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Full Length Article

Expression Profiling of Different Signaling Markers during PBMC Apoptosis by Porcine Parvovirus

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Abstract

Porcine parvovirus (PPV) is an important pathogen in reproductive failure. PPV-induced apoptosis has been indicated the apoptosis-related genes change resulting in reproduction failure. However, the underlying regulatory mechanism was still not fully elucidated in porcine peripheral blood mononuclear cells (PBMCs). In this study, Flow cytemeter and quantitative real-time PCR (qRT-PCR) were used to investigate the apoptosis induced by PPV infection and detect the changes of some apoptosis related genes expression in the course of infection under controlled conditions. Results showed that porcine PBMCs infected with PPV showed an exponential increase in PPV replication, particularly at 48 h post-infection (hpi) and 72 hpi. Similarly, flow cytometry results observed that PPV infection led to the increased percentage of apoptotic cells in PBMCs reaching to 70.7% at 48 hpi, then decreased to 38.9% at 72 hpi. Moreover, qRT-PCR results showed that the mRNA abudance of *TNF-α*, *P53*, *Bcl-2*, *Caspase-8* and *FasL* associated with apoptosis in porcine PBMCs by PPV infection were significantly upregulated at 1hpi, and then decreased slowly, whereas *TNFR-2*, *PBR* and *Bcl-xl* had an obviousincrease at 48 hpi (11.5-fold), 24 hpi (11.5-fold) and 72 hpi (3.6-fold), respectively. Taken together, these results revealed that PPV infection significantly induced cell apoptosis through regulating the P53 and TNF signaling pathway in porcine PBMCs, which provides a molecular basis for elucidating the function of apoptosis in the interaction of PPV with host cells.

Keywords: Apoptosis; P53 signaling pathway; Porcine parvovirus; Peripheral blood mononuclear cells

Introduction

Porcine parvovirus (PPV), a DNA virus belonging to the genus parvovirus, the subfamily and family of parvovirinae, was first isolated from sows in Germany (Cságola *et al*., 2012; Zhang *et al*., 2019), and its virions are characterized by non-enveloped and contain approximately 5-6 kilobases (kb), single-stranded linear DNA genomes (Xiao *et al*., 2013). Many studies confirmed that PPV can lead to reproductive failure in pregnant sows, which characterized by embryonic and fetal death, mummification, stillbirth and delayed estrus (Ren *et al*., 2013; Zhou *et al*., 2017; Zhang et al., 2019), resulting in a reduction in size of swine house and a serious economic loss in the swine industry (Ren *et al*., 2013; Chen *et al*., 2015).

Typically, PPV replicates in infected cells and induces a specific cytopathic effect (CPE) in the host cell. However, the interaction between the PPV and cells is not well recognized. Virus-infected cells through apoptosis, necrosis, or to destroy part of a cell lysis mediated by the innate immune system and the adaptive immune system, but the virus evolved various strategies to manipulate the apoptotic response of the host, to ensure its continued reproduction (Everett and Mcfadde, 2001). Viruses that induce apoptosis in infected cells, such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), infectious bursal disease virus (IBDV) (Vasconcelos and Lam, 1994), human immunodeficiency virus (HIV) (Mehto *et al*., 2015), equine arteritis virus (EAV) (Metz *et al*., 2016), influenza virus (Ludwig *et al*., 2010), transmissible gastroenteritis virus (TGEV) (Ding *et al*., 2018) have been discovered, but there is no report whether PPV infection induces apoptosis in porcine peripheral blood mononuclear cells (PBMCs).

