PI3K/SHIP2/PTEN pathway in cell polarity and hepatitis C virus pathogenesis

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Abstract

Hepatitis C virus (HCV) infects hepatocytes, polarized cells in the liver. Chronic HCV infection often leads to steatosis, fibrosis, cirrhosis and hepatocellular carcinoma, and it has been identified as the leading cause of liver transplantation worldwide. The HCV replication cycle is dependent on lipid metabolism and particularly an accumulation of lipid droplets in host cells. Phosphoinositides (PIs) are minor phospholipids enriched in different membranes and their levels are tightly regulated by specific PI kinases and phosphatases. PIs are implicated in a vast array of cellular responses that are central to morphogenesis, such as cytoskeletal changes, cytokinesis and the recruitment of downstream effectors to govern mechanisms involved in polarization and lumen formation. Important reviews of the literature identified phosphatidylinositol (PtdIns) 4-kinases, and their lipid products PtdIns(4)P, as critical regulators of the HCV life cycle. SH2-containing inositol polyphosphate 5-phosphatase (SHIP2), phosphoinositide 3-kinase (PI3K) and their lipid products PtdIns(3,4)P2 and PtdIns(3,4,5)P3, respectively, play an important role in the cell membrane and are key to the establishment of apicobasal polarity and lumen formation. In this review, we will focus on these new functions of PI3K and SHIP2, and their deregulation by HCV, causing a disruption of apicobasal polarity, actin organization and extracellular matrix assembly. Finally we will highlight the involvement of this pathway in the event of insulin resistance and nonalcoholic fatty liver disease related to HCV infection.

Key words: Hepatitis C virus; Phosphoinositide 3-kinase; SH2-containing inositol polyphosphate 5-phosphatase; Epithelial cell polarity; Phosphoinositides

Core tip: Chronic hepatitis C virus (HCV) infection leads to liver cirrhosis and cancer. HCV infection modulates the lipid metabolism. Phosphoinositides are minor phospholipids that are also modified by HCV infection. phosphatidylinositol (PtdIns)(3,4,5)P3 is mainly formed by phosphoinositide 3-kinase (PI3K), and

can be dephosphorylated by SH2-containing inositol polyphosphate 5-phosphatase (SHIP2) to generate PtdIns(3,4)P2. In this review, we will discuss the effects of SHIP2 and PI3K on the formation of cell polarity and how their expression and activation are modulated by HCV infection, leading to the disruption of cell polarity. This pathway is also discussed in the event of insulin resistance and nonalcoholic fatty liver disease related to HCV infection.

INTRODUCTION

Chronic Hepatitis C virus (HCV) infection leads to cirrhosis that will develop complications such as liver failure and liver cancer[1]. The principal target of HCV is hepatocytes, which are highly polarized cells, their plasma membranes being separated by tight junctions into apical (canalicular) and basolateral (sinusoidal) domains[2,3]. After it enters the hepatocytes, the life cycle of HCV is very closely linked to cell lipid metabolism. The very low density lipoprotein (VLDL) pathway is required for the assembly and secretion of new viral particles[4,5]. However, the effect of lipid droplets (LD) on the replication of HCV is becoming increasingly clear[6]. HCV induces an accumulation and change to the cellular distribution of LDs, moving from a dispersed profile in the cytoplasm of non-infected cells to their perinuclear localization in infected cells. This relocation permits the interaction of LDs with viral proteins and genomes[7]. While much is known about the role of lipoproteins and LDs in the HCV life cycle, studies are only now emerging on the modulation of phosphoinositides (PI) by HCV infection[8].

PIs are phosphorylated derivatives of phosphatidylinositol (PtdIns). They are minor phospholipids (10%-20%) on the inner surface of the lipid bilayer and an important constituent of the cell membrane. The phosphorylation and dephosphorylation of PIs is achieved by various isoforms of PI kinases and PI phosphatases, distributed in a specific way in the cell, this result in the distribution of different PIs in cell compartments. These complex reactions are mediated by 19 kinases and 28 phosphatases that have been identified in mammals[9,10]. Figure 1 illustrates the phosphorylation and dephosphorylation cycles of different monophosphate, diphosphate and triphosphate PIs, as well as the most widely studied kinases and phosphatases. PIs are secondary messengers responsible for transmitting receptor signals to the effectors that induce a cellular response. PIs interact with these effectors *via* specific binding domains that are known to interact with the membrane, either by specific recognition of the membrane components or through attraction by its properties such as charge,

structure, curvature and amphiphilicity[11]. As well as acting as precursors of secondary messengers, PIs are spatiotemporal regulators of several target proteins involved in vesicular trafficking [such as PtdIns(4)P and PtdIns(3)P] and cytoskeletal rearrangement [PtdIns(4,5)P2], by which they control cell polarity, migration, proliferation and differentiation [PtdIns(3,4)P2] and PtdIns(3,4,5)P3[12,13].

