論文の内容の要旨

論文題目　Proposal for re-classification of saffold virus as a new species by comprehensive genetic analysis

(サフォルドウイルスの網羅的遺伝子解析による新たなウイルス種としての再分類の提唱)

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Saffold virus (SAFV) is currently placed along with Theiler's murine encephalomyelitis virus (TMEV) and Theiler's like rat virus (TRV) in the Theilovirus species of the Cardiovirus genus in family *Picornaviridae*. Rodents serve as the natural host for most of cardioviruses whereas the presumed host for SAFV is humans. The prototype strain of Saffold virus type 1 (SAFV-1) was discovered by viral metagenomics in 2007. Since then, a number of clinical and epidemiological studies signifying the prevalence of SAFV in human population have been published. Phylogenetic analysis suggests the presence of eight distinct genetic SAFV lineages. The genotype classification is based on the similar criteria used for enteroviruses. SAFV is considered to be associated with asymptomatic infections that occur early in life and could also involve respiratory, gastrointestinal and nervous systems. In south Asia, SAFV-2 to -8 from stool specimen of acute flaccid paralysis (AFP) case patients have been identified. AFP surveillance is used to detect paralytic illness due to many causes, including paralytic poliomyelitis caused by wild polioviruses. For that purpose, AFP cases are notified and investigated by collecting stool specimens. Phenotypic characterization of SAFV is still limited in part due to inefficient virus replication in cell culture except some SAFV strains.

I aimed to determine the prevalence and genetic diversity of SAFV in AFP patients. I also tried to redefine the exact relationship of SAFV among themselves and to the other members of Cardiovirus genus. New comprehensive genetic analyses would improve the classification of SAFV.

Materials and Methods

I investigated the stool specimens of 943 AFP cases from Afghanistan and Pakistan by conventional nested RT-PCR based on 5'UTR and complete VP1 region. Full genome sequencing was performed for all eleven genotypes' representative strains by primer walking and long PCR. Phylogenetic analysis, geographical distribution, recombination mapping and pairwise distance distributions were investigated. All the positive stool supernatants were inoculated initially in LLC-MK2 (Rhesus monkey kidney) and HeLa-C (Human cervical

epithelia expressing Sendai virus C-protein) cell lines. Viral replication was monitored by cytopathic effect (CPE) and quantitative real time reverse transcription-PCR (RT-PCR) assay of cell culture supernatants for SAFV.

Results

I detected 88 SAFV using RT-PCR in stool specimen collected from children with AFP. Characterization based on VP1 region revealed 71 SAFV belonging to eleven different genotypes. I determined the complete genome sequence of eleven representative SAFV genotypes. The phylogenetic analysis based on the entire capsid region confirmed type specific clustering which confirmed the reliability of the VP1 based genotype identification. In the non-capsid region the clustering was type exclusive and phylogenetic incongruence was quite high in the SAFV cluster, suggesting local intratypic recombination especially in the P2 and P3 regions. Recombination analysis of species Theiloviruses by several different approaches showed maximum similarity in the UTRs and minimum in the structural region especially VP1. Mapping of recombination showed a mosaic pattern with multiple breakpoints in the non-capsid region among different SAFV genotypes only. No evidence of intertypic recombination could be found. Analysis of pairwise distances distribution among the VP1 sequences revealed genotype threshold at 9% and species threshold at 52% amino acid pairwise distances. To examine the virus replication phenotype in cell culture, I established a sensitive and efficient quantitative real time RT-PCR assay. Quantitative real time PCR assay showed low or inefficient replication for most of SAFV while some of the Japanese strains belonging to SAFV-2 and SAFV-3 could grow well in cell culture.

Discussion

Type identification and new genotype assignment were based on the complete VP1 sequences of all 71 SAFV because few phylogenetic conflicts were found between VP1 and P1 region of SAFV and other Theiloviruses. Pakistani SAFV defined clusters with their respective type in P1 region while exclusive clustering of SAFV observed in the non-capsid part of genome meant geographical isolation of these viruses. Availability of extensive and diverse sequence data in the present study ensured the reliability of recombination analysis. Despite the use of various recombination analyses, I was not able to find any evidence of recombination between Theiloviruses and SAFV. High genetic diversity, phylogenetic incongruence leading to frequent recombination and distribution pattern of pairwise distances based on VP1 were the main criteria for the discrimination of SAFV genotypes in this study. However, SAFV genotypes shared >70% in sequence identity to almost all viral proteins except VP1 from other members of Theilovirus species. Other rodent theiloviruses were more heterogeneous therefore it could be difficult to treat them as genetic types along human SAFV, with scores ranging from 52-60% amino acid pairwise distances. Based on my findings, SAFV may constitute a separate species containing highly diverse human viruses.

Conclusion

Comprehensive genetic analysis showed that SAFV have high prevalence and genetic diversity in both Afghanistan and Pakistan. Phylogenies based on the non-structural region indicated frequent intratypic recombination. I established the genotyping classification criteria by determining new inter- and intra-type thresholds but it was difficult to treat the rodent theiloviruses as genetic types since they were more heterogeneous compared to SAFV. Therefore, based on genetic diversity, recombination and natural host, I propose the re-classification of SAFV as a new separate species.