Apoptosis can be induced by three pathways including the intrinsic pathway, the endoplasmic reticulum (ER) stress pathway and the extrinsic pathway. For the intrinsic pathway, many intracellular stresses cause up-regulation of Bax and Bak levels, which initiates the intrinsic pathway. Apoptosis is the process by which cells undergo systematic self-destruction under various stimuli (Sauerwald *et al*., 2011). The process of apoptosis is mainly carried out by a cysteine proteases family called caspases (Thornberry and Lazebnik, 1998) and regulated by members of the *Bcl-2* protein family (e.g. Bcl-2 and Bcl-XL), the TNF-R

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superfamily (e.g. TNF-R1, Fas and TNF-R2), Fas-ligand (FasL) p53, a nuclear transcription factor (Reed *et al*., 1996; Sauerwald *et al*., 2011; Zhang *et al*., 2015; Zhou *et al*., 2017), and the cysteine proteases (Caspase-8). Some cytokines (e.g. TNF-α) also can trigger off apoptosis phenomena (Zhou *et al*., 2017). Our previous study has shown that PPV can induce the production of inflammatory cytokine factors in infected cells via regulating toll-like receptor 9 and NF-κB signaling pathways, however, there is less information about the cellular pathways involved in PPV - infected apoptosis and specific proteins of porcine PBMCs (Zhou *et al*., 2017).

In the present study, we are interested in finding out the apoptosis induced by PPV in porcine PBMCs in vitro. We used Flow cytometry and quantitative real-time PCR (qRT-PCR) to investigate the apoptosis induced by PPV infection and detect the changes of some apoptosis-related genes expression in the course of infection under controlled conditions. The percentage of apoptotic cells and the gene expression at different times were compared in this study. The objective is to understand the mechanism of PPV infection in cell modern and to provide a molecular basis of PPV infection.

Materials and Methods

Virus and Animals

The PPV standard strain HN-4 was isolated by our laboratory and porcine kidney (PK)-15 cells used for PPV propagation were obtained from the China Institute of Veterinary Drug Control (Beijing, China), and a PPV-permissive cell line derived from PK-15 cell line (Wei *et al*., 2009). The virus titer was determined by using a plaque assay.

Porcine peripheral blood was collected from a healthy castrate boar, 1-year-old, genetic line pigs (line 2) raised at the Swine research Farm in Henan, China, which derived from combination of Landrace and Large White breeds. The porcine serum samples were tested by ELISA and PCR, and results showed that PPV, PRV, PRRSV, PCV2, JEV, and HCV were all negative.

PBMCs Sample Preparation

PBMCs were separated from heparinized peripheral blood on Ficoll-Hypaque gradients by the method of Paul *et al*. (1979). The collected PBMCs were then transferred to a 50-mL corning tube. The PBMCs were washed and centrifuged at 1500 rpm for 10 min at room temperature. After centrifugation, the pellet was resuspended in RPMI-1640 (GIBCOBR) supplemented with 10% heated-inactivated fetal bovine serum (FBS, Hyclone) for concentration measurement.

Infection of PBMCs with PPV

The virus was cultured and titrated in PK-15 cells as previously described (Zhou *et al*., 2017). The PBMCs were diluted to a final volume of 2×10^5 cells/mL with supplemented RPMI-1640 medium, plated into six-well cell-culture dishes (1 mL) plates, incubated at 37°C under a humidified 5% CO_2 atmosphere, and washed with PBS twice, inoculated with PPV at a multiplicity of infection (m.o.i.) of 1 (MOI=1), and then incubated in the presence of fresh RPMI-1640 medium with 1% FBS at 37 °C in 5% CO_2 incubator for indicated times.

Cell Apoptosis Analysis by Flow Cytometry

Cells analyzed by flow cytometry were isolated from PBMCs line infected with PPV at 0 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h post-infection (hpi), and corresponding time points control PBMCs line were collected for investigating the apoptosis induced by PPV. Flow cytometry was used to detect apoptotic cells using DNA staining protocol stained with Annexin V-FITC and propidium iodide (PI) (Annexin V-FITC/PI detection kit, Hoechst 33258). The cell cultures were washed with PBS twice, then digested by 0.25% trypsin (without EDTA). After washing in PBS again, we centrifuged the cells, discard the supernatant, and diluted with binding buffer to a final density of 1106 cells/mL. Added Annexin V-FITC and PI, incubate the cells at room temperature for 15 min. Cells were analyzed with Becton-Dickinson FACS to determine the percentage of apoptotic cells in the cell cycle.