Given the importance of PI metabolism to cellular signaling and trafficking events, numerous intracellular pathogens modulate and exploit PIs in order to ensure their survival and efficient intracellular replication[14,15]. A considerable body of literature has addressed the modulation of PIs by HCV[8]. Changes to the localization of PtdIns(4)P and activation of PI4KⅢα following HCV infection have been identified as being key to membrane network formation and viral replication[1,16,17]. In this review we will focus on the roles of phosphoinositide 3-kinase (PI3K), SH2-containing inositol polyphosphate 5-phosphatase (SHIP2) and phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and their lipid products in the establishment of plasma membrane polarity. We will also discuss how HCV infection modulates these polarity mechanisms to invade host cells and replicate. Finally, we will consider the involvement of the PI3K/PTEN/SHIP2 pathway in insulin resistance and nonalcoholic fatty liver disease (NAFLD) related to HCV infection.

HCV

HCV is an enveloped virus with linear, single-stranded RNA contained in a capsid protein called core. Following entry of the virus into a host cell and uncoating of the viral genome, the translation of viral RNA produces a polyprotein that will be degraded to form three structural proteins (core, E1 and E2), six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) and p7 protein. The structural and non-structural proteins will mate with viral RNA to form new viral particles. This viral assembly occurs near the endoplasmic reticulum, and then new viral particles are released from the cell through fusion with the cell membrane[18,19]. Chronic infection with HCV, especially in cases of cirrhosis or advanced fibrosis, remains the leading cause of hepatocellular carcinoma (HCC) worldwide through the modulation of different pathways such as inflammation, proliferation and differentiation, and DNA damage[20]. Effective treatments for HCV have recently been developed, and over 95% of patients can now be cured[21,22], but pathologies such as cancer induced by HCV infection remain a health problem for those already infected. Interactions between viral proteins and host cell mechanisms therefore remain central to understanding the pathogenesis of HCV.

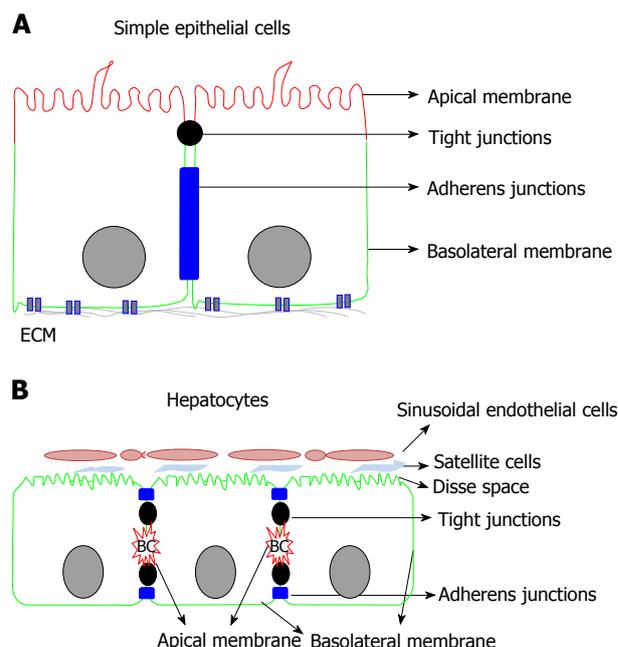## POLARITY IN THE LIVER AND THE ENTRY OF HCV

The polarity of epithelial cells is a property that is ess-
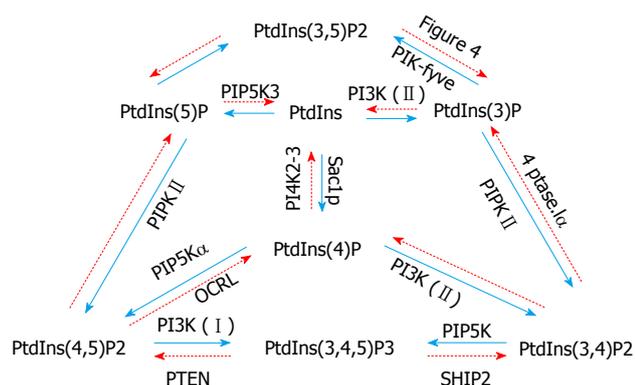
Figure 1 Phosphorylation and dephosphorylation cycles of different phosphoinositides. Monophosphate, diphosphate and triphosphate phosphoinositides are represented, as are the most widely studied kinases (in blue) and phosphatases (in red) that regulate their expression. PtdIns: Phosphatidylinositol; PI3K: Phosphoinositide 3-kinase; SHIP2: SH2-containing inositol polyphosphate 5-phosphatase; PTEN: Phosphatase and tensin homologue deleted on chromosome 10; OCRL: Inositol polyphosphate 5-phosphatase.

Figure 2 Comparison between simple epithelial cells polarity and hepatocytes polarity. A: The epithelial cell polarity presents a differentiation of the apical membrane (in red) to the light, and the basolateral membrane (in green) in contact with the extracellular matrix (ECM). Apical and basal membranes are separated with tight junctions (in black) and adherens junctions (in blue); B: The hepatocytes present a more complex polarity, with several apical membranes for the same cell forming bile ducts. The basolateral membrane is in contact with Disse space allowing the exchange with blood vessels.

ential to maintaining the structure and function of the epithelium. A polarized epithelium provides an effective barrier against biological pathogens. It is characterized by an asymmetric formation of the plasma membrane, and the distribution of different intracellular organelles and cytoskeletons, including the proteins and lipids they contain. This asymmetry is created according to an apical-basal axis which reveals asymmetry of the cell from the basal pole in contact with the extracellular matrix (ECM) to the apical pole interfacing with the lumen of the tissue. The establishment of apical-basal polarity involves several steps, the first of them being contact between the cell with the ECM and also with adjacent cells by tight and adherens junctions[23] (Figure 2A). Numerous pathogens, including viruses and bacteria, are capable of modulating the regulators of cell polarity in order to infect and then replicate in host cells[14-24].

