Virus Research 179 (2014) 147–152

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

The DNA replication, virogenesis and infection of canine minute virus in non-permissive and permissive cells

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ARTICLE INFO

Article history:
Received 20 July 2013
Received in revised form 22 October 2013
Accepted 22 October 2013
Available online 12 November 2013

Keywords:
Bocavirus
Canine minute virus
DNA replication
Virogenesis
Infection
Non-permissive cells
Permissive cells

ABSTRACT

Canine minute virus (CnMV), a kind of autonomous parvovirus, is a member of genus bocavirus in parvovirdae family. In our previous study, we constructed and obtained infectious clones of CnMV, analyzed genome characteristics, RNA transcription profile, and revealed some molecular mechanisms of cytopathic effect of target cells. The purpose of this study was to investigate DNA replication, virogenesis and infectious tropism of CnMV in non-permissive and permissive cells. We demonstrated that the genomic DNA of CnMV, besides WRD cells, could replicate significantly in some non-permissive cells (CrFK, EBtR and COS-7) following transfection with infectious clone of CnMV, pI-MVC. Moreover, by using Western blotting and immunofluorescence, we found that the NS1 protein of CnMV was obviously expressed in both 293, CrFK, EBtR and COS-7 cells transfected with pI-MVC. Meanwhile, two-rounds of reinfection on WRD cells (blind passage) of the transfected cell lysates in CrFK, EBtR and COS-7cells tranfected with pI-MVC showed that pI-MVC could produce infectious virions in these types of non-permissive cells. Furthermore, it is confirmed that CnMV only infected WRD cells (permissive cells for CnMV), could not infect any non-permissive cells including CrFK, EBtR, COS-7, HK293, A549 and A9 cells. Taken together, for the first time, we have demonstrated that bocavirus CnMV DNA could replicate and form infectious progeny virus in some non-permissive cells. And what is more, unlike other parvoviruses, CnMV did not infect some non-permissive cells, although the DNA replication of CnMV occurred in these cells.

1. Introduction

The genus bocavirus is non-enveloped single-stranded DNA (ss-DNA) virus, which belongs to the subfamily Parvovirinae of the Parvoviridae family (Tattersall, 2006). Known members of bocavirus include bovine parvovirus (BPV), canine minute virus (CnMV) and human bocaviruses 1–4 (HBoV1–4) (Allander et al., 2005; Khamrin et al., 2013). Recently, new bocavirus species were isolated from gorilla, swine, cat and California sea lions, named gorilla bocavirus (GBoV) (Kapoor et al., 2010), porcine bocavirus (PBoV) (Blomström et al., 2009; Li et al., 2012), feline bocavirus (FBoV) (Lau et al., 2012) and sea lions bocavirus (Li et al., 2011). The first kind of bocavirus, named BPV, was identified in samples from calves with diarrhea in 1961 (Chen et al., 1988), while CnMV was first isolated from canine fecal samples in 1970 (Binn et al., 1970). HBoV was first described in pooled nasopharyngeal aspirates from children with respiratory infections in 2005 (Allander et al., 2005), and subsequently, HBoV2, HBoV3, and HBoV4 were discovered, sharing a mean similarity of 80% with HBoV1 (Allander et al., 2005; Jartti et al., 2012). All infectious parvoviral virions are non-enveloped, contain a single-stranded, self-priming, linear DNA genome with palindromic telomeres, and the size of genome is ~5 kb. They comprise 70% to 80% protein, with the remainder being DNA, and are uniquely dense and compact, with molecular masses. BPV is the first study in genus bocavirus, and much of our knowledge of bocavirus is obtained from the studies of BPV (Chen et al., 1988; Qiu et al., 2007). We have previously reported that the full-length sequence of infectious CnMV, named pI-MVC (GenBank accession no. FJ214110), and the genome was composed of 5402 nucleotides. Analysis of CnMV sequence showed 43% identity to that of BPV, while 52.6% identity to that of HBoV (Sun et al., 2009).

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0168-1702/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2013.10.019

Each kind of parvoviruses, generally, has its natural susceptible cells, named permissive cells. The permissive cell lines are usually derived from corresponding parvoviruses host. For instance, the A9 (mouse fibroblast) and EL4 (T-cell lymphoma) cells are susceptible to MVMp (minute virus of mice) and MVMi respectively (Tattersall and Bratton, 1983; Adeyemi and Pintel, 2012). The swine testis (ST) cells are susceptible to PPV (porcine parvovirus) (Ridpath and Mengeling, 1988; Choi et al., 1990; Oraveerakul et al., 1992). Each parvovirus can infect its permissive cells, conduct viral DNA replication and generate virions in later, such as MVM, PPV and human parvovirus B19 that only infects its permissive cells. Meanwhile, some parvovirus, besides its own permissive cells, can also infect other mammalian cells, viral DNA replications occur to form viruses. AAV2 infects virtually all mammalian cells, as long as the helper virus infects the host (Urabe et al., 2002). MVM not only replicates in mice cell lines, but also grows and replicates well in a variety of human cell lines, particularly in 324K cells (Tattersall and Bratton, 1983). CPV-2a, 2b and 2c have also penetrated the feline host-range, and they are able to infect and replicate in cats, causing diseases (Hueffer et al., 2003).

