



# Hepatitis C virus NS5A protein promotes the lysosomal degradation of diacylglycerol 0-acyltransferase 1 (DGAT1) via endosomal microautophagy

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(課程博士関係)

学 位 論 文 の 内 容 要 旨

Hepatitis C virus NS5A protein promotes the lysosomal  
degradation of diacylglycerol O-acyltransferase 1 (DGAT1)  
via endosomal microautophagy

C型肝炎ウイルス NS5A 蛋白質はエンドソーム・ミクロオートファジーを介して  
DGAT1 蛋白質を分解する

神戸大学大学院医学研究科医科学専攻  
微生物感染症学講座 感染制御学  
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PUTU YULIANDARI

## SUMMARY

### Background:

Approximately 56 million people worldwide are estimated to be infected with hepatitis C virus (HCV), and ~20% of them develop liver cirrhosis or hepatocellular carcinoma. HCV is an enveloped, positive-sense single-stranded RNA virus that belongs to the Flaviviridae family, the Hepacivirus genus. The HCV genome consists of a 9.6-kb RNA encoding a polyprotein of 3,010 amino acids. The polyprotein is cleaved into three structural proteins and seven nonstructural proteins. Recent advances in HCV research have resulted in the development of novel anti-HCV therapeutics, i.e., direct-acting antivirals (DAAs), which have dramatically improved the treatment of chronic hepatitis C. However, the emergence of resistance-associated substitutions raises new concerns.

We reported that HCV infection promotes the lysosomal degradation of hepatocyte nuclear factor-1 $\alpha$  (HNF-1 $\alpha$ ) via chaperone-mediated autophagy (CMA) through an NS5A-mediated association of HNF-1 $\alpha$  with cellular chaperone heat shock cognate 70 kDa (HSC70) protein; we demonstrated that lysosome-associated membrane protein type 2A (LAMP-2A) is required for the degradation of HNF-1 $\alpha$ . However, little is known about the roles of HCV-induced lysosomal degradation in the HCV life cycle and viral pathogenesis. In this study, we searched for a novel substrate of an HCV-induced lysosomal degradation pathway by examining the NS5A-interacting proteins that carry the KFERQ motif, which is important for the association with HSC70.

### Methods:

1. To investigate whether HCV NS5A protein interacts with DGAT1 protein, we cotransfected pCAG-FLAG-DGAT1 together with pEF1A-NS5A-Myc-His6 into Huh-7.5 cells. To further examine whether DGAT1 is colocalized with NS5A protein, we performed a proximity ligation assay (PLA).
2. To determine whether protein degradation is involved in the HCV-induced reduction of DGAT1 protein, we assessed the potential role of proteasomal or lysosomal protease on DGAT1 protein. We treated the cells with a proteasome inhibitor, MG132, a lysosomal protease inhibitor, ammonium chloride (NH<sub>4</sub>Cl), or a macroautophagy inhibitor, 3-methyladenine (3-MA).
3. To determine whether the putative KFERQ motif on DGAT1 protein is required for the interaction with HSC70, we constructed pCAG-FLAG-DGAT1 Q149A (encoding a substitution of Q to A at the position of aa 149).
4. To examine the subcellular colocalization of DGAT1 and NS5A in HCV-infected cells,

we performed immunofluorescence staining. We used LysoTracker as a marker for lysosome, Rab7 as a marker for late endosome, and LC3 as a marker of autophagosome.

5. To determine whether DGAT1 is degraded via CMA or eMI, we made stable knockdown of LAMP-2A cells, a specific receptor for the CMA pathway, or stable VPS4A- and VPS4B-knockdown cells for eMI pathway.