DNA Isolation

The genomic DNA of PPV was extracted with a method as previously described (Sambrook and Russell, 2001). After freezing and thawing three times, 400 μL supernatant of PBMCs was digested with 10 ul of Proteinase K at 55ºC for 1 h. The digestion was extracted with the same volume of phenol-chloroform (1:1, v/v), centrifuged at 12 000 rpm/min for 5 min, and the supernatant was transferred to a new Eppendorf tube. Isopropannol (200 μL) was added, and then incubated at -20ºC for 1 h. Centrifuged and discarded the supernatant, and washed with 75% ice-cold ethanol, then dried in a laminar flow cabinet. The precipitate of DNA was dissolved in 20 μL of sterile water and then stored at -20ºC for later use.

RNA Isolation and Reverse Transcription

Samples were collected as the previous time-point. The cell cultures were washed with PBS twice, digested by 0.25% trypsin (without EDTA), centrifugalized the washed cells, and stored at -80ºC until needed. Isolation of total RNA from cells using an RNA-isolation kit (E.Z.N.A Total RNA Kit Ⅰ) for analysis of cytokine mRNA expression. RNA was reverse transcribed into cDNA and mRNA encoding cytokines was quantitatively expressed by real-time RT-PCR.

Quantitative Real-time PCR (qRT- PCR)

Specific primers were designed using Primer 5 software according to the GenBank sequence. The sequences of

primers were listed in the following Table 1. qRT-PCR was performed with *TransStart* Tip Green qPCR Kit (TransBionovo Co., Ltd, Beijing, China) in Step One-Plus™ Real-Time PCR System (Applied Biosystems, CA, USA). The procedure was as follow: 1 cycle of 30 sec at 95ºC, and 40 amplification cycles of 5 sec at 95ºC and 30 sec at 58ºC, of dissociation stage. β-actin was used as an internal control.

Statistical Analysis

The significance of differences between groups was analyzed by one-way analysis of variance (ANOVA) or by Student's t-test with Bonferroni correction. Moreover, all numerical data comes from at least three separate experiments. The results are presented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. NS, no significance.

Results

PPV Infected PBMCs

To examine the susceptibility to PPV, PBMCs were infected with PPV, and infection was assessed by extracting DNA from infected cells lysates (Fig. 1). After qRT-PCR detecting and comparative analysis with β-actin, the genomic DNA of PPV in PBMCs at 1h was defined as 1×, and the DNA multiplication multiple at different times were obtained.

As shown in Fig. 1, PPV replicated in PBMCs after infection at different times and the level was increased with time changes. PPV DNA was detected at 1 hpi with low level, and the increase of PPV DNA proliferated was quickly peaked at 24 hpi and 72 hpi and the level was ten times more than 1 hpi.

PPV Induced Apoptosis of PBMCs

Using fluorescein annexin V-FITC combined with PI staining cells to observe the apoptotic rate of PPV-infected porcine PBMC. The results showed that apoptosis was induced in PBMC between 0 and 72 h after infection with PPV. The scatter diagram obtained from the flow cytometric analyses of PBMCs infected with PPV (Fig. 2) and the results showed that PPV induced apoptosis in PBMCs. The data presented in Fig. 2 and demonstrated that 3～48 h after infection, 29.2～70.7% of PBMCs showed signs of apoptosis and the percentage of apoptotic cells increased with the incubation time, and the frequency of apoptotic cells continued to increase and reach 70.7% at 48 hpi, then decreased to 38.9% at 72 hpi.

