様式３

論　文　内　容　の　要　旨

〔　題　　名　〕A de-ubiquitinating enzyme USP15 participates in the propagation of hepatitis C virus
(脱ユビキチン化酵素USP15はC型肝炎ウイルスの増殖に関与する)

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Hepatitis C virus (HCV) is a major causative agent of chronic liver diseases including steatosis, cirrhosis and hepatocellular carcinoma (HCC). Current therapy such as combinating pegylated-interferon (IFN) and ribavirin (RBV) achieved about 50% sustained virological response (SVR) in genotype 1 patients with high viral load. As alternative therapeutics, drug-acting antivirals (DAA) such as viral protease or polymerase inhibitors had recently developed. Clinical trials data showed DAA treatment critically eliminated HCV in over 80% of chronic patient. However, DAA-resistant virus had been already reported, suggesting that another therapeutics that don't emerge drug-resistance virus are needed.

Ubiquitylation is a post-translational protein modification that can regulate protein function in eukaryotes. It covalently attached substrate proteins by using E1, E2 and E3 enzymes. Firstly, E1 enzyme conjugates an ubiquitin in ATP dependent manner, and then ubiquitin is activated. Activated ubiquitin is transferred to E2. E3 ubiquitin ligases recognize its substrate protein and ubiquitin, and covalently conjugate ubiquitin to substrate protein. By now, two E1s and 10 E2s were identified and E3 ligases were identified as hundreds. On the other hands, de-ubiquitinases (DUBs) catalyze the opposite reaction to ubiquitylation, which means the release of ubiquitin from substrate. DUBs are consisted of about 100 proteins. DUBs regulate multi-cellular functions and its deregulation is involved in many diseases such as cancer and immune disorders. However, it remains unclear which DUBs control HCV life cycle.

I set up a screening system to identify DUBs to regulate HCV replication. Huh7.5.1 cells were infected with retrovirus expressing shRNA against 65 different DUBs and established stable knockdown Huh7.5.1 cells. Screening data suggested that shUSP15 inhibited HCV RNA in the same level as shPI4KA reported to be involved in HCV replication. Interestingly, the mRNA level of USP15 was upregulated by HCV infection. Therefore, I focused on USP15. To study more details of the involvement of USP15 with HCV replication, I generated USP15 deficient Huh7 cells by CRISPR/Cas9 systems. The levels of HCV RNA and HCV titer in USP15 deficient Huh7 were over 100 times less than those of Huh7. However, The replication efficiency of Japanese encephalitis virus (JEV) or Hepatitis B virus (HBV) did not change between USP15 deficient cells and Huh7. These data suggested that USP15 contributes to HCV specific replication.

Immunofluprescent microscopy data suggested that USP15 localized with lipid droplets. Lipid droplets had been reported to be important for HCV encapsidation and virus release. In the USP15 deficient Huh7 cells, the amount of lipid droplets was severely reduced. The PAT family proteins are capable to bind intracellular lipid droplets and regulate lipid droplets biogenesis. Among the PAT family proteins, I found ADRP was ubiquitinated and a substrate of USP15.

These data suggested that HCV infection upregulated the expression of USP15, and then USP15 may regulate lipid droplets through alteration of the distribution or components of lipid droplets.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

平成26年8月29日に公聴会及び本審査を開催した。申請者が論文内容について説明を行い、その後、論文内容に関する質疑応答を行った。

肝臓は生体内で最も大きい臓器であり、解毒作用、グルコース代謝、脂質代謝等、さまざまな機能を有している。したがって、肝機能傷害は生体に多大なダメージを与える。また、我が国の癌死の第四位が肝癌である。C型肝炎ウイルス(HCV)はフラビウイルス科に属しており、ヒトに感染すると高率に慢性化し、脂肪肝、肝硬変、そして肝細胞癌を発症する。肝癌全体の8割がHCV感染に起因しており、HCV感染の制御は肝癌征圧において極めて重要なテーマである。申請者は、近年その性質が明らかになりつつある脱ユビキチン化酵素群に注目し、HCVの増殖に関与している脱ユビキチン化酵素を探索し、USP15とUSP20を同定した。申請者は中でも、USP15に焦点を絞り解析を行った。

申請者の研究により、USP15は肝臓内で脂質代謝に関与し、HCVの複製を亢進させる役割を担うことを明らかとした。これらの成績は、USP15阻害剤が抗HCV薬となり得ること、また、USP15のこれまでに報告のなかった新たな機能の発見に繋がることから、博士学位論文審査は合格とした。