Enterovirus subverts autophagy through cleavage of fusion adaptor proteins and selective autophagy receptors

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Background: Myocarditis is an inflammatory disease of the heart often caused by viral infection, particularly the enteroviruses, such as coxsackievirus B3 (CVB3). Autophagy, an evolutionarily-conserved intracellular degradation pathway, targets misfolded proteins, damaged organelles, and invading pathogens for lysosomal clearance. Although traditionally considered a non-selective degradative process, it's now clear that autophagy can mediate targeted clearance of protein aggregates/damaged organelles via selective autophagy receptors, which harbor highly conserved ubiquitin-associated and LC3 interacting domains. Contrary to previous understanding of autophagy as an anti-viral pathway, we and others have shown that the cellular autophagic machinery can be hijacked by enterovirus to disrupt its degradative capacity (or autophagic flux) and promote the accumulation of autophagosomes that serve as membrane scaffolds for viral replication. Moreover, we discovered that two selective autophagic receptors, namely p62/sequestosome 1 and neighbor of BRCA1 gene 1 (NBR1), are cleaved upon CVB3 infection, resulting in not only loss-of-function, but also the generation of dominant-negative fragments that further impair selective clearance of ubiquitinated protein aggregates. Despite these intriguing findings, the exact mechanism by which CVB3 inhibits autophagic flux and disrupts protein/organelle quality control is not fully understood. We hypothesize that CVB3 infection impairs the autophagic pathway through virus-encoded proteinases that specifically target autophagic proteins required for autophagosome-lysosome fusion and/or selective cargo recruitment, ultimately leading to cardiac dysfunction by facilitating viral replication and via preventing the clearance of toxic protein aggregates/damaged organelles.

Methods & Results: Our previous *in vivo* findings that CVB3-infected mouse hearts display an abnormal accumulation of autophagosomes and misfolded proteins/damaged mitochondria, and the in vitro evidence that CVB3 infection inhibits autophagic flux, suggest that the fusion process of autophagy is disrupted during infection. To delineate the possible mechanism involved, we focused on proteins previously reported to be involved in autophagosome fusion. Notably, we found that the autophagosomal SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptor) protein SNAP29 (synaptoxomal-associated protein 29) and the tethering protein PLEKHM1 (pleckstrin homology domain containing protein family member 1), two critical proteins known to regulate autophagosome-lysosome fusion, were cleaved upon CVB3 infection. Further *in vivo* (in cells transfected with protease constructs) and in vitro (using recombinant proteases) cleavage assays demonstrated that CVB3-encoded proteinase 3Cpro, not 2Apro or caspases, is responsible for these cleavages. Combining a bioinformatics approach with site-directed mutagenesis, we identified the cleavage sites on SNAP29 (Q161) and PLEKHM1 (Q668), respectively, leading to impaired SNARE complex formation. Moreover, we showed that gene-silencing of SNAP29 and PLEKHM1 inhibited autophagic flux, resulting in a significant increase in viral growth, likely due to enhanced accumulation of autophagosomes that provide sites for viral RNA replication and assembly. Finally, we also identified the autophagic receptor protein, NDP52 (nuclear domain 10 protein 52), as a bona fide substrate of viral proteinase 3Cpro. The cleavage of NDP52 takes place at Q139, separating the N-terminal LC3-interacting region from the C-terminal ubiquitin-binding domain. The functional significance of NDP52 cleavage is currently under investigation.

Conclusion: We identified a novel underlying mechanism by which enterovirus, through viral encoded proteinases, subverts the host autophagic pathway to promote viral propagation and cause cardiac damage. Our findings in this study provide strong evidence of a potential therapeutic benefit by targeting the autophagy-virus interface.

Speaker Biography

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