Biotin Labeling of Proximal Proteins of ATG2A in Human Cells

Junnan MA, 47206339

Abstract

Autophagy is a dynamic intracellular catabolic system widely conserved among eukaryotes that allows cells to maintain homeostasis. The hallmark of it is the biogenesis of a double-membrane organelle termed an autophagosome which packages intracellular materials and deliver them to lysosome in mammalian cells or vacuoles in plant and yeast cells for degradation.

Identification of Atg (autophagy-related) genes and proteins set up the stage of unveiling the rules governing autophagosome biogenesis. Among the more than 20 identified Atg proteins, Atg2 plays an essential role in the process of phagophore expansion and autophagosome formation.

Atg2 mutant was first discovered in yeast seminal autophagy screens in 1993 (Tsukada et al., 1993). But it was not until recently its precise role as membrane tether, lipid transporter and apoptosis executor was established (Chowdhury et al., 2018; Matoba et al., 2020; Tang et al., 2017). Human ATG2A is one of the two orthologs of yeast Atg2. It mediates the phagophore-endoplasmic reticulum (ER) association and interacts with WIPI4, TOM40 and GABARAP family proteins (Chowdhury et al., 2018; Tang et al., 2019; Kotani et al., 2018). Moreover, it cooperates with ATG9 to transfer phospholipids for phagophore expansion like yeast Atg2 (Matoba et al., 2020; Nishimura et al., 2020).

However, most of the above ATG2A related protein interaction studies were performed in vitro and they are only a drop in the bucket because there are approximately 2~4 million proteins per cubic micron in bacteria, yeast and mammalian cells.

As a result, this research aims to employ a new method termed Proximity-dependent Biotin Labeling to promiscuously label potential interacting partners of ATG2A in vivo in the process

of its translocation to phagophore formation sites, rooting to the phagophore membrane terminus, and mediating the phagophore-ER association.

This method relies on the labeling of neighboring proteins of a protein of interest with biotin utilizing biotin ligases such as engineered ascorbate peroxidase (APEX2) and *E. coli* biotin ligase TurboID (Lam et al., 2015; Branon et al., 2018). Biotin is a water-soluble natural coenzyme that strongly bind avidin or streptavidin. This high affinity of biotin for avidin and streptavidin enables the capture of weak or transient, as well as soluble and insoluble protein interactions.

In this research, I constructed the pEGFP-hATG2A-APEX2 and transformed it to HEK293 cells, followed by treating the transfected cells with 2.5mM biotin-phenol and 0.5mM hydrogen peroxide. Subsequently, I harvested the cells and prepared cell lysates to perform Western Blotting using horseradish peroxidase conjugated streptavidin. Compared with cell lysates from cells transfected with pEGFP-C1-hATG2A, those from pEGFP-hATG2A-APEX2 expressing cells showed multiple bands. Consequently, I recognized 17 typical candidates of interacting partners of ATG2A (Figure).

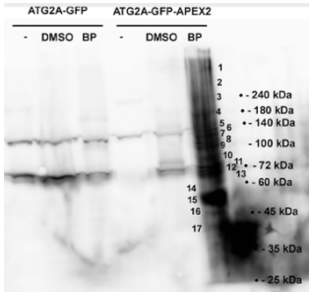

Figure. Western Blotting recognized 17 candidates of ATG2A interacting partners

Among these 17 biotin labeled proteins, I will confirm the existance of the formerly published WIPI4, TOM40, GABARAP and ATG9 by performing Western Blotting using antibodies of them. Then I will purify these biotinylated proteins utilizing streptavidin beads and identify new interacting partners of ATG2A by the method of Immunoprecipitation and Mass Spectrometry.