Cell-dependent susceptibility to Porcine circovirus ORF3-induced apoptosis

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Introduction

Porcine circovirus associated disease (PCVAD) has caused considerable economic impact worldwide since its first description on a Canadian pig farm in the 1990s. PCVAD, a wasting and immunosuppressive disease in post-weaned pigs, is caused by Porcine circovirus type 2 (PCV2). Despite the availability of effective vaccines, little is known about the molecular pathogenesis of the disease. Belonging to the Circoviridae family, PCV2 and PCV1 are closely-related viruses with a 1.7kb, ambisensed and circular ssDNA genome 1. While PCV2 is pathogenic, PCV1 is not. Investigating the differences in the genomic composition of these viruses will provide clues to the molecular pathogenesis of PCVAD. ORF3 is a good candidate as a determinant of PCV pathogenicity because PCV1 and PCV2 ORF3 share only 60.6% amino acid (aa) sequence identity in the translated region. In addition, a consistent single nucleotide (nt) substitution in the PCV2ORF3 coding region resulted in a stop codon, leading to PCV2ORF3 that is half the length of PCV1ORF3 (104aa versus 206aa). PCV2ORF3 was shown to induce apoptosis and a mutant virus devoid of ORF3 expression did not induce apoptosis in Cos-7 and PK-15 cell lines 2. Abrogation of ORF3 expression appeared to reduce PCV2 virulence in a specific-pathogen-free (SPF) piglet model 3. Due to the differences between PCV1 and PCV2 ORF3 and what is known about PCV2ORF3, it is hypothesized that ORF3 determines the pathogenicity of PCV. Thus, the objective of this study is to compare and contrast the pro-apoptotic capability of PCV1 and PCV2 ORF3.

Materials and Methods

PCV1 and PCV2 ORF3 genes were cloned into mammalian expression peGFP-C1 vector to create fusion proteins with eGFP at the N’terminus. Flow cytometry was used to assess cell viability and apoptosis. Immunoblot was used to detect protein expression and PARP cleavage associated with apoptosis. Z-Asp-CH2-DCB was used as a caspase inhibitor. Three cell lines were used in this study: human epithelial 293T, porcine kidney epithelial PK-15 and porcine primary kidney (PPK) cells. Statistical analysis was performed, using ANOVA and Tukey’s multiple comparison tests with p<0.05 considered as statistically significant.

Results and Discussion

In all three cell lines, eGFPPCV1ORF3 significantly induced higher cell death. Meanwhile, eGFPPCV2ORF3 significantly induced higher cell death only in PK-15 cells. It was also consistently observed that eGFPPCV1ORF3 was a more potent inducer of cell death than eGFPPCV2ORF3.

During apoptosis, a programmed cell death process, phosphatidylserine molecules become externalized to the outer surface of the cell membrane. This externalization is important for macrophage recognition, phagocytosis and clearance of apoptotic cells without causing an inflammatory response 4. In addition, caspases become activated and cleave PARP molecules, DNA repair enzymes, leading to apoptosis 5. In this study, significantly higher percentage of phosphatidylserine externalization as well as the presence of a cleaved PARP fragment was observed in cells transfected with eGFPPCV1ORF3. Moreover, addition of increasing concentration of a caspase inhibitor reduced the level of cell death induced by eGFPPCV1ORF3. Hence, cell death induced by PCV1ORF3 occurs via a caspase-dependent apoptotic pathway, similar to what was reported for PCV2ORF3 2.

While PCV1 does not cause disease, PCV2 causes PCVAD. Their distinctly different ORF3 protein was hypothesized to be the factor for their different pathogenicity. Although both PCV1 and PCV2 ORF3 can induce cell death via apoptosis, it is not completely surprising because they share 60.6% aa sequence identity. However, PCV1ORF3 appears more potent than PCV2ORF3 in inducing cell death despite the lack of cytopathic effects in cells infected with PCV1 6. It is speculated then that PCV1ORF3 expression is absent or is tightly regulated by other viral protein(s), incapacitating PCV1 from causing PCVAD in pigs. Different cell death profiles seen with different cell lines and even within the same cell population suggest a cellular-dependent response to ORF3-induced apoptosis.

References