GHENT UNIVERSITY

FACULTY OF VETERINARY MEDICINE
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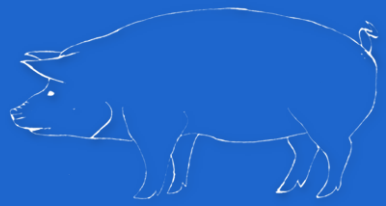
INVITATION

PUBLIC DEFENSE OF THE DOCTORAL THESIS

Interplay between porcine reproductive syndrome virus and cellular entry mediators



Jiexiong Xie
April 18th, 2019

Promoter

Prof. Dr. Hans J. Nauwynck
Faculty of Veterinary Medicine, UGent

Members of the Examination Committee

Prof. Dr. Richard Ducatelle
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Chairman of the Examination Committee

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Department of General Internal Medicine of UGhent Hospital

Prof. Dr. Xavier Saelens
VIB-UGent Center for Medical Biotechnology

Dr. Nathalie Vanderheijden
Faculty of Veterinary Medicine, UGent

Curriculum Vitae

Jiexiong Xie was born on 1st May 1988 in Hunan, China. He started his research on PRRSV from 2007 in the Faculty of Veterinary Science in Guangxi University where he obtained his Bachelor degree in June 2011. In September of the same year, he continued his master study in the department of Veterinary Medicine of South China Agricultural University. He worked on the project "Genetic variation analysis of PRRSV in Southern China from 2010-2013 and antiviral research by silencing PRRSV Nsp9 with siRNA". He obtained his master degree in June 2014 with several peer reviewed publications.

In 2014, he started his PhD research under supervision of Prof. Hans Nauwynck in the Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University on the project of "Interplay between porcine reproductive and respiratory syndrome virus (PRRSV) and cellular entry mediators". He is author and co-author of several publications in international peer-reviewed journals and gave presentations during several international conferences.

When and where

The public defence will take place on

Thursday 18th April at 17.00
Auditorium Hoogbouw (Entrance 24)
Faculty of Veterinary Medicine, UGent
Salisburylaan 133, 9820 Merelbeke

A reception with drinks and traditional Chinese food will follow in the Museum of Anatomy ☺

Registration

If you plan to attend the reception, please confirm your presence before 15th April by e-mail to jiexiong.xie@ugent.be

Summary of the Thesis

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important infectious diseases of pigs. The etiological agent is a single-stranded RNA virus named PRRS virus (PRRSV). It is endemic in all swine-producing countries and evolved rapidly since its emergence in 1980s. Highly virulent strains were consecutively reported in both Asia and European countries as well as in the United States. The virus can persist in animals for a long period and the infection with PRRSV may lead to impaired immunity, which make this virus a big threat to the pig industry. Although a lot of efforts have been made in combatting this virus, still no highly effective vaccines or antiviral strategies are available at the moment. It will be essential for the development of novel vaccines and effective antivirals in the future to have a better understanding of the interactions between viruses and the cellular entry mediators

The first study mainly focussed on identifying the new Siglec entry mediators. PRRSV has a restricted cell tropism *in vitro*. Entry mediators determine the cell susceptibility for PRRSV. Two major receptors sialoadhesin (Siglec-1) and CD163 have been extensively investigated. Previous research indicated that Siglec-1 is not the only Siglec entry mediator for PRRSV. In search for the alternative Siglec-1-like receptor, Siglec molecules identified in pigs (Siglec-1, Siglec-3, Siglec-5, Siglec-10) were cloned and the functions of these molecules were analyzed. The results revealed that besides Siglec-1, Siglec-10 is able to bind red blood cells in a sialic acid dependent manner. Virus production for both type 1 and type 2 strains in PK-15 cells co-transfected with Siglec-10 and CD163 was significantly higher compared to cells only transfected with CD163. A much higher production was observed when cells were treated with sialidase. Introducing the mutation R119E at the sialic acid binding site of Siglec-10 (Siglec-10^{R119E}) blocked the increase of virus production in the CD163 and Siglec-10 co-transfected cells. These results show that Siglec-10, similar to Siglec-1 is joining forces with CD163 during the infection of PRRSV in a sialic acid dependent manner. To further clarify the functional role of Siglec-10, binding and internalization assays were performed and a clear binding and internalization of PRRSV particles were observed in Siglec-10 but not Siglec-10^{R119E} transiently transfected cells. In conclusion, Siglec-10, like Siglec-1 is able to mediate the attachment and endocytosis of PRRSV, depending on the sialic acid-binding activity of the N-terminal Ig domain.

Upon the discovery of Siglec-10 as a new receptor of PRRSV, a PK-15 cell line recombinantly expressing Siglec-10 and CD163 (PK-$15^{S10\text{-}CD163}$) was established in the second study. The established PK-$15^{S10\text{-}CD163}$ cell line as well as the PK-15 cell line recombinantly expressing Siglec-1 and CD163 (PK-$15^{S1\text{-}CD163}$) were used to compare the replication of 7 genotype 1 subtype 1 strains (LV, 94V360, 07V063, 08VA, 13V091, 13V117 and 17V035), 2 genotype 1 subtype 3 strains (Lena and SU1-Bel) and 5 genotype 2 strains (VR2332, MN-184, SDSU-73, NADC30). All of the tested strains were growing well in both cell lines. A much higher virus production efficiency was observed in PK-$15^{S10\text{-}CD163}$ compared to PK-$15^{S1\text{-}CD163}$. The variability in growth characteristics of all the virus strains tested could be partially be explained by the usage of the receptor. In summary, the established cell line can be a good candidate for virus isolation as well as virus production for inactivated PRRSV vaccines.

At present, vaccines are mostly produced in the (monkey) MARC-145 cell line, requiring adaptation of the virus for its efficient replication. In the third study, a triple mutation (V88F, M94I, F95L) was demonstrated to be related to the MARC-145 adaptation. Multiple PRRSV1 strains were serially passaged on MARC-145 cells. The sequences of passaged virus were compared to those of the wild type viruses. GWAS analysis revealed consistent amino acid substitutions in GP2a (V88F, M94I, F95L) of MARC-145 cell-adapted strains. The identified substitutions were further investigated by introducing those amino acid changes into two PRRSV1 (13V091 and IVI-1173) infectious clones. Comparison of the replication kinetics in MARC-145 cells demonstrated the highest viral replication for both the 13V091 strain (+2.2 log10) and the IVI-1173 strain (+1.7 log10) when the triple substitution was present. This suggests that the simultaneous introduction of three mutations at positions 88, 94 and 95 is a determining factor in PRRSV1 adaptation to MARC-145 cell line. The results obtained within this study provide the molecular basis for a more efficient way in the development and production of PRRSV vaccines

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