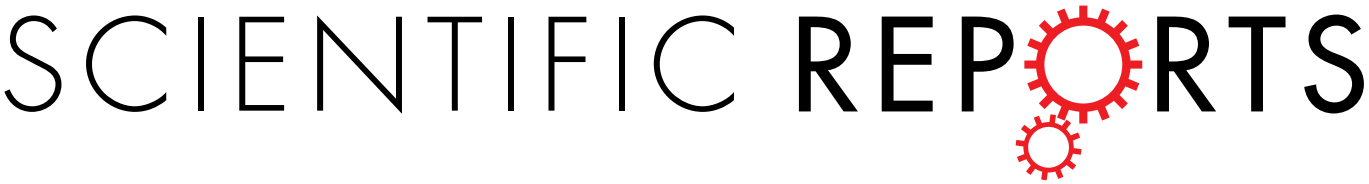

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AIM2 Inflammasome-Mediated Pyroptosis in Enterovirus A71-Infected Neuronal Cells Restricts Viral Replication

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Encephalomyelitis is a well-known complication of hand, foot, and mouth disease (HFMD) due to Enterovirus 71 (EV71) infection. Viral RNA/antigens could be detected in the central nervous system (CNS) neurons in fatal encephalomyelitis but the mechanisms of neuronal cell death is not clearly understood. We investigated the role of absent in melanoma 2 (AIM2) inflammasome in neuronal cell death, and its relationship to viral replication. Our transcriptomic analysis, RT-qPCR, Western blot, immunofluorescence and flow cytometry studies consistently showed AIM2 gene up-regulation and protein expression in EV-A71-infected SK-N-SH cells. Downstream AIM2-induced genes, CARD16, caspase-1 and IL-1β were also up-regulated and caspase-1 was activated to form cleaved caspase-1 p20 subunits. As evidenced by 7-AAD positivity, pyroptosis was confirmed in infected cells. Overall, these findings have a strong correlation with decreases in viral titers, copy numbers and proteins, and reduced proportions of infected cells. AIM2 and viral antigens were detected by immunohistochemistry in infected neurons in inflamed areas of the CNS in EV-A71 encephalomyelitis. In infected AIM2-knockdown cells, AIM2 and related downstream gene expressions, and pyroptosis were suppressed, resulting in significantly increased virus infection. These results support the notion that AIM2 inflammasome-mediated pyroptosis is an important mechanism of neuronal cell death and it could play an important role in limiting EV-A71 replication.

Enterovirus A71 (EV-A71) is a human RNA virus that belongs to the species A group, *Enterovirus* genus and *Picornaviridae* family. The virion is about 30 nm and contains a single-stranded, positive-sense RNA genome of approximately 7.5 kb. EV-A71 causes sporadic and epidemic hand, foot and mouth disease (HFMD), a common infectious disease most frequently seen in young children aged 5 and below[1–3]. Since its initial isolation and identification in 1969[4], numerous large outbreaks of HFMD have been reported worldwide[5–13]. EV-A71-associated HFMD is occasionally associated with central nervous system (CNS) complications, such as aseptic meningitis, acute flaccid paralysis and encephalomyelitis[14–19]. Based on autopsy findings in fatal cases of EV-A71 encephalomyelitis, it is clear that CNS neurons are the main viral targets since neuronal degeneration/necrosis and neuronophagia were readily observed. Moreover, viral antigens and RNA localized almost exclusively to these cells[20,21]. Thus, viral-induced cell death or viral cytolysis in neurons plays a major role in neuropathogenesis[22,23].

Classically, neuronal cell death may result from apoptosis and necrosis[24]. Nonetheless, recent advances in understanding of cell death mechanisms suggest that apart from apoptosis, other complex mechanisms such as pyroptosis, autophagy and necroptosis may be involved in viral infection[25–28]. Even though both pyroptosis and necroptosis are programed cell death mechanisms and promote inflammation, these pathways differ in their initiators; pyroptosis is induced via inflammasomes and caspase-1 activation, while necroptosis involves receptor-interacting protein kinase 3[29]. Moreover, both mechanisms are distinct from autophagy that causes activation of microtubule-associated protein 1A/1B-light chain 3 and formation of autophagosomes. Studies have shown that EV-A71 infection can cause apoptosis in cell lines such as rhabdomyosarcoma, human neuroblastoma (SK-N-SH, SK-N-MC and SH-SY5Y) and human glioblastoma cells[30–34]. Specifically, protein expression

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