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Characterization of cellular furin content as a potential factor determining the susceptibility of cultured human and animal cells to coronavirus infectious bronchitis virus infection

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ABSTRACT

In previous studies, the Beaudette strain of coronavirus infectious bronchitis virus (IBV) was adapted from chicken embryo to Vero, a monkey kidney cell line, by serial propagation for 65 passages. To characterize the susceptibility of other human and animal cells to IBV, 15 human and animal cell lines were infected with the Vero-adapted IBV and productive infection was observed in four human cell lines: H1299, HepG2, Hep3B and Huh7. In other cell lines, the virus cannot be propagated beyond passage 5. Interestingly, cellular furin abundance in five human cell lines was shown to be strongly correlated with productive IBV infection. Cleavage of IBV spike protein by furin may contribute to the productive IBV infection in these cells. The findings that IBV could productively infect multiple human and animal cells of diverse tissue and organ origins would provide a useful system for studying the pathogenesis of coronavirus.

Introduction

Entry of viruses into host cells by crossing the plasma membrane is an essential step for successful viral replication. This process is initially mediated by the interaction of a viral protein with its corresponding host cell receptor(s). This interaction is therefore one of the prime determinants for the host cell specificity of a virus. Cellular receptors identified for coronavirus, important pathogens of human and many other animal species, include aminopeptidase N (APN) for alphacoronaviruses transmissible gastroenteritis virus (TGEV), human coronavirus (HCoV) 229E, canine coronavirus (CCoV) and feline infectious peritonitis virus (FIPV) (Delmas et al., 1992; Tresnan et al., 1996; Yeager et al., 1992), the carcinoembryonic antigen–cell adhesion molecular (CEACAM) for mouse hepatitis virus (MHV) (Coutelier et al., 1994; Dveksler et al., 1991; Godfraind et al., 1995; Williams et al., 1991), and angiotensin-converting enzyme 2 (ACE2) and CD209L for severe acute respiratory syndrome coronavirus (SARS-CoV) and HCoV-NL63 (Hofmann et al., 2005; Jeffers et al., 2004; Li et al., 2003). CD209L was initially identified as an attachment factor for human immunodeficiency virus (Curtis et al., 1992; Geijtenbeek et al., 2000), but was subsequently found to be a co-receptor for hepatitis C virus (Pohlmann et al., 2003), cytomegalovirus (Halary et al., 2002), dengue virus (Tassaneetrithep et al., 2003) and SARS-CoV (Jeffers et al., 2004).

Coronavirus infectious bronchitis virus (IBV), a prototype coronavirus, is the etiological agent of infectious bronchitis which impairs the respiratory and urogenital tracts of chicken (Cavanagh, 2007). The receptor(s) for IBV in its native or adapted host cells remains unknown, although IBV might use APN as a receptor in vitro (Miguel et al., 2002). In addition, the widely distributed sialic acid was suggested to be important for the primary attachment of IBV to host cells (Winter et al., 2006). In a more recent paper, DC-SIGN-like lectins, including L-SIGN, were shown to be able to increase susceptibility to infection by IBV in otherwise refractory cells (Zhang et al., 2012).

In a recent study, furin was shown to be responsible for proteolytic activation of S protein at a novel $RRRR_{690}$/S motif in the S2 region (Yamada and Liu, 2009). This cellular proprotein convertase is a calcium-dependent serine protease that circulates between *trans*-Golgi network (TGN), plasma membrane, and early endosome by association with exocytic and endocytic pathways (Bosshart et al., 1994; Molloy et al., 1994). It cleaves a wide variety of protein precursors after the C-terminal arginine (R) residue in the preferred consensus motif (RXR(K)R/R (K, lysine; X, any amino acid). Introduction of mutations into the $RRRR_{690}$/S motif and use of specific furin inhibitors demonstrated that furin may play an important role in furin-dependent entry, cell–cell fusion and infectivity of IBV in cultured cells (Yamada and Liu, 2009). Similar observations were also reported by Belouzard et al. (2009).

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Coronaviruses generally have a limited host range. However, recent evidence showed that several coronaviruses could break the host barrier and become zoonotic. For example, the novel SARS-CoV was found to cross host species from animal to human (Guan et al., 2003). HCoV-OC43 could infect cells from a large number of mammalian species, although the virus was propagated exclusively in suckling mouse brains (Butler et al., 2006). To study the mechanisms underlying the host cell specificity and susceptibility to coronavirus and coronavirus–host interactions, the Beaudette strain of IBV was adapted from chicken embryo to a monkey kidney cell line, Vero, by continuous propagation for 65 passages (Shen et al., 2003, 2004). This adaptation was accompanied by mutations in the spike (S) protein (Fang et al., 2005, 2007). In this study, infection of 15 cell lines of different species and tissue origins with the Vero cell-adapted IBV shows that four out of the 15 cell lines were able to support continuous and productive IBV infection with high efficiency. Meanwhile, strong correction between cellular furin abundance and productive IBV infection was found in five human cell lines infected with IBV.

Results

Susceptibility of different human and animal cell lines to Vero-adapted IBV

The susceptibility of 15 human and animal cell lines to IBV infection was tested by infecting cells with the 65th passage (p65) of Vero-adapted IBV. The tissue and species origins of these cell lines are listed in Table 1. Based on the preliminary observations from pilot tests, the susceptibility of these cells to IBV could be divided into two groups, the more susceptible and the more resistant groups. The more susceptible cell lines, including H1299, Huh7, HepG2, Hep3B and HCT116, were infected with IBV at a multiplicity of infection of approximately 0.1 and harvested at 0, 12, 16, 24, 36 and 48 h post-infection, respectively. The more resistant cells, including A549, MRC-5, DLD-1, DLD-1Amix, CHO, U937, HeLa, BHK-21, 293T, and Cos-7, were infected with IBV at a multiplicity of infection of approximately 0.3, a higher MOI to ensure successful infection of these cells, and harvested at the same time points as the former group.

