstream targets of the insulin signaling pathway, phosphorylation status of Fox01 Ser^{256} was examined in this study. Our results suggested that there was a decreased phosphorylation level of Fox01 Ser^{256} in the HCV NS5A transfected cells compared to control cells. Furthermore, the same pattern of decreased phosphorylation of GSK3β was observed in the transfected cell line.

Taken together, our data reveal that HCV NS5A is potentially able to modulate the normal insulin signaling pathway at various cellular points and favors the gluconeogenic pathway.

PEPCK is the rate limiting step of gluconeogenesis as it dictates the fate of gluconeogenesis by converting

oxaloacetate to phosphoenol pyruvate and is greatly linked with the phenomenon of insulin resistance. CREB is the main transcription factor that governs the transcriptional level of PEPCK and favors gluconeogenesis. In addition, some recent data have linked obesity and insulin resistance with the upregulation of TNF-α[42-45]. Our data elucidate that HCV NS5A has a strong role in the enhancement of gluconeogenesis by the way of increased expression of key gluconeogenic genes, *i.e.*, PEPCK and G6P, with the concomitant increased expression of related transcription factors and inflammatory cytokine CREB and TNF-α, respectively.

In this study, we have demonstrated that HCV NS5A favors serine phosphorylation of IRS-1, which is critically involved in the modulation of downstream insulin signaling pathway (Figure 6). Furthermore, downstream targets of the insulin signaling pathway (Akt Thr^{308}, Fox01 and GSK3β) undergo a decreased level of serine phosphorylation that is involved in the inhibition of glycogen synthesis and favors gluconeogenesis, thereby imparting its role in the induction of hepatic insulin resistance. Hence, we characterized a mechanism through which HCV NS5A can modulate various cellular check points and leads toward hepatic insulin resistance.

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COMMENTS

Background

Hepatitis C virus (HCV) is a lethal blood borne pathogen targeting hepatocytes and causes chronic infection in the majority of the infected individuals. Some studies reveal that chronic HCV infection attenuates the insulin signaling pathway, which can lead to glucose intolerance and the development of insulin resistance. So far, HCV core protein has been clearly shown to induce insulin resistance through the modulation of signaling pathways and upregulation of gluconeogenesis. Up till now, there is no conclusive study that reveals the potential of HCV non structural protein 5A (NS5A) in the induction of insulin resistance through the modulation of insulin receptor substrate-1 (IRS-1) protein.

Research frontiers

HCV targets hepatocytes where various HCV proteins get replicated and favor various pathological conditions like insulin resistance, a step towards type 2 diabetes mellitus. The hotspot of this research article is the identification of HCV NS5A as a potential candidate for the development of insulin resistance. This study reveals that HCV NS5A modulates the insulin signaling pathway, thereby leading to increase gluconeogenesis.

Innovations and breakthroughs

The underlying molecular mechanisms of insulin resistance in response to HCV infection are poorly understood. Previous studies have demonstrated the effect of HCV core and envelop proteins in insulin signaling in human hepatocytes. However, the role of HCV NS5A in insulin resistance is not known. Their data clearly indicate the role of NS5A in insulin resistance through increased phosphorylation of IRS-1Ser^{307} and decreased phosphorylation of AktThr^{308}, Fox01Ser^{256}, and GSK3βSer^{9}.

Applications

This study suggests that HCV NS5A has multiple cellular targets that should be prevented in order to reduce disease pathogenesis. Furthermore, it highlights the fact that HCV NS5A specific inhibitors should be synthesized that can reduce the chance of disease progression and morbidity rate.

Terminology

Insulin resistance: A complicated metabolic disorder that refers to the pre-diabetic phase with the modulation of insulin signaling at various cellular checkpoints like insulin receptors, IRS and impairment of homeostasis; Gluconeogenesis: Synthesis of glucose from non-glucose moieties; Fibrosis: A pathological condition in which excessive fats are deposited over liver; Cirrhosis: A pathological condition in which liver shrinks.

Peer-review

This paper describes the influence of HCV NS5A on serine phosphorylation of insulin receptor substrate-1, Fox01 and GSK-3β, and the mRNA levels of key gluconeogenic enzyme genes. The paper reports a potentially interesting and an important study.

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