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Short communication

Productive infection of human hepatocellular carcinoma cells by porcine circovirus type 1

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ABSTRACT

Porcine circovirus type 1 (PCV1), a small DNA virus in pigs, recently gained its notoriety when commercial human rotavirus vaccines were discovered to be contaminated by infectious PCV1. Here we report, for the first time, definitive evidence of productive PCV1 infection in a subclone of human hepatocellular carcinoma cell line (Huh-7, subclone 10-3). Infectious virus was detected in the lysates of infected Huh-7 cells by immunofluorescent assay (IFA) and can be serially passaged in Huh-7-S10-3 cells. The growth kinetic of PCV1 in Huh-7-S10-3 cells was determined in a one-step growth curve using IFA and a quantitative PCR assay. PCV1 achieved a lower infectious titer in Huh-7-S10-3 human cells compared to the titer normally achieved in porcine PK-15 cells from published studies. While the direct relevance to vaccine safety of PCV1 growth in human hepatocellular carcinoma cells is unclear, these data should be considered in further evaluation of vaccines and other products that could contain infectious PCV1.

Porcine circovirus type 1 (PCV1) is a small non-enveloped virus with a single-stranded circular DNA genome of approximately 1.7 kb in size. PCV1 was originally discovered as a contaminant of a porcine kidney cell line (PK-15) [1], and PCV1 infection is widespread in pig populations worldwide. Although PCV1 does not cause any clinical disease in pigs [2,3], a variant strain, PCV type 2 (PCV2), is associated with a devastating global disease in swine, especially when found during coinfections with other porcine pathogens [4]. It is generally believed that PCV1 has a narrow host range that is restricted to pigs, although evidence of possible human infection by PCV1 has also been reported. PCV1 antibodies were reportedly detected in human serum samples in Germany [5], although two subsequent studies failed to reproduce the finding of PCV1 antibodies in humans [6,7]. Using a consensus-primer PCR, PCV1 DNA was not detected in more than 1100 human blood, serum, urine, or tissue samples [8]. However, recently PCV1 and PCV2 DNA were detected from approximately 5.3% (13/247) of human stool samples from both healthy people and individuals with acute gastroenteritis in Minnesota, although this finding may likely reflect dietary consumption of PCV-contaminated pork products [9]. It has been reported that several human cell lines such as Hep2, 293, and Chang liver cells do support limited PCV1 replication and gene expression, however the infection in these human cells by PCV1 is non-productive since supernatants of the infected cells did not contain infectious virus [10]. In this present study, we evaluated the susceptibility of Huh-7 human hepatocellular carcinoma cells to PCV1 infection, and demonstrated, for the first time, that the human Huh-7 cells support a low level but productive PCV1 infection.

The Huh-7 cell line was originally isolated in Japan [11], and the Huh-7-S10-3 cells used in this study were from subclone 10-3 (a gift of Dr. Suzanne U. Emerson, NIAID, NIH) [12]. Other human cell lines including HeLa (ATCC CCL-2, passage 10) and HepG2/C3A (ATCC CRL-10741, passage 7) were also tested. The PCV1 used in this study was isolated from persistently infected porcine kidney cells (PK-15; ATCC CCL-31). All cell lines used in the study were tested by PCR and IFA with PCV1- and PCV2-specific antibodies and shown to be free of both PCV1 and PCV2 (data not shown). The species identity was confirmed for each of the human cell lines using a PCR assay specific for the cytochrome C oxidase subunit 1 (COX-I) gene as previously described [13]. Porcine-specific PCR primers were designed in order to amplify porcine COX-I: forward primer 5′-TTTGGTGCCTGAGCAGGAATAG-3′; reverse primer 5′-TATACGTGGAAAGGCCATATCG-3′. DNA extracted from HeLa, Huh-7-S10-3, and HepG2/C3A cells all had the expected bands when the PCR was performed using human-specific primers, while porcine PK-15 cells were negative (data not shown). Conversely, porcine PK-15 cells had the expected band after PCR amplification using the porcine-specific primers, while all human cell lines were negative (data not shown). These results confirm that the human cell lines used in this study were indeed of human origin, and that no contaminating porcine cells were present.

