Study on viral pathogenicity and disease control of positive-stranded RNA viruses

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Abstract

Understanding viral pathogenicity is key to establishing disease control. Emerging and re-emerging infectious viral diseases, including Dengue fever, Zika, COVID-19, and Classical swine fever are caused by positive-stranded RNA viruses. Thus far, I have been studying these determinants of positive-stranded RNA viruses.

Classical swine fever virus (CSFV), belonging to the genus *Pestivirus* in the family *Flaviviridae*, naturally infects only swine. A live attenuated vaccine virus generated by multiple passages in non-host cells was readapted to pigs through serial *in vivo* passages. The virus after the 11^{th} passages in pigs acquired the pathogenicity. Using the recombinant viruses and *in vitro* assay, Amino acids in the envelope protein E2, non-structural proteins N^{pro}, and NS4B acted synergistically in acquiring pathogenicity, suggesting the host specificity plays a crucial role in virulence determinants[1)]. Consequently, I investigated viral host specificity.

Pestiviruses and flaviviruses encode secretory proteins E^{rns} and NS1, respectively, in their genomes. In contrast, the hepatitis C virus, also a member of the *Flaviviridae* family, does not encode such glycoproteins and instead utilizes secretory apolipoproteins for virus particle formation. Using *in vitro* transcribed CSFV RNA lacking the E^{rns}, CSFV particle formation was restored in cells expressing not only E^{rns} but also apolipoproteins/NS1 proteins[2)]. These results demonstrated that secretory glycoproteins are essential for flavivirus particle formation and determine tissue tropism. To accelerate research, I have initiated the further development of reverse genetics and animal models.

By applying the split NanoLuc reporter HiiBiT, recombinant flaviviruses carrying the HiBiT peptide showed growth comparable to the parental strain, exhibited strong luminescence, and enabled *in vivo* tracking. Additionally, a rapid method for generating all types of positive-sense RNA viruses, bypassing the construction of infectious cDNA clones and virus isolation, was developed. This method has been effectively utilized in analyzing SARS-CoV-2 variants[3)].

References

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