The principal target of HCV is hepatocytes, which are polarized cells in the liver. They communicate with the bloodstream *via* their basal surface which interfaces with sinusoidal endothelial cells, and they secrete bile from the apical surface, which faces and forms the bile canaliculi. The apical and basolateral domains of hepatocytes are separated by tight junctions that ensure integrity of the canaliculi and the secretory function of hepatocytes. The space between hepatocytes and sinusoidal endothelial cells is called the "space of Disse" containing hepatic stellate cells, which are mesenchymal cells responsible for much of the scarring that accompanies chronic liver injury[25] (Figure 2B). The polarization of hepatocytes is necessary to sustain the liver's architecture, and restrict infection by pathogens and the development of diseases. HCV disrupts this polarized architecture by modulating polarity proteins in order to ensure its entry and replication. In fact, HCV exploits tight junction proteins such as Claudin-1[26], occludin[27] and Interferon-induced transmembrane protein 1[28]. On the other hand, cell polarity and well maintained junctions significantly restrict HCV entry into Huh7 and HepG2 cells[29] by

reducing the expression of CD81 in the membrane and impairing the dynamics of scavenger receptor class B type I (SR-B1)[30]. These findings highlight the important role of tight junctions and cell polarity in HCV entry[31,32], but pose the question as to whether HCV infection manipulates PIs in order to deregulate cell polarity, thus leading to pathogenesis. Indeed, the few studies that have focused on the link between HCV and epithelial apicobasal polarity strongly suggested that polarization restricts HCV entry into epithelial cells[33-35], so the role of PIs remains a challenging issue.

PI3K ACTIVITY, CELL POLARITY AND HCV INFECTION

PI3Ks phosphorylate the hydroxyl group 3-position of the inositol ring of PtdIns. The PI3K family of enzymes contains three different classes (I , II and III), based on their substrate specificity and molecular structure[36]. PtdIns(3)P can be formed by PI3K- II and PI3K-III, and PtdIns(3,4)P2 is generated by the activity of PI3K- II . The activity of PI3K- I produces PtdIns(3,4,5)P3 which, once produced at the membrane, exercises its vital role of second messenger by recruiting different proteins containing a pleckstrin homology (PH) domain. The protein kinase Akt possesses a PH domain which was the first to be discovered and displays high affinity binding to phosphoinositides. The interaction between Akt and PtdIns(3,4,5)P3 induces a conformational change in

the Akt structure which permits its phosphorylation by phosphoinositide-dependent kinase 1 (PDPK1) at threonine 308, and the mammalian target of rapamycin complex 2 (mTORC2) at serine 473 (Figure 2). Activated Akt is responsible for triggering numerous cellular signaling pathways involved in proliferation, survival, apoptosis and autophagy[12-37]. PI3K and its lipid product PtdIns(3,4,5)P3 have been identified as key regulators of apicobasal polarity[38,39]. Watton and Downward showed that the adhesion of epithelial cells to the extracellular matrix provides protection from apoptosis *via* the activation of PI3K and Akt/PKB. They confirmed the localization of PI3K at the basolateral membrane producing PtdIns(3,4,5)P3[38]. Another study also revealed PI3K activation after interaction of the cell with the ECM and cell-cell contact. They showed that E-cadherin, which is responsible for cell-cell junctions, joins the p85 sub-unit of PI3K and activates PI3K/Akt to generate PtdIns(3,4,5)P3 during the early stages of cellular polarization[40]. Gassama-Diagne *et al*[39] studied the localization of PtdIns(3,4,5)P3 at the basolateral membrane in madin darby canine kidney (MDCK) po-larized cells. This study confirmed that the formation of PtdIns(3,4,5)P3 at the basolateral membrane is essential to the initiation of basolateral polarization through the activation of Rac1. In fact, the experimental addition of exogenous PtdIns(3,4,5)P3 at the apical pole of polarized MDCK cells on transwell filters led in five minutes to the formation of PtdIns(3,4,5)P3 and basolateral protein-rich protrusions above the apical surface, from which apical proteins were excluded[39]. In MDCK 3D culture on matrigel, the formation of basolateral protein-rich protrusions was observed after three minutes of treat-ment with exogenous PtdIns(3,4,5)P3. The location and PI3K activity of these protrusions is important because the action of the PI3K inhibitor LY294002 inhibits the formation of protrusions, even when treating the cells with PtdIns(3,4,5)P3. By testing different ATP-competitive isoform-selective inhibitors of PI3K on the apicobasal polarity of MDCK grown in a 3D culture on Matrigel. Peng *et al*[41] recently showed that treatment with the p110δ inhibitors IC87114 and CAL-101 inverted the cell polarity of cysts displaying the apical marker Podocalyxin in contact with the ECM. The treatment of cells with other inhibitors such as PI-103, a multi-targeted inhibitor of p110α/β/δ/γ, and AS-605240 which targets p110γ, led to the formation of either multi-lumen or lumen-free cysts. Taken together, these data indicate that the P110δ isoform plays a role in establishing the apicobasal polarity axis. Next, the p110δ isoform was localized at the basolateral membrane of polarized cysts, and colocalized with the ECM receptor dystroglycan. The depletion of p110δ at the basolateral membrane disrupted laminin and type IV collagen assembly by down-regulating β1-integrin, which is a transmembrane protein with a specific role in ECM assembly and remodeling[42]. Overall, these findings revealed the role of epithelial p110δ in the orientation of cell polarity and lumen formation by

regulating ECM assembly and interactions[41].

