Mini-Review

Reporter Classical Swine Fever Virus: A Powerful Tool to Study Virus Biology

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Abstract | Classical swine fever virus (CSFV) is generally a noncytopathogenic virus in cell cultures. The development of reporter CSFV has provided an excellent option to directly detect viral replication without the use of secondary labeling. This mini-review discusses the applications of reporter CSFV in the study of CSFV biology, including efficiently screening the antivirals against CSFV and viral receptors, conveniently tracking the viral proteins in live cells as well as improving diagnostic methods and vaccines for classical swine fever.

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Classical swine fever virus (CSFV) is the etiological agent of classical swine fever (CSF), which is an economically important disease of pigs in many countries. CSFV, a single-stranded positive-sense RNA virus, represents the genus *Pestivirus* within the family *Flaviviridae* (Meyers et al., 1996). The genome of CSFV comprises a large open reading frame (ORF) flanked by 5'- and 3'-untranslated regions. The ORF encodes a 3,898-amino-acid polyprotein, which is processed into 11 proteins (N^{pro}, C, E^{rns}, E1, E2, p7, NS2-3, NS4A, NS4B, NS5A and NS5B) (Moser et al., 1999). Generally, CSFV does not cause cytopathic effects in cell culture.

Commonly used detection methods for CSFV, such as immunostaining, cannot meet the demands for rapid detection of viral replication, high-throughput screening for antivirals, as well as tracking viral proteins or virus transport in cells/animals in real time. The reverse genetics systems of CSFV allows us to engineer the viral genome for generating reporter CSFV as a powerful tool. The development of reporter CSFV represents a significant advance in the study of CSFV biology.

Efficient screening of antivirals against CSFV

Reporter CSFV is an efficient tool for screening novel antiviral siRNAs and interferon-stimulated genes (ISGs) which will be useful for developing antivirals against viral infections. To date, few anti-CSFV ISGs have been reported, with only myxovirus resistance protein 1 (Mx1) identified to have an antagonistic effect on CSFV (Yan et al., 2014). We have used reporter CSFV in combination with other approaches, such as RNA interference (RNAi), to screen anti-CSFV ISGs (Wang et al., 2016; Li et al., 2016), which is time-saving and cost-effective. We also used a recombinant CSFV expressing the firefly luciferase (Fluc) (CSFV-N^{pro}Fluc) to screen antiviral siRNAs

(Shen et al., 2014). Furthermore, the antiviral effects of screened out ISGs or siRNAs have been verified through the wild-type virus. However, their antiviral mechanisms need further investigation in the future.

Screening of the viral receptor

Currently, the mechanism of CSFV entry into host cells is unknown. Identification of cellular receptors with facilitate to clarify the mechanisms of virus entry into the host cells. Using a set of siRNAs against a number of genes encoding porcine cell membrane proteins and the reporter virus CSFV-N^{pro}Fluc, we have identified the laminin receptor (LamR) as a cellular attachment receptor for CSFV (Chen et al., 2015). However, the entry receptor(s) for CSFV has not been screened out. We need to improve the screening strategy in order to obtain the entry receptor(s) of CSFV.

Convenient tracking of the viral proteins in live cells

Reporter CSFV provides an ideal tool to monitor the dynamics of viral infection *in vitro* and *in vivo* due to eliminating the need for secondary labeling, which represents a significant advance in the study of the virus biology. CSFV N^{pro} protein has been successfully tracked in real time through the tetracysteine (TC) tag fused to the N^{pro} protein (Li et al., 2014). In the future, we will track the CSFV structural proteins during virus particle assembly *in vitro* and *in vivo*. For example, we are now focusing on the live imaging of CSFV expressing the Fluc in rabbits in order to clarify the adaptation mechanism of CSFV lapinized vaccine C-strain.

Improvement of diagnostic methods and vaccines for CSF

In addition, reporter CSFV can be used to improve the diagnostic methods and vaccines for CSFV. Due to high sensitivity and operational simplicity for detection of the reporters, reporter CSFV can be used in rapid neutralization tests. EGFP-tagged CSFV has been applied for rapid detection of anti-CSFV neutralizing antibodies in sera, which is easier to perform and less time-consuming than neutralization immunofluorescence test (NIFT) (Li et al., 2013). In addition, a recombinant CSFV expressing bacterial chloramphenicol acetyltransferase (CAT) gene was a useful tool for quantitative analysis of viral replication and gene expression by measuring CAT enzyme activity (Moser et al., 1998).

Currently, CSF control and eradication programs need a genetically marked vaccine allowing serological differentiation of infected from vaccinated animals (DIVA). CSFV has been used to express the EGFP gene by bicistronic strategy to develop CSFV as a potential viral vector (Stettler et al., 2002). Moreover, the recombinant vSMEGFP-HCLV3′UTR in the context of the CSFV Shimen strain was generated by inserting EGFP to create a candidate vaccine with a positive marker (Li et al., 2016).

Other potential applications

Elucidating various aspects of CSFV-host interactions is essential for a comprehensive understanding of pathogenesis. Compared with the most frequently used techniques for screening virus-host interactions, the approaches based on reporter CSFV can recapitulate the virus life cycle. Therefore, we will develop CSFV expressing the split-Gluc (Gluc1 and Gluc2) or the split-GFP reporter to identify CSFV-host interactions.

The CSFV mutants are usually generated for studying viral replication, virulence, as well as viral protein functions. Our recent work is focused on the generation of CSFV mutants expressing a reporter gene in order to conveniently evaluate viral replication.

Limitations and prospectives

Reporter CSFV has been used to address a number of research questions. However, there are some problems associated with the reporter CSFV. Firstly, to date, only N^{pro} has been reported to allow the insertion of reporter genes (Moser et al., 1998; Li et al., 2013, 2014, 2016; Shen et al., 2014; Wang et al., 2016). Secondly, some reporter genes may not be expressed correctly in the context of some CSFV strains. For example, the EGFP was fluorescent when fused to the N^{pro} protein of the CSFV Shimen strain but not that of C-stain. Finally, the insertion of a reporter gene may compromise viral replication. For instance, a recombinant CSFV expressing the Fluc showed a deceased growth compared with the wild-type virus (Shen et al., 2014).

In the future, other CSFV proteins particularly the structural proteins allowing the introduction of reporter genes need to be defined, which will help us to track virus particles. Furthermore, for particular applications, a reporter gene with a small size in combination with the separate expression strategy by introduction of the 2A self-cleaving peptide of picornaviruses is a promising approach for the stable expression of the reporter gene while minimizing the effects on the viral growth.

In summary, despite some known limitations, reporter CSFV has been proved to be a promising tool for studing virus pathobiology and has more far-reaching benefits in various studies, including screening cellular receptor(s), identifying antivirals against viral infections, tracking viral infections *in vitro* and *in vivo* in real time, as well as improving vaccines for CSF.

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