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医学系研究科分子病態医学専攻

学 位 論 文 名 Lipid rafts play an important role in the early stage of severe acute respiratory syndrome-coronavirus life cycle.
(SARS コロナウイルスの複製初期課程における脂質ラフトの重要な役割)

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論 文 内 容 の 要 旨

Objective

Lipid rafts are special microdomains of the cell membrane where sphingolipids, cholesterol and associated proteins are enriched. Accumulating evidence suggests that many pathogens, especially viruses, require lipid rafts at multiple stages of their life cycles. Viruses utilize lipid rafts as platform for entry, assembly or budding. Some viruses incorporate lipid rafts in their envelopes. Recently, the requirement for lipid rafts in the replication of coronaviruses (CoV), such as murine hepatitis virus (MHV), has been reported. Although a recent report showed that SARS-CoV envelope protein was partially localized on lipid rafts and its transmembrane domain was required for maintenance of the membrane permeabilizing activity, there is no direct evidence demonstrating the significance of lipid rafts during the replication of SARS-CoV. In the present study, we aimed to investigate whether lipid rafts are required during the replication of SARS-CoV.

Materials and Methods

SARS-CoV, Frankfurt-1 strain, was prepared from the culture medium of infected Vero E6 cells. For the depletion of cholesterol, Vero E6 cells were treated with methyl- β -cyclodextrin (M β CD). Cholesterol concentration in cell lysates after treatment were examined using the Amplex Red Cholesterol Assay system. For cell viability assay, Vero E6 cells treated with M β CD were subjected to viability assay by using a cell proliferation assay kit. For investigation of the significance of lipid rafts in SARS-CoV infection, Vero E6 cells were either treated with M β CD or left untreated, and then they were infected with SARS-CoV. The virus productions were compared by TCID₅₀/ml. Virus antigen expressions were investigated by immunofluorescence assay (IFA). Virus mRNA levels were compared by Northern blot analysis. Furthermore, membrane flotation assay was performed to show the co-localization of lipid rafts with SARS-CoV receptor, angiotensin converting enzyme 2 (ACE-2).

Results

The effect of cholesterol depletion on host cells was investigated. The result showed that treatment of Vero E6 cells by 10 mM M β CD decreased about 40% of cholesterol after the treatment. There was no apparent difference in the cell numbers between treated and untreated Vero E6 cells, indicating no cytotoxicity at a concentration of 10 mM M β CD. Virus productions were strongly impaired by pretreatment of Vero E6 cells with M β CD. Interestingly, cholesterol depletion at post-entry stage showed only mild effect on viral production. IFA showed that viral antigen expressions were also suppressed by depletion of cholesterol by M β CD, while treatment at post-entry stage showed moderate effects. Northern blot analysis revealed that viral mRNAs at 3 hours after adsorption were significantly affected by M β CD treatment, indicating that the amount of virus entry was reduced. In addition, membrane flotation analysis revealed that ACE-2 was not localized in the raft membrane of Vero E6 cells.

Conclusions

These results suggested that lipid rafts are required in the life cycle of SARS-CoV. The treatment at post-entry stage showed only mild effect, suggesting that cholesterol were mainly important during the early stage of SARS-CoV replication. ACE2 was not localized on raft membranes. Further detailed studies are needed to clarify this inhibitory mechanism.

論文審査の結果の要旨

ウイルスのライフサイクルにおいて、初期の吸着・侵入過程は最も重要なステップである。この過程において、ウイルス粒子の宿主細胞表面での相互作用については不明な点が多い。脂質ラフト構造は細胞膜の構造の一つであり、ウイルス感染においてもこれまでに様々な知見が報告されているが、SARS コロナウイルス (SARS-CoV) についてはその重要性について明らかになっていなかった。

申請者は、脂質ラフト構造を破壊することで、ウイルスの産生能が顕著に低下することを明らかにし、これがウイルス感染の初期過程に対しての影響であることを示した。また、このことは細胞表面の受容体分子に対しての影響であり、ウイルス粒子と受容体分子の結合以降に関係することを明らかにした。

以上のことから、申請者は SARS-CoV の感染初期過程と脂質ラフト構造の関連性についての新しい知見を明らかにしたことから、学位の授与に値すると考えられる。