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Establishment of a virulent full-length cDNA clone for Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

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Abstract

As MERS-CoV infectious clone is an important platform for studying the virus protein function and virus-host interaction. In the present study, the infectious cDNA clone of Vero cell adapted MERS-CoV (MERS-CoV-HKU) was generated with class II restriction site BsmBI based seamless clone technology. Its genome was split into a panel of 12 contiguous fragments and following amplification by PCR flanked with BsmBI. The fragments were further assembled into bacterial artificial chromosomes (BAC) using unique junctions formed by digestion of endonuclease BsmBI with two rounds of cloning. Two BsmBI restriction sites inside the genome were removed by silent mutation and set as the molecular marker of icMERS-CoV-HKU. Finally, A full-length of MERS-CoV-HKU, on the downstream of the CMV promoter in BAC, was recovered.

Comparing with wild type HCoV-EMC/2012 strain, the MERS-CoV-HKU recovered by infectious clone showed high titers on Vero and Huh7 cells. By genetic analysis, it was found genetic modification on nonstructural proteins (nsp2, nsp3, nsp14, and nsp16) and structural proteins (Spike, ORF5, and N). The synonymous mutations were placed in nsp2 (A2169C), nsp3 (C6172T, A6954G), nsp14 (C19514T), nsp16 (T21423G), S (A22367G, T24093A) and, N(T29149A). Nonsynonymous mutations were found in nsp3 (C6172T, A6954G), S (C25217T), and N (A29574T), which leads to amino acids mutation in nsp3(T198I, D1702Y), S (S1251F), and N (S192T). Surprisingly, three amino acids 'SHY' insertion and four nucleotides 'TTTA' deletion was found in S2 and ORF5 region, respectively.

According to the previous study, the RBD of BatCoV-HKU4 and BatCoV-HKU25 can recognize human DPP4 receptor but the RBD of BatCoV-HKU5 could not. To elucidate the relationship between MERS-CoV and BatCoVs, the MERS-CoV infectious clone and chimeric MERS-CoV infectious clones were generated in which the MERS RBD region was replaced by that of BatCoV-HKU4, BatCoV-HKU5, and BatCoV-HKU25. Recombinant MERS-CoV infectious clones were further assessed its replication on Vero and Huh7 cell lines.

Our recombinant MERS-CoV infectious clone has been shown its crucial role in studying coronavirus interspecies transmission and pathogenesis.

Keywords: MERS-CoV, infectious clone, interspecies transmission.

Biography:

Mr. Zhu Longchao is a PhD candidate from The University of Hong Kong, China. Currently, He is studying the mechanism of virus interspecies transmission and the host-virus interaction. He obtained his Mphil degree of Biochemistry and Molecular Biology from South Chain Agriculture University in 2011 and work in Veterinary Research Institute of Guangdong Academy of Agricultural Sciences. Later, he went to South Dakota University and Kansas State University, U.S. and worked as a visitor scholar for 2 years. In 2016, he started his PhD program in The University of Hong Kong University..

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