Super-resolution imaging of macroautophagy in astrocytes

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Autophagy is a regulated pathway ending in lysosomal degradation of intracellular material, including organelles. It maintains cell homeostasis and enables cell survival under stress. Astrocytes are a type of glial cells, providing homeostatic support in the brain. They are essential for neuron function and also participate in synaptic signalling. However, the exact role of autophagy in astrocytes in different conditions, including reactive gliosis and viral infections are not known. Studying macroautophagy is challenging because it is a multistep process, involving structures with dimensions below the optical resolution. Plasmid constructs that encode fluorescent-tagged LC3 (microtubule-associated protein 1A/1B-light chain 3), a marker of autophagosomal membranes, have been successfully used to distinguish autophagosomes and autolysosomes in single cells. In our experiments we tested the efficacy of plasmid construct encoding LC3 in evaluating autophagic activity of murine astrocytes. To discern the autophagic intermediates with dimensions below limits imposed by diffraction, we have used super-resolution structured illumination microscopy (SIM). Therefore, murine astrocytes in culture were transfected with LC3-coding plasmid constructs and treated with known autophagy inhibitors (putatively affecting different stages of autophagy) and/or activators. Astrocytes expressing the constructs were examined with SIM. To estimate autophagic activity, we determined the total number of autophagic compartments and the fractions of autophagosomes and autolysosomes in single astrocytes. The results show that astrocytes expressing fluorescent-tagged LC3 have responded in expected ways to known autophagy activators and inhibitors. We conclude that murine astrocytes expressing fluorescent-tagged LC3 in combination with SIM, represent a suitable approach for the future studies of autophagic activity in reactive astrocytes and in the presence of different flaviviruses.