A growing body of evidence in the literature has revealed that PIs and their metabolic enzymes are essential to HCV replication at different stages of the cell cycle[16,43,44]. Some studies validated activation of the PI3K/Akt pathway following infection by HCV[45]. Epidermal growth factor receptor (EGFR), which activates the PI3K/Akt pathway, was recently shown to be a co-factor for HCV entry in a cell[46]. Moreover, Street *et al*[47] demonstrated an interaction between the viral protein NS5A and the p85 subunit of PI3K. This interaction is responsible for activating the p110 subunit of PI3K, which induces the formation of PtdIns(3,4,5)P3. The same study also showed that the NS5A protein induces the phosphorylation of Akt at tyrosine 308, thus causing anti-apoptotic activity. Other than its role in cell proliferation, transcription and migration, PI3K is also responsible for membrane expression of the SR-B1 receptor in HepG2 cells, promoting viral entry into the cells[48]. Furthermore, the NS4B protein induces lipogenesis in infected cells by activating the Akt pathway[49]. It has been shown that HCV core protein is expressed at the basal membrane of polarized cells, which leads to a deregulation of actin organization and affects focal contacts by increasing the expression of phosphorylated paxillin at the basal membrane[50]. The same study showed that the dere-gulation of actin is due to RhoA inhibition and Rac1 activation in cells expressing HCV core protein. These results also suggest that HCV core disrupts cell adhesion, inducing a reorganization of the actin cytoskeleton and a loss of cell polarity. Is PI3K involved in HCV core expression, thus inducing a disruption of cell adhesion? Many studies have revealed activation of the PI3K/Akt pathway and the formation of PtdIns(3,4,5)P3 following HCV infection[8,45,51,52]. This work focused on virus entry and replication and the epithelial to mesenchymal tran-sition, but the effect of PI3K expression on the loss of cell polarity induced by HCV infection was not investigated. Nevertheless, the phenotype of cysts from MDCK cells expressing HCV core protein was of a multi-lumen type[50] which differed markedly from that of the inverted polarity cysts obtained from MDCK cells treated with the p100δ inhibitors IC87114 and CAL-101, but was similar to those from MDCK cells treated with the p110γ and p110β inhibitors AS-605240 and TGX115, respectively[41]. This observation reveals a potential role for p110γ and p110β in the loss of cell polarity induced by HCV infection. Interestingly, Peng *et al*[41]. studied an MDCK phenotype involving an over-expression of the PI3K p110 subunit. These cysts displayed a marked ECM assembly (laminin and type IV collagen) at the basal membrane and a loss of cell polarity, and they were flatter than control cells. This result allows us to advance the hypothesis that p110δ may be over-expressed in the context of HCV infection, leading to an accumulation of ECM which is responsible for cirrhosis. Taken together, these findings suggest a potential effect of PI3K and PtdIns(3,4,5)P3 on the dere-gulation of cell polarity induced by HCV infection.

SHIP2 ACTIVITY, CELL POLARITY AND HCV INFECTION

The level of PtdIns(3,4,5)P3 is maintained through its dephosphorylation by the phosphatases SHIP1/2 and PTEN to produce PtdIns(3,4)P2 and PtdIns(4,5)P2, respectively[53] (Figure 3). A recent study highlighted the role of PtdIns(3,4)P2 and SHIP2 as additional determinants of basolateral membrane formation[50]. In non-polarized cells, SHIP2 is localized in the perinuclear and cytoplasmic domains. After stimulation with serum, SHIP2 may be localized at focal contacts in the plasma membrane[53]. In 3D cultured MDCK cells, Awad *et al*[50] demonstrated a basolateral localization of SHIP2 and its lipid product PtdIns(3,4)P2. The enzymatic activity of SHIP2 gives rise to PtdIns(3,4)P2 and is essential for cell polarization. Indeed, SHIP2 inhibition by siRNA, and exogenous expression of the catalytic mutant of SHIP2 (D607A)[54,55] lead to a deregulation of cell polarity and the formation of multi-lumen cysts. Indeed, PtdIns(3,4)P2 is capable of binding Dlg1, the master regulator of basolateral polarity[56]. The inhibition of SHIP2 leads to a delocalization of the basolateral polarity proteins β-catenin, Scribble and Dlg1 from cell-cell contacts; moreover, their expression is markedly reduced. Overall, these data suggest that SHIP2 is required for the localization and expression of the baso-lateral complex proteins Dlg1 and Scribble in order to maintain cell morphogenesis[50]. Moreover, the Rho family of GTPases, and particularly RhoA and Rac1 which regulate the formation of stress fibers and lamelipodia, respectively[57], play a pivotal role in cell polarity[58]. It has been reported that SHIP2 increases the activation of RhoA in epithelial cells[50]. This activation is required for the polarization and migration of glioma cells[59]. Awad *et al*[50] confirmed that SHIP2 is an additional target for HCV infection. Their study examined the expression of SHIP2 and PtdIns(3,4)P2 in MDCK cells grown in a 3D culture on Matrigel, and in a 2D culture on transwell filters. MDCK cells expressing HCV core protein displayed a reduction of SHIP2 and PtdIns(3,4)P2 expression at the basal membrane. Interestingly, HCV core protein was localized specifically at the basal membrane in contact with the ECM. Together, these findings indicate that HCV core protein is able to subvert SHIP2 expression in order to disrupt cell membrane morphology[50]. In these cells, the down-regulation of SHIP2 and PtdIns(3,4)P2 leads to down-regulation of the expression of Dlg1 and Scribble at the basolateral membrane. These disturbances to the expression of polarity proteins lead accordingly a loss of apicobasal cell polarity and the formation of multi-lumen cysts. HCV core expression also displays a loss of RhoA activation, in the same way as SHIP2 depleted cells. Interestingly, an over-expression of SHIP2 cDNA in HCV core-expressing cells has been seen to restore single lumen formation, RhoA activation and cell polarity. Taken together, these data indicate that HCV core is able to subvert SHIP2 in order to disrupt cell polarity and infected polarized cells[50].

