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学位論文内容の要旨
Abstract of the dissertation

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Subcellular localization of nucleocapsid protein of severe fever with thrombocytopenia syndrome virus (SFTSV) and characterization of quasi-species of SFTSV

(重症熱性血小板減少症候群ウイルス(SFTSV)の核蛋白の細胞内局在および SFTSV の多種性の解析)

< abstract >

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne bunyavirus that causes novel zoonotic diseases in Asian countries. In this study, I aimed to understand the interaction of viral N, L proteins, and viral RNA (vRNA) and their roles in the RNP complex formation. A minus-sense reporter gene-eGFP, or luciferase, was constructed with untranslated regions of the M segment at both ends using T7 promoter-expression vector for the minigenome system as a functional alternative to vRNA. Sub-cellular localization studies showed that the N protein interacts with vRNA and shows translational activity with RdRp, an L protein localized in the ER-Golgi intermediate compartment (ERGIC) and Golgi apparatus when co-expressed with GP. On the other hand, mutant N protein without interaction with vRNA either couldn't localize in the ERGIC or Golgi apparatus.

Interestingly, co-expression of GP showed decreased replication activity. These results suggest a necessity for functional RNP complex formation with vRNA to locate N protein to the ERGIC and Golgi complex and viral assembly. The findings of this study provide valuable insights into the life cycle of SFTSV, which will lead to the detection of antiviral targets.

YG1 strain is the first isolated SFTSV strain in Japan. One variation on Gn (Y328H) was the major population among patient blood. Other variations on Gc (R624W) and L (N1891K) were under detection in patient blood but affected low-pH-dependent cell fusion

activity, polymerase activity, and cytopathic effect (CPE). However, the role of individual mutations in quasispecies was still unclear. I generated viruses with specific mutations with 1 to 3 substitutions of Y328H, R624W on GP, and N1891K on L protein. Then I analyzed recombinant viruses' plaque formation, cell fusion, CPE, and cell death. Mutations Y328H and R624W on the GP alone increased the cell fusion ability. Y328H on the GP was found to increase the plaque size. Pseudotyped VSV of Y328H showed a reduction of efficiency of virus entry, but R624W mutations compensated for it. Viruses with the N1891K mutation on L alone exhibited significant CPE with pinhole-sized plaques. Induced CPE was inhibited by pan-caspase inhibitor Z-VAD-FMK, suggesting caspase-dependent cell death.
On the other hand, Caspase 1 and Caspase 3 were also induced by non-CPE-inducing virus infection. These results indicated that N1891K mutations were involved in the failure to suppress cell death. Furthermore, the parental non-CPE-inducing virus showed suppression of Actinomycin D-induced cell death. Moreover, the N1891K mutant virus competed and was defeated by ten times lower amounts of the parental virus. Despite high polymerase activity, N1891K mutation on L was not advantageous for virus survival. This result suggests combining mutations may increase mutant viruses' viability, leading to new pathogenic viruses' appearance.
The knowledge of the pathogenesis of SFTSV allows us to evaluate various intervention strategies aimed at mitigating the risk of transmission of SFTSV by having a holistic approach to solving emerging/re-emerging viral diseases in the future, as suggested by the One Health approach.

483 words.