Related Cytokines mRNA Expression in PPV Infected PBMCs

PBMCs were collected at 0 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h for extracting RNA of cells. The pro-infection cells were chosen as the reference sample for each individual samples. After qRT-PCR detecting and comparative analysis with β-actin, the mRNA of β-actin in PBMCs at 0 h was defined as 1×, and the RNA multiplication multiple at different time were obtained. The influence of PPV on apoptosis related gene mRNA expression in porcine PBMCs is shown in Fig. 3. The results showed that PPV infection in PBMCs tended to up-regulate of the genes mRNA expression at different times.

Of the level of genes tested, the mRNA expression of Bcl-2, FasL, Caspase-8, P53 and TNF-α were peaked at 1 hpi, and then decreased slowly. Bcl-2, FasL and TNF-α were also increased after 24 hpi. Although the tendency of expression is similar, the changes are quite different. TNF-α expression was significantly increased in the 1 hpi, reaching 3.9-fold, and the expression was significantly below the control at 24 hpi, and then reached 2.1-fold in the 72 hpi. The mRNA expression of TNFR-2 was peaked at 48 hpi and reached 11.5-fold. P53 mRNA expression showed a slightly increase at 1 hpi and 72 hpi, and decreased at 12 and 24 hpi (0.4-fold and 0.49-fold). PBR expression increased from 1 hpi to 48 hpi, and markedly increased at 24 h reaching 12.9-fold, and a slightly decrease in 72 hpi. Bcl-2 expression was a slight increase in 1 hpi, and then decreased from 3 hpi to 72 hpi, and the bottom was 0.28-fold at 24 hpi. Bcl-xL expression was significantly declined to 0.16-fold at 24 hpi, and significantly increased to 3.6-fold at 72 hpi. Caspase-8 expression was significantly increased in 1 hpi reaching 19.3-fold, and decreased between 3 hpi to 12 hpi, no obviously changes in 48 hpi and 72 hpi. FasL expression was increased significantly in 1 hpi reaching 5.7-fold, and decreased at 12 and 24 hpi (0.47-fold and 0.02-fold, respectively).

Discussion

Apoptosis is an effective mechanism in the process of virus-infected cells by which the viruses can disseminate progeny while limit the induction of inflammation and immune response (Koyama *et al*., 2000; Sauerwald *et al*., 2011; Zhang *et al*., 2019). The results presented in the current study indicated that PPV infection induced apoptosis. And the percentage of apoptosis in PBMCs from 0h to 48hpi increased with the incubation times.

To learn more information on PPV-induced apoptosis, we detected these cytokines and related genes expression (including *TNF-α*, *caspase-8*, *Bcl-2*, *Bcl-XL*, *TNF-R1*, *Fas*, *TNF-R2*, *FasL* and *p53*). TNF-α and FasL belong to a small subset of pro-apoptotic protein ligands in the TNF superfamily, which cells are capable of activating extracellular apoptosis death pathways during binding to cell surface homologous death receptors (Gonzalvez and Ashkenaz, 2010). TNF-α expression was significantly increased at 1 hpi, reaching 3.9-fold. And the expression of TNFR-2 peaked to 11.5-fold at 48 hpi. The receptor for TNF-α signaling belongs to the TNF receptor (TNFR) superfamily and it is involved in many biological effects processes, including inflammation, apoptosis, and other programmed

Table 1: List of primers used in Real-time PCR assays for expression profiling of different genes and their respective amplicon sizes

Gene name	Forward primer 5' to 3'	Reverse primer 5' to 3'	Size of amplicon (bp)	Gene ID
PPV	AGTTCAATGATTTCAATG	TTAACCAACATTCCCAT	203	NC001560
β-actin	GGACTTCGAGCAGGAGATGG	GCACCGTGTTGGCGTAGAGG	233	XM_021086047.1
TNF-α	TCACCACGCTCTTCTGC	CCCTCGGCTTTGACATT	166	NM_214022.1
TNFR2	CACCAGGCTTCCGAATACAA	ACGCAGAAACCGAGTTCCA	105	NM_001097441.2
Bcl-2	TGCGACTTTGCCGAGAT	CCCAGCCTCCGTTATCC	259	NM214285
Bcl-xl	GAGCGTAGACAAGGAGATGC	CGACTGAAGAGCGAACCC	237	XM_021077298.1
FasL	TCCCTGTCCAGCTTCTGTG	CTGCATTGTGGTCCCTCTT	152	NM_213806.1
p53	TTGAGGTGCGTGTTTGTG	GCAGACTGGGCATCCTTC	259	NM_213824.3
Caspase-8	AGAGGCATCAGAAGCAGT	TGGTCCAAGTTTCGGTAG	255	XM_021074714.1
PBR	AGCTCACGCAATGTCCTCG	AGAGGGCTCACCTGATACCA	332	NM_213753.1

