FACULTEIT DIERGENEESKUNDE
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INVITATION

PhD PUBLIC DEFENCE

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Activation of the ERK1/2 signalling molecule by the gE glycoprotein of pseudorabies virus

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Where?

Thursday, February 15th, 2018 at 17h

Kliniekauditorium D
Faculteit Diergeneeskunde
Universiteit Gent
Salisburylaan 133, Merelbeke

After the defence there will be a small reception to which you are cordially invited to attend.

UNIVERSITEIT GENT

Curriculum Vitae

Maria José Setas Lopes Pontes was born in Mirandela, Portugal, on 17th of October 1986.

In 2004, Maria entered University of Minho, Braga, Portugal, graduating in 2008 with a Master degree in Applied Biology. For her master thesis research, Maria attended Wageningen University & Research (WUR), Wageningen, The Netherlands. She continued her research at the same institution after graduating, as a research assistant.

In September 2009, Maria enrolled in the Doctoral Program of Ghent University, Ghent, Belgium.

Her research around the effects of pseudorabies glycoprotein E on the ERK1/2 signalling pathway resulted in two first author peer-reviewed papers published in international journals.

During her research time, Maria presented her work, both via poster- and oral-presentation, at national and international congresses.

Summary of the PhD thesis

The glycoprotein E (gE), an envelope protein conserved amongst the *Alphaherpesvirinae* subfamily members, has been credited with multiple functions throughout the years. The role of gE in virulence, viral intercellular and neuronal spread, virion assembly, species-specific Fcγ receptor-like activity and antibody-induced capping has been reported by several authors, including our group. Herpesviruses are highly competent in manipulating cellular signalling cascades to their advantage. The ERK1/2 MAPK signalling pathway, a central signalling cascade, represents a particularly attractive target for

viruses since this signalling axis controls various fundamental cellular events.

Chapter 1 introduces pseudorabies virus (PRV), and provides a description of gE and its role during PRV infection. This introduction chapter also covers the ERK1/2 signalling pathway and its involvement in the cellular events relevant for this thesis, cell motility and cell survival. Finally, an overview is given the interactions of different herpesviruses with the ERK1/2 signalling pathway.
The main objective of the present thesis was to investigate whether PRV and particularly gE interfere with the ERK1/2 signalling machinery. An additional aim was to assess some of the biological effects resulting from such interference with the ERK1/2 signalling pathway, which may contribute to viral replication and spread (**Chapter 2**).

Immune evasion is one of the key elements to the success of herpesviruses propagation and survival in their hosts. In **Chapter 3**, the effect of PRV and gE on the ERK1/2 signalling pathway in T lymphocytes was investigated. Since this signalling cascade is involved in key aspects of T cell activation, PRV may benefit from the modulation of this pathway as part of its virus immune evasion strategies. In **Chapter 3a**, we describe that PRV WT infection leads to the phosphorylation, and therefore activation of ERK1/2 in the Jurkat T cell line. Additionally, we observed that PRV interfered with ERK1/2 activation mediated by CD3/TCR signaling, the classical route used for T cell activation. These results suggest that, besides triggering ERK1/2 activation, PRV actively regulates the ERK1/2 cascade by blocking other external stimuli from activating ERK1/2. Going forward, infecting Jurkat T cells with an isogenic PRV mutant that lacks gE expression (PRVΔgE) did not cause ERK1/2 phosphorylation. Infection with an isogenic mutant lacking the cytoplasmic domain of gE (PRV gEΔcd), however, did not affect PRV-mediated ERK1/2 activation. Taken together, these results show that PRV-induced ERK1/2 activation depends on gE, but does not require its cytoplasmic domain. A recombinant version of the extracellular domain of gE protein caused a rapid and transient activation of ERK1/2 in Jurkat T cells, confirming that gE affects ERK1/2 phosphorylation through its extracellular domain,

In Chapter 3b, we validated that PRV gE also leads to ERK1/2 activation in primary porcine T lymphocytes. Indeed, gE was found to trigger ERK1/2 signaling in primary T lymphocytes in assays using recombinant gE, swine testicle (ST) cells that were transiently transfected with gE or PRV WT versus ΔgE virions. Primary porcine T lymphocytes have been shown, by us and other labs, to display limited susceptibility to productive PRV infection *in vitro* and *in vivo*. We co-cultured inoculated primary T lymphocytes with ST cells, which are highly susceptible to PRV infection. With this experiment, we confirmed earlier findings that apparently noninfected lymphocytes are able to transmit the virus to more virus-susceptible cells. However, gE (and therefore gE-induced ERK1/2 signaling) appears not to affect virus transmission from T cells to susceptible cells. Thus, we investigated whether the ability of PRV gE to trigger ERK1/2 activation would have any biological consequences on T lymphocyte behavior. Homotypic T cell aggregation correlates with T cell activation and formation of T cell aggregates upon contact with either infected cells or virus has been described before, albeit not in the context of herpesvirus infection. PRV WT caused an increase in T lymphocyte aggregation, whereas PRVΔgE was significantly impaired in triggering cell aggregation. Addition of an inhibitor of ERK1/2 signalling abrogated the formation of large cell aggregates. T cell aggregation has been described to correlate with T cell migration. Inoculation of primary T lymphocytes with PRV resulted in an increased cell migration and, in line with our results on T cell aggregation, gE contributed to some extent to PRV-induced migration of T lymphocytes and inhibition of ERK1/2 signalling abrogated cell migration.

ERK1/2 is not only involved in T cell activation, aggregation and motility, but also in promoting cell survival. **Chapter 4** describes that PRV also causes ERK1/2 activation in epithelial porcine kidney (PK15) cells and that this may affect cell survival signaling pathways. Comparison of PK15 cells infected with PRV WT or PRV ΔgE showed that gE is also involved in ERK1/2 phosphorylation in this cell type. We observed that PRV WT infection triggered degradation of the pro-apoptotic protein Bim and that this process was suppressed in PRVΔgE-infected cells. Addition of an ERK 1/2 inhibitor during PRV WT infection prevented Bim degradation, confirming that PRV-induced ERK 1/2 activation results in Bim degradation. Interestingly, when PK15 cells were inoculated with PRV gEΔcd, we could observe a more rapid ERK 1/2 phosphorylation and a more dramatic Bim degradation than in PRV WT. This result may be explained by an earlier and increased gE cell surface expression observed with the PRV gEΔcd strain, possibly because of the lack of endocytosis motifs in the gE protein in this virus.
In **Chapter 5** we discuss further our findings, highlighting hypothetical possibilities how this novel role of gE may contribute to survival and propagation of PRV within the host population, as well as its possible contribution to functions described in previous reports. Moreover, we discuss the mechanism via which gE may activate ERK1/2, as well as suggest a cellular receptor as a putative binding partner for this glycoprotein.
In conclusion, we describe for the first time that gE manipulates the cellular machinery of host cells by modulating the ERK1/2 signalling pathway in both T lymphocytes and epithelial cells. This may lead to several downstream events, including changes in cellular behaviour like T cell activation and increased motility, or the interference with the apoptotic signalling network. We look forward to future research that will further unravel the importance of these findings and answer pending questions including a further assessment of the identity and role of putative cellular binding partners of gE in ERK1/2 signalling, exploring PRV/gE-mediated ERK1/2 manipulation in other relevant cellular models including neuronal cells, as well as during in vivo PRV pathogenesis.