A Molecular Reporter for Monitoring Autophagic Flux in Nervous System In Vivo

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Resumen

The relevance of autophagy in neuronal health has been extensively reported in a plethora of conditions affecting the nervous system, such as neurodegenerative diseases, cancer, diabetes, and tissue injury, where altered autophagic activity may contribute to the pathological process. Autophagy is a dynamic pathway involving the formation of a membrane surrounding and enclosing cargoes that are delivered to lysosomal compartments for degradation. Cargoes can include large protein aggregates, organelles, or even pathogens. Traditionally, autophagy assessment relies on the measurement of LC3-II protein levels or the visualization of LC3-positive puncta. However, these approaches represent a static measurement of autophagy markers, making difficult the dissection of the actual changes in the autophagy process (activation, inhibition, or no effects), due to the dynamic regulation of LC3 viral levels. To circumvent this limitation, we previously developed an adeno-associated vector (AAV) to deliver a molecular autophagy sensor to the neuronal compartment in vivo. Here, we describe the detailed design and methods to use an engineered AAV harboring the monomeric tandem mCherry-GFP-LC3 to determine autophagic fluxes in the nervous system. Key methodological details to succeed in the use of this reporter are provided.

Palabras clave

KeyWords Plus:TRAUMATIC BRAIN-INJURY; ADENOASSOCIATED VIRUS; SPINAL-CORD; GENE DELIVERY; INTRACEREBROVENTRICULAR INJECTION; NEURODEGENERATIVE DISEASES; TRANSGENE EXPRESSION; HYPERTENSIVE-RATS; AAV VECTORS; MICE

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