Title

RSAD2 and AIM2 modulate CV-A16 and EV-A71 replication in neuronal cells in different ways that may be associated with their 5' non-translated regions

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Running title: RSAD2, AIM2 and 5'NTR modulates viral replication

Abstract

Coxsackievirus A16 (CV-A16) and Enterovirus A71 (EV-A71) are closely related enteroviruses that cause the same hand, foot and mouth disease but neurological complications occur only very rarely in CV-A16 compared to EV-A71 infections. To elucidate host responses that may be able to explain these differences, we performed transcriptomic analysis and qRT-PCR in CV-A16 infected neuroblastoma cells (SK-N-SH) which showed that the radical s-adenosyl methionine domain containing 2 (RSAD2) was the highest up-regulated gene in the anti-microbial pathway. Increased RSAD2 expression was correlated with reduced viral replication while RSAD2 knockdown cells were correlated with increased replication. EV-A71 replication showed no apparent correlation to RSAD2 expressions. Absent in melanoma 2 (AIM2) which is associated with pyroptosis cell death was upregulated in EV-A71 infected neurons but not in CV-A16 infection, suggesting that the AIM2 inflammasome played a significant role in suppressing EV-A71 replication. Chimeric viruses derived from CV-A16 and EV-A71 but containing swapped 5' non-translated regions (5'NTR) showed that RSAD2 expression/viral replication and AIM2 expression/viral replication patterns may be linked to the 5'NTRs of parental viruses. Differences in secondary structure of internal ribosomal entry sites within the 5'NTR may be responsible for these findings. Overall, our results suggest that CV-A16 and EV-A71 elicit different host responses to infection, which may help explain the apparent lower incidence of CV-A16 associated neurovirulence in HFMD outbreaks compared to EV-A71 infection.

Importance

Although Coxsackievirus A16 (CV-A16) and Enterovirus A17 (EV-A71) both cause hand, foot and mouth disease, EV-A71 has emerged as a leading cause of non-polio, enteroviral fatal encephalomyelitis among young children. The significance of our research is in the identification of the possible differing and novel mechanisms of CV-A16 and EV-A71 inhibition in neuronal cells that may impact on viral neuropathogenesis. We further showed that viral 5'NTRs may play significant roles in eliciting different host response mechanisms.

Keywords

Coxsackievirus A16, Enterovirus 71, SK-N-SH, AIM2, RSAD2, 5' non-translated region

Introduction

Coxsackievirus 16 (CV-A16) and Enterovirus 71 (EV-A71) are human enteroviruses that belong to the *Enterovirus* genus, species A group, in the *Picornaviridae* family. These small non-enveloped, ~30 nm viruses, each has a positive-sense RNA genome of approximately 7.5kb. The RNA genome consists of a single open reading frame flanked by non-translated regions (NTR) at the 5' and 3' ends, and a variable length poly-A tail located at the 3'NTR (1). The 5'NTR consists of cloverleaf-like structures called internal ribosomal entry sites (IRES), which are involved in RNA replication, and are important internal initiators of translation. Highly conserved among human enteroviruses (2), the CV-A16 and EV-A71 5'NTRs have a nucleotide homology of 84% (3). Both their genomes contain genes VP1-VP4 that encode for structural capsid proteins and genes 2A–3D that encode for non-structural proteins (4).

Both CV-A16 and EV-A71 cause the same sporadic and epidemic hand, foot and mouth disease (HFMD), commonly seen in young children. Nonetheless, HFMD due to CV-A16 is far less frequently associated with central nervous system (CNS) complications than EV-A71, although some cases of aseptic meningitis, encephalitis and rhombencephalitis have been reported (5-7). Our previous *in-vitro* study (8) and another study by Chan *et al* (9) have shown that CV-A16 could infect human neuroblastoma cell lines. Neuronal infection and replication in a mouse model of CV-A16 infection have also been demonstrated (10-12). In contrast, neurological complications following HFMD due to EV-A71 is well known and well documented (13-18).

The observed difference in neurovirulence may be due to genomic differences between CV-A16 and EV-A71 in the 5'NTR, analogous to another enterovirus, poliovirus (19). Studies have shown that point mutations in the 5'NTR IRES of poliovirus (102/103 nucleotides), affected viral replication in neuronal cells and infectivity in mice (20-23). A mutation at the 148 nucleotide of the