Function of lipid droplets in the production of infectious particles

As mentioned earlier, lipid droplet plays an important role in the production of infectious HCV particles. For this role, one could think the following possibilities: (1) the lipid droplet generates an aggregate together with NPC and RC for the production of infectious virus particles, (2) it provides a factor required for infectivity to the virus particles, and (3) it incorporates virus particle into a vesicle transport system of lipid or lipid related materials to export infectious virus outside the cells. The lipid droplets and their surrounding environment including NPC may be utilized for particle formation because virus-like particles are observed around the lipid droplets by electron microscopy.[18] However, as shown in the model (Fig. 6), non-infectious virus particles can be produced even when the HCV NS proteins are not aggregated with lipid droplets. Therefore, lipid droplets seem to be non-essential organelles involved in the assembly of viral particle. Regarding the second possibility, the buoyant density of infectious virus particles was lower than those of noninfectious virus particles by 0.03 g/ml. This suggests that virus particles produced around the Core-coated lipid droplets have a composition of viral protein and nucleic acid different from that of non-infectious particles which are produced from environment other than that produced with association of the Core-coated lipid droplet, to give rise to virus with lighter density. Or, viruses produced from the lipid droplet-associated environment are composed of, or have associated with components with lower density, which is essential for infectivity. The third proposed role of lipid droplets attracts attention to a new function of lipid droplets as a transporting machinery of virus embedded vesicle. It has been known that the LD is surrounded by a monolayer of phospholipids and dynamically moves through the cytoplasm interacting with other organelles, including mitochondria, peroxisomes, endosomes, and the endoplasmic reticulum (ER).[29] It is possible that these interactions facilitate the transport of lipids and lipid-associated proteins to other organelles. Thus, it is also possible that these dynamic movements of the lipid droplet facilitate transport of virus particles to where secretion of virus can take place.

A model of production of infectious HCV

When HCV in the blood stream of patients is analyzed by density-gradient centrifugation, virus is observed as a heterogeneous population with different densities.[30] The reasons for this heterogeneity may include the association of HCV with blood components, such as lipoprotein and others, rather than differences in the viral protein or nucleic acid composition. It has been shown that HCV associates with lipoproteins including very low-density lipoprotein (VLDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL).[30] In addition, the possibility that virus particles associate with antibodies against the virus itself, rheumatoid factor or cryoglobulin has also been reported. However, the significance of these associations with blood components in the HCV replication cycle is still unknown and many questions remain unanswered. It has been suggested that endocytosis of the *Flaviviridae* viruses such as HCV and bovine viral diarrheal virus was mediated by low density lipoprotein (LDL) receptors on cultured cells.[31] Recently, it has been shown that the association with VLDL has an important role in the secretion of virus particles as well as in the acquisition of infectivity.[32–34]

VLDL is a particle that consists of a hydrophobic core of neutral lipid surrounded by a monolayer of amphipathic hospholipids and free cholesterol in which apolipoproteins reside.[35] VLDL synthesis is mediated by the function of microsomal triglyceride transfer protein (MTP), which regulates the association of triglyceride with apolipoproteins B-100 to synthesize VLDL precursor. Cholesterol ester and additional triglyceride complexed with apolipoproteins E are incorporated while the VLDL precursor is simultaneously transformed to the matured form of VLDL. In HCV-producing cells, it is possible that VLDL precursor accumulates in the microenvironment of the lumen of ER where Core-coated lipid droplets associate with ER membranes rich in RC and NPC, which may increase the amount of the precursor VLDL and allow to associate with HCV which is also budded into ER lumen. It is presumed that HCV/VLDL complexes are secreted to outside of the cell via the Golgi pathway. Importance of lipoproteins to infectivity of HCV is suggested by the fact that an inhibitor of MTP suppresses release of

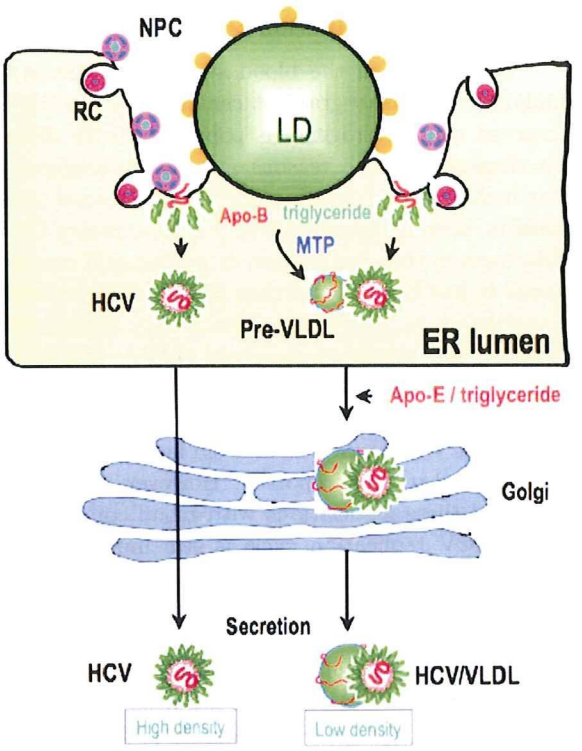

Fig. 8. Model of the predicted role of the Core-coated lipid droplet in the production of infectious HCV. The association of the Core-coated lipid droplet with NPC-and RC-rich ER may enhance the interaction of HCV and VLDL. VLDL is generated frequently and enhanced in the micro-environment where lipid droplets associate with the ER. This increased concentration may increase the frequency of the association of HCV and VLDL. HCV/VLDL is released as an infectious particle with a low density, whereas HCV particles that are not associated with VLDL are secreted into the culture medium as noninfectious, dense particles. However, this model does not discriminate the possibility that noninfectious virus particle also associates with or is integrated with some lipoprotein like structure.

infectious virus particle.[32),33)] In cells treated with MTP, it was observed that despite the suppression of infectious viruses released into culture medium, intracellular infectious virus particles have a slightly higher density than those released into culture medium. Another possibility for the result obtained by MTP inhibitor experiment is that MTP inhibitor may affect production or function of other cellular factor(s) that associates with HCV or regulates production of infectious HCV.

