Microbiol. Biotechnol. Lett. (2017), 45(2), 93–100
http://dx.doi.org/10.4014/mbl.1706.06001
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Review

Cell Culture Models of Human Norovirus: the End of the Beginning?

Minh Tue Nguyen[1], Mi-Kyung Park[2], Sangdo Ha[3], In-Soo Choi[4], Changsun Choi[5], and Jinjong Myoung[1]*

[1]*Korea Zoonosis Research Institute and Department of Bioactive Material Sciences, Chonbuk National University, Jeonju 54896, Republic of Korea*
[2]*School of Food Science and Biotechnology, Kyungpook National University, Daegu 41566, Republic of Korea*
[3]*School of Food Science and Technology, Chung-Ang University, Anseoung 17546, Republic of Korea*
[4]*Department of Infectious Disease, College of Veterinary Medicine, Konkuk University, Seoul 05029, Republic of Korea*
[5]*Department of Food and Nutrition, Chung-Ang University, Anseong 17546, Republic of Korea*

Received: June 6, 2017 / Accepted: June 7, 2017

Human norovirus (hNoV) infection accounts for the vast majority of virus-mediated gastroenteritis cases worldwide. It causes self-limiting acute illnesses in healthy individuals lasting for a few days, however, in immunocompromised patients, hNoV can establish chronic and potentially fatal infections. Since its discovery in 1968, much effort had been made to develop cell culture and animal infection models to no avail. Only recently, some promising breakthroughs in the development of in vitro infection models have been made. Here, we will contrast and compare those models and discuss what further needs to be done to develop a reliable and robust cell culture model.

Keywords: **Human norovirus, cell culture model, human intestinal enteroid**

Introduction

Noroviruses are small non-enveloped viruses belonging to Caliciviridae family [1] with single-stranded, positive-sense non-segmented ribonucleic acid (RNA) genome [2]. HNoV was first discovered in an elementary school in Norwalk, Ohio in 1968 by its association with a disease outbreak of "winter vomiting" and the gastroenteritis was confirmed to have a viral etiology using immune-electron microscopy in 1972 [3, 4]. Norovirus genome is organized into three open reading frames (ORF1, 2 and 3) with the exception of that of murine norovirus (four ORFs). Non-structural proteins are encoded by ORF1 while two structural proteins (VP1 and VP2) are encoded by ORF2 and ORF3, respectively [5]. VP1 is the major capsid protein and forms a shell protecting the RNA genome while VP2 is a small basic protein associated with VP1.

HNoV is responsible for diarrhea worldwide affecting people of all age groups [6]. It is remarkably stable in the environment and extraordinarily contagious as it was claimed that less than scores of virus particles are enough to establish a successful infection [7]. Moreover, viral shedding has been reported to continue for several weeks even after the abatement of disease symptoms [8]. HNoVs account for more than half the outbreaks and up to 40% of sporadic cases of acute gastroenteritis worldwide [9]. Recent estimation argues that in developed countries, roughly a million of clinic visits and 64,000 cases of inpatient hospitalization of the young are related with hNoV infections annually while in developing countries hNoV infection is responsible for 200,000

***Corresponding author**
Tel: +82-63-900-4055, Fax: +82-63-900-4012
E-mail: Jinjong.myoung@jbnu.ac.kr

deaths of children <5 years of age mainly due to their inaccessibility to proper hydration and clinical care [10]. Gastroenteritis symptoms last for 24 to 72 hours and people usually recover without any serious long-term problems [11, 12]. However, norovirus illness can become serious for the young and the old, and especially immunocompromised patients [13]. Person-to-person transmission seems to be the major route (roughly 90%) of viral spreads in norovirus-related gastroenteritis outbreaks [14, 15] as well as sporadic cases [16, 17] and the transmission mode can be both fecal-oral and vomit-oral.

Prior to the recent advent of in vitro norovirus replication systems [18, 19] and the cloning of hNoV genome [20], mechanisms of hNoV-mediated pathogenesis with emphasis on virus-host interactions have been sought by analyzing immunological parameters of human patients or volunteers or the surrogate murine norovirus (MuNoV)-infected animals [21–26]. Historically, much effort had been made to establish susceptible cell lines for hNoVs, however, no cell lines, of human and other species, demonstrated signs of infection in vitro. And as such lack of cell culture models has imposed the main barrier to detailed delineation of hNoV replication strategies in infected cells.

Conventional in vitro cell culture systems

Since its discovery nearly 50 years ago, many laboratory efforts have been made to develop an in vitro infection model for hNoVs in cultured cell lines [27–30]. A large number of human cell lines of intestinal origin, such as I-407, HT-29, Caco-2, AGS, Kato-3, HCT-8, Detroit 562, HuTu-80, and of other tissues, including but not limited to A549, CCD-18, Detroit 551, Hep-2, HEC, HeLa, RD, and 293, were tested with different supplements (insulin, DMSO, and butyric acid) under various culture conditions to examine if they supported hNoV infection, however, no clear indications of infection were evident [27, 31–41]. The unavailability of an hNoV culture model has made the MuNoV an attractive surrogate model virus for the study of hNoV pathogenesis as the MuNoV is the only norovirus that can replicate efficiently in vitro in cell lines of macrophage and dendritic cell origin and is competent to establish either acute or chronic infections in mice depending on the viral genotype by penetrating the intestinal epithelial barrier through microfold (M) cells [42–46]. However, it is not clear yet whether hNoV has the same tropism in vivo and in vitro as no cell lines of the cell lineage are capable of supporting hNoV infection and/or replication as the MuNoV does [28].