death processes (Muppidi *et al*., 2004). Our result indicated that TNF-α and TNFR mRNA related tightly to the apoptosis in PPV infected PBMCs. Fas-ligand triggers apoptosis by activating Fas, a cell surface molecule homologous to the tumor necrosis factor-alpha (TNF-α) receptor (Liu *et al*., 2000). Fas-ligand (FasL) interactions can mediate apoptosis in PBMCs (Perlman *et al*., 2015). The gene expression of *FasL* increased significantly at 1 hpi reaching 5.7-fold. The change of FasL's expression indicated that FasL may involve the early apoptosis in PBMCs infected with PPV.

Both in vivo and in vitro studies suggest that *Bcl-2* plays a role in preventing apoptosis (Frenzel *et al*., 2009). In addition, several viral Bcl-2 homologs have a direct and proximal effect on mitochondrial function when expressed independently of any other viral proteins in the cell (Everett and Mcfadden, 2001). In our study, *Bcl-2* expression was slightly increased at 1 hpi, and then decreased from 3 hpi to 72 hpi. Bcl-xL expression increased significantly to 3.6-fold at 72 hpi. It is suggested that Bcl-2 and Bcl-xL may inhibit apoptosis in the early and late phases of PPV- infected PBMCs, respectively.

Tumor suppressor gene p53 is involved in the induction of apoptosis during cell division (Jordán *et al*., 1997), and p53 can be used as a nuclear sensor to detect genotoxic damage or inappropriate transcriptional activation caused by multiple viruses. Moreover, Blocking of p53 activation can markedly inhibit the occurrence of apoptosis by inhibiting the TGEV-induced FasL expression, Bcl-2 reduction, Bax and cytochrome c redistribution (Huang *et al*., 2013). In this study, P53 expression was a slight increase at 1 hpi and 72 hpi, no significantly changes happened at 3 hpi and 48 hpi, and decreased at 12 and 24 hpi which reached 0.4-fold and 0.49-fold respectively.

Caspase-8 is a member of the cysteine protease and is involved in apoptosis and cytokine processing. Many studies have indicated that caspase-8 has been confirmed to be activated during the erythrocyte apoptosis infecting human parvovirus B19 (Sol *et al*., 1999; Chen *et al*., 2010). In this study, caspase-8 expression was significantly increased in 1 hpi and 24 hpi reaching 19.3-fold and 2.1-fold differently. The peak of caspase-8 gene expression are accordance with FasL and TNF-α gene expression in our study, and the results indicated that FasL and TNF-α may require for caspase-8 activation in PPV - infected PBMCs.

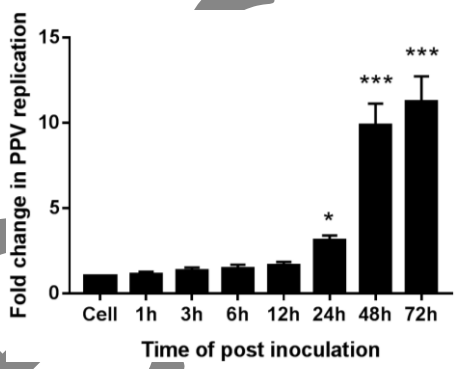

Fig. 1: PPV replication in PBMCs after infection at different times
PBMCs were collected at 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72h for extracting the genomic DNA of PPV. All data are shown as mean ± SD of three independent experiments. *$p < 0.05$, ***$p<0.001$ relative to mock-infection cells