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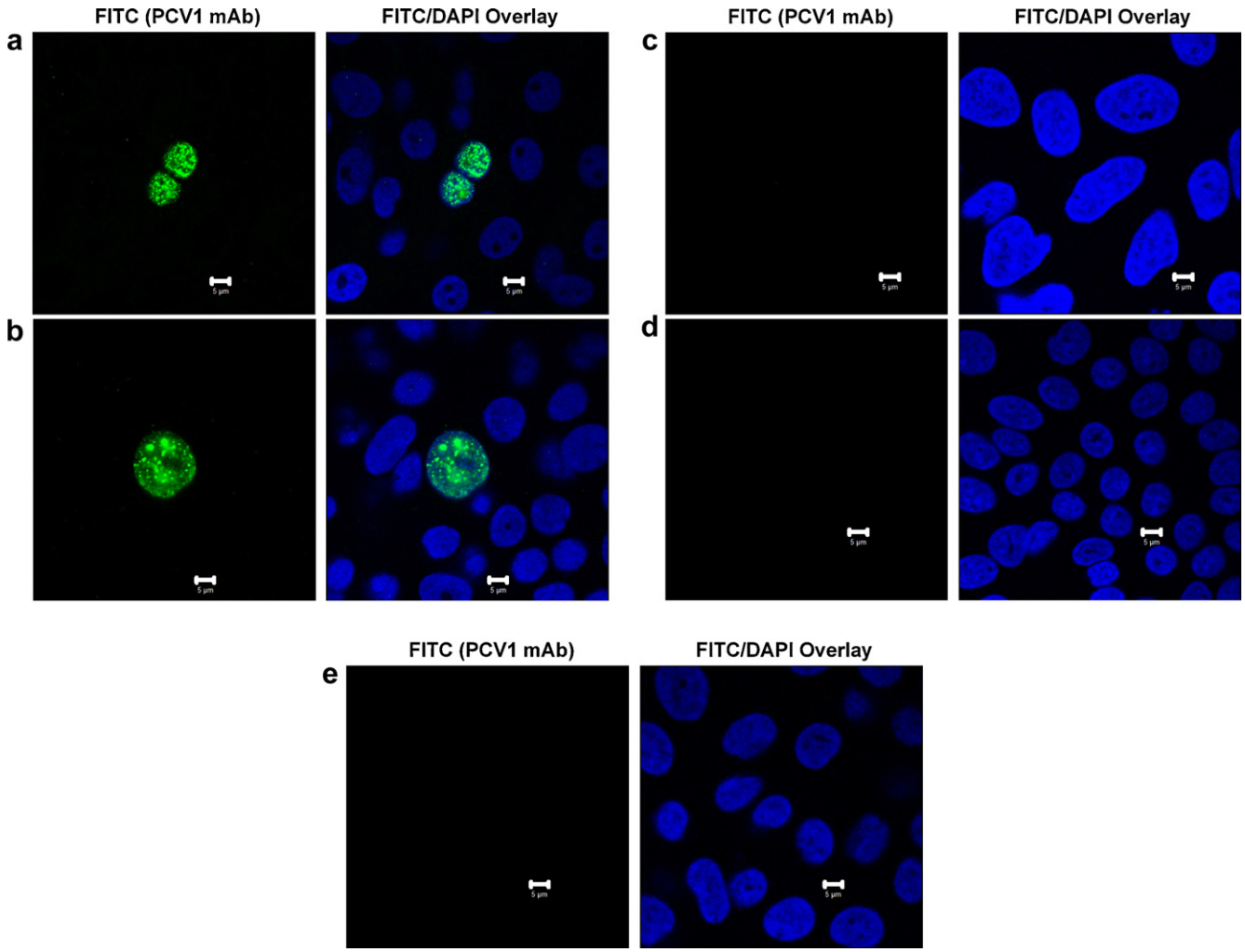