3-dimensional cell culture model

A large number of previous studies have demonstrated the application of 3-dimensional (3D) models for studying the pathogenesis of a variety of microbes, including gastroenteritis pathogens [47–52]. It is reasonable to construe that cellular behavior in vitro in a two-dimensional (2-D) culture is very different from that of in vivo, and thus it is not surprising to see that conventional routine 2-D cultures in a cell monolayer could not recapitulate desirable and adequate differentiation of epithelial cells at basal and apical cell surfaces [53, 54]. Recently, some 3D organoid models, which emulate the virus-host microenvironment, were reported to promote successful hNoV replication using INT-407 and Caco-2 cells, a human embryonic small intestinal epithelial cell line [55] and a human epithelial colon rectal adenocarcinoma cell line [56], respectively. To build a 3-D model, target cells are cultivated as in a conventional 2-D culture to a certain density in monolayers before incubated with porous collagen-coated beads in a bioreactor, called rotating wall vessel (RWV), which was first developed by NASA. Cells are attached to the beads and form cell-bead complexes, left allowed to grow under microgravity in a RWV. Resulting 3-D cells generally epitomize important aspects of cellular differentiation and tissue organization, which parallel those of the body in vivo [52]. By adopting 3-D culture techniques, Straub *et al.* established in vitro cell culture models using Int-407 and Caco-2 which were rendered susceptible to hNoV GI and GII [55, 56]. Infected cells exhibited signs of cytopathic effect (CPE) and seemed to produce hNoV particles in the culture. However, extensive scrutiny of the utility of the 3-D cell culture model for hNoV infection is entailed as there have been disparities reported by an independent research group regarding susceptibility of the model to hNoV based on real-time PCR or immunofluorescence assay [29, 57], and the latter group suggested that observed CPE reported by Straub *et al.* presumably stemmed from the toxicity of contaminating

LPS in the virus stock prepared from fecal samples.

hNoV infection in BJAB cells

Several pieces of evidence demonstrated that B cells might be one of the targets of noroviruses in vivo. First of all, B cells were stained positive for MuNoV when interferon-deficient or interleukin 10-deficient mice were inoculated with the virus [58, 59]. On the other hand, MuNoV infection and replication significantly decreased in mice where functional B cells were absent such as in *Rag1*$^{-/-}$ mice (lacking both B and T cells) and μ–deficient mice (lacking B cells only) [60]. Furthermore, Bok *et al.* also reported that duodenal B cells of chimpanzees infected with hNoV were stained positive for hNoV capsid protein [61]. More recently, Jones *et al.* demonstrated MuNoV infection in the B cells of Peyer's patches and the expression of a viral nonstructural protein attested to bona fide viral infection and replication in the cell type [62, 63]. Taken together, these data strongly suggest that B cells are a legitimate target in vivo. Indeed, Jones *et al.* first demonstrated that murine B cell lines, M12 and WEHI-231, were susceptible to both acute (MuNoV-1) and chronic (MuNoV-3) strains of MuNoV. Moreover, a human B cell line (BJAB) was shown to support hNoV (GII.4-Sydney) entry and replication only when co-infected with an enteric bacteria, *Enterobacter cloacae* [62, 63]. It is surprising but not totally unprecedented to see that enteric bacteria play an important role in viral infections as exemplified in other viral infections: rotavirus, poliovirus, and mouse mammary tumor virus [64–70]. The same was true to MuNoV infection as extended oral administration of antibiotics significantly reduced MuNoV infection and replication, strongly suggesting a role of enteric bacteria. A prominent difference was that hNoV displays absolute requirement of bacterial co-infection as filtered virus-containing stool samples could not establish infection in BJAB cells while MuNoV can infect the target cells (macrophages and dendritic cells) in the absence of enteric bacteria. It is not clear yet whether both noroviruses constrain the presence of enteric bacteria for viral tropism in B cells in vivo. Alternatively, those two viruses have evolutionarily diverged out, evincing differential requirement for efficient infection of target cells. Of note, not all enteric bacteria enabled norovirus infection in BJAB cells as co-infection with *Escherichia coli*, deficient of HBGA-like substances on the surface, could not confer viral susceptibility on the cells, highlighting the critical role of HBGA in viral infections [71]. Interestingly, not only intact enteric bacterial co-infection but also addition of synthetic soluble H antigen alone could facilitate viral entry into BJAB cells. In fact, a number of studies have implicated HBGA as a (co)-receptor for hNoV: i) genetic, biochemical, and immunological studies demonstrated that HBGA is required for hNoV infection [72–80], ii) when HBGA binding was inhibited, hNoV infection was curtailed [81], and iii) presence of carbohydrate attachment factors, which are widely expressed on the bacterial surface [82], was prerequisite for efficient hNoV infection [83–85]. On the other hand, potential implications of this phenomenon could shake our conventional view of HBGA as a receptor for human norovirus. One may imagine that a soluble form of a cellular receptor would antagonize, rather than promote, viral infection. Therefore, it would be tempting to postulate that there is another receptor which binds to HBGA-hNoV complexes, allowing them to be endocytosed into target cells. In this regard, it is intriguing to note that a proteinaceous receptor has recently been identified for MuNoV [86].