It is worth mentioning that PBR is a widely expressed, evolutionarily conserved mitochondrial apoptosis regulatory protein. It is endowed with a variety of biological functions, including the regulation of reactive oxygen species and the regulation of apoptosis. (Casellas *et al*., 2002). Moreover, the mitochondrial peripheral benzodiazepine receptor (mPBR) is involved in a functional structure called a permeability transition pore that also controls cell apoptosis. The binding of mPBR to Fas triggers a prototypic pathway that induces apoptosis (Decaudin *et al*., 2002). In this study, PBR expression increased 24 hpi reaching 12.9-fold. PBR levels increased from 1 hpi till 48 hpi, and a slight decrease in 72 hpi, and the level of PBR were related to the virus replification. Our results showed that PPV can promote apoptosis by induced high expression of PBR in PBMCs.

Conclusions

The present results suggest that PPV induced PBMCs apoptosis in vitro and the percent of apoptosis cells was correlated to viruses' replication. The changes of related genes expression also indicated that the apoptosis induced by PPV, which was the mechanism of PPV infection host cells in order to avoid the elimination by immune system. Further studies are required to demonstrate the relationship between TNF-α and Bcl-2 mRNA expression and their role in PPV infection-induced apoptosis.

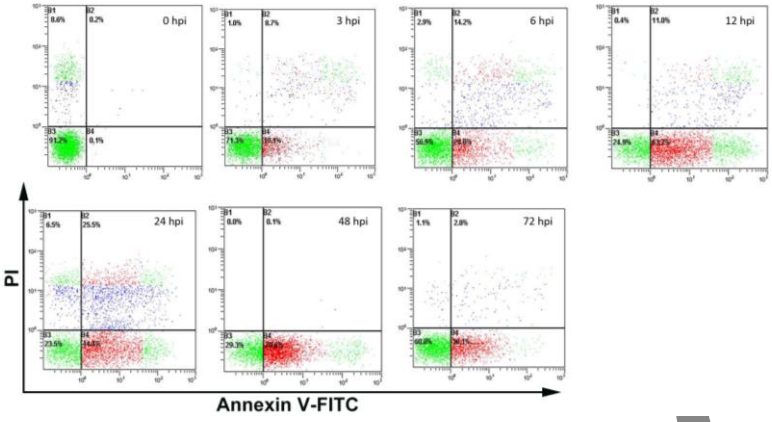

Fig. 2: The percentage of apoptotic cells in PBMCs cultures after infection with PPV. PPV induced apoptosis of PBMCs by staining cells with a combination of fluorescein annexin V-FITC and PI, the percentage of apoptosis in PBMCs from 0 hpi to 48 hpi was increased with the incubation times, and reached 70.7% at 48 hpi, then decreased to 38.9% at 72 hpi

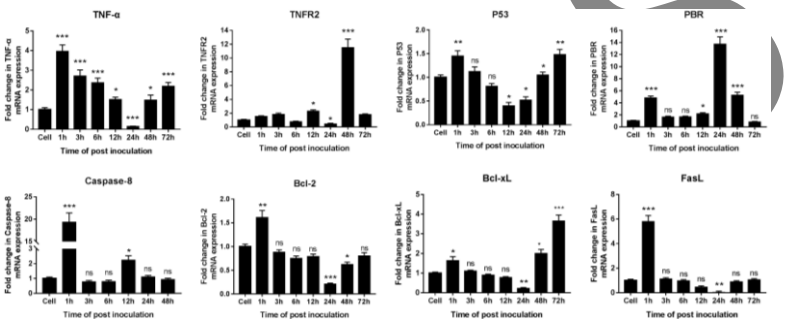

Fig. 3: The mRNA expression of apoptosis-related genes in porcine PBMCs after PPV infection