The BPV DNA replication has been studied most often in primary bovine fetal lung (BFL) cells (Lederman et al., 1983; Metcalf et al., 1990), bovine fetal spleen cells(BFS) (Paris and Bates, 1976; Burd et al., 1983), bovine turbinate (BT) cells (Lederman et al., 1984; Qiu et al., 2007) and EBTr (Embryo bovine tracheal) cells (Johnson et al., 2004; Abdel-Latif et al., 2006). An infectious clone of BPV was reported by Shull BC in 1988 (Shull et al., 1988). However, it was no longer available. There is rarely reported cell tropism, DNA replication, virions generation and infectious ability of BPV in any non-permissive cells. HBoV was recently identified, and its full sequence of genome (including palindromic repeats), cell tropism, or permissive cells are not completely recognized. Although Dijkman et al. (2009) had reported that HBoV could be propagated in pseudostratified human airway epithelium, but the numbers of virion were too lower to be collected, purified, and gained high sum of virus. Fortunately, Huang et al. (2012) reported that they obtained a full-length HBoV1 clone (pIHBoV1), and tested its infectivity in polarized CuFi-HAE (human airway epithelial cell line), but MOI (of ~750 gc/cell) used for infection was so high.

In our previous study, we have cloned and sequenced the whole of CnMV genome, including 5′- and 3′-terminal palindromes (Sun et al., 2009). Moreover, we also successfully constructed and obtained recombinant infectious clones of CnMV (pI-MVC), and got much invaluable information about DNA replication and a few molecular mechanisms of pathogenicity in WRD (Walter Reed canine cell/3873D) cells (Sun et al., 2009; Chen et al., 2010). Based on the similarities between CnMV and HBoV (or BPV) both in the organization and sequence of the genomes and in the symptoms of diseases they caused. We thought whether the plasmid pI-MVC could generate viral DNA replication and formed viruses in any non-permissive cells, and could CnMV infect other non-canine derived mammalian cells and generate virions in later? In this study, we first identified that genomic DNA of CnMV could replicate significantly in some non-permissive cells, apart from WRD cells; Importantly, CnMV could produce complete infectious virions in some non-permissive cells. Moreover, we confirmed that, except for WRD cells, CnMV could not infect any non-permissive cells (including some cells, which supported CnMV virions generation).

2. Materials and methods

2.1. Cells and virus

The CnMV used in this study, the original strain GA3, was isolated at the School of Veterinary Science, Cornell University. It was grown in the WRD cell line through two passages (CnMV GA3 P2). Both the virus and the cell line were gifts from Colin Parrish at the James A. Baker Institute of Cornell University. Virus was cultured and quantified as previously described (Sun et al., 2009). All cell lines, CrFK, EBtR, COS-7, HeLa, A549, HEK293, 324K and A9 were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum in 5% CO_2 at 37 °C.

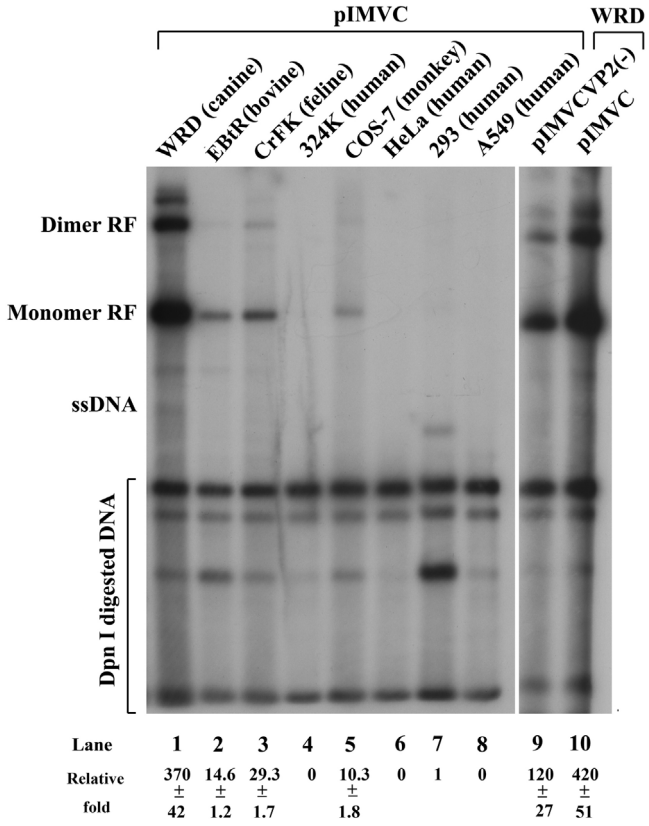

Fig. 1. DNA replication of CnMV in non-permissive and permissive cells. The infectious clone of CnMV, pI-MVC, was transfected into different cell types as indicated (lanes 1–10). VP(−) mutant was transfected into WRD as a control of single-burst cycle replication of CnMV DNA (lane 9). Three days post-transfection, Hirt DNA was prepared and digested with DpnI. The blot was probed with the CnMV *NSCap* probe. Detected bands are indicated with respective designations to the left. Quantifications of the abundance of RF DNA were standardized relatively to DpnI-digested plasmid DNA. The relative abundances (fold) that appeared in comparison with RF DNA generated from 293 cells (lane 5) are shown as the average ± the standard deviation. At least three separate experiments were performed.

2.2. Antibody and CnMV infectious clone

The C-terminal sequence of NS1 (nt 2389 to 2724) was inserted into the pGEX-4T-3 vector (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) for constructing pGEX-NS1 expression vector. Then the anti-NS1 antiserum was produced by immunizing Sprague-Dawley rats with purified GST-NS1 as described previously (Sun et al., 2009). The CnMV infectious clone, pI-MVC, was constructed, as described previously (Sun et al., 2009).

2.3. Infection and transfection

WRD or other cells (described above) were seeded 1 day prior to infection or transfection. CnMV at the indicated multiplicity of infection (MOI; FFU/cell) was added to the culture right after the medium was refreshed. Transfection of pI-MVC was performed by using the Lipofectamine and Plus reagent (Invitrogen) following the manufacturer's instructions.