However simple it may sound, hNoV infection in BJAB cells raise a number of questions. First, the major caveats of the use of unfiltered stool sample as a virus stock include variable reproducibility of BJAB cell infection in different laboratories [63]. Furthermore, the higher titers of hNoV were inoculated, the lower levels of viral production were observed, which likely hints on the presence of viral replication inhibitors in the stool sample. Second, although exact causes of variability have yet to be identified, the source and quality of fetal bovine serum and the state of BJAB cells, such as clumping activity, could be incriminated for low reproducibility of viral infection among different laboratories. Last but not in the least, *E. cloacae* co-infection rather inhibited hNoV infection in gnotobiotic pigs [87], a well-established hNoV animal model [88–90]. Thus, the role of HBGA-expressing bacteria on hNoV infection may not be generalized in different species. Of note, based on analyses by real-time PCR or immunohistochemistry, no signs of hNoV infection in B cells of gnotobiotic pigs were evident [87], suggesting that B cells may not be the main target of hNoV in the swine model. The same may be true to hNoV infection in humans. As B cell-deficient

patients were still shown to be susceptible to hNoV [91, 92], B cells seem to be dispensable for hNoV infection and pathology. The susceptibility and roles of B cells for hNoV biology need to be extensively investigated in the future studies.

hNoV infection in stem cell-derived human enteroids

Saxena *et al.* developed human intestinal enteroids (HIEs) to study pathophysiology of human rotavirus [93], which has narrow host range and low levels of replicability in transformed cell lines. After differentiation, HIEs contained various physiologically active intestinal epithelial cells, including enterocytes, Paneth and goblet cells. The model successfully supported human rotavirus infection and was shown to recapitulate some of rotavirus-induced pathologies such as luminal expansion upon endotoxin treatment and induction of viroplasms and lipid droplets following viral infection. Very recently, similar enteroid cultures were applied to hNoV infection and were demonstrated to support hNoV entry and replication in a monolayer established from stem cells derived from duodenum, jejunum, and ileum [94]. Unlike infection in BJAB cells described above, infected HIEs display clear signs of cytopathic effects (cellular rounding and death) upon hNoV inoculation while UV-inactivated virus did not induce similar cellular destruction, which argues for authentic virus-mediated pathology rather than that of contaminating LPS in the virus stock. Viral progeny production was confirmed by transmission electron microscopy, showing typical morphology of hNoV virus particles [3, 4]. Furthermore, viral replication was documented by expression of structural (VP1) and nonstructural proteins (RdRp, NTPase) or double-stranded RNA's, an intermediate during hNoV RNA replication [95] when assessed by confocal microscopy [96, 97].

Of note, replication of some HNoV strains, such as G1.1, GII.3, and GII.17, required the pretreatment of bile salt on both virus stocks and HIEs. Bile is known to have diverse functions on cells such as a natural surfactant, a modulator of varying signaling pathways, and an aid of lipid digestion and absorption [98, 99]. However, interestingly, bile pretreatment on HIEs cells was not necessary but could enhance the infectivity of other strains (e.g. GII.4). Besides, the presence of secretor status in HIEs also affects strain-specific hNoV susceptibility. Expression of human fucosyltransferase 2 (FUT2, known as secretor-positive genotype), which transfers a fucose to the HBGA precursor in gastrointestinal cells, correlates with viral infectivity with most GII.4 hNoVs [79, 100–102]. Ettayebi *et al.* also reported that all secretor-positive jejunal HIEs supported viral replication of various hNoV strains evident within 96 hours to 6 days post-infection (d.p.i) [94]. On the other hand, only hNoVs GII.3 could infect HIEs of secretor-negative individuals, which closely reflects epidemiological characteristics of hNoV [100]. Successful establishment of hNoV-susceptible enteroid models allowed assessment of effectiveness of suggested methods for hNoV inactivation, such as gamma irradiation and heat treatment (60℃ from 15–60 min). Inoculation of HIEs with inactivated GII.3 and GII.4 did not result in viral growth, demonstrating utilities of HIEs for testing effectiveness of a variety of other anti-hNoV treatments in the coming years. Despite the paucity of availability of patient-derived intestinal stem cell-derived enterocytes, employment and cultivation of HIEs will undoubtedly advance our understanding of hNoV-mediated pathogenesis.

