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CELL DEATH

The single-membrane diet

> LC3–GFP was directly recruited to the entotic vacuoles independently of auto-phagosome formation.

Autophagy usually entails the recycling of cytoplasm and/or organelles that have been engulfed in double-membrane structures known as autophagosomes. Florey *et al.* show that elements of the autophagy machinery are also involved in a non-canonical degradation pathway, in which lysosomes are targeted to single-membrane vesicles.

Entosis is a mechanism whereby live cells are engulfed by their neighbours and, after an extended period, undergo non-apoptotic cell death, for example to prevent tumour growth. The authors investigated whether autophagy was involved in the death of these cells by following green fluorescent protein (GFP)-tagged light chain 3 (LC3; which is important for autophagosome formation) in cells undergoing entosis. LC3–GFP from the cytosolic pool localized to entotic vacuole membranes, and electron microscopy revealed that these structures had single membranes and no visible autophagosome contents. This suggested that LC3–GFP was directly recruited to the entotic vacuoles independently of autophagosome formation. Knockdown experiments showed that this recruitment depended on the autophagy pathway components VPS34 (also known as PIK3C3), autophagy 5 (ATG5) and ATG7 (which are essential for LC3 lipidation) but not on FIP200 (which is required for autophagosome formation).

Further work revealed that lysosomes bind and fuse with entotic vacuoles after LC3 recruitment, leading to non-apoptotic cell death, and that ATG5 is required in the host cell for these events to occur. Inhibition of autophagy proteins in MCF10A cells growing on soft agar decreased the level of entotic cell death and increased colony growth compared with control cells, a result that supports a role for entosis in preventing uncontrolled proliferation.

The authors saw that LC3 was also recruited to entotic vacuoles containing apoptotic cells, so they examined LC3 recruitment to the vesicles of macrophages, a type of phagocyte. They found that LC3 was recruited to single-membrane phagosomes containing apoptotic cell 'corpses', leading to their rapid degradation, and also to macropinocytic vacuoles. Again, this recruitment was dependent on ATG5, presumably for LC3 lipidation, but independent of autophagosome formation.

Finally, the authors confirmed their findings *in vivo* by following the LC3 homologue LGG-1 during *Caenorhabditis elegans* embryo development. LC3 localized to apoptotic cell phagosomes, and corpse degradation was defective when its recruitment was inhibited.

This study shows that autophagy proteins have a role in a non-canonical pathway involving the recruitment of LC3 to single-membrane vesicles for lysosome fusion. This seems particularly important for entotic cell death, which can prevent abnormal proliferation of matrix-deprived cells and may inhibit tumour formation. It will be interesting to see whether tumour cells actively prevent entotic cell death, perhaps through inhibition of LC3 recruitment to entotic vacuoles.

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ORIGINAL RESEARCH PAPER Florey, O. *et al.* Autophagy machinery mediates macroendocytic processing and entotic cell death by targeting single membranes. *Nature Cell Biol.* 16 Oct 2011 (doi:10.1038/ncb2363)