Recently, we found that Apo-E interactd with HCV NS5A (Hishiki *et al.*, unpublished data). Moreover, Apo-E knockdown cells suppressed the production of infectious virus in a culture medium.[36)] while Apo-A1 knockdown cells did not (Hishiki *et al.* unpublished data). Taking this data together with others, it is suggested that Apo-E as well as Apo-B are important host factors, at least in part, involved in endowing infectivity to HCV.

A model of infectious virus particle production that reflects our experimental results, which indicate that the association of Core-coated lipid droplets with the ER membrane is important for infectious virus particle production, is shown in Fig. 8. In this model, we hypothesize that the association of lipid droplets with the ER membrane increases the production efficiency of VLDL precursor in micro environmental space of the lumen. This higher concentration of VLDL precursor may facilitate the association with HCV which is also budded into ER lumen. But it is unclear whether the formation of this complex occurs actively or passively. Since NS5A binds with Apo-B[20)] as well as Apo-E, it is possible that the NS proteins promote interaction of VLDL with HCV. Alternatively, integration of Apo-E and/or Apo-B to micro-environmental membranous structure where HCV particle buds may also be likely. In this case Apo-B and/or Apo-E should be integrated as a membranous component of HCV membranous structure. Although involvement of VLDL for infectious HCV production was suggested, it remains to be clarified whether or not VLDL itself associates with HCV before virus release to culture medium. There is no direct evidence to show this association in an *in vitro* experiment.

It has been reported that HCV infection is mediated by various cellular receptors and the LDL receptor (LDLR) is one of them.[31),34)] VLDL or its derivative(s) in the VLDL/HCV complex, or Apo-B and -E in HCV may act as a ligand of LDLR on surface protein of hepatocyte.

Increase in lipid droplets in HCV-infected cells

It has been shown that the lipid droplet is an important cellular organelle that produces infectious HCV.[18)] Then, how does HCV infection affect lipid droplets in the cell? As described above, the structure of the membrane monolayer of the lipid droplet is modified in HCV-replicating cells; multilayered membrane structure on the surface of the lipid droplet are observed often and, moreover, lipid droplets are surrounded by an excess membranous structure.

In addition, the amount of lipid droplets increases in HCV-replicating cells.[18] Enhanced production of the lipid droplet seems to be mediated by the expression of Core, which is suggested by the following experiment. When autonomous HCV replicon lacking Core production was expressed in HuH7 cells, no enhanced expression of lipid droplet was observed by confocal microscopy analysis of stained cells with BODIPY 493/503 (Invitrogen), a marker for the lipid droplet. In contrast, cells expressing the full HCV replicon showed substantial enhanced production of lipid droplet. Enhancement of lipid droplet production was also observed by ectopic expression of Core in cells bearing HCV replicon lacking expression of the Core. Therefore, it was concluded that HCV infection increases the amount of lipid droplets and that the Core is required for this increase. The amount of intercellular lipids depends on a balance between lipid synthesis, degradation, and lipid transport to the outside of the cell. The Core enhances lipid synthesis by activating SREBP, a transcription factor necessary for lipid synthesis, as well as by suppressing MTP activity slightly. NS5A is also reported to suppress MTP activity some extent. These roles of Core and NS5A are probably significant for balancing intracellular lipid level in HCV infected cells.

Relationship between abnormal cellular metabolism of lipid in HCV-infected cells and HCV related hepatic disease

Many questions about the molecular basis between infection/replication of HCV and the development of chronic hepatitis, hepatic cirrhosis, and liver cancer remain unsolved. It is believed that chronic hepatitis results from the continuous destruction and regeneration of hepatic cells. It has been suggested that continuous immuno-mediated destruction of liver plays a central role in liver pathogenesis by HCV infection. On the other hand, many HCV-infected patients can experience complicated diabetes due to inhibition of insulin signal transduction and can also experience abnormal metabolism, including increased liver lipid content, because of abnormal lipid accumulation. In addition, these disorders are thought to exacerbate liver diseases.[37)–39)]

Transgenic mice producing HCV core protein develop steatosis and subsequent liver cancer.[40)] This suggests that abnormal lipid metabolism caused by the HCV core protein can lead to the development of liver cancer at least in mouse model system. The physiological significance of the mechanism by which the HCV core protein controls lipid metabolism became clear when lipid droplets were shown to be essential in the production of infectious virus particles in infected cells. In other words, HCV requires activation of lipid metabolism in the host cell to produce its progeny efficiently, which is, at least in part, a requisite of abnormal metabolism of lipid that may be linked to subsequent liver diseases in the host.

Conclusions

HCV establishes persistent infection and causes chronic hepatitis, which causes the development of hepatic cirrhosis and liver cancer in some patients. Therefore, it is very important to prevent infection and disease progression. A better understanding of the molecular mechanisms of virus replication would help to establish preventative measures. Recent studies have uncovered a mechanism of HCV replication in which lipid droplets are used to produce infectious virus. This finding may contribute to uncovering the link between HCV infection/replication and liver pathogenesis. A detailed analysis of the mechanism of HCV replication will further increase our understanding of the interaction between the virus and host cell, to explain disease development.

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