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Rapid communication

Microglial mitophagy mitigates neuroinflammation in Alzheimer's disease

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ABSTRACT

In neurons, defective mitophagy results in accumulation of damaged mitochondria, and finally leading to various neurodegenerative diseases, including Alzheimer's disease (AD). However, how mitophagy is defective in AD as well as how defective mitophagy contributes to AD is not fully understood. We give commentary on recent progress of this topic, highlighting the importance of mitophagy not only in neurons, but also in microglia, in forestalling pathology and cognitive decline in different animal models of AD.

Accumulation of dysfunctional mitochondria in neurons is a hallmark of various neurodegenerative diseases including Alzheimer's disease (AD) (summarized in (Kerr et al., 2017)). The role of mitophagy, a process that removes damaged mitochondria, in AD, is not completely understood, whether it be at the mechanistic level or in the development and/or progression of the disease. A recent study by Fang et al. (2019) reports compromised mitophagy in patients and animal models of AD. In particular, they demonstrate facilitating mitophagy inhibits the development of AD-associated behavioral and pathological hallmarks, namely extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles composed of phosphorylated tau (pTau) in animal models. Moreover, the study reinforces the contribution of microglial activation and deficient phagocytotic activity in AD-associated neuroinflammation; they show that impaired mitophagy causes microglial activation (Fig. 1a). Together, these results provide a novel insight into the 'defective mitophagy hypothesis of AD' and the 'mitochondrial cascade hypothesis of AD' (Kerr et al., 2017; Swerdlow et al., 2014); implicating enhancement of mitophagy as a potential therapeutic intervention strategy (Kingwell, In press).

Mitochondrial dysfunction in neurons and AD pathology contribute to exacerbation of one another in a self-propagating vicious cycle. However, the underlying mechanism orchestrating these events remain elusive. Mitochondrial quality is tightly regulated by mitophagy (mitochondria-specific autophagy), in which damaged mitochondria are degraded and recycled (Fang, 2019; Fang et al., 2014, 2017; Fivenson et al., 2017; Kerr et al., 2017; Palikaras et al., 2018; Rubinsztein et al., 2011). Fang et al. (2019) identified accumulation of damaged mitochondria in AD patient brain post-mortem brain samples that coincided with lower basal mitophagy events and decrease in phosphorylation of proteins (i.e. TBK1 and ULK1) involved in the initiation mitophagy relative to healthy control counterparts (Lazarou et al., 2015). These findings were verified in AD patient iPSC-derived cultured neurons. Thus, the authors went to test the hypothesis whether restoration of mitophagy holds the potential to delay or inhibit the progression of AD associated phenotype. They approached this by the use of an in-house small molecule library and their established neuronal mitophagy screening *C. elegans*-based platform. Based upon which Fang et al., identified the NAD^+ precursor nicotinamide mononucleotide (NMN), urolithin A (UA) and actinonin (AC) as robust neuronal mitophagy inducers that have previously been shown in other cell types or organs (Fang et al., 2014; Ryu et al., 2016; Sun et al., 2015). In particular UA and AC were demonstrated in human neuronal cell lines to promote mitophagy via increasing the levels of proteins of the mitophagy machinery namely, PINK1 and phosphorylated ULK1. Consistently, genetic upregulation of *PINK1* alleviates Aβ pathology and mitochondrial dysfunction in an APPSwInd mouse model (Du et al., 2017), confirming the importance of the PINK1 pathway in maintaining a healthy brain.

In order to test the potential of mitophagy facilitation in an *in-vivo* system, Fang et al. utilized well-established animal models of AD. Firstly, starting in a simple yet powerful *C. elegans* models of AD that overexpress pan neuronal $A\beta_{1\text{-}42}$ (CL2355 and CL2241) or pro-aggregating tau (BR5270) the authors elucidated relatively lower basal mitophagy, which coincided with cognitive deficits. Treatment with NMN, UA, or AC not only induced mitophagy, but also inhibited the memory loss and reduced Aβ levels in CL2355 strain upon UA