## **Results:**

1. The immunoprecipitation analysis revealed that the NS5A protein was coimmunoprecipitated with DGAT1 protein. The PLA showed a strong signal in the presence of both FLAG-DGAT1 and NS5A-Myc-His6. These results suggest that NS5A interacts with DGAT1 in Huh-7.5 cells. We mapped the DGAT1-binding domain on NS5A protein and found that the region spanning from aa 1 to aa 213 is important for the interaction with DGAT1. We also mapped the NS5A-binding domain on DGAT1 protein and found that the region spanning from aa 157 to aa 399 is important for the interaction with NS5A.
2. Using the lysosomal protease inhibitor NH<sub>4</sub>Cl, we demonstrated that DGAT1 is degraded via an NS5A-dependent lysosomal degradation pathway. Treatment with MG-132 or 3-MA did not recover DGAT1 protein level, suggesting that proteasome degradation and macroautophagy are not involved in the reduction of DGAT1 protein.
3. We observed a putative KFERQ motif in the region spanning from aa 149 to 153 (<sup>149</sup>QVEKR<sup>153</sup>) on DGAT1 protein. The results of the coimmunoprecipitation analysis demonstrated that endogenous HSC70 interacted with FLAG-DGAT1, but not with FLAG-DGAT1 Q149A mutant. These results suggest that HSC70 interacts with DGAT1 via the KFERQ motif.
4. Immunofluorescence staining showed that the complex of DGAT1 protein and NS5A protein was colocalized in the late endosome and the lysosome.
5. DGAT1 protein was recovered in VPS4B knockdown cells. On the other hand, DGAT1 protein was not recovered in VPS4A and LAMP-2A knockdown cells. These results suggest that HCV induces the lysosomal degradation of DGAT1 protein via eMI and that VPS4B plays an important role in eMI.

## **Discussion:**

Diacylglycerol O-acyltransferase 1 (DGAT1) is an important enzyme in the final step of triglyceride synthesis. DGAT1 plays a crucial role in HCV infection by recruiting the HCV core protein onto the surface of cellular lipid droplets. In this study, we observed

that DGAT1 is degraded via the lysosomal degradation pathway. We also demonstrated that HCV NS5A protein promotes the lysosomal degradation of DGAT1 protein via endosomal microautophagy (eMI), but not chaperone-mediated autophagy (CMA).

The interaction between HSC70 and the target protein is necessary for two selective lysosomal autophagy pathways: CMA and eMI. In CMA, the protein complex formed by HSC70 interacts with LAMP-2A, causing the target protein to unfold and degrade in the lysosome. In eMI, HSC70 interacts with phosphatidylserine (PS) of the endosomal membrane. A substrate protein for eMI is sequestered by the formation of an invagination in the surface of the endosomal membrane, which is mediated by tumor susceptibility gene (TSG) 101 as an endosomal sorting complex required for transport (ESCRT I) and three proteins: vacuolar protein sorting-associated protein (VPS) 4A, VPS4B, and Alix.

Our present findings provide evidence suggesting that HCV promotes the lysosomal degradation of DGAT1 protein via eMI. We are currently seeking to determine the physiological significance of HCV-induced DGAT1 degradation in the HCV life cycle. In our preliminary result, we observed that overexpression of DGAT1 protein resulted in decrease of extracellular HCV infectivity titers, although there were no significant differences in intracellular and extracellular HCV RNA levels and intracellular HCV infectivity titers between control cells and DGAT1-overexpressed cells (data not shown). We speculate that removal of too much DGAT1 protein or quality control of DGAT1 protein via eMI may be important for the efficient production of infectious HCV particles. Further research is necessary to elucidate a pathophysiological role of the HCV-induced lysosomal degradation of DGAT1 protein via eMI.

Taken together, these results suggest that HCV NS5A protein interacts with HSC70 and DGAT1, thereby promoting the lysosomal degradation of DGAT1 via eMI in a VPS4B-dependent manner. To our knowledge, this is the first report clarifying the molecular mechanism of eMI induced by HCV infection.

In conclusion, we propose that HCV NS5A interacts with HSC70 and recruits HSC70 to DGAT1, thereby promoting the lysosomal degradation of DGAT1 via eMI. Further investigations of the HCV-induced selective degradation of host proteins via CMA and eMI may contribute to our understanding of the pathogenesis of HCV.

論文審査の結果の要旨			
受付番号	甲 第 3210 号	氏 名	PUTU YULIANDARI
論文題目 Title of Dissertation	<p>C型肝炎ウイルス NS5A 蛋白質はエンドソーム・ミクロオートファジーを介して DGAT1 蛋白質を分解する</p> <p>Hepatitis C virus NS5A protein promotes the lysosomal degradation of diacylglycerol O-acyltransferase 1 (DGAT1) via endosomal microautophagy</p>		
審査委員 Examiner	<p>主 査 児玉 裕三 Chief Examiner</p> <p>副 査 上田 佳秀 Vice-examiner</p> <p>副 査 青井 貴之 Vice-examiner</p>		

(要旨は1, 000字～2, 000字程度)

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The candidate, having completed studies on HCV, with a specialty in the viral strategy by the use of endosomal microautophagy and having advanced the field of knowledge in the area of Virology, is hereby recognized as having qualified for the degree of Ph.D. (Medical).