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Non-canonical ATG8 conjugation in ESCRT-driven membrane remodeling processes

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Abstract

ATG8 family proteins have the unique ability to conjugate to membrane lipids. Initially identified as a hallmark of autophagy, ATG8 lipidation is emerging as an important regulator of a growing list of non-degradative cellular functions. In this thesis we developed and applied novel chemical genetic approaches to perturb dynamic membrane remodeling processes and induce non-canonical ATG8 conjugation in cells. We investigated novel roles of ATG8 in membrane deformation processes carried out by the Endosomal Sorting Complex Required for Transport (ESCRT) machinery.

In **Paper I,** using a high-throughput phenotypic screening assay, we developed a collection of pseudo-natural product-based compounds which potently induce ATG8 lipidation in mammalian cells. The most potent compound, Tantalosin, induces ATG8 lipidation which is insensitive to simultaneous inhibition of autophagosome-lysosome fusion, suggesting a non-canonical function of Tantalosin-induced ATG8 conjugation.

In **Paper II** we investigated the molecular target of Tantalosin. We found that Tantalosin targets the ESCRT-III protein IST1 and inhibits IST1-CHMP1B copolymer formation. This inhibition results in the impairment of transferrin receptor (TfR) recycling resulting in the rapid accumulation of the receptor in early/sorting endosomes. At the same time, Tantalosin induces non-canonical ATG8 conjugation on stalled sorting endosomes containing TfR. This conjugation is dependent on the ATG16L1-ATG5-ATG12 complex which is recruited to stalled endosomes via ATG16L1-V-ATPase interaction.

In **Paper III** and **Paper IV** we studied the induction of non-canonical ATG8 lipidation in response to endolysosomal membrane damage. We used two established membrane damaging agents: *V. Cholerae* cytotoxin MakA and the lysosomotropic compound, LLOMe. In **Paper III** we demonstrated that, at low pH, MakA assembles into small pores in endosomal membranes which are recognized by the ESCRT membrane repair machinery. Non-canonical ATG8 lipidation in response to MakA-induced pore formation is mediated by V-ATPase activity. In **Paper IV** we identified a novel player in the lysosomal damage response – TECRP1. TECPR1 is recruited to damaged membranes where it forms an alternative ATG16L1-independent E3 ligase complex with the ATG5-ATG12 conjugate and plays a role in the restoration of lysosomal integrity after damage.

Keywords
ATG8 conjugation, Endosomal Sorting Complex Required for Transport, membrane remodeling, chemical genetics

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