PTEN AND PTDINS(4,5)P2, CELL POLARITY AND HCV INFECTION

PTEN, the other phosphatase which antagonizes PI3K, is also implicated in cell polarity. Martin-Belmonte *et al*[60] identified PtdIns(4,5)P2 as a key regulator of the apical membrane. During the early stages of cyst formation, PtdIns(3,4,5)P3 and PtdIns(4,5)P2 are co-localized at the plasma membrane of non-polarized cells, while PtdIns(4,5)P2 becomes concentrated at the apical surface of polarized cells. The role of PIP5K in apical membrane trafficking, by which synthesizing PtdIns(4,5)P2, has recently been reported and confirmed the possible production of PtdIns(4,5)P2 at the apical membrane[61,62]. Meanwhile, PTEN regulates the apical recruitment of Par3, Par 6, Cdc42 and annexin 2 (Anx2), and is required for lumen development[63]. In addition, it has been shown that Par3 membrane targeting is dependent on the binding of its PDZ domain to PtdIns(4,5)P2, the product of PTEN[64,65]. The inhibition of PTEN by siRNA, or by a specific inhibitor bpV(pic), prevents the formation of a single central lumen in the cysts and causes a defective segregation of PtdIns(3,4,5)P3 and PtdIns(4,5)P2. This study also identified the fact that PTEN binds Anx2, which is responsible for the recruitment of Cdc42 and hence of apical aPKC, causing polarization of the apical membrane[60].

HCV replication is dependent on PtdIns(4,5)P2[66] the lipid product of PTEN. HCV infection leads to a down-regulation of PTEN, triggering an acceleration of lipid droplet formation and insulin resistance[67,68]. The down-regulation of PTEN causes a malformation of the apical domain in an MDCK 3D culture[60], suggesting that the multi-lumen phenotype in MCDK cells expressing HCV core protein may be caused not only by RhoA down-regulation[50] but also by modifying PTEN expression. It has been shown that the core protein of HCV genotype 3a Core decreases the expression of PTEN by blocking the translation of messenger RNA and causing an accumulation of lipid droplets in the cells. In addition, PTEN over-expression in these cells is capable of reducing the accumulation of lipid droplets. This study therefore suggests that this down-regulation of PTEN by HCV infection is a critical mechanism leading to steatosis and its progression toward fibrosis and hepatocellular carcinoma[67].

INSULIN RESISTANCE AND HCV INFECTION

Type Ⅱ diabetes, and more generally insulin resistance, is very common in the context of chronic HCV infection, as has been established by several recent epidemiological studies[69-71]. Other studies have also shown that HCV infection can cause insulin resistance by the phosphorylation of IRS-1 at a serine residue (Ser307) followed by a decreased phosphorylation of Akt Thr(308), Fox01 Ser(256) and GSK3β Ser(9), downstream players
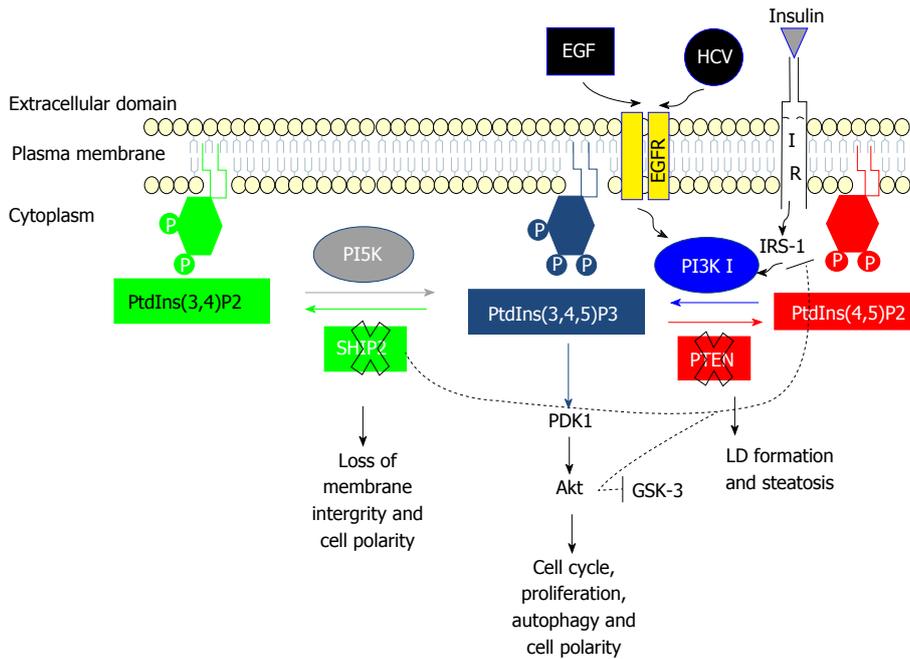
Figure 3 PI3K/Akt pathway and its activation by hepatitis C virus infection. When EGF binds to EGFR this will activate PI3K I. PI3K activation will increase the level of PtdIns(3,4,5)P3 to phosphorylate Akt and induce cell proliferation and cell polarity. Hepatitis C virus (HCV) is able to bind EGFR at its entry into a cell, thus increasing PI3K activation and the level of PtdIns(3,4,5)P3, the latter being sustained by the down-regulation of SHIP2 and PTEN. The down-regulation of SHIP2 by HCV induces a loss of membrane integrity and cell polarity, while the down regulation of PTEN induces lipid droplet (LD) formation and steatosis. PtdIns: Phosph-atidylinositol; PI3K: Phosphoinositide 3-kinase; SHIP2: SH2-containing inositol polyphosphate 5-phosphatase; PTEN: Phosphatase and tensin homologue deleted on chromosome 10; EGFR: Epidermal growth factor receptor.