Porcine PBMCs were infected for different time periods with PPV at MOI of 1. Total RNA in mock and PPV-infected cells at different times (1, 3, 6, 12, 24, 48 and 72 hpi) was isolated and quantitated using qRT-PCR as described in the methods. All data are shown as mean ± SD of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$ relative to mock-infection cells.ns, not significant

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References

Casellas, P., S. Galiegue and A.S. Basile, 2002. Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem. Intl.,* 40: 475–486

Chen, A.Y., E.Y. Zhang, W. Guan, F. Cheng, S. Kleiboeker, T.M. Yankee and J. Qiu, 2010. The small 11 kDa nonstructural protein of human parvovirus B19 plays a key role in inducing apoptosis during B19 virus infection of primary erythroid progenitor cells. *Blood*, 115: 1070–1080

Chen, Y., Y. Dong, Y. Jiao, L. Hou, Y. Shi, T. Gu, P. Zhou, Z. Shi, L. Xu and C. Wang, 2015. In vitro, antiviral activity of germacrone against porcine parvovirus. *Arch. Virol.,* 160: 1415–1420

Cságola, A., M. Lőrincz, D. Cadar, K. Tombacz, I. Biksi and T. Tuboly, 2012. Detection, prevalence and analysis of emerging porcine parvovirus infections. *Arch. Virol.,* 157: 1003–1010

Decaudin, D., M. Castedo, F. Nemati, A. Beurdeley-Thomas, G. De Pinieux, A. Caron, P. Pouillart, J. Wijdens, D. Rouillard, G. Kroemer and M.F. Poupon, 2002. Peripheral benzodiazepine receptor ligands reverse apoptosis resistance of cancer cells in vitro and in vivo. *Cancer Res.,* 62: 1388–1393

Ding, L., J. Li, W. Li, Z. Fang, N. Li, Q. Guo, H. Qu, D. Feng and M. Hong, 2018. p53 mediated IFN-β signaling to affect viral replication upon TGEV infection. *Vet. Microbiol.*, 227: 61–68

Everett, H. and G. Mcfadden, 2001. Viruses and apoptosis: meddling with mitochondria. *Virology*, 288: 1–7

Frenzel, A., F. Grespi, W. Chmelewskij and A. Vilunger, 2009. Bcl2 family proteins in carcinogenesis and the treatment of cancer. *Apoptosis,* 14: 584–596

Gonzalvez, F. and A. Ashkenazi, 2010. New insights into apoptosis signaling by Apo2L/TRAIL. *Oncogene,* 29: 4752–4765

Koyama, A.H., A. Adachi and H. Irie, 2000. Physiological significance of apoptosis during animal virus infection. *Intl. Rev. Immunol.,* 22: 341–359

Liu, H.C., Z.Y. He, C.A. Mele, L.L. Veeck, O. Davis and Z. Rosenwaks, 2000. Expression of apoptosis-related genes in human oocytes and embryos. *J. Assist. Reprod. Genet.,* 17: 521–533

Ludwig, S., S. Pleschka, O. Planz and T. Wolff, 2010. Ringing the alarm bells: signalling and apoptosis in influenza virus infected cells. *Cell Microbiol.,* 8: 375–386

Mehto, S., C. Antony, N. Khan, R. Arya, A. Selvakumar, B.K. Tiwari, M. Vashishta, Y. Singh, S. Jameel and K. Natarajan, 2015. Mycobacterium tuberculosis and human immunodeficiency virus type 1 cooperatively modulate macrophage apoptosis via toll like receptor 2 and calcium homeostasis. *PloS One,* 10: e0131767

Metz, G.E., I. Galindo, M.M. Abeyá, M.G. Echeverria and C. Alonso, 2016. Intrinsic, extrinsic and endoplasmic reticulum stress-induced apoptosis in RK13 cells infected with equine arteritis virus. *Virus Res.*, 213: 219–223

