〔課程-2〕

審査の結果の要旨

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The dissertation describes the proposal for re-classification of Saffold viruses (SAFV), a recently recognized group of human cardioviruses of the *Picornaviridae* family, as a new species through comprehensive genetic analysis. It describes the detection of 88 SAFV using RT-PCR in 943 stool samples collected from children with acute flaccid paralysis (AFP) in Pakistan and Afghanistan. It is the largest to-date characterization of the circulating SAFV which was based on the complete VP1 sequence. The complete VP1 sequences were used to propose VP1-based criteria for genotyping SAFV. These criteria were then used to recognize 11 genotypes. Comprehensive genetic analyses were performed after the determination of the complete genome sequences of the representative strains of eleven genotypes. SAFV genotypes were found to recombine at many locations while no recombination was detected between SAFV and TMEV/TRV all of which belong to the species *Theilovirus*. Most SAFV strains showed a non-replicative phenotype while some Japanese strains of SAFV-2 and SAFV-3 had replicative phenotypes in cell culture. Several important findings about SAFV have been described which are as follows.

1. Phylogenies based on the VP1 region showed the presence of eleven different lineages of SAFV which meant high genetic diversity among SAFV compared to the other members of the species. No major phylogenetic conflicts were observed between VP1 and capsid P1 region therefore both genotype identification and assignment were based on the complete VP1 region of all 71 SAFV. Pairwise distance methods also confirmed the presence of three new genotypes as they were divergent from existing SAFV genotypes and other theiloviruses.

2. The construction of phylogenies based on the complete genome sequences showed that the Pakistani SAFV defined clusters with their respective type in P1 region. The phylogenetic incongruence was high and clustering of SAFV was exclusive in the non-capsid region of the genome which meant geographical isolation of these viruses. SAFV clustered separately from all other theiloviruses throughout the genome and were related to TRV in the non-capsid region.

3. Recombination analysis of the *Theilovirus* species using several different approaches showed maximum similarity in the untranslated regions and minimum in the structural region especially VP1. It also showed the presence of multiple significant ($p<0.01$) breakpoints in the non-capsid region. The phylogenies constructed from the resulting fragments showed the separate clustering of SAFV from other theiloviruses which confirmed that there was no evidence of any recombination among SAFV and other members of the species *Theilovirus*.

4. The analysis with pairwise distance distribution of VP1 sequences were used to establish the genotyping criteria and species demarcation. All of the well separated distributions of pairwise distances in the VP1 region suggested that different genotypes of SAFV might be easily defined and segregated for classification purpose by determining the genotyping and species thresholds. The genotype threshold was observed at 9% and species threshold at 52% amino acid pairwise distances.

This report provides new insights into the prevalence and genetic classification of SAFV. This work plays an important role in the development of the research of SAFV and is considered worthy of the award of the degree.