Virology 521 (2018) 69–76

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Pyroptosis induced by enterovirus A71 infection in cultured human neuroblastoma cells

Xiaojuan Zhu[a,1], Tao Wu[a,1], Ying Chi[a], Yiyue Ge[a], Bin Wu[a], Minghao Zhou[a], Fengcai Zhu[a], Minjun Ji[b,c,*], Lunbiao Cui[a,c,**]

[a] Institute of Pathogenic Microbiology, Key Laboratories of Enteric Pathogenic Microbiology (Ministry of Health), Jiangsu Provincial Center for Disease Control and Prevention, 172 JiangSu Road, Nanjing 210009, China
[b] Department of Pathogen Biology, Nanjing Medical University, Nanjing 211166, China
[c] Key Laboratory of Infectious Diseases, Nanjing Medical University, Nanjing 211166, China

ARTICLE INFO

Keywords:
Enterovirus A71
Caspase-1
Pyroptosis

ABSTRACT

Enterovirus A71 (EV-A71) infection can cause hand, foot and mouth disease (HFMD), and even fatal meningoencephalitis. Unfortunately, there is currently no effective treatment for EV-A71 infection due to the lack of understanding of the mechanism of neurological diseases. In this study, we employed SH-SY5Y human neuroblastoma cells to explore the roles of caspase-1 in neuropathogenesis. The expression and activity of caspase-1 were analyzed. The potential immuneconsequences mediated by caspase-1 including cell death, lysis, DNA degradation, and secretion of pro-inflammatory were also examined. We found the gene expression levels of caspase-1, IL-1β, IL-18 and active caspase-1 were markedly increased in the SH-SY5Y cells at 48 h post EV-A71 infection. The cell death, lysis, and DNA degradation were also increased during infection, which could be significantly alleviated by caspase-1 inhibition. These observations provided additional experimental evidence supporting caspase-1-mediated pyroptosis as a novel pathway of inflammatory programmed cell death.

1. Introduction

Hand, foot, and mouth disease (HFMD) is a common infectious enterovirus disease in young children, particularly in those less than seven years old (Lin et al., 2016). Among the enteroviruses, enterovirus A71 (EV-A71) and coxsackievirus A16 (CA16) are the two main causative agents of HFMD (Wang et al., 2014). EV-A71 and CA16 infections manifest the clinically similar lesions on the skin and oral mucosa, but serious neurological complications (such as encephalitis, aseptic meningitis, and poliomyelitis-like paralysis) almost always occur in EV-A71 infection, rarely in CA16 infection (Cui et al., 2010; McMinn et al., 2001; Yogarajah et al., 2017b). The neurological complications of EV-A71 infection may cause permanent paralysis or death. Although many studies suggest that fatalities associated with EV-A71 infection are the result of central nervous system (CNS) inflammation characterized by inflammatory cell infiltration and activation, cytokine overproduction, and neuronal viral cytolysis (Chang et al., 2015; Chen et al., 2004; Jiang et al., 2012; Koroleva et al., 2014; Lin et al., 2015; McMinn et al., 2001; Wang et al., 2003; Yao et al., 2012; Zhang et al., 2012), the molecular mechanisms underlying EV-A71 infection associated neuroinflammation and neurodegeneration remain unclear. In order to develop effective antiviral therapies against EV-A71, it is important to understand the pathogenesis of EV-A71 infection.

Current evidences suggest necrosis, programmed cell death such as apoptosis and autophagy might play an important role in neuropathologic change during EV-A71 infection. However, blocking apoptosis and autophagy could not completely inhibit cytopathic effect (CPE) which suggested that EV-A71 infection might cause some other forms of cell death (Lee et al., 2014; Lin et al., 2015; Xi et al., 2013; Zhang et al., 2014). Recently pyroptosis has been found to be a novel form of programmed cell death, which is characterized by caspase-1 activation, DNA breakages without laddering, cell swelling, plasma membrane rupture and release of intracellular contents of pro-inflammatory cytokines (Adamczak et al., 2014; Bergsbaken et al., 2009). Caspase-1 was firstly recognized as a protease that processed the inactive precursors of IL-1β and IL-18 into mature inflammatory cytokines (Fantuzzi and Dinarello, 1999). In addition, the release of pro-inflammatory cytokines and DNA fragmentation mediated by caspase-1 would eventually result

* Corresponding author at: Department of Pathogen Biology, Nanjing Medical University, Nanjing 211166, China.
** Corresponding author at: Key Laboratory of Infectious Diseases, Nanjing Medical University, Nanjing 211166, China.
E-mail addresses: jiminjun@njmu.edu.cn (M. Ji), lbcui@jscdc.cn (L. Cui).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.virol.2018.05.025
Received 13 February 2018; Received in revised form 4 May 2018; Accepted 29 May 2018