Muppidi, J.R., J. Tschopp and R.M. Siegel, 2004. Life and Death Decisions: Secondary Complexes and Lipid Rafts in TNF Receptor Family Signal Transduction. *Immunity*, 21: 461–465

Paul, P.S., W.L. Mengeling and B.T. Jr, 1979. Replication of porcine parvovirus in peripheral blood lymphocytes, monocytes, and peritoneal macrophages. *Infect. Immun.,* 25: 1003–1007

Perlman, H., L.J. Pagliari, N. Nguyen, K. Bradley, H. Liu and R.M. Pope, 2015. The Fas-FasL death receptor and PI3K pathways independently regulate monocyte homeostasis. *Eur. J. of Immunol.,* 31: 2421–2430

Reed, J.C., T. Miyashita, S. Takayama, H.G. Wang, T. Sato, S. Krajewski, C. Aime-sempe, S. Bodrug, S. Kitada and M. Hanada, 1996. Bcl-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. *J. Cell Biochem.,* 60: 23–32

Ren, X., Y. Tao, J. Cui, S. Suo, Y. Cong and P. Tijssen, 2013. Phylogeny and evolution of porcine parvovirus. *Virus Res.,* 178: 392–397

Sambrook, J. and D.W. Russell, 2001. *Molecular Cloning: A Laboratory Manual,* pp: 6.4–6.12, 3rd edition. New York: Cold Spring Harbor Laboratory Press

Sauerwald, T.M., A. Lewis and H. Dorai, 2011. Apoptosis: The Signaling Pathways and Their Control. *Comprehensive Biotechnol.,* 300: 483–494

Sol, N., J.J. Le, I. Vassias, J.M. Freyssinier, A. Thomas, A.F. Prigent, B.B. Rudkin, S. Fichelson and F. Morinet, 1999. Possible interactions between the NS-1 protein and tumor necrosis factor alpha pathways in erythroid cell apoptosis induced by human parvovirus B19. *J. Virol.,* 73: 8762–8770

Thornberry, N.A. and Y. Lazebnik, 1998. Caspases: Enemies Within. *Science*, 281: 1312–1316

Vasconcelos, A.C. and K.M. Lam, 1994. Apoptosis induced by infectious bursal disease virus. *J. Gen. Virol.,* 75: 1803–1806

Wei, Z.Y., X.B. Wang, X.D. Ning, Y.B. Wang, H.Y. Zhang, D.F. Wang, H.Y. Chen and B.A. Cui, 2009. Nitric oxide inhibits the replication cycle of porcine parvovirus in vitro. *Arch. Virol.,* 154: 999–1003

Xiao, C.T., P.F. Gerber, L.G. Giménez-Lirola, P.G. Halbur and T. Opriessnig, 2013. Characterization of porcine parvovirus type 2 (PPV2) which is highly prevalent in the USA. *Vet. Microbiol.,* 161: 325–330

Zhang, J., J. Fan, Y. Li, S. Liang, S. Huo, X. Wang, Y. Zuo, D. Cui, W. Li, Z. Zhong and F. Zhong, 2019. Porcine Parvovirus Infection Causes Pig Placenta Tissue Damage Involving Nonstructural Protein 1 (NS1)-Induced Intrinsic ROS/ Mitochondria- Mediated Apoptosis. *Viruses*, 11: 389

Zhang, H., Y. Huang, Q. Du, X. Luo, L. Zhang, X. Zhao and D. Tong, 2015. Porcine parvovirus infection induces apoptosis in PK-15 cells through activation of p53 and mitochondria-mediated pathway. *Biochem. Biophys. Res. Commun.,* 456: 649–655

Zhou, Y., X.H. Jin, Y.X. Jing, Y. Song, X.X. He, L.L. Zheng, Y.B. Wang, Z.Y. Wei and G.P. Zhang, 2017. Porcine parvovirus infection activates inflammatory cytokine production through Toll-like receptor 9 and NF-κB signaling pathways in porcine kidney cells. *Vet. Microbiol.,* 207: 56–62

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