論文の内容の要旨

論文題目　Mechanisms of Attenuated HCV Replication with NS3 Protease Derived from A Different Genotype

(異なる遺伝子型由来の NS3 プロテアーゼによる C 型肝炎ウイルス増殖変化の機構)

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Hepatitis C virus (HCV) was identified in 1989 by immunoscreening of an expression library with serum from a patient with post-transfusion non-A, non-B hepatitis. HCV is an enveloped RNA virus belonging to the *Hepacivirus* genus of the *Flaviviridae* family. The HCV genome consists of a positive single-stranded RNA of approximately 9.6 kb, and encodes a large polyprotein of about 3030 amino acids. The viral protein is processed by cellular and viral proteases, resulting in structural proteins (core, E1, E2), and nonstructural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B).

About 170 million people worldwide are infected with hepatitis C virus (HCV), and HCV causes chronic liver disease at a high rate, including cirrhosis and hepatocellular carcinoma. HCV NS3 protease is essential for polyprotein processing and viral replication. NS3 protease has been considered an attractive target for HCV antiviral drugs. HCV has 6 genotypes, and only genotype 2 replicates efficiently and produces infectious viral particles in a human hepatoma cell line. However, because there is no infectious virus production system for genotype 1b, it is difficult to study new antiviral treatments for patients infected with genotype 1.

In this study, we constructed a chimeric subgenomic replicon with the backbone of the genotype 2a JFH-1 strain and NS3 protease region derived from the genotype 1b Con1 strain to examine the compatibility of NS3 protease. We found several adaptive mutations important for higher replication efficiency by colony formation assay. We introduced adaptive mutations into the full-length virus cDNA construct to examine whether the full-length clones with the adaptive mutations could replicate efficiently and produce infectious virus. In transient transfection assays, although the full-length chimera did not have the viral replication efficiency or produce infectious viral particles, adaptive mutations obtained from this study restored the viral replication efficiency. However, these adaptive mutations could not recover infectious viral particle formation and secretion.

Only a small amount of HCV core protein was detected in the culture media of cells transfected using JFH-1/N3P_Con1 chimeras with adaptive mutations. To examine whether secreted core proteins were parts of infectious viral particles in the transient transfection assay, we determined the infectivity of the culture media and analyzed them by sucrose density gradient. JFH-1 wild type had clear major peaks of HCV core protein and RNA at 1.15 mg/ml, but chimeric JFH-1/N3P_Con1 with T64N + T73P + M631V had broad peaks of HCV core protein and RNA at 1.15 mg/ml. HCV particles produced in the cell culture usually show a major pea at 1.15 mg/ml. These results suggest that chimeric virus might have weak virus

particle formation or release efficiency.

To further clarify the mechanism of virus particle formation defects in adaptive mutation constructs, we determined the intracellular sublocalization of lipid droplets (LDs) and core proteins by immunofluorescence staining assay. It has been reported that sublocalization of core protein on the surface of LDs is essential for HCV infectious virus particles. In the majority of cells expressing the JFH-1 wild type, LDs accumulated in the perinuclear region, and core proteins co-localized to the surface of LDs. On the other hand, LDs and HCV core of chimeric JFH-1/N3P_Con1 virus showed identical localization in the perinuclear region. Core proteins were scattered, not localized surrounding the LDs, and LDs were small as in immature forms. This result suggests that the proper localization of core protein to produce viral particles is disrupted in the adaptive mutation RNA-transfected cells.

We then determined the cleavage efficiency of protease by a Huh7OK1/TG-Luc cell line system. This cell line also contains the C-terminal region of IPS-1, which is efficiently cleaved by HCV NS3/4A protease upstream of the transmembrane region. We are thus able to determine the cleavage efficiency of protease by luciferase assay. Chimeric JFH-1/N3P_Con1 without adaptive mutations was not active same as JFH-1 GND, but chimeric JFH-1/N3P_Con1 with T64N + T73P + M631V mutations was approximately 3 times lower than JFH-1 wt at 96 h after transfection. This result indicates that the

different replication efficiency by adaptive mutations is caused by a different cleavage efficiency of NS3 protease.

To analysis the effect of NS3 protease inhibitor in this JFH-1/N3P_Con1 with adaptive mutations and JFH-1 wild type, we used the specific NS3 protease inhibitor, VX-950. VX-950 has been shown to be effective in increasing SVR rates when used with peginterferon alfa and ribavirin in patients with chronic HCV genotype 1 infection. VX-950 was more effective against NS3 protease derived from genotype 1b than that of genotype 2a. From this result, we established the assay system of NS3 protease inhibitors for genotype 1b.

In this study, we produced a JFH-1/Con1 full-length chimera that can replicate at the same levels as JFH-1 wild type in a transient assay. And protease inhibitor, VX-950, was more effective against NS3 protease derived from genotype 1b than that of genotype 2a. From this result, we established the assay system of NS3 protease inhibitors for genotype 1b. We expect this will be a useful assay system for protease inhibitors. Moreover, we can use this chimera for characterization of NS3 protease function compared to JFH-1 wild type.