The results showed that almost all cell lines tested could be infected by virus stocks prepared from IBV-infected Vero cells in passage 1. These cells were then continuously infected with the freezing/thawing preparations of IBV from the same cell lines infected with IBV. IBV was found to be able to propagate efficiently to at least passage 20 (p20) only in four lines derived from human cancer cells, i.e., H1299, Huh7, Hep3B and HepG2 (Table 1). In other cell lines, viral infectivity was found to be lost in passages 2–5 (Table 1). Fig. 1a–c shows the expression of IBV nucloecapsid (N) protein in the 15 cell lines infected by passages 1–3 of IBV, which were harvested at 24 h post-infection. One exception is HCT116 cell line, in which productive infection with low efficiency was observed after 20 passages (Table 1).

Table 1
Continuous propagation of IBV in 16 susceptible cell lines.

Cell line	Tissue	Organism	Productive infection[a]
H1299	Lung	Human	Yes
A549	Lung	Human	No
MRC-5	Lung	Human	No
Huh7	Liver	Human	Yes
HepG2	Liver	Human	Yes
Hep3B	Liver	Human	Yes
HCT116	Colorectal	Human	Yes/no
DLD-1	Colorectal	Human	No
DLD-1A	Colorectal	Human	No
Vero	Kidney	Monkey	Yes
BHK	Kidney	Hamster	No
293T	Kidney	Human	No
Cos-7	Kidney	Monkey	No
CHO	Ovarian	Hamster	No
HeLa	Cervical	Human	No
U937	Blood	Human	No

[a] Continuous propagation of IBV up to at least 20 passages.

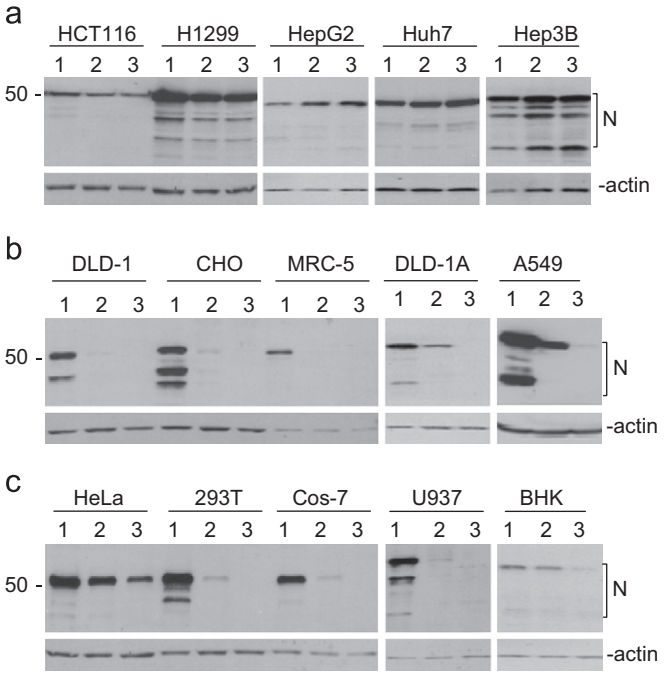

Fig. 1. Infection of 15 human and animal cell lines with IBV. (a) Western blot analysis of the expression of IBV N protein in IBV-infected HCT116, H1299, HepG2, Huh7 and Hep3B cells. Cells were infected with passages 1–3 of IBV and harvested at 24 h post-infection. Cell lysates were prepared and separated on SDS-10% polyacrylamide gels. The expression of IBV N protein was analyzed by Western blot with anti-N antibodies, after the proteins were separated by SDS-PAGE. The membrane was also probed with anti-actin monoclonal antibody as a loading control. (b) Western blot analysis of the expression of IBV N protein in IBV-infected DLD-1, CHO, MRC-5, DLD-1A and A549 cells. Cells were infected with passages 1–3 of IBV and harvested at 24 h post-infection. Cell lysates were prepared and separated on SDS-10% polyacrylamide gels. The expression of IBV N protein was analyzed by Western blot with anti-N antibodies, after the proteins were separated by SDS-PAGE. The membrane was also probed with anti-actin monoclonal antibody as a loading control. (c) Western blot analysis of the expression of IBV N protein in IBV-infected HeLa, 293T, Cos-7, U937 and BHK cells. Cells were infected with passages 1–3 of IBV and harvested at 24 h post-infection. Cell lysates were prepared and separated on SDS-10% polyacrylamide gels. The expression of IBV N protein was analyzed by Western blot with anti-N antibodies, after the proteins were separated by SDS-PAGE. The membrane was also probed with anti-actin monoclonal antibody as a loading control.

Characterization of viral structural protein expression and syncytium formation in productive IBV infection of six human and animal cell lines

The expression kinetics of IBV structural proteins, S, membrane (M) and N, in H1299, Huh7, Hep3B, HepG2 and HCT116 cells were analyzed by Western blot with polyclonal antibodies. As a positive control, Vero cells were first infected with IBV at a multiplicity of infection of approximately 1 and analyzed. As shown in Fig. 2a, analysis of IBV-infected Vero cells with anti-S antibodies detected the full-length glycosylated (S*), the full-length unglycosylated (S) and the S1/S2 cleavage forms of the protein from 12 h post-infection. Analysis of the same cell lysates with anti-N antibodies detected the full-length N protein (N) from