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Autophagy: Identification of MTMR5 as a neuron-enriched suppressor

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Abstract

A puzzle of autophagy in neurons is that, unlike in other cells, it is not robustly induced by inhibition of mammalian target of rapamycin (mTOR). A new study now solves this conundrum and establishes that myotubularin-related phosphatase 5 limits the induction of neuronal autophagy by mTOR inhibitors.

Macroautophagy (hereafter referred to as autophagy) captures cytoplasmic cargo into autophagosomes for clearance in lysosomes[1]. This pathway is critically important for neuronal function and survival. In fact, knockout of key autophagy genes causes neurodegeneration in mice, and mutations in autophagy genes are linked to neurodegenerative disorders in humans[2,3]. Moreover, proteins that are prone to aggregation in neurodegenerative diseases are substrates for autophagy, sparking strong interest in autophagy as a therapeutic target[2]. Thus, a key question in the field is: how can autophagy in neurons be manipulated to enhance clearance of protein aggregates and promote neuronal viability in neurodegenerative disease? Conventional methods of inducing autophagy have limited effects in neurons compared with non-neuronal cells such as astrocytes[4–7]. However, the factors that confer resistance to these autophagy inducers in neurons have remained unknown. In a new study published in this issue of *Current Biology*, Chua *et al.*[8] solve this conundrum and identify myotubularin-related phosphatase 5 (MTMR5) as an autophagy suppressor that is enriched in neurons (Figure 1A,B).

To study how autophagy is regulated in neurons compared with non-neuronal cells, Chua *et al.*[8] used an induced pluripotent stem cell (iPSC) line edited with CRISPR–Cas9 to tag endogenous LC3, a marker for autophagic organelles, with EGFP at the amino terminus. These iPSCs were then differentiated into glutamatergic forebrain-like neurons (iNeurons), astrocytes (iAstrocytes), or skeletal muscle cells (iMuscle cells), and autophagy levels were compared across these cell types in response to pharmacological inhibition of mammalian target of rapamycin (mTOR) with Torin1. mTOR is a serine/threonine kinase of the phosphatidylinositol 3-kinase (PI3K)-related kinase family that negatively regulates

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autophagy by phosphorylating and suppressing the function of various targets, including ULK1 and ATG13, components of the ULK1 complex, which initiate autophagosome formation, and TFEB, a transcription factor that activates the expression of autophagy and lysosomal genes[9]. Inhibition of mTOR prevents these inhibitory phosphorylation events, which induces autophagy in various cell types, but has surprisingly only modest effects in neurons[5,6,10]. Indeed, Chua *et al.*[8] found that Torin1 robustly increased the number of autophagosomes in iAstrocytes, iMuscle cells, and the parental iPSC line, but not in iNeurons (Figure 1B). Torin1 effectively reduced mTOR activity in iNeurons, as evidenced by reduced phosphorylation of downstream targets (such as S6 and 4E-BP1, factors that promote protein synthesis), but was not sufficient to stimulate autophagosome formation. Thus, unlike iPSCs, iAstrocytes, and iMuscle cells, iNeurons are resistant to autophagy induction by mTOR inhibition, indicating the existence of neuron-specific mechanisms for regulating autophagy.

To elucidate the molecular determinants regulating autophagy in neurons, Chua *et al.*[8] compared the rates of synthesis and turnover of mRNA transcripts in iNeurons, parental iPSCs and fibroblasts. They found that the transcript *SBF1*, encoding the protein MTMR5, was synthesized at similar rates but had greater stability in iNeurons than in fibroblasts and undifferentiated iPSCs. Measurements of *SBF1* mRNA and MTMR5 protein confirmed fivefold higher expression levels in iNeurons compared with the parental iPSC line. MTMR5 is a member of the MTMR family of phosphatases, which remove the 3-phosphate from membrane phosphoinositides, thereby converting phosphatidylinositol 3-phosphate (PI3P) and PI(3,5)P_2 into PI and PI5P, respectively[11]. Since PI3P serves as a platform for the assembly of autophagy initiation complexes[1], MTMRs can function as autophagy suppressors[12,13]. MTMR5 is catalytically inactive as a phosphatase, but dimerizes with and enhances the phosphatase activity of MTMR2[14]. Accordingly, Chua *et al.*[8] find that *MTMR2* transcript expression is also enriched in iNeurons compared with the parent iPSCs. Thus, MTRMR5–MTMR2 heterodimers emerge as a candidate that might restrict the induction of autophagy by mTOR inhibition in neurons.

