Determining the Unique Cellular Targets of Enterovirus-D68 Proteases 3C and 2A

Noah A. Pollack[1], Michael Wagner[1], and William T. Jackson[1]
(1) University of Maryland Baltimore, Baltimore MD

Introduction

Enterovirus-D68 (EV-D68) is a member of the enterovirus family and as such is a non-enveloped single stranded positive sense RNA virus. The viral RNA encodes for a single polyprotein that is then proteolytically cleaved by the viral proteases 3C and 2A resulting in 4 structural and 7 non-structural proteins. Some of the non-structural proteins can remain and have roles in intermediate roles before cleavage. These include 2BC and 3CD. 2A and 3C of polio virus have been shown to cleave various cellular proteins including SQSTM1 and innate immune proteins. More recently, further evidence suggests EV-D68 3C cleaves the cellular proteins related to autophagy SQSTM1 and SNAP29. However, little has been studied regarding the cellular targets of EV-D68 2A. Many of the nuances of the viral replication process are still being studied, however, it is known that the cellular autophagy machinery is required for proper viral replication. Studies demonstrate that double membrane vesicles, with hallmarks of autophagosomes, are formed in cells infected with PV. Previous work has identified cellular proteins critical to viral induction and utilization of the autophagy machinery, but little work has been done to investigate the role of viral proteins. Evidence suggests the viral proteases play a role in regulation of cellular autophagy during infection. EV-D68 infection induces cellular autophagy, however the produced autophagosomes do not fuse with the lysosome. We propose that EV-D68 viral proteases 3C and 2A cleave specific cellular proteins to regulate the induced autophagic machinery and prevent fusion with the lysosome.