Fig. 1. Confocal microscopy of Huh-7-S10-3 human hepatocellular carcinoma cells infected with PCV1. PCV1-infected PK-15 (a), Huh-7 subclone 10-3 (Huh-7-S10-3) (b), HeLa (c), HepG2/C3A (d), and mock-infected Huh-7-S10-3 cells (e), were stained with a mouse anti-PCV1 capsid monoclonal antibody, followed by staining with a FITC-labeled goat anti-mouse antibody (KPL; Gaithersburg, MD) and mounted with Vectashield mounting medium with DAPI (Vector Labs; Burlingame, CA). Infected cells were visualized using a Zeiss LSM-510 confocal microscope at 488 nm (505/550 emission filter) to detect the PCV1 capsid protein, and 364 nm (385/470 emission filter) to detect DAPI staining.

To test permissivity for PCV1 infection, each cell type was inoculated with PCV1 at a multiplicity of infection (MOI) of 0.1 for 4 h at 37 °C followed by washing three times with Hank's buffered salt solution (HBSS) and incubation for 72 h at 37 °C. Infected cells were visualized by IFA with a PCV1-specific monoclonal antibody as previously described [14]. PCV1-specific fluorescent signals were detected in the nuclei of infected Huh-7-S10-3 and PK-15 cells, and occasionally in the cytoplasm, indicating that the Huh-7-S10-3 cells are permissive to PCV1 infection (Fig. 1a, b). An uncloned Huh-7 cell line from a different source (a gift from Dr. Jianming Hu of Penn State University) was also tested and shown to be permissive to PCV1 infection (data not shown). The HeLa and HepG2/C3A cells similarly inoculated with PCV1 had no detectable fluorescent signal (Fig. 1c, d).

In order to confirm that the Huh-7 cells support productive PCV1 infection, three serial passages were performed in both PK-15 cells (passage 15) and Huh-7-S10-3 (passage 20) cells. Briefly, infected cells were subjected to three freeze/thaw cycles at −80 °C, and the supernatant was clarified by centrifugation at 2500 × *g* for 10 min. This cell lysate was used in the subsequent infection of fresh cells for 4 h at 37 °C, followed by three washes with HBSS and incubation for 72 h at 37 °C. After harvesting the virus by three freeze/thaw cycles and reinfection of fresh cells, PCV1-specific positive signals were detected in the infected Huh-7-S10-3 and PK-15 cells by IFA, indicating that infectious viral particles were released during each passage of infection (data not shown). The PCV1 recovered after passage 3 in Huh-7-S10-3 cells was sequenced for its complete genome, and no mutation was identified in the virus genome during the 3 passages in Huh-7-S10-3 cells (data not shown).

To determine the growth kinetics of PCV1 in Huh-7-S10-3 cells and further confirm productive infection, a one-step growth curve was performed (Fig. 2). Briefly, Huh-7-S10-3 cells (passage 6) at 50% confluency were infected with PCV1 at an MOI of 0.5 for 4 h at 37 °C, followed by washing three times with HBSS. Cells in quadruplicate wells were harvested by three freeze/thaw cycles at −80 °C starting from 4 h post-infection (hpi) and thereafter every 12 h through 96 hpi. The PCV1 infectivity titers in cell lysates were determined in PK-15. Briefly, serial tenfold dilutions (10^{-1}–10^{-3}) of each quadruplicate cell lysate collected at each time point were produced, and each dilution was used to infect 6 wells (500 μl inoculum per well) in a 48-well plate containing PK-15 cells at 50% confluency. After 4 h at 37 °C, media was replaced and cells were incubated 72 h at 37 °C. IFA was performed with a PCV1 monoclonal antibody as previously described, and each well was examined to detect the presence of any positive fluorescent nuclei. Tissue culture infectious dose 50% ($TCID_{50}$) was calculated using