Enterovirus A71 Triggers Autophagosome-lysosome Fusion to Facilitate Viral Replication

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Background/Objective
Autophagy is a cellular process which allows degradation of cellular components by lysosomes. Viruses have evolved unique strategies to evade or subvert autophagy machinery. Some viruses inhibit autophagy induction while others utilize autophagy to promote their replication and virion maturation. In enterovirus A71 (EV-A71) infection, the autophagy machinery mediates the production of the virus in vitro and in vivo. However, the role of autophagosome-lysosome fusion (a key step in late autophagy) during EV-A71 infection remains unknown.

Method
Human rhabdomyosarcoma (RD), human embryonic kidney 293 (HEK-293), and neuroepithelioma (SK-N-MC) cells were infected with EV-A71. We monitored LC3 modification, a marker of autophagy, in cells treated with inhibitors of vesicle acidification such as chloroquine, ammonium chloride and bafilomycin A1 that block autophagosome-lysosome fusion. siRNAs were also used to target factors involved in autophagosome-lysosome fusion, namely syntaxin-17, a SNARE complex mediating vesicular fusion, and LAMP1, the transmembrane protein of lysosome. The effects of these inhibitors and siRNAs on viral RNA replication, viral titer and viral proteins were assayed by Taqman qPCR, plaque assay and Western blotting, respectively. To identify the viral proteins of EV-A71 that induce autophagic maturation, the non-structural proteins 2B, 2C, 2BC, 3A and 3B were transfected into stable HEK-293/LC3 cells.

Result
We found that autophagosome-lysosome fusion was important for EV-A71 replication in all three cell lines. Blockage of autophagosome-lysosome fusion with inhibitors of vesicle acidification and siRNAs reduced virus titers, viral RNA and viral protein production. The 2BC non-structural protein of EV-A71, but not 2B or 2C alone, modulates autophagosome-lysosome fusion in transfected HEK-293 cells.

Conclusion
The autophagosome-lysosome fusion process in the late maturation stage of autophagy is important for EV-A71 replication.