in the insulin signaling pathway[72,73]. These data raise the question whether insulin resistance is the cause of liver steatosis in patients with chronic HCV infection, or the consequence of viral molecular expression. Indeed, Shintani *et al*[74] showed that insulin resistance preceded the onset of steatosis in transgenic mice expressing HCV core protein, suggesting that insulin resistance was not a consequence of hepatic steatosis in these animals. Another study confirmed that the pathophysiology of fatty liver-associated chronic hepatitis C differed in patients infected with genotypes 1 and 3, showing that insulin resistance in genotype 1 patients is the cause rather than the consequence of hepatic steatosis and fibrosis, and suggesting that elevated circulating insulin levels are a risk factor for fibrosis through steatosis induced by insulin resistance. In genotype 3-infected patients, steatosis was related to HCV viral load[75]. These findings suggest that antiviral therapy in genotype 1-infected patients will not be sufficient. But does an improvement in metabolic syndrome increase the success rates of antiviral therapy? Walsh *et al*[76] confirmed that in patients with chronic HCV viral genotype 1, an increased expression of factors inhibiting interferon signaling could be a mechanism by which obesity reduces the biological response to IFN-α. In 2006, Tarantino *et al*[77] also confirmed that by improving metabolic syndrome, a lowering of the body mass index could play a key role in reducing the importance of metabolic co-factors and improving the foundations for a good antiviral response. For this reason, insulin sensitizers such as metformin are known to improve the response to HCV treatment and have been associated with a lower risk of developing HCC. Very recently, a clinical trial was initiated by the Ottawa Hospital Research Institute to evaluate the effects of metformin on liver fibrosis in HCV-HIV co-infected and HCV mono-infected patients suffering from insulin resistance. If metformin proves to be effective in reducing liver fibrosis in this patient population, it will represent a well-tolerated, easy-to-administer, inexpensive therapy that could protect against negative HCV outcomes. This study will also provide an opportunity to evaluate the impact of insulin resistance and hyperglycemia on viral clearance in HCV-infected patients treated with interferon-free regimens[78].

PI3K/SHIP2/PTEN PATHWAY AND INSULIN RESISTANCE

Akt activity is essential for the translocation and fusion of glucose transporter 4 (GLUT4) to the plasma membrane of cells in the skeletal muscle and adipose tissue. In turn, GLUT4 plays a crucial role in the absorption of glucose. Akt induced by insulin signaling is also critical to the regulation of gluconeogenesis and glycolysis in the liver. When binding insulin to its receptor, SHIP2 also binds to the cell membrane and negatively regulates insulin signaling. An over-expression of SHIP2 in adipocyte 3T3-L1 cells inhibits insulin signaling, and expression of the catalytic mutant SHIP2 enhances the activity of Akt induced by insulin and thus generates glucose uptake and glycogen synthesis[79]. In 2001, Clément *et al*[80] developed transgenic mice deficient in SHIP2.