Strikingly, modulation of MTMR5 expression can tune autophagy levels. Overexpression of MTMR5 in iPSCs attenuated the induction of autophagy by Torin1. Thus, MTMR5 is sufficient to suppress autophagy and increase resistance to Torin1-mediated induction of autophagy in iPSCs, similar to iNeurons. Conversely, knockdown of MTMR5 (or its binding partner MTMR2) in iNeurons unlocked a sensitivity to Torin1, enabling iNeurons to increase autophagy in response to Torin1 (Figure 1A). This effect required the class III PI3K VPS34, which phosphorylates PI to generate PI3P, a phospholipid important for autophagosome biogenesis. PI3P levels would be reduced by high levels of MTMR5–MTMR2 heterodimers[15].

To further define the effects of MTMR5 and MTMR2 knockdown on autophagy induction in iNeurons by Torin1, Chua *et al.*[8] measured the levels of p62/SQSTM1, a receptor for selective autophagy that recruits ubiquitinated substrates to the autophagosome[16]. In this process, p62 is incorporated into autophagosomes and is degraded. The authors found that knockdown of MTMR5 or MTMR2 reduced steady-state levels of p62. Moreover, blocking lysosome function captured more p62 in the absence of MTMR5 or MTMR2 compared with

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the control. Thus, more p62 is routed to the lysosome for degradation in the absence of MTMR5 or MTMR2, indicating increased autophagic flux. These findings are consistent with mTOR inhibition more effectively inducing autophagy in neurons in the absence of MTMR5 or MTMR2.

Chua *et al.*[8] also investigated the effect of MTMR5 or MTMR2 knockdown on the turnover of TDP-43, an RNA-binding protein involved in the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. For this experiment, the authors fused TDP-43 to Dendra2, a photoconvertible tag used to optically label the TDP-43 population at the start of the experiment and track its lifetime. They found that knockdown of MTMR5 enhanced the turnover of TDP-43, an effect that could be blocked largely by inhibiting lysosome function. Thus, reducing MTMR5 stimulates proteolytic clearance of TDP-43. Similar effects were observed with knockdown of MTMR2. Interestingly, knockdown of MTMR5 was sufficient to achieve these effects under baseline conditions, and treatment with Torin1 did not further enhance the proteolytic degradation of TDP-43. Thus, the optical pulse labeling of autophagy substrates enabled higher sensitivity for discerning changes in autophagic flux.

Importantly, this study provides key insights into the mechanistic complexity underlying cell-type-specific regulation of autophagy in neurons compared with non-neuronal cells. The authors suggest that the regulation of autophagy is influenced by a cell-type-specific stoichiometry of inhibitory factors, including the MTMR5:MTMR2 ratio, antagonized by autophagy-promoting factors (Figure 1B). For example, the parental iPSC line has lower levels of *SBF1* and *MTMR2* transcripts, and their corresponding proteins, than iNeurons. The parental iPSC line also has higher levels of transcripts for autophagy-promoting factors (e.g. *TFEB*, *ATG5*, and *SQSTM1*). In combination, these features may enable iPSCs to be more sensitive to autophagy stimulation by Torin1. iAstrocytes, however, express moderate levels of MTMR5, yet are able to robustly activate autophagy in response to Torin1. One possible reason for this effect is that iAstrocytes express lower levels of MTMR2 mRNA and protein compared with iNeurons, and higher levels of autophagy-promoting factors compared with iNeurons. Interestingly, iMuscle cells express the most MTMR2 protein of the cell types examined, yet display the largest induction of autophagy with Torin1. This effect may be explained by the significantly lower expression levels of *SBF1* and MTMR5 in iMuscle cells compared with iNeurons, which would result in reduced activity of MTMR2. Thus, levels of both MTMR5 and MTMR2 appear to play a role in regulating autophagy in different cell types. Further complexity is revealed upon examination of iMotor Neurons. Unlike iNeurons, iMotor Neurons show induction of autophagy with Torin1, indicating distinct mechanisms for autophagy regulation that are specific to neuronal subtypes. Interestingly, iMotor Neurons express similar levels of *SBF1* as iNeurons. However, the expression of autophagy-promoting factors in iMotor Neurons is strikingly higher than in iNeurons. Thus, the relative stoichiometry of autophagy-promoting and -inhibitory factors may underlie the cell-type-specific sensitivities of autophagy induction to various stimuli. Moreover, the precise combination of factors contributing to this ratio is likely cell-type specific.

