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Autophagy enables retromer-dependent plasma membrane translocation of SLC2A1/GLUT1 to enhance glucose uptake

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Macroautophagy sustains core metabolic functions during starvation or stress by promoting intracellular catabolism and nutrient recycling. In addition to salvaging metabolic intermediates, autophagy has been linked to the control of diverse metabolic pathways, including glucose metabolism. During oncogenic transformation, the genetic deletion of multiple different autophagy-related (*ATG*) genes all result in attenuated glycolytic flux and anchorage-independent growth. However, until now, the precise mechanisms through which the core autophagy machinery facilitates glycolysis have remained unclear. To rigorously dissect the role of autophagy in glucose metabolism, we investigated 2 models distinguished by their high rates of glycolysis and increased levels of autophagy—$HRAS^{G12V}$ oncogene-transformed cells and nontransformed cells undergoing hypoxia. We uncovered that autophagy deficiency, achieved via the genetic ablation of *ATG5* or *ATG7*, significantly compromises glucose uptake and glycolytic flux in both $HRAS^{G12V}$-transformed and hypoxic cells.

SLC2A1/GLUT1, a member of the SLC2 family of transmembrane glucose transporters, has wide tissue distribution and is overexpressed in diverse tumor tissues. The kinetics of SLC2A1 trafficking i.e., its internalization from the plasma membrane surface, followed by its endosomal sorting and recycling back to the plasma membrane, is instrumental in maintaining glucose homeostasis. We performed confocal microscopy and flow cytometry studies to interrogate SLC2A1 expression. During $HRAS^{G12V}$ transformation and hypoxia, enhanced glucose uptake correlates with increased plasma membrane localization of SLC2A1 in autophagy-proficient cells. Importantly, the pharmacological SLC2A1 inhibitor, WZB117, significantly suppresses hypoxia-induced glucose uptake, underscoring the functional relevance of this specific glucose transporter in supporting autophagy-dependent glycolytic flux. To interrogate how autophagy affects SLC2A1 trafficking we performed pulse-chase assays, via flow cytometry, in cells overexpressing FLAG-SLC2A1. Whereas SLC2A1 internalization is unaffected by autophagy status, its recycling to the plasma membrane is significantly compromised in autophagy-deficient cells. Live cell imaging of cells expressing GFP-SLC2A1 corroborated that SLC2A1 is localized in tubulovesicular endosomes and migrates to the plasma membrane in response to acute glucose withdrawal. In contrast, upon genetic autophagy deletion, SLC2A1 is missorted into late endolysosomes, evidenced by the punctate colocalization of SLC2A1 with LAMP1 in immunofluorescence studies. Thus, a functional autophagy pathway is required for the efficient recycling of SLC2A1 from endosomes to the plasma membrane surface.

The trafficking of a growing number of membrane transporters, including SLC2A1, is mediated by the SNX-BAR retromer assembly, which is comprised of the core vacuolar sorting trimer (VPS26-VPS29-VPS35) in association with the sorting nexins, SNX1-SNX2 and SNX5-SNX6. The VPS26-VPS29-VPS35 trimer of the retromer complex mediates cargo binding and selectivity. RNAi depletion of VPS35 significantly attenuates SLC2A1 trafficking to the plasma membrane surface in autophagy-competent cells, with a concomitant increase in SLC2A1 localization within late endolysosomes. These results suggest that in the absence of a functional retromer complex, SLC2A1 cannot be trafficked back to the plasma membrane and is thereby redirected to the late endosomes. The integrity of the core retromer assembly and its recruitment to endosomal membranes are both crucial for its proper function. Immunoprecipitation studies indicated that the mutual interaction of VPS26, VPS29 and VPS35 is unaffected by autophagy status. In contrast, immunostaining showed that recruitment of these retromer components to endosomal membranes is significantly compromised in autophagy-deficient cells, indicating that the autophagy pathway is required for retromer localization at endosomes.

We next sought to understand the mechanism by which autophagy dictates retromer-dependent SLC2A1 trafficking. TBC1D5 is a RabGAP protein for RAB7A that functions as a key negative regulator of the retromer complex. Moreover, TBC1D5 can bind to both MAP1LC3 and GABARAP family proteins via a canonical LC3-interacting region and has been reported to relocalize from the retromer complex to $MAP1LC3^{+}$ autophagosomes during saline starvation.

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We uncovered that glucose withdrawal significantly reduces TBC1D5 interactions with VPS35, while concomitantly increasing TBC1D5-MAP1LC3 complex formation in autophagy-competent cells. In contrast, the TBC1D5-VPS35 molecular interaction is still maintained in glucose-starved autophagy-deficient cells. Interestingly, steady state protein levels of TBC1D5 are unaffected by glucose withdrawal or autophagy inhibition, suggesting this RabGAP is not significantly degraded via autophagy. Rather, autophagosome formation during metabolic stress appears to shuttle TBC1D5 away from the retromer complex toward MAP1LC3^{+} compartments. We next confirmed the functional importance of autophagy-dependent TBC1D5 shuttling for retromer function and glucose uptake. RNAi-mediated TBC1D5 depletion in autophagy-deficient cells restores retromer recruitment to endosomal membranes and rescues the exocytic recycling of SLC2A1 to the plasma membrane. Taken together, we propose that in response to stresses that augment glycolytic demand or reduce extracellular glucose availability, autophagy induction promotes the relocation of TBC1D5 to MAP1LC3^{+} autophagosomes, thereby relieving its inhibition of the retromer and promoting SLC2A1 trafficking to the plasma membrane.

In summary, our findings reveal a novel interconnection between autophagy and the early endosomal pathway that dictates both SLC2A1 trafficking and extracellular glucose uptake. Notably, in addition to SLC2A1, autophagy deficiency prevents the surface expression of SLC16A1/MCT1, a lactate transporter and retromer-dependent cargo, in hypoxic cells. Because a plethora of nutrient transporters and cell surface receptors undergo retromer-dependent trafficking, moving forward it will be important to identify the larger repertoire of retromer-dependent cargoes whose cell surface expression is modulated by autophagy status. Identification of such targets will broaden our understanding of autophagy in the control of extracellular nutrient uptake and export in response to diverse metabolic stresses.

Disclosure of potential conflicts of interest

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