Reverse Genetics-based Approaches to Attenuate Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

Yanyan Ni

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Xiang-Jin Meng, Chair
Zachary N Adelman
Tanya LeRoith
P. Christopher Roberts
Lijuan Yuan

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Chapter 2

Establishment of a DNA-launched infectious clone for a highly pneumovirulent strain of type 2 porcine reproductive and respiratory syndrome virus: Identification and *in vitro* and *in vivo* characterization of a large spontaneous deletion in the nsp2 region

Yan-Yan Ni[1], Yao-Wei Huang[1], Dianjun Cao[1], Tanja Opriessnig[2], and Xiang-Jin Meng[1*]

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ABSTRACT

A highly pneumovirulent strain of porcine reproductive and respiratory syndrome virus (PRRSV), ATCC VR2385, was isolated from a pig exhibiting typical PRRS in the early 90's. While passaging the virus in monkey kidney cells, we identified a large spontaneous deletion of a 435-bp in the nsp2 gene. To assess the biological significance of this spontaneous deletion, we first determined the full-length genomic sequence of this virus and established a DNA-launched infectious clone of the passage 14 virus containing the 435-bp nsp2 deletion (designated as pIR-VR2385-CA). The full-length viral genome engineered with two ribozyme elements at both ends was placed under the control of the eukaryotic CMV promoter. The infectious virus was successfully rescued from pIR-VR2385-CA DNA-transfected BHK-21 cells. To characterize the biological and pathological significance of this large nsp2 deletion, we subsequently constructed another DNA-launched infectious clone, pIR-VR2385-R, in which we restored the deleted 435-bp nsp2 sequence back to the pIR-VR2385-CA backbone. The growth characteristics of the two rescued viruses (VR2385-CA and VR2385-R) were compared, and the results showed that the VR2385-CA virus with the nsp2 deletion replicated more efficiently *in vitro* (1.0-1.5 log titer higher) than the VR2385-R virus with the restored nsp2 sequence but the VR2385-CA virus exhibited a significantly reduced serum viral RNA load *in vivo*. A comparative pathogenicity

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Chapter 3

Attenuation of porcine reproductive and respiratory syndrome virus by molecular breeding of the virus envelope genes from genetically divergent strains

Yan-Yan Ni, Tanja Opriessnig, Lei Zhou, Dianjun Cao, Yao-Wei Huang, Patrick G. Halbur, and Xiang-Jin Meng

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ABSTRACT

Molecular breeding via DNA shuffling can direct the evolution of viruses with desired traits. By using a positive-strand RNA virus, porcine reproductive and respiratory syndrome virus (PRRSV), as a model, rapid attenuation of the virus is achieved in this study by DNA shuffling of the viral envelope genes from multiple strains. The GP5 envelope genes of 7 genetically divergent PRRSV strains and the GP5-M genes of 6 different PRRSV strains were molecularly bred by DNA shuffling and iteration of the process, and the shuffled genes were cloned into the backbone of a DNA-launched PRRSV infectious clone. Two representative chimeric viruses, DS722 with shuffled GP5 genes and DS5M3 with shuffled GP5-M genes, were rescued and shown to replicate at a lower level and formed smaller plaques *in vitro* when compared to its parental virus. An *in vivo* pathogenicity study revealed that pigs infected with the two chimeric viruses have significant reductions in viral RNA loads in sera and lungs, and in gross and microscopic lung lesions, indicating attenuation of the chimeric viruses. Furthermore, pigs vaccinated with the chimeric virus DS722, but not with DS5M3, still induced protection against PRRSV challenge at a level similar to that of its parental virus. Therefore, this study reveals a

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