FACULTEIT DIERGENEESKUNDE
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UNIVERSITEIT GENT

INVITATION

PUBLIC DEFENSE OF THE DOCTORAL THESIS

A new co-culture system of primary porcine enterocytes/myofibroblasts to investigate the replication characteristics of porcine enteric viruses in their target cell

Tingting Cui

PROMOTER

Prof. Dr. Hans J. Nauwynck
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Curriculum Vitae

Tingting Cui was born in 1988 in Sichuan, China. In 2007, she was admitted to Sichuan Agricultural University in China and studied Veterinary Medicine for her Bachelor degree. After graduation in 2011, she was enrolled as a master student in the department of Preventive Veterinary Medicine of Sichuan Agricultural University. She worked on the project “Isolation and identification of porcine rotavirus”. She obtained her Master degree in June, 2014. In the same year, she started her PhD research under supervision of Prof. Hans Nauwynck in the Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University on the project of “A new co-culture system of primary porcine enterocytes to investigate the replication characteristics of porcine enteric viruses in their target cell”. She is author and co-author of several publications in international peer-reviewed journals.

When and Where

The public defence will take place on
Wednesday, December 18th at 17.00
Auditorium Hoogbouw (Entrance 24)
Faculty of Veterinary Medicine, UGent
Salisburylaan 133, 9820 Merelbeke

The PhD defence will be followed by a reception with drinks and traditional Chinese food

Registration

If you plan to attend the reception, please confirm your presence before 15th December by e-mail to tingting.cui@ugent.be

Members of the Examination Committee

Prof. Dr. Eric Cox
Faculty of Veterinary Medicine, UGent
Chairman of the Examination Committee

Dr. Lowiese Desmarets
Center for Infection and Immunity of Lille, France
Secretory of the Examination Committee

Prof. Dr. Dominiek Maes
Faculty of Veterinary Medicine, UGent

Prof. Dr. Koen Chiers
Faculty of Veterinary Medicine, UGent

Prof. Dr. Jelle Matthijnssens
Department of Microbiology and Immunology, KULeuven

Prof. Dr. Chris Van Ginneken
Department Veterinary Sciences, UAntwerp

Summary of the Thesis

Porcine enteric viruses are the main pathogens that cause intestinal diarrhea, and the associated dehydration may lead to death and considerable economic losses in pig industry. The most common causal agents for pigs are rotavirus, porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV). Rotavirus belongs to the genus rotavirus within the family *Reoviridae*. PEDV and TGEV belong to the *alphacoronaviruses*. Although rotavirus, PEDV and TGEV mainly infect the mature small intestinal villous enterocytes, most research on these viruses was conducted on non-intestinal cell lines, such as MA104, ST and Vero cells. The lack of target intestinal enterocyte cultures hampered a good understanding of the virus replication mechanisms and impedes the development of preventive and curative control strategies. In this thesis, we developed a co-culture system of porcine enterocytes and porcine myofibroblasts, which were susceptible to rotavirus, PEDV and TGEV. Concerning the infection of rotavirus, PEDV and TGEV in primary enterocytes, we found that a basolateral receptor may exist for rotavirus because rotavirus preferentially infects enterocytes at the basolateral side, and that both PEDV and TGEV are capable to infect both APN positive and negative enterocytes, indicating the existence of non-APN receptor for coronaviruses.

The first study mainly focused on the development of a co-culture system with porcine intestinal enterocytes and myofibroblasts. Because of the difficulties of direct cultivation of primary porcine enterocytes, a co-cultivation system of enterocytes and subepithelial myofibroblasts was developed, as subepithelial myofibroblasts secrete extracellular matrix and growth factors contributing to the attachment, proliferation and differentiation of epithelial cells. The co-cultures of primary porcine enterocytes (ileocytes and colonocytes) maintained their enterocyte properties and their susceptibility to enteric viruses. First, it was demonstrated that the co-cultured ileocytes and colonocytes were susceptible to an archival rotavirus strain RVA/pig-tc/BEL/RV277/1977/G1P[7] and different other rotavirus genotypes (fecal samples containing G5P[7], G5P[13], G9P[23], G4P[6]). Next, TGEV Purdue infected both ileocytes and colonocytes whereas Miller only infected ileocytes. Last, PEDV CV777 Vero adapted and non-adapted (fecal suspension) strains could infected co-cultured ileocytes but not colonocytes. In conclusion, a novel co-culture of porcine enterocytes with myofibroblasts was established, which can be used for the investigation of the replication of enteric viruses.

Upon the new co-culture system, the polarity of rotavirus replication in target enterocytes and the effect of intestinal epithelial integrity on rotavirus infection were studied. The results clearly demonstrated that the treatment of enterocytes with EGTA, a drug that chelates calcium and disrupts the intercellular junctions, (i) significantly enhanced the infection of rotavirus in primary enterocytes, (ii) increased the binding of rotavirus to enterocytes, but (iii) greatly blocked internalization of rotavirus. After internalization, the rotavirus was resistant to EGTA treatment. Moreover, rotavirus preferentially infected enterocytes at the basolateral surface. In summary, our findings demonstrated that the integrity of intestinal epithelium is crucial in the host's innate defense against rotavirus infection. In addition, the intercellular receptor is basolaterally located and the disruption of intercellular junctions facilitates the binding of rotaviruses to their receptor at the basolateral surface. Future work could focus on the identification of this receptor.

Afterwards, the role of APN in PEDV and TGEV infections in primary porcine enterocytes was studied. After 7 days cultivation, more enterocytes presented microvilli and expressed APN and were more susceptible to PEDV and TGEV than after 3 days of cultivation. APN expression of enterocytes was also enhanced by the treatment of differentiation

factors. A significant increase of PEDV and TGEV infections was correlated with the higher expression of APN, which was indicative for an important role of APN in porcine coronavirus infections. However, by double immunofluorescence staining, we found that PEDV and TGEV could infect both APN positive and negative cells. Non-adapted and Vero-adapted PEDV and TGEV Miller showed a two- to seven-fold higher infectivity in APN positive cells than in APN negative cells. In contrast, the multiple passaged TGEV Purdue replicated better in APN negative cells than APN positive cells. These results show that APN is not a unique cellular receptor for porcine coronavirus. Subsequently, the role of sialic acid (SA) for TGEV infection in primary enterocytes was analyzed. Treatment of cells with neuraminidase (NA) had no effect on infection efficiency. In contrast, treatment of TGEV with NA significantly enhanced the infection which shows that TGEV is masked by SA and that removal of SA from virion increases the TGEV infectivity.