Conclusion

Lack of robust cell culture and small animal models for hNoV infection has imposed a significant barrier to proper understanding of pathophysiology of hNoV since its discovery in 1968. In this regards, the recent development of the two in vitro cell culture models for hNoV has been met with great enthusiasm from the scientific community as it holds great promise for inspecting cellular, as well as viral, factors that affect the susceptibility of the cell culture models to the virus. In fact, by employing those two cell culture models, the critical role of bile on infectivity of some strains of hNoV is revealed and indispensability of HBGA for hNoV infection is confirmed. Although some aspects of hNoV entry were examined, much remains yet to be analyzed and scrutinized to fathom depths and widths of hNoV biology in target cells, including but not limited to searching for the proteinaceous cellular receptor(s), mechanisms of commensal bacteria-mediated infection, and activation status of various cellular signaling pathways. Better understanding of hNoV biology will pave the way for the development of effective antivirals and preventive vaccines.

http://dx.doi.org/10.4014/mbl.1706.06001

Acknowledgments

This research was supported by a research grant from the Ministry of Food and Drug Safety (14162-973).

References

1. Baltimore D. 1971. Viral genetic systems. *Trans. N. Y. Acad. Sci.* **33**: 327-332.
2. Green KY. 2007. Caliciviridae: the noroviruses, pp. 949-980. *In* Knipe DM and Howley PM (eds.), Fields virology, 5th ed., vol. 1. Lippincott, Williams & Wilkins, Philadelphia, PA.
3. Kapikian AZ. 2000. The discovery of the 27-nm Norwalk virus: an historic perspective. *J. Infect. Dis.* **181 Suppl 2**: S295-302.
4. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. *J. Virol.* **10**: 1075-1081.
5. Prasad BV, Hardy ME, Dokland T, Bella J, Rossmann MG, Estes MK. 1999. X-ray crystallographic structure of the Norwalk virus capsid. *Science* **286**: 287-290.
6. Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, *et al.* 2014. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet. Infect. Dis.* **14**: 725-730.
7. Teunis PFM, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, *et al.* 2008. Norwalk virus: How infectious is it? *J. Med. Virol.* **80**: 1468-1476.
8. Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, *et al*. 2008. Norwalk virus shedding after experimental human infection. *Emerg. Infect. Dis.* **14**: 1553-1557.
9. Esposito S, Ascolese B, Senatore L, Codeca C. 2014. Pediatric norovirus infection. *Eur. J. Clin. Microbiol. Infect. Dis.* **33**: 285-290.
10. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD. 2008. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg. Infect. Dis.* **14**: 1224-1231.
11. Newman KL, Moe CL, Kirby AE, Flanders WD, Parkos CA, Leon JS. 2016. Norovirus in symptomatic and asymptomatic individuals: cytokines and viral shedding. *Clin. Exp. Immunol.* **184**: 347-357.
12. Lee RM, Lessler J, Lee RA, Rudolph KE, Reich NG, Perl TM, Cummings DA. 2013. Incubation periods of viral gastroenteritis: a systematic review. *BMC Infect. Dis.* **13**: 446.
13. Bok K, Green KY. 2012. Norovirus gastroenteritis in immunocompromised patients. *N. Engl. J. Med.* **367**: 2126-2132.
14. Lopman BA, Adak GK, Reacher MH, Brown DW. 2003. Two epidemiologic patterns of norovirus outbreaks: surveillance in England and wales, 1992-2000. *Emerg. Infect. Dis.* **9**: 71-77.
15. Yen C, Wikswo ME, Lopman BA, Vinje J, Parashar UD, Hall AJ. 2011. Impact of an emergent norovirus variant in 2009 on norovirus outbreak activity in the United States. *Clin. Infect. Dis.* **53**: 568-571.
16. de Wit MA, Koopmans MP, van Duynhoven YT. 2003. Risk factors for norovirus, Sapporo-like virus, and group A rotavirus gastroenteritis. *Emerg. Infect. Dis.* **9**: 1563-1570.
17. Phillips G, Tam CC, Rodrigues LC, Lopman B. 2011. Risk factors for symptomatic and asymptomatic norovirus infection in the community. *Epidemiol. Infect.* **139**: 1676-1686.
18. Asanaka M, Atmar RL, Ruvolo V, Crawford SE, Neill FH, Estes MK. 2005. Replication and packaging of Norwalk virus RNA in cultured mammalian cells. *Proc. Natl. Acad. Sci. USA* **102**: 10327-10332.
19. Katayama K, Hansman GS, Oka T, Ogawa S, Takeda N. 2006. Investigation of norovirus replication in a human cell line. *Arch. Virol.* **151**: 1291-1308.
20. Lambden PR, Caul EO, Ashley CR, Clarke IN. 1993. Sequence and genome organization of a human small round-structured (Norwalk-like) virus. *Science* **259**: 516-519.
21. Wyatt RG, Dolin R, Blacklow NR, DuPont HL, Buscho RF, Thornhill TS, *et al.* 1974. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. *J. Infect. Dis.* **129**: 709-714.
22. Parrino TA, Schreiber DS, Trier JS, Kapikian AZ, Blacklow NR. 1977. Clinical immunity in acute gastroenteritis caused by norwalk agent. *N. Engl. J. Med.* **297**: 86-89.
23. Johnson PC, Mathewson JJ, DuPont HL, Greenberg HB. 1990. Multiple-challenge study of host susceptibility to norwalk gastroenteritis in US adults. *J. Infect. Dis.* **161**: 18-21.
24. Agus SG, Dolin R, Wyatt RG, Tousimis AJ, Northrup RS. 1973. Acute infectious nonbacterial gastroenteritis: Intestinal histopathology: histologic and enzymatic alterations during illness produced by the norwalk agent in man. *Ann. Int. Med.* **79**: 18-25.
25. Jean J, Morales-Rayas R, Anoman MN, Lamhoujeb S. 2011. Inactivation of hepatitis A virus and norovirus surrogate in suspension and on food-contact surfaces using pulsed UV light (pulsed light inactivation of food-borne viruses). *Food Microbiol.* **28**: 568-572.
26. Park GW, Linden KG, Sobsey MD. 2011. Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. *Lett. Appl. Microbiol.* **52**: 162-167.
27. Duizer E, Schwab KJ, Neill FH, Atmar RL, Koopmans MP, Estes MK. 2004. Laboratory efforts to cultivate noroviruses. *J. Gen. Virol.* **85**: 79-87.
28. Lay MK, Atmar RL, Guix S, Bharadwaj U, He H, Neill FH, *et al.* 2010. Norwalk virus does not replicate in human macrophages or dendritic cells derived from the peripheral blood of susceptible humans. *Virology* **406**: 1-11.
29. Herbst-Kralovetz MM, Radtke AL, Lay MK, Hjelm BE, Bolick AN, Sarker SS, *et al.* 2013. Lack of norovirus replication and histo-blood group antigen expression in 3-dimensional intestinal epithelial cells. *Emerg. Infect. Dis.* **19**: 431-438.
30. Papafragkou E, Hewitt J, Park GW, Greening G, Vinjé J. 2013. Challenges of culturing human norovirus in three-dimensional organoid intestinal cell culture models. *PLoS One* **8**: e63485.