They showed that adult mice with the heterozygous SHIP2 mutation increased their glucose tolerance and insulin sensitivity, which was also associated with an increase in recruitment of the glucose transporter GLUT4. Homozygous mice with a SHIP2 deficiency experienced severe neonatal hypoglycemia and died within three days. These results show that SHIP2 is a potent negative regulator of insulin signaling and insulin sensitivity *in vivo*. A second SHIP2 deficient mouse model was studied by Sleeman *et al*[81] in 2005. They found that SHIP2 deficient mice were viable, with normal glucose levels and normal tolerance to insulin. However, they were very resistant to putting on weight when fed a high-fat diet[81]. Although these two models had different phenotypes, the results suggest that SHIP2 is a key regulator of glucose, and its inhibition would be useful regarding efforts to improve diet-induced obesity. Furthermore, transgenic mice expressing catalytically-inactive SHIP2 have displayed altered lipid metabolism and insulin secretion[82]. In addition transgenic mice over-expressing SHIP2 WT are obese and suffer from hepatic insulin resistance[83]. These results show that the inhibition of SHIP2 may influence lipid metabolism and insulin signaling. For these reasons, the use of antisense oligonucleotides against SHIP2 in model diabetic rats produced a rapid improvement in insulin sensitivity[84]. Taken together, these *in vivo* studies suggest that an inhibition of SHIP2 expression may be effective in the treatment of type 2 diabetes. The relationship between SHIP2 and insulin resistance has also been studied in patients with type Ⅱ diabetes whose SHIP2 gene (*INPPL1*) had a deletion of the 3' extremity. This mutation enhanced the expression of SHIP2 in the adipose tissue and skeletal muscles of diabetic patients, causing insulin resistance[85]. Overall, these findings suggest that SHIP2 is a key regulator of glucose homeostasis and could be targeted when treating diseases that affect insulin metabolism such as diabetes type Ⅱ. This central role of SHIP2 as a negative regulator of insulin signaling encouraged Sumie *et al*[86] to investigate changes to SHIP2 expression in HCC patients with HCV infection. They showed that the cumulative survival rate was significantly lower in the glucose intolerance group than that in the normal glucose tolerance group, and that the level of SHIP2 expression fell in a context of HCC when compared to that seen in non-tumor tissues. This study therefore indicated a prognostic role for glucose tolerance and SHIP2 expression in HCC patients with HCV infection.

PTEN is the second phosphoinositide phosphatase that negatively regulates insulin signaling[83-87]. Studies using 3T3-L1 adipocytes clearly demonstrated that PTEN over-expression inhibited the production of insulin-induced PtdIns(3,4)P2 and PtdIns(3,4,5)P3, the activation of Akt/PKB, the translocation of GLUT4 to the cell membrane and glucose uptake[88,89]. By contrast, the down-regulation of PTEN by small interfering RNAs enhanced Akt/PKB activation and glucose uptake in response to insulin[90]. Furthermore, an over-expression of catalytically-inactive or dominant-negative PTEN mutants also indicated that it is the lipid phosphatase activity of PTEN which is necessary to down-regulate Akt/PKB signaling and glucose uptake in response to insulin[89-91]. Finally, all these studies showed that the PI3K/Akt pathway offers a target to improve steatosis and insulin resistance during the development of NAFLD. Different treatments such as Silibinin and FAM3A (cytokine-like gene family) activate PI3K p110α/Akt signaling in order to ameliorate hepatic gluconeogenesis and lipogenesis[92,93]. Flanovol Quercetin is another treatment with favorable effects on the progression of NAFLD, acting *via* the PI3K/Akt pathway. Treatment with quercetin has been shown to reduce oxidative/nitrosative stress and inflammation, and genes related to lipid metabolism displayed a tendency to normalize in both *in vivo* and *in vitro* models[94].

PI3K/SHIP2/PTEN PATHWAY AND NAFLD

NAFLD is often associated with HCV infection. NAFLD is frequently described as encompassing a histological spectrum from nonalcoholic fatty liver to simple hepatic steatosis (SHS) plus a characteristic pattern of steatohepatitis [nonalcoholic steatohepatitis (NASH)]. HCV infection also gives rise to liver steatosis. So is the PI3K/SHIP2/PTEN pathway implicated in NAFLD in a context of HCV infection? In the liver, insulin controls lipid metabolism through its cell surface receptor and intracellular mediators such as PI3K and serine-threonine kinase Akt. It has been shown that insulin inhibits apoB100 secretion through the activation of PI3K. And insulin signaling *via* PI3K inhibited the maturation of VLDL lipoprotein particles by preventing lipidation of the VLDL precursor[95]. For this reason, a disruption of phospholipid metabolism is present in NAFLD. Indeed, a recent study demonstrated that plasma phospholipids differed between liver biopsies from NAFLD patients and healthy subjects. Phosphatidylinositol levels were higher in SHS and NASH patients compared with healthy controls[96]. Another study also identified the role of dietary phosphatidylinositol (and particularly phosphatidylcholine and phosphatidylserine) in preventing NAFLD in a rat model of metabolic syndrome[97]. Furthermore, in transgenic mice with hepatic steatosis and developing a tumor, alongside an abnormal accumulation of fatty acids, the study demonstrated activation of the Akt/mTOR pathway, and a reduction in the expression of tumor suppressor genes, including *Pten*. This confirmed that an accumulation of fatty acids may have a role in promoting *in vivo* hepatic tumorigenesis under constitutive activation of the PI3K pathway[98]. Another study confirmed that high unsaturated fatty acid levels significantly decreased PTEN mRNA expression in hepatic cells by means of a mechanism involving the sequential activation of mTOR and NFB, which were found to form a complex in cultured cells[99] which led to a significant alteration of PTEN expression. It is important to remember that inflammatory cytokines such as transforming growth factor β, tumor necrosis factor α, interleukin-6
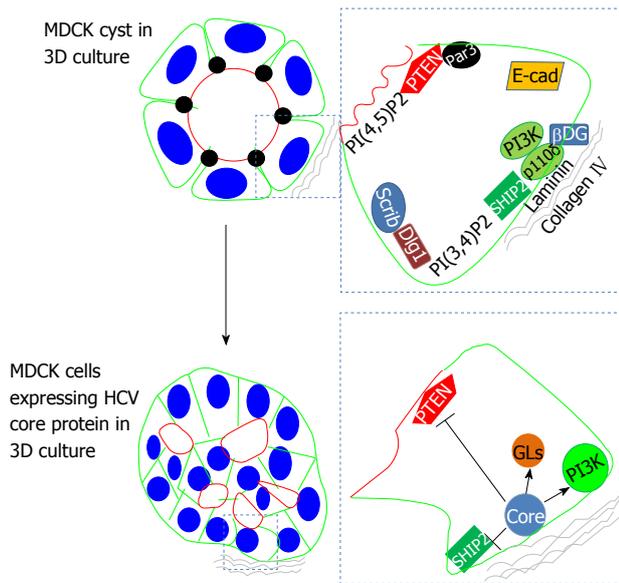
Figure 4 Effects of hepatitis C virus infection on cell polarity. In a 3D culture, MDCK form a cyst comprising a monolayer of cells around a lumen (the basolateral membrane is indicated in green, the apical membrane in red and the tight junctions in black). The right-hand column shows a zoom of a polarized cell. PI3K is activated by E-cadherin. The p110δ subunit is activated by ECM (Laminin and collagen IV). SHIP2 and PI(3,4)P2 at the basal membrane are responsible for the Dlg1/Scribble complex at the basolateral membrane. PTEN interacts with Par3 at the tight junctions and its lipid product PtdIns(4,5)P2 is localized at the apical membrane. The bottom column represents the multi-lumen phenotype of cysts expressing HCV core protein. The presence of this core protein at the basolateral membrane activates PI3K and inhibits SHIP2 and PTEN, leading to a loss of cell polarity and an accumulation of lipid droplets (LDs). HCV: Hepatitis C virus; PtdIns: Phosphatidylinositol; PI3K: Phosphoinositide 3-kinase; SHIP2: SH2-containing inositol polyphosphate 5-phosphatase; PTEN: Phosphatase and tensin homologue deleted on chromosome 10; MDCK: Madin darby canine kidney; DG: Dystroglycan.

