Mitophagy and NAD^+ inhibit Alzheimer's disease

Evandro F. Fang[1*]

Department of Clinical Molecular Biology, University of Oslo and Akershus University Hospital, 1478 Lørenskog, Norway

*Corresponding author**:** Evandro F. Fang (e.f.fang@medisin.uio.no)

Our latest publication on the inhibition of Alzheimer's disease (AD)[1] through mitophagy consolidates the 'Defective mitophagy hypothesis of AD etiology'[2]. AD affects over 45 million people worldwide and there is no effective treatment or cure. The disease leads to progressive loss of cognition, and pathological hallmarks of AD include aggregates of amyloid-β peptides extracellularly and microtubule-associated protein tau intracellularly[2, 3]. However, there is no conclusive link between these pathological markers and cognitive symptoms[3]. Anti-AD drug candidates have repeatedly failed, which led us to investigate other molecular etiologies to guide drug development[4]. Mitochondria, known as the 'powerhouse' of the cell, produce the majority of cellular ATP, affect Ca^{2+} and redox signaling, and promote developmental and synaptic plasticity[5, 6]. Mitochondrial dysfunction and accumulation of damaged mitochondria are common in brain tissues from AD patients and transgenic AD animal models, but the underlying molecular mechanisms are not fully understood[2, 7]. Damaged mitochondria are removed through multiple pathways, the major two being mitochondrial autophagy (mitophagy) and the ubiquitin proteasome pathway[8-10]. Mitophagy is essential for efficient clearance of damaged mitochondria to maintain mitochondrial homeostasis, ATP production, and neuronal activity and survival[8-10]. These pieces of evidence converge on the 'Defective mitophagy hypothesis of AD etiology', and the current cross-species study provides strong support for this hypothesis[1, 2].

The takeaway of our study is that neuronal mitophagy is indeed impaired in both AD patient samples and AD animal models. Compared with normal controls, postmortem hippocampal brain tissues from AD patients show an accumulation of damaged mitochondria as well as reduced mitophagy. The mechanism underlying this may be an impaired initiation of the mitophagic machinery, evinced by lower phosphorylated (S172)-TBK1 and lower phosphorylated (S555)-ULK1[1]. This mechanism is seen in both familial (APP) and sporadic (APOE4) AD patient iPSC-derived cortical neurons. For further mechanistic and interventional studies, we generated transgenic nematodes (*C. elegans*) expressing the DCT-1 mitophagy receptor fused with GFP together with the autophagosomal marker LGG-1 fused with DsRed in neurons of both the $A\beta_{1-42}$(CL2355) and the Tau(BR5270) strains. Both $A\beta_{1-42}$(CL2355) and Tau(BR5270) nematodes display defective energy metabolism, shown by a lower oxygen consumption rate and reduced mitophagy under normal and stress (paraquat treatment) conditions[1]. Furthermore, impaired mitochondrial function, accumulation of damaged mitochondria, and reduced mitophagy are shown in the hippocampal tissues of an Aβ-related APP/PS1 AD mouse model[1]. Collectively, these cross-species results unambiguously indicate impaired mitophagic machinery in AD.

If defective mitophagy is a contributing factor to AD, then restoration of mitophagy should inhibit disease phenotypes. We established an *in vivo* drug-screening platform using nematodes to identify potent neuronal mitophagy inducers. We have identified three potent neuronal mitophagy-inducing agents, the NAD^+ precursor nicotinamide mononucleotide (NMN)[6, 11], urolithin A (UA[12]), and actinonin (AC; an antibiotic that induces mitophagy through a specific mitochondrial ribosomal and RNA decay pathway[13]). UA and AC are robust mitophagy

inducers, both inducing expression of a wide spectrum of mitophagy/autophagy proteins, including full-length PINK1, Parkin, OPTN, p-ULK1, LC3B-II, Beclin1, Bcl2L13, AMBRA1, and FUNDC1[1]. Our genetic mutation studies in the $A\beta_{1-42}$(CL2355) nematodes show that UA- and AC-dependent memory improvement depends on PINK1 and PDR-1(the nematode orthologue of mammalian Parkin) but not DCT-1[1]. However, genetic mutation studies in the Tau(BR5270) AD nematodes indicate UA-induced memory improvement is dependent on both PINK1 and PDR-1, while AC-induced memory improvement is only dependent on PINK1[1]. Reasons in the difference in AC-dependent molecular pathways of mitophagy in Aβ and Tau nematodes are unclear so far, and further exploration of confounding factors is necessary.