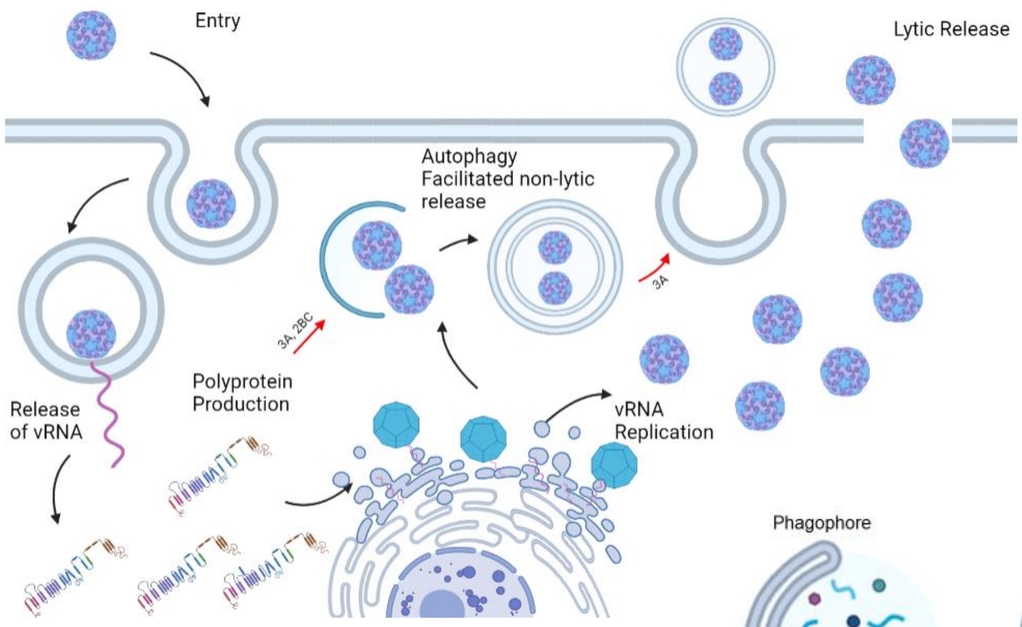

Figure 1. Schematic of EV-D68 replication cycle. Made with Biorender

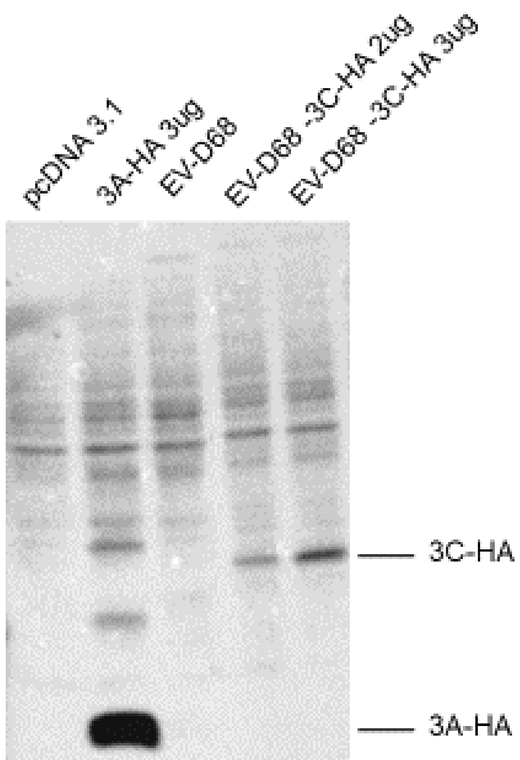

Figure 3. Expression of HA-tagged EV-D68 3C. HeLa cells were transfected with either 2ug or 3ug of the labeled expression vector using lipofectamine 2000 or infected with EV-D68 at MOI=20. Western blot was stained with anti-HA (Cell signaling technologies)

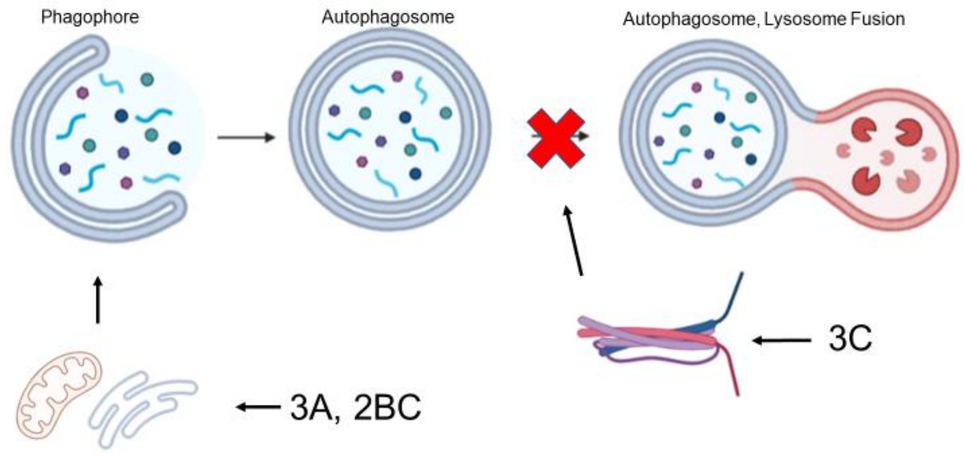

Figure 2. Schematic of proposed EV-D68 viral regulation of autophagy. Made with Biorender

Expression and Validation of EV-D68 3C-HA

To investigate the individual cellular proteins related to autophagy EV-D68 3C cleaves, we generated expression vectors containing either a wild type or HA epitope tagged 3C protein (20kDA). The generation of an epitope-tagged construct was necessary to detect protein expression due to a lack of commercially available antibodies against EV-D68 3C. To validate the protein expression of the 3C-HA plasmid, HeLa cells were transfected to visualize a dose dependent increase of protein expression. To control for HA antibody staining a previously validated EV-D68 3A-HA construct (10kDa) was transfected. Staining of the blot revealed firstly a strong band at 10kDa indicative of strong 3A-HA expression as expected (Figure 2). There were also two distinct 20kDa bands present in the lanes corresponding to 3C-HA transfection (Figure 2). These results strongly support that the generated EV-D68 3C-HA constructs express in HeLa cells.