31. Cubitt WD, Barrett ADT. 1984. Propagation of human candidate calicivirus in cell culture. *J. Gen. Virol.* **65**: 1123-1126.
32. Gauthier R, Harnois C, Drolet JF, Reed JC, Vezina A, Vachon PH. 2001. Human intestinal epithelial cell survival: differentiation state-specific control mechanisms. *Am. J. Physiol Cell Physiol.* **280**: C1540-1554.
33. Goke M, Kanai M, Podolsky DK. 1998. Intestinal fibroblasts regulate intestinal epithelial cell proliferation via hepatocyte growth factor. *Am. J. Physiol.* **274**: G809-818.
34. Hughes JH. 1993. Physical and chemical methods for enhancing rapid detection of viruses and other agents. *Clin. Microbiol. Rev.* **6**: 150-175.
35. Joshi SS, Jackson JD, Sharp JG. 1985. Differentiation inducing effects of butyrate and DMSO on human intestinal tumor cell lines in culture. *Cancer. Detect. Prev.* **8**: 237-245.
36. Lamartina S, Roscilli G, Rinaudo D, Delmastro P, Toniatti C. 1998. Lipofection of purified adeno-associated virus Rep68 protein: toward a chromosome-targeting nonviral particle. *J. Virol.* **72**: 7653-7658.
37. Maitreyi RS, Broor S, Kabra SK, Ghosh M, Seth P, Dar L, Prasad AK. 2000. Rapid detection of respiratory viruses by centrifugation enhanced cultures from children with acute lower respiratory tract infections. *J. Clin. Virol.* **16**: 41-47.
38. Pintó RM, Diez JM, Bosch A. 1994. Use of the colonic carcinoma cell line CaCo-2 for in vivo amplification and detection of enteric viruses. *J. Med. Virol.* **44**: 310-315.
39. Quaroni A, Tian JQ, Goke M, Podolsky DK. 1999. Glucocorticoids have pleiotropic effects on small intestinal crypt cells. *Am. J. Physiol.* **277**: G1027-1040.
40. Seno M, Takao S, Fukuda S, Kanamoto Y. 1991. Enhanced Isolation of influenza virus in conventional plate cell cultures by using low-speed centrifugation from clinical specimens. *Am. J. Clin. Pathol.* **95**: 765-768.
41. Svensson L, Finlay BB, Bass D, von Bonsdorff CH, Greenberg HB. 1991. Symmetric infection of rotavirus on polarized human intestinal epithelial (Caco-2) cells. *J. Virol.* **65**: 4190-4197.
42. Wobus CE, Karst SM, Thackray LB, Chang K-O, Sosnovtsev SV, Belliot G, *et al.* 2004. Replication of norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLOS Biol.* **2**: e432.
43. Wobus CE, Thackray LB, Virgin HW. 2006. Murine norovirus: a model system to study norovirus biology and pathogenesis. *J. Virol.* **80**: 5104-5112.
44. Elftman MD, Gonzalez-Hernandez MB, Kamada N, Perkins C, Henderson KS, Núñez G, Wobus CE. 2013. Multiple effects of dendritic cell depletion on murine norovirus infection. *J. Gen. Virol.* **94**: 1761-1768.
45. Gonzalez-Hernandez MB, Liu T, Payne HC, Stencel-Baerenwald JE, Ikizler M, Yagita H, *et al.* 2014. Efficient norovirus and reovirus replication in the mouse intestine requires microfold (M) cells. *J. Virol.* **88**: 6934-6943.
46. Gonzalez-Hernandez MB, Liu T, Blanco LP, Auble H, Payne HC, Wobus CE. 2013. Murine norovirus transcytosis across an in vitro polarized murine intestinal epithelial monolayer is mediated by M-like cells. *J. Virol.* **87**: 12685-12693.
47. Carterson AJ, Höner zu Bentrup K, Ott CM, Clarke MS, Pierson DL, Vanderburg CR, *et al.* 2005. A549 Lung epithelial cells grown as three-dimensional aggregates: Alternative tissue culture model for pseudomonas aeruginosa pathogenesis. *Infect. Imm.* **73**: 1129-1140.
48. Noel G, Baetz NW, Staab JF, Donowitz M, Kovbasnjuk O, Pasetti MF, Zachos NC. 2017. A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. *Scient. Rep.* **7**: 45270.
49. Honer zu Bentrup K, Ramamurthy R, Ott CM, Emami K, Nelman-Gonzalez M, Wilson JW, *et al.* 2006. Three-dimensional organotypic models of human colonic epithelium to study the early stages of enteric salmonellosis. *Microbes Infect.* **8**: 1813-1825.
50. LaMarca HL, Ott CM, Honer Zu Bentrup K, Leblanc CL, Pierson DL, Nelson AB, *et al.* 2005. Three-dimensional growth of extravillous cytotrophoblasts promotes differentiation and invasion. *Placenta* **26**: 709-720.
51. Long JP, Hughes JH. 2001. Epstein-barr virus latently infected cells are selectively deleted in simulated-microgravity cultures. *In Vitro Cell Dev. Biol. Anim.* **37**: 223-230.
52. Nickerson CA, Goodwin TJ, Terlonge J, Ott CM, Buchanan KL, Uicker WC, *et al.* 2001. Three-dimensional tissue assemblies: novel models for the study of salmonella enterica serovar typhimurium pathogenesis. *Infect. Imm.* **69**: 7106-7120.
53. Zhang S. 2004. Beyond the Petri dish. *Nat. Biotechnol.* **22**: 151-152.
54. Nickerson CA, Richter EG, Ott CM. 2007. Studying host-pathogen interactions in 3-D: organotypic models for infectious disease and drug development. *J. Neuroimm. Pharmacol.* **2**: 26-31.
55. Straub TM, Höner zu Bentrup K, Coghlan PO, Dohnalkova A, Mayer BK, Bartholomew RA, *et al.* 2007. In vitro cell culture infectivity assay for human noroviruses. *Emerg. Infect. Dis.* **13**: 396-403.
56. Straub TM, Bartholomew RA, Valdez CO, Valentine NB, Dohnalkova A, Ozanich RM, *et al.* 2011. Human norovirus infection of caco-2 cells grown as a 3-dimensional tissue structure. *J. Water Health.* **9**: 225-240.
57. Takanashi S, Saif LJ, Hughes JH, Meulia T, Jung K, Scheuer KA, Wang Q. 2014. Failure of propagation of human norovirus in intestinal epithelial cells with microvilli grown in three-dimensional cultures. *Arch Virol.* **159**: 257-266.
58. Mumphrey SM, Changotra H, Moore TN, Heimann-Nichols ER, Wobus CE, Reilly MJ, *et al.* 2007. Murine norovirus 1 infection is associated with histopathological changes in immunocompetent hosts, but clinical disease is prevented by STAT1-dependent interferon responses. *J. Virol.* **81**: 3251-3263.
59. Basic M, Keubler LM, Buettner M, Achard M, Breves G, Schroder B, *et al.* 2014. Norovirus triggered microbiota-driven mucosal inflammation in interleukin 10-deficient mice. *Inflamm. Bowel Dis.* **20**: 431-443.
60. Zhu S, Regev D, Watanabe M, Hickman D, Moussatche N, Jesus