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Accumulating evidence indicates that autophagy serves a variety of functions in neurons depending on neuronal subtype and developmental stage. How do these findings from Chua *et al.*[8] relate to the diversity of functions for autophagy in neurons? The unique complement of autophagy-promoting versus -inhibitory factors across neuronal subtypes confers a differential sensitivity of autophagy to diverse stimuli. Moreover, these subtype-specific stoichiometries may render a differential susceptibility of neurons to the progression of neurodegenerative disease. For example, Chua *et al.*[8] suggest that autophagy-promoting factors such as TFEB may have a more central role in regulating autophagy in iMotor Neurons. In support of this model, Cunningham *et al.*[17] found that dysfunction of the autophagy–lysosomal pathway underlies motor neuron degeneration in models of ALS, and these phenotypes were partially caused by a defect in the nuclear import of TFEB. Autophagy can also be regulated by mTOR-independent pathways, such as calcineurin-mediated dephosphorylation of TFEB to induce nuclear translocation of TFEB[18]. But the contributions of these pathways to autophagic activity in neurons and different neuronal subtypes remain to be explored. Another outstanding question that emerges from this study is why is the regulation of autophagy in neurons so complex? It may be that the unique properties of neurons (such as their post-mitotic state and exceptionally long lifetime) require them to be more selective in the volume and identity of material to be degraded[19]. Furthermore, evidence suggests that neuronal proteostasis is regulated by crosstalk with neighboring astrocytes[20]. Future studies will need to elucidate the impact of astrocytes on the regulation of autophagy in neurons to control proteostasis in the brain. In sum, the study by Chua *et al.*[8] illuminates the complexity of regulating autophagy across cell types. This knowledge will help define more targeted therapeutic approaches to mitigate neurodegeneration by manipulating autophagy in a manner specific to neuron subtype. In this way, autophagy could be upregulated specifically in the selectively vulnerable neuronal populations characteristic of each neurodegenerative disease.

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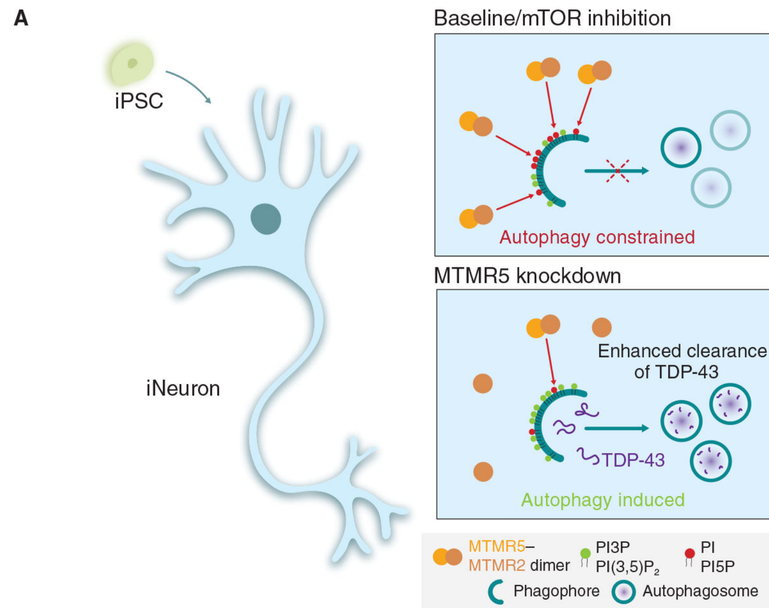

B Balance between inhibitory MTMR5:MTMR2 and autophagy-promoting factors by cell type:

	iNeuron	iPSC	iAstrocyte	iMuscle
Autophagy induction in response to mTOR inhibition	–	++	++	++++
MTMR5	+++++	+	++	+
MTMR2	++	+	+	+++++
Autophagy-promoting factors (*TFEB, ATG5, SQSTM1*)	+	++	+++	ND

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Figure 1. Autophagy regulation by MTMR5 and MTMR2 in iNeurons and other cell types.

(A) In iNeurons differentiated from iPSCs, MTMR5 and MTMR2 antagonize the formation of autophagosomes, thereby desensitizing iNeurons to mTOR inhibition. This suppression is a result of the formation of MTMR5–MTMR2 heterodimers that reduce PI3P levels, thereby preventing the assembly of autophagy-initiating factors (top panel). Reduction of MTMR5 expression allows for autophagy induction and enhanced clearance of cargoes such as TDP-43 (bottom panel). (B) Cell-type-specific stoichiometries between factors that inhibit and promote autophagy may account for the differential regulation of autophagy in response to mTOR inhibition in iNeurons compared with iPSCs, iAstrocytes and iMuscle cells. iNeurons do not upregulate autophagy in response to mTOR inhibition and have higher levels of MTMR5–MTMR2 and lower levels of autophagy-promoting factors compared with the other cell types examined. By contrast, iPSCs, iAstrocytes, and iMuscle cells induce autophagy in response to mTOR inhibition and this effect may be due to varying proportions of lower levels of autophagy-inhibitory factors relative to higher levels of autophagy-promoting factors. ND, not determined.