How does NAD^+ induce mitophagy/autophagy? NAD^+-dependent inhibition of cognitive loss in AD is indeed dependent on mitophagy, as knockout of any of the three mitophagy genes (*pink1*, *pdr-1*, and *dct-1*) eliminates cognitive benefit in both the $A\beta_{1-42}$(CL2355) and the Tau(BR5270) AD nematodes[1]. Since NAD^+ is a co-factor for other important proteins, such as the sirtuins (SIRT1 to SIRT7), CD38, sterile alpha and TIR motif-containing 1 (SARM1), and Poly (ADP-ribose) polymerase (PARPs), there is a possibility of the involvement of different pathways (**Figure 1**). The NAD^+-dependent deacetylase SIRT1 can upregulate autophagy/mitophagy through multiple pathways: by deacetylating the autophagy machinery proteins Atg5, Atg7, and Atg8 (LC3)[14]; by increasing the expression of Beclin1, a key member of the autophagy initiation PI3K III nucleation complex[15]; by deacetylating LC3 at K49 and K51 in the nucleus, enabling its nucleocytoplasmic transportation and binds on Atg7 for autophagy[16]; and by increasing the expression of autophagic proteins Rab7, LC3, Atg12, BNIP3 through deacetylation and acetylation of the transcriptional factors FOXO1 and FOXO3[17-19]. Rab7, a small GTPase, interacts with the UVRAG-Vps16 complex to enhance the maturation of autophagosomes and endosomes[20]. The NAD^+-SIRT1 pathway may also increase mitophagy through stimulation of autophosphorylation and activation of ataxia telangiectasia mutated (ATM) which induces mitophagy via a LKB1-AMPK-TSC2 pathway[11, 21, 22]. There are three mitochondrial sirtuins, SIRT3, SIRT4, and SIRT5. Recently, it has been shown that NAD^+ replenishment induces the mitochondrial SIRT3-PGAM5-FUNDC1-dependent mitophagy[23]. nuclear SIRT6 and SIRT7 induce autophagy through inhibition of mTOR[24, 25]. While the NAD^+-consuming enzyme CD38 plays a necessary role in autophagic fusion with lysosomes[26], a new discovered NAD^+-consuming enzyme SARM1 facilitates mitophagy via formation of a PINK1-SARM1- tumor necrosis factor receptor–associated factor 6 (TRAF6) complex to stabilize PINK1 on depolarized mitochondria[27]. Interestingly, SARM1 is required for activation of an injury-induced axon degeneration[28], thus SARM1 may be involved in both neuroprotection and neuronal death. NAD^+ also induces expression of the anti-inflammatory cytokine IL-10 which induces mitophagy through mTOR inhibiton[1, 29, 30]. However, NAD^+ is also involved with the TCA cycle, OXPHOS, and β-oxidation, so how these metabolic pathways interact with mitophagy are elusive. Noticeably, increased NAD^+ may also inhibit autophagy/mitophagy through SIRT2[31], SIRT4[32], SIRT5[33], and PARPs[6]. A possible balance of robust NAD^+-dependent mitophagy induction and a mild NAD^+-dependent mitophagy inhibition presents a still robust mitophagy induction (**Figure 1**).

Collectively, our study strongly indicates defective mitophagy in AD with mitophagy reduction as a possible major driver of AD pathology. It is important to point out that defective mitophagy solely may not suffice to induce AD, genetic factors (e.g., mutations of *APP* and *PS1*, *APOE4* variant, and Tau) and environmental challengers may work together to induce and exacerbate AD. Many questions need to be addressed regarding this hypothesis: What are the additional molecular mechanisms of defective mitophagy in AD? What are the connections between mitophagy, Aβ, and pTau, and which occurs first? Nonetheless, manipulation of mitophagy might therefore be an attractive therapeutic target for AD patients. NAD^+ precursors, including NMN and nicotinamide riboside (NR), are promising drug candidates in view of their natural existence in human body as well as their safety and efficiency in preclinical trials[34, 35]. Large randomized, double-blind, placebo-controlled studies of neuronal mitophagy inducers on AD patients are necessary.

