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Preview

New insights into the autophagy-NAD axis in brain disease

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Sun et al. demonstrate that defects in autophagy cause nicotinamide adenine dinucleotide (NAD) depletion and neurotoxicity.[1] Restoring NAD levels rescues cytotoxicity in autophagy-deficient neurons, providing a potential therapy for neurodegenerative and lysosomal storage diseases associated with autophagy defects.

Autophagy is involved in neuronal development and survival, as well as brain aging and disease. Neurons are more dependent on autophagy than other cell types because damaged organelles and misfolded proteins cannot be diluted through cell division. Several studies support the essential cytoprotective role of autophagy in the brain, which is most likely mediated by the selective clearance of toxic protein aggregates.[2,3] However, the mechanistic link between autophagy defects and brain disease remains poorly understood.

To address this question, Sun et al.[1] developed human stem cell-based platforms with mutations that block early or late stages of the autophagy-lysosome pathway. First, they generated autophagy-deficient human embryonic stem cells (hESCs) by knocking out *ATG5*, which is essential for autophagy induction. Previous studies have shown that inducible knockout of the key autophagy gene *ATG5* leads to the aggregation of a variety of misfolding-prone proteins and a neurodegenerative phenotype in mice.[2,3] Conversely, *ATG5* overexpression extends lifespan and is associated with anti-aging phenotypes and improved motor function in mice.[3] Interestingly, *ATG5* missense mutations cause a syndrome characterized by congenital ataxia, mental retardation, and developmental delay.[3] In the current study, $ATG5^{-/-}$ hESC-derived neurons showed increased apoptosis and elevated levels of cleaved caspase-3 and cytotoxicity. Unbiased metabolomic analysis revealed the depletion of metabolites related to glycolysis, the tricarboxylic acid cycle, nucleotides, and amino acids in autophagy-deficient neurons. Interestingly, NAD levels were also significantly decreased in these neurons.

NAD is a ubiquitous coenzyme involved in a variety of cellular processes, including bioenergetics, genomic stability, mitochondrial homeostasis, adaptive stress responses, and cell survival. Although NAD plays a key role in autophagy regulation, the mechanisms governing this process remain poorly understood.[4] Sun et al. showed that restoring intracellular NAD levels improved mitochondrial bioenergetics and viability in $ATG5^{-/-}$ neurons. In a previous study, the authors showed that this approach promoted survival in the induced pluripotent stem cell (iPSC)-derived neurons of patients with Niemann Pick type C1 (NPC1) disease, a neurodegenerative lysosomal storage disorder associated with autophagy defects.[5] Consistent with the current study, NPC1 iPSC-derived neurons exhibited decreased NAD levels. As NAD supplementation restores protein aggregation induced by autophagy depletion in $ATG5^{-/-}$ neurons, improving proteostasis may rely on ATG5-independent alternative macroautophagy.[6] While several therapeutic strategies aimed at modulating autophagy are currently being investigated for the treatment of neurodegenerative diseases, these findings suggest that NAD supplementation could prevent brain pathology by boosting alternative autophagy. NAD supplementation also improves mitochondrial proteostasis, which influences cytosolic aggregation-prone protein homeostasis.[7] Previous studies in a *C. elegans* model of Alzheimer's disease (AD) showed that knockdown of mitochondrial autophagy-related genes (*pink-1*, *pdr-1*, or *dct-1*) abrogates the benefits of NAD augmentation on behavioral deficits,[8] suggesting that NAD may modulate selective autophagy. A similar pivotal role for mitophagy has been shown in AD mice and AD patient-derived iPSCs.[8] Future studies should further address the mechanisms underlying the role of NAD in selective autophagy.

Because the loss of autophagy is associated with increased DNA damage and oxidative stress, the authors propose that the hyperactivation of NAD-consuming enzymes such as SIRTs and PARPs depletes NAD(H) in autophagy-deficient neurons. To test this hypothesis, the authors performed experiments based on the chemical inhibition of SIRTs and PARPs and showed that the chemical inhibition of SIRTs and PARPs rescued NAD(H) levels and viability in $ATG5^{-/-}$ neurons. Future work should examine the specific roles of distinct NADases in autophagy-deficient models.

In summary, the current study sheds light on a possible mechanistic link between autophagy deficiency and neuronal cell death and proposes NAD replenishment as a therapeutic intervention in brain disorders associated with autophagy defects. Recent studies have demonstrated that the administration of NAD boosters is safe and efficiently increases NAD levels in healthy volunteers.[9] While further

clinical studies are needed to determine the proper dose and the long-term effects of these approaches, additional questions should be addressed. Aging is accompanied by a decline in autophagic activity, as well as mitochondrial bioenergetics and NAD levels. Interestingly, NAD also regulates immune function in inflammatory conditions, and age-related chronic inflammation has been correlated with a decline in NAD levels.[10] While most studies have addressed autophagic mechanisms in neurons, it would be of great interest to determine how these mechanisms affect microglial function. Microglia play an important role in the clearance of misfolded proteins, and they contribute to the senescent phenotype in the aging brain. Answers to these questions will help researchers understand the cell-type-specific contributions of metabolic pathways to age and disease and better design tailored therapeutic strategies.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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