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# *Goose parvovirus* and the protein NS1 induce apoptosis through the AIF-mitochondrial pathway in goose embryo fibroblasts

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ABSTRACT

In this study, the effects of *Goose parvovirus* (GPV) infection as well as the possible role of NS1 protein on apoptosis induction in goose embryo fibroblast (GEF) cells were examined. Flow cytometry analysis and TUNEL assays revealed that GPV infection and NS1 transfection induced significant apoptosis in GEF cells compared to what was observed in mock-infected cells. Interestingly, the increase in the rate of apoptosis detected in GPV-infected GEFs was accompanied by an increased viral load in the cells. In addition, the apoptotic pathway was mediated by apoptosis-inducing factors (AIFs) and internal factors that influence the release of AIFs. The results indicated that the mitochondrial membrane potential was decreased, and AIF expression was increased in the nucleus ($P < 0.01$). Reactive oxygen species (ROS) increased gradually within 48 h ($P < 0.001$). Cathepsin D activities were also increased ($P < 0.05$). The results demonstrated that the AIF-mediated pathway is a new mitochondrial apoptotic pathway and that mitochondrial depolarization, ROS content, and cathepsin D activities are the key factors influencing apoptosis in GEF cells.

## 1. Introduction

Derzsy's disease is a highly contagious disease caused by a pathogen named goose parvovirus (GPV). This disease affects goslings and young muscovy ducks by the age of 3 weeks (Irvine and Holmes, 2010). This disease mainly causes acute hemorrhagic necrotic enteritis, acute septicemia and hepatitis, and it is associated with substantial loss in the goose industry. GPV belongs to *Dependovirus* within the family *Parvoviridae*. Its genome is composed of single-stranded, linear DNA of approximately 5100 nucleotides (nt) in length, and it contains two open reading frames (ORFs). The left reading frame encodes two types of nonstructural proteins (NS1 and NS2), and the right reading frame encodes structural proteins (VP1, VP2 and VP3) (Wang et al., 2014). Among these proteins, NS1 has a total length of approximately 1884 nt, encodes 627 amino acids with has a size of approximately 83 kDa (Chen et al., 2018). NS1 protein is mainly involved in viral replication and regulatory functions (Liu et al., 2014). NS1 protein belongs to the superfamily 3 (SF3) helicase and has multiple functions in the early stages of viral replication, such as being involved in DNA replication, DNA helicases or nicking enzymes, regulation of gene expression, regulation of genomic replication and induction of cytopathic changes (Mansillasoto et al., 2009). In recent years, many scholars have also found that the NS1 protein of parvovirus can inhibit tumor growth and play a role as an oncolytic factor (Marchini et al., 2015). NS2 protein is not cytotoxic, but it can enhance the cytotoxicity of NS1 protein.

Apoptosis is different from cell necrosis. Apoptosis is not a passive process; rather, it is an active process that includes gene activation, expression and regulation. It is not a phenomenon of pathological self-injury but a death process that actively seeks to generate a better living environment. A large number of scholars have shown that parvovirus infection can induce apoptosis, and its main functioning protein is the nonstructural protein (NS1), but different viruses have different apoptotic signaling pathways. Generally, apoptosis is divided into two pathways: the exogenous receptor pathway, which activates the

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