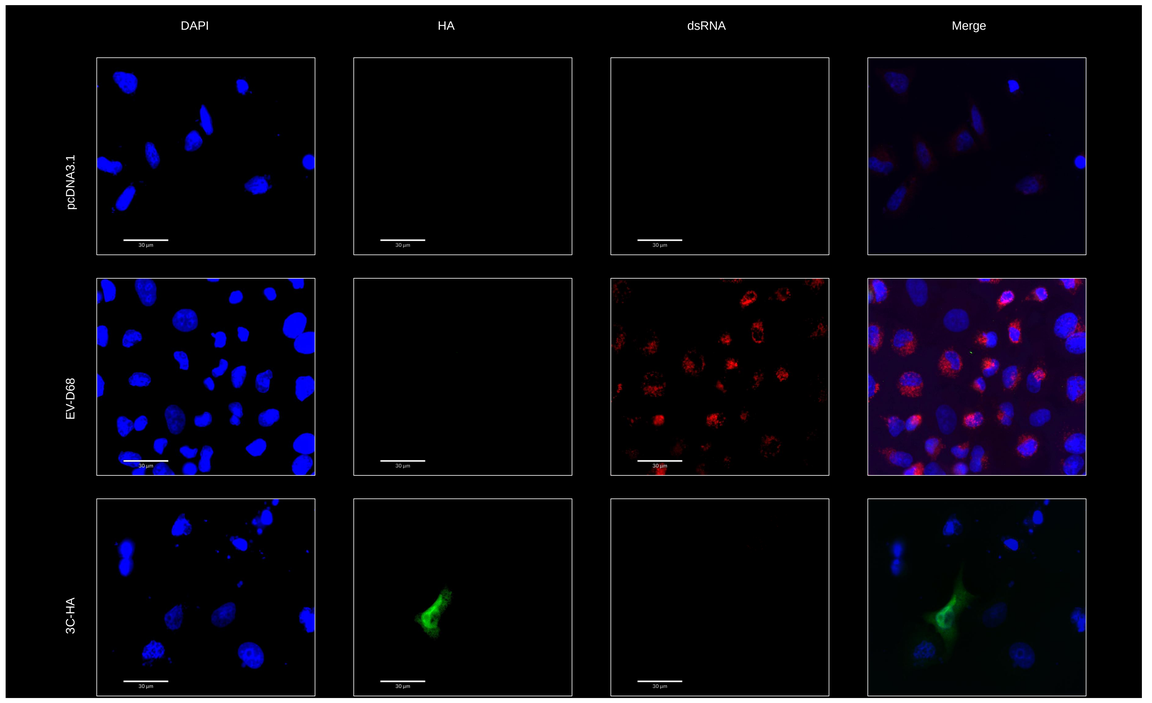

Figure 4. Localization of EV-D68 3C-HA expression in HeLa cells. HeLa cells were either transfected with 3ug DNA or infected with EV-D68 MOI=20. Cells were stained for DNA (blue), double stranded RNA (red), and HA (green).

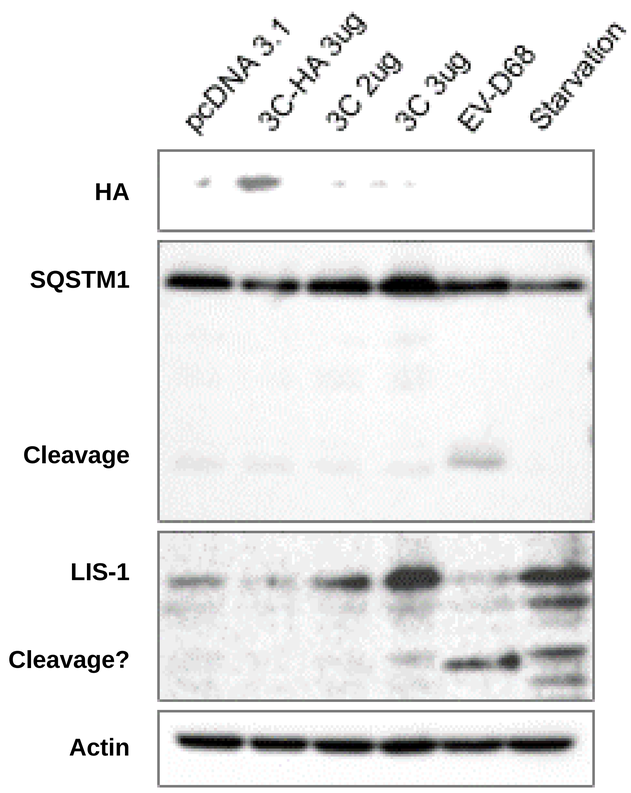

Figure 5. Expression of HA-tagged and untagged EV-D68 3C. HeLa cells were transfected with either 2ug or 3ug of the labeled expression vector using lipofectamine 2000 or infected with EV-D68 at MOI=20. Starved cells were deprived of essential amino acids for four hours Western blot was stained with anti-HA (Cell signaling technologies)

Conclusions

- EV-D68 Protein was cloned into pcDNA3.1
- Expression of 3C-HA was confirmed
- Expressed EV-D68 3C-HA localizes to the cytoplasm in HeLa cells
- Expressed 3C-HA is catalytically active and cleaves SQSTM1 and Lis-1

Acknowledgements

- Jackson Lab (https://www.picorna.com/)
- University of Maryland, Baltimore School of Medicine
- This work was funded by the NIH grant RO1-AI104928