DM, *et al.* 2013. Identification of immune and viral correlates of norovirus protective immunity through comparative study of intra-cluster norovirus strains. *PLoS Pathog.* **9**: e1003592.
61. Bok K, Parra GI, Mitra T, Abente E, Shaver CK, Boon D, *et al.* 2011. Chimpanzees as an animal model for human norovirus infection and vaccine development. *Proc. Natl. Acad. Sci. USA* **108**: 325-330.
62. Jones MK, Watanabe M, Zhu S, Graves CL, Keyes LR, Grau KR, *et al.* 2014. Enteric bacteria promote human and mouse norovirus infection of B cells. *Science* **346**: 755-759.
63. Jones MK, Grau KR, Costantini V, Kolawole AO, de Graaf M, Freiden P, *et al.* 2015. Human norovirus culture in B cells. *Nat. Protoc.* **10**: 1939-1947.
64. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, *et al.* 2011. Intestinal microbiota promote enteric virus replication and systemic pathogenesis. *Science* **334**: 249-252.
65. Kane M, Case LK, Kopaskie K, Kozlova A, MacDearmid C, Chervonsky AV, Golovkina TV. 2011. Successful transmission of a retrovirus depends on the commensal microbiota. *Science* **334**: 245-249.
66. Robinson CM, Jesudhasan PR, Pfeiffer JK. 2014. Bacterial lipopolysaccharide binding enhances virion stability and promotes environmental fitness of an enteric virus. *Cell. Host. Microbe.* **15**: 36-46.
67. Baldridge MT, Nice TJ, McCune BT, Yokoyama CC, Kambal A, Wheadon M, *et al.* 2015. Commensal microbes and interferon-lambda determine persistence of enteric murine norovirus infection. *Science* **347**: 266-269.
68. Ha S, Choi IS, Choi C, Myoung J. 2016. Infection models of human norovirus: challenges and recent progress. *Arch. Virol.* **161**: 779-788.
69. Grimprel E, Rodrigo C, Desselberger U. 2008. Rotavirus disease: impact of coinfections. *Pediat. Infect. Dis. J.* **27**: S3-S10.
70. Wilks J, Beilinson H, Golovkina TV. 2013. Dual role of commensal bacteria in viral infections. *Immunol. Rev.* **255**: 10.1111/imr.12097.
71. Miura T, Sano D, Suenaga A, Yoshimura T, Fuzawa M, Nakagomi T, *et al.* 2013. Histo-blood group antigen-like substances of human enteric bacteria as specific adsorbents for human noroviruses. *J. Virol.* **87**: 9441-9451.
72. Marionneau S, Ruvoën N, Le Moullac–Vaidye B, Clement M, Cailleau–Thomas A, Ruiz–Palacois G, *et al.* 2002. Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology* **122**: 1967-1977.
73. Tan M, Jiang X. 2014. Histo-blood group antigens: a common niche for norovirus and rotavirus. *Expert. Rev. Mol. Med.* **16**: e5.
74. Huang P, Farkas T, Marionneau S, Zhong W, Ruvoen-Clouet N, Morrow AL, *et al.* 2003. Noroviruses bind to human ABO, Lewis, secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. *J. Infect. Dis.* **188**: 19-31.
75. Sestak K. 2014. Role of histo-blood group antigens in primate enteric calicivirus infections. *World J. Virol.* **3**: 18-21.
76. Hutson AM, Atmar RL, Graham DY, Estes MK. 2002. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J. Infect. Dis.* **185**: 1335-1337.
77. Hutson AM, Airaud F, LePendu J, Estes MK, Atmar RL. 2005. Norwalk virus infection associates with secretor status genotyped from sera. *J. Med. Virol.* **77**: 116-120.
78. Karst SM. 2010. Pathogenesis of noroviruses, emerging RNA viruses. *Viruses* **2**: 748-781.
79. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, *et al.* 2003. Human susceptibility and resistance to Norwalk virus infection. *Nat. Med.* **9**: 548-553.
80. Marionneau S, Airaud F, Bovin NV, Le Pendu J, Ruvoen-Clouet N. 2005. Influence of the combined ABO, FUT2, and FUT3 polymorphism on susceptibility to Norwalk virus attachment. *J. Infect. Dis.* **192**: 1071-1077.
81. Zhang X-F, Tan M, Chhabra M, Dai Y-C, Meller J, Jiang X. 2013. Inhibition of histo-blood group antigen binding as a novel strategy to block norovirus infections. *PLoS One* **8**: e69379.
82. Almagro-Moreno S, Boyd EF. 2010. Bacterial catabolism of nonulosonic (sialic) acid and fitness in the gut. *Gut. Microbes.* **1**: 45-50.
83. Bally M, Rydell GE, Zahn R, Nasir W, Eggeling C, Breimer ME, *et al.* 2012. Norovirus GII.4 virus-like particles recognize galactosylceramides in domains of planar supported lipid bilayers. *Angew. Chem. Int. Ed. Engl.* **51**: 12020-12024.
84. Rydell GE, Nilsson J, Rodriguez-Diaz J, Ruvoen-Clouet N, Svensson L, Le Pendu J, Larson G. 2009. Human noroviruses recognize sialyl Lewis x neoglycoprotein. *Glycobiology* **19**: 309-320.
85. Tamura M, Natori K, Kobayashi M, Miyamura T, Takeda N. 2004. Genogroup II noroviruses efficiently bind to heparan sulfate proteoglycan associated with the cellular membrane. *J. Virol.* **78**: 3817-3826.
86. Orchard RC, Wilen CB, Doench JG, Baldridge MT, McCune BT, Lee Y-CJ, *et al.* 2016. Discovery of a proteinaceous cellular receptor for a norovirus. *Science* **353**: 933-936.
87. Lei S, Samuel H, Twitchell E, Bui T, Ramesh A, Wen K, *et al.* 2016. Enterobacter cloacae inhibits human norovirus infectivity in gnotobiotic pigs. *Scientif. Rep.* **6**: 25017.
88. Cheetham S, Souza M, Meulia T, Grimes S, Han MG, Saif LJ. 2006. Pathogenesis of a genogroup ii human norovirus in gnotobiotic pigs. *J. Virol.* **80**: 10372-10381.
89. Jung K, Wang Q, Kim Y, Scheuer K, Zhang Z, Shen Q, *et al.* 2012. The effects of simvastatin or interferon-α on infectivity of human norovirus using a gnotobiotic pig model for the study of Antivirals. *PLoS One* **7**: e41619.
90. Bui T, Kocher J, Li Y, Wen K, Li G, Liu F, *et al.* 2013. Median infectious dose of human norovirus GII.4 in gnotobiotic pigs is decreased by simvastatin treatment and increased by age. *J. Gen. Virol.* **94**: 2005-2016.
91. Brown JR, Gilmour K, Breuer J. 2016. Norovirus Infections Occur in B-Cell-Deficient Patients. *Clin. Infect. Dis.* **62**: 1136-1138.
92. Green KY. 2016. Editorial commentary: noroviruses and B cells.