and interleukin-1, which are produced in the course of NAFLD, also significantly alter PTEN expression, as has been shown in non-liver cells. These studies offer an interesting link between insulin resistance and steatosis, which may also explain (at least in part) the high risk of developing HCC associated with diabetes and obesity[100]. PTEN deregulation has also been demonstrated during *in vivo* studies. First, a study of heterozygous PTEN deletion was confirmed as inducing atypical adenomatous liver hyperplasia[101]. Subsequently, a genetic inhibition of PTEN expression, specifically in the liver of rodents, was shown to trigger liver steatosis, insulin hypersensitivity and HCC[102]. Because of the lack of PTEN activity, there may be an increase in fatty acid uptake by hepatocytes, and in fatty acid synthesis[103]. Hepatocyte-specific PTEN deficient mice display similar histological features to human NASH patients. These hepatocytes display enhanced lipid accumulation, inflammatory changes and hyperoxidation, and also develop into HCC[104]. Therefore, an impairment of PI3K/PTEN signaling could be involved in some NASH/HCC cases in humans. These results are very compatible with the down-regulation of PTEN in HCV infection, which leads to an acceleration of lipid droplet formation and insulin resistance[67,68]. Taken

together, these studies have suggested a role for PTEN in regulating lipogenesis in liver cells; however, less information is available on the effects of another lipid phosphatase, SHIP2, on lipid and lipoprotein metabolism in the liver. A very recent study found a molecular link between SHIP2 expression and metabolic dyslipidemia using the over-expression or suppression of the *SHIP2* gene in HepG2 cells. SHIP2 over-expression led to higher lipid production and secretion *via* apoB100 secretion and de novo lipogenesis[105]. Another study confirmed that PBX-regulating protein 1 enhances *Ship2* transcription, leading to hepatic lipogenesis and steatohepatitis in mice. However, Prep1 hypomorphic heterozygous [Prep1 (i/+)] mice displayed lower SHIP2 levels, and significantly decreased serum triacylglycerol levels and the liver expression of fatty acid synthase[106]. We have discussed above the down-regulation of SHIP2 in HCV core-expressing cells, so is this down-regulation of SHIP2 the cause of lipid droplet accumulation in these cells? Overall, these findings confirm that SHIP2 is responsible for hepatic lipogenesis and secretion. To conclude, the PI3K/SHIP2/PTEN pathway, which is markedly deregulated in the context of HCV infection, activates Akt causing an over-expression of fatty acids, leading subsequently to liver steatosis, insulin hypersensitivity and HCC.

CONCLUSION

PI3K and SHIP2 have been widely studied for their roles in intracellular signaling and membrane trafficking. However, their membrane segregation and the effects of their enzymatic activity on the establishment of cell polarity are only now starting to be investigated. Recent studies have defined the manipulation of these PI enzymes and their lipid products by the hepatitis C virus, so that it can enter and replicate in epithelial host cells (Figure 4). The PI3K/PTEN/SHIP2 pathway is now better understood in the context of HCV infection, inasmuch as it induces changes to cell polarity and lipid metabolism which can generate several pathologies such as insulin resistance, liver steatosis, NAFLD and HCC.

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