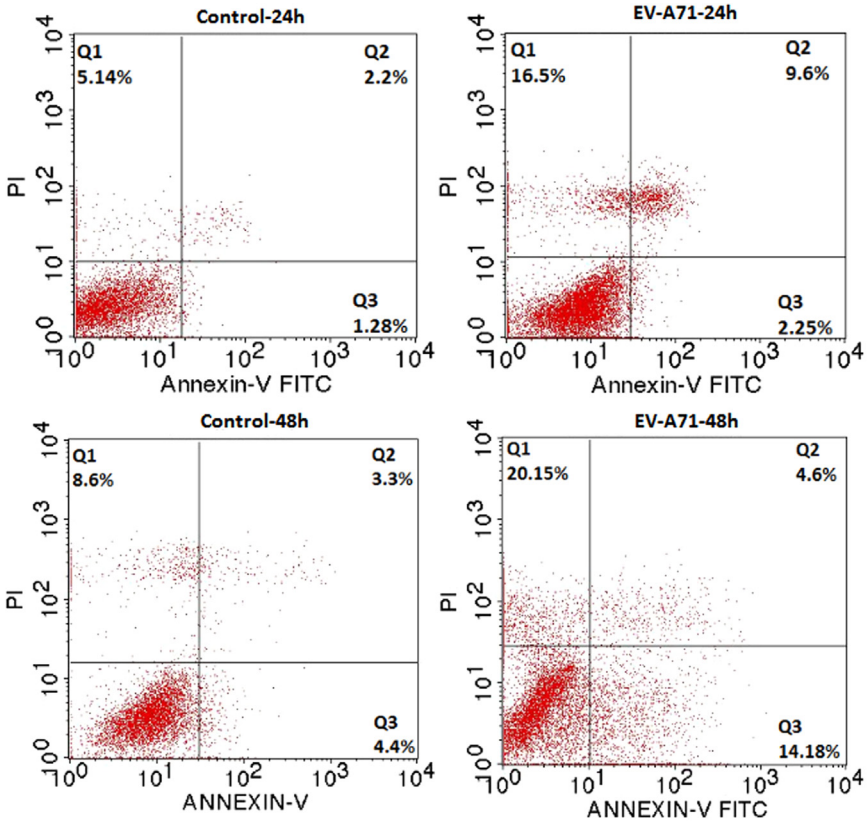

Fig. 1. Flow cytometric analysis of Annexin-V-FITC (fluorescein isothiocyanate) and propidium iodide (PI) staining in SH-SY5Y cells. The numbers in each image reflect the percentage of the cells in each quadrant. Among which, Q1: other forms of cell death (Annexin-V-FITC)-/PI +); Q2: the late apoptotic/necrotic cells (Annexin-V-FITC) + /PI +); Q3: the early apoptotic cells (Annexin-V-FITC) + /PI-). The significantly increased number of positive cells stained with only PI (Q1) indicating other forms of cell death (Q1) such as pyroptosis occurred.

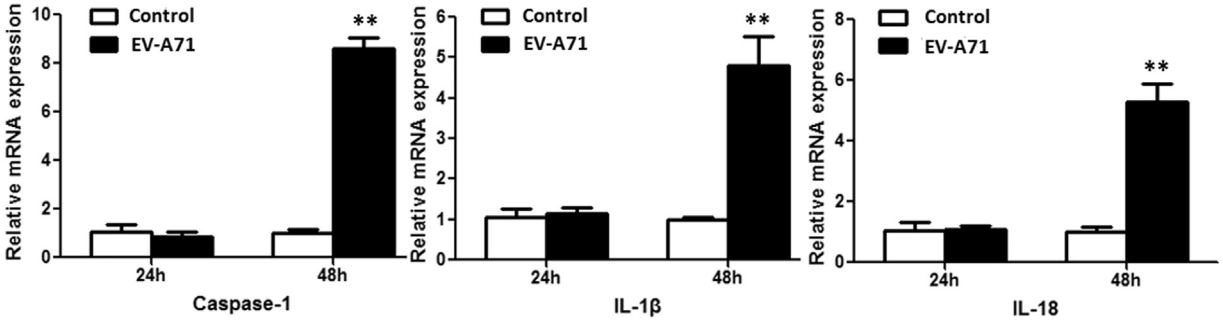

Fig. 2. Real-time PCR detection of gene expression levels in SH-SY5Y cells. Expression levels of target genes were normalized to the mRNA level of β-Actin. EV-A71 infection increased the gene expression of caspase-1, IL-1β and IL-18 at 48 h post infection compared with uninfected cells. Values represent the mean of triplicate samples ± SD. * * indicates $P < 0.01$.

in cell osmotic lysis and induce pyroptosis. An increasing number of studies have revealed that numerous viral infections, including dengue virus, hepatitis C virus, and human immunodeficiency virus, are closely associated with pyroptosis in their pathogenic processes (Doitsh et al., 2014; Kofahi et al., 2016; Tan and Chu, 2013). Recent studies have revealed EV-A71 may activate NLRP3 and AIM2 (absent in melanoma 2) inflammasomes in various cells and mice (Wang et al., 2015, 2018; Yogarajah et al., 2017a) indicating pyroptosis might be involved in the pathogenesis of EV-A71 infection. However, whether pyroptosis participates in EV-A71-induced neuronal cell death has not been clarified. In this study, using EV-A71 infected human neuroblastoma cells model, we investigated the role of pyroptosis in neuronal pathological alterationduring EV-A71 infection.

2. Materials and methods

2.1. Cell

The SH-SY5Y human neuroblastoma cells were purchased from the Cell Resources Center of Shanghai Institute of Life Science, Chinese Academy of Sciences (Shanghai, China). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gabico, USA) supplemented with 10% fetal bovine serum (FBS) (Gabico, USA) and 1% penicillin/streptomycin (Gabico, USA). The SH-SY5Y cells were cultured at 37 °C in a humidified atmosphere of air containing 5% CO_2.

2.2. Virus and virus infection

The EV-A71 strain 2008-43-16 (Genbank accession no. KP266572) which was isolated from a child who suffered HFMD accompanying encephalitis was used in this study. Viruses were amplified in rhabdomyosarcoma (RD) cells and identified through quantitative real-time