Clin. Infect. Dis. **62**: 1139-1140.
93. Saxena K, Blutt SE, Ettayebi K, Zeng XL, Broughman JR, Crawford SE, *et al.* 2015. Human intestinal enteroids: a new model to study human rotavirus infection, host restriction, and pathophysiology. *J. Virol.* **90**: 43-56.
94. Ettayebi K, Crawford SE, Murakami K, Broughman JR, Karandikar U, Tenge VR, *et al.* 2016. Replication of human noroviruses in stem cell-derived human enteroids. *Science* **353**: 1387-1393.
95. Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR. 2006. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. *J. Virol.* **80**: 5059-5064.
96. Monaghan P, Simpson J, Murphy C, Durand S, Quan M, Alexandersen S. 2005. Use of confocal immunofluorescence microscopy to localize viral nonstructural proteins and potential sites of replication in pigs experimentally infected with foot-and-mouth disease virus. *J. Virol.* **79**: 6410-6418.
97. Paddock SW. 1999. Confocal Microscopy: Methods and Protocols, vol. 122, Humana Press, Totowa (Meth Mol Biol).
98. Hofmann AF. 2007. Biliary secretion and excretion in health and disease: current concepts. *Ann. Hepatol.* **6**: 15-27.
99. Hofmann AF. 1977. Enterohepatic circulation of bile acids and biliary lipid secretion. *Minerva Med.* **68**: 3011-3017.
100. Ruvoën-Clouet N, Belliot G, Le Pendu J. 2013. Noroviruses and histo-blood groups: the impact of common host genetic polymorphisms on virus transmission and evolution. *Rev. Med. Virol.* **23**: 355-366.
101. Thorven M, Grahn A, Hedlund K-O, Johansson H, Wahlfrid C, Larson G, Svensson L. 2005. A homozygous nonsense mutation (428G⟶A) in the human secretor (FUT2) gene provides resistance to symptomatic norovirus (GGII) infections. *J. Virol.* **79**: 15351-15355.
102. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB. 1995. Sequence and expression of a candidate for the human Secretor blood group alpha(1,2)fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. *J. Biol. Chem.* **270**: 4640-4649.