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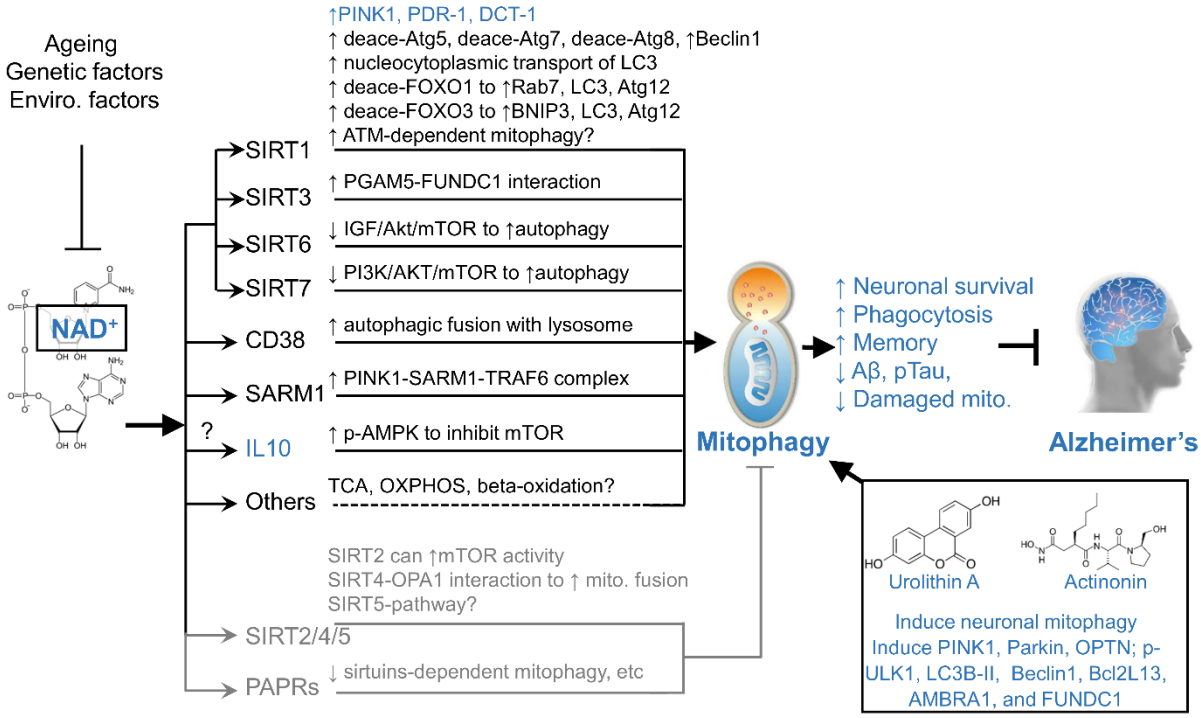

Figure 1. Schematic representation of how NAD⁺, urolithin A, and actinonin induce mitophagy, and to inhibit Alzheimer's disease (AD). CNAD⁺ levels are reduced with ageing as well as affected by genetic and environmental (enviro.) factors. NAD⁺ is a co-factor of sirtuins (SIRT1 to SIRT7), CD38, sterile alpha and TIR motif-containing 1 (SARM1), and Poly (ADP-ribose) polymerase (PARPs). The nuclear SIRT1, SIRT6, SIRT7, mitochondrial SIRT3, CD38, and SARM1 induce mitophagy/autophagy. NAD⁺ may induce mitophagy through other pathways such as through the induction of the mitophagy inducer IL10 and the fundamental metabolic pathways. Increased NAD⁺ may also inhibit autophagy/mitophagy through cytoplasmic SIRT2, mitochondrial SIRT4, mitochondrial SIRT5, and the DNA damage sensor PARPs. One reasonable explanation is that a robust NAD⁺-dependent mitophagy induction and a mild NAD⁺-dependent mitophagy inhibition gives an outcome of a remaining robust induced mitophagy. UA and AC are robust mitophagy inducers, both inducing expression of mitophagy/autophagy proteins such as PINK1, Parkin, OPTN, p-ULK1, LC3B-II, Beclin1, Bcl2L13, AMBRA1, and FUNDC1.Results marked in blue are from the current study. Results marked in dark (induction of mitophagy) and gray (inhibition of mitophagy) are from previous publications. See manuscript for details and references.

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