

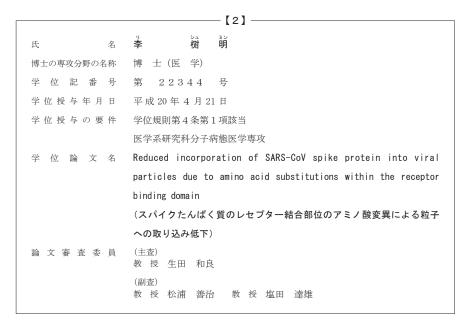
Title	Reduced incorporation of SARS-CoV spike protein into viral particles due to amino acid substitutions within the receptor binding domain
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論文内容の要旨

Objective

The severe acute respiratory syndrome (SARS) coronavirus (CoV) was identified as the etiological agent for an acute infectious respiratory disorder. SARS-CoV is an enveloped, positive-strand RNA virus with a ~30 kb genome

that encodes replicase (1a and 1b), spike (S), envelope (E), membrane (M), nucleocapsid (N), and several accessory proteins. It was reported that cell clone #21, isolated from persistently infected cells, was a long-term producer of infectious viral particles at a high rate for more than one year. Electron microscopic and Western blotting analyses demonstrated that the incorporated number of S protein on viral particles from #21 cells was lower than that from acutely infected cells. In this study, we focused on the significance of amino acid substitutions in structural proteins for reduced S incorporation into virions in #21.

Materials and Methods

The Vero E6 cell line was used to propagate SARS-CoV (Frankfurt-1 strain). The full-length sequence of the whole viral genome in #21-derived SARS-CoV was determined. Virus-like particles (VLPs) by co-expression with S, E, M and N-expressing plasmids were generated. VLPs were purified from the culture supernatant of transfected Vero E6 cells by 20-60% discontinuous sucrose gradient ultracentrifugation and pelletting down through 20% sucrose. VLPs collected in the pellet were resuspended in 100 ·l of PBS for Western blotting with the anti-V5 monoclonal antibody. A computer program was used to analyze the structure of wild-type S and #21-derived S proteins.

Results

Six amino acid substitutions were identified: Y442C, L472F, V594F, H641Y and P794S in the S gene and T6A in the M gene. H641Y substitution was also detected in the original wild-type virus (Frankfurt-1 strain) from acutely infected cells. The amino acid substitution of M protein resulted in disruption of the putative N-glycosylation site. Reconstitution analysis using the VLP formation system confirmed that a lower amount of S protein was incorporated into #21 VLPs than wild-type VLPs, although the intracellular expression level of each structural protein was comparable. Further mutational analysis of the S gene showed that the first two mutations (Y442C and L472F) were critical for determining incorporation efficiency. Structural analysis of S protein using a computer program indicated that Y442C and L472F substitutions might reduce binding affinity between the S protein and receptor ACE2. Y442C substitution also reduced the binding efficiency to S protein with an anti-S monoclonal antibody which possesses neutralizing activity by Western blotting.

Conclusion

Two amino acid substitutions, Y442C and L472F, of the S protein were shown to be related to the reduced incorporation of this protein into virions that could affect the #21 phenotype, i.e., reduced affinity to receptor ACE2 and anti-S neutralizing antibody, and the different intracellular distribution. The two amino acid substitutions may have led to improper conformation of the S protein in the endoplasmic reticulum/Golgi network, and this could have affected the decreased S proteins on the cell surface and viral membrane.

SARSコロナウイルスの in vitro 感染試験により樹立した持続感染細胞(#21クローン)は急性期のウイルス感染細胞と同程度のウイルス産生が認められる一方で、外被たんぱく質であるスパイク(S) タンパク質の粒子への取り込み量が低下していることが以前より指摘されていたが、そのメカニズムについては不明であった。

申請者は、この#21細胞クローンよりウイルスゲノムを抽出し、全ゲノム塩基配列を決定した。その結果、構造タンパク質であるSタンパク質に4か所および膜タンパク質に糖鎖付加欠損を伴う1アミノ酸の変異が認められた。Viral-like particles (VLPs)を用い、各変異を導入したウイルス粒子再構成試験により、Sタンパク質の取り込み低下は、膜タンパク質の糖鎖欠損とは関係がなく、Sタンパク質のレセプター結合部位内の2つのアミノ酸変異が関与していることを明らかにした。また、この2つのアミノ酸置換がSタンパク質とレセプターあるいは中和抗体との結合性を低下させる可能性が示唆された。

申請者はリバースジェネティックス法によるVLP再構成システムを開発し、VLP構成タンパク質の定量を行うことにより、SARSコロナウイルス外被タンパク質の粒子への取り込み量低下の分子メカニズムについての新しい知見を明らかにした。このことはSARSコロナウイルスの粒子形成機構を理解する上で重要な発見であると思われる。

以上のことから、本論文は学位の授与に値すると考えられる。