국문초록

인간노로바이러스의 세포배양 기술개발: 새로운 시작?

응웬황민뒈[1], 박미경[2], 하상도[3], 최인수[4], 최창순[5], 명진종[1]*
[1]*전북대학교 인수공통전염병연구소, 생리활성소재과학과*
[2]*경북대학교 농업생명과학대학 식품공학부*
[3]*중앙대학교 식품공학부*
[4]*건국대학교 수의과대학 수의미생물학교실*
[5]*중앙대학교 식품영양학과*

인간노로바이러스는 바이러스성 식중독 원인의 대부분을 차지한다. 노로바이러스가 건강한 성인에 감염하면 설사 등의 병변을 몇 일간 일으키다 대부분 별다른 처치 없이도 치유되는 경우가 대부분이다. 그러나 면역기능이 약화된 환자에게 감염한 경우, 만성감염 내지 치명적 감염도 가능한 것으로 보고 되고 있다. 1968년에 처음 노로바이러스가 보고된 이후 세포 감염 모델과 소동물 감염 모델을 만들고자 하는 시도가 이어져 왔으나 대부분 실패하였다. 그러나 최근들어 세포감염 모델 개발에 있어 주목할 만한 기념비적인 연구들이 이루어졌다 것이 고무적이다 할 수 있다. 이번 총설에서는 새로 개발된 감염 모델들의 특징과 장단점을 살펴보고, 이를 더욱 개선할 수 있는 방향에 대하여 살펴보고자 한다.

http://dx.doi.org/10.4014/mbl.1706.06001