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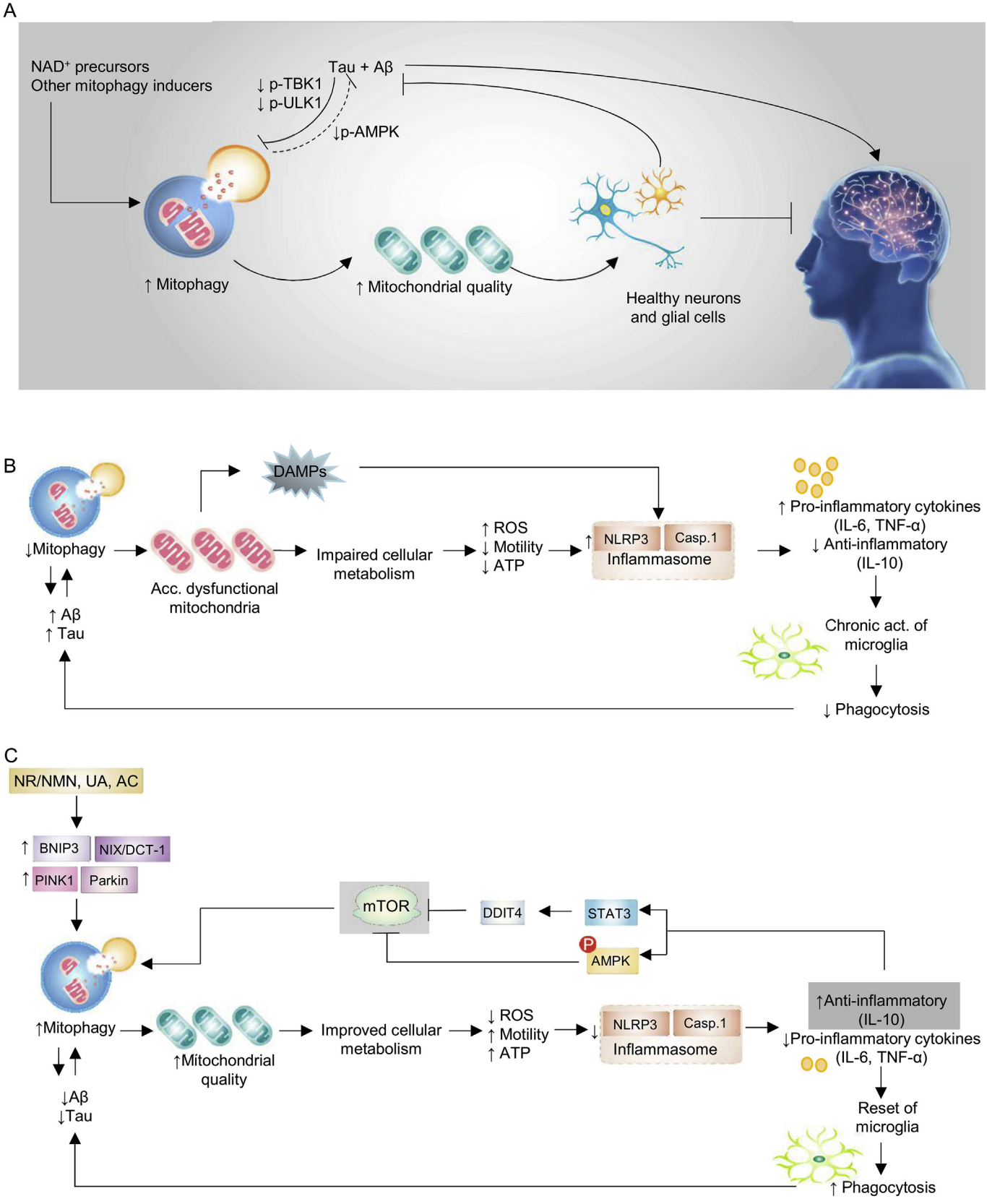

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Fig. 1. Mitophagy inhibits neuroinflammation and increases microglial phagocytosis. **A.** An overview of a causative role of defective mitophagy in AD progression. A crosstalk between defective mitophagy and AD-associated pathological hallmarks Aβ and pTau is presented. **B.** In AD, compromised mitophagy results in an upregulation of neuroinflammation, chronic activation of microglia and decreased phagocytosis leading to increased proteinopathies (Aβ and pTau). In addition to induce impaired metabolism, accumulated damaged mitochondria activate damaged-associated molecular patterns (DAMPs, also known as mitochondrial alarmins), including increased release of mtDNA, ATP, TFAM, succinate, cardiolipin, N-formyl peptide (fMLP), and cytochrome C. On entering cytoplasm, mitochondrial DAMPs become pro-inflammatory and initiate innate and adaptive immune responses, including activating the NLRP3 inflammasome, followed by Caspase-1 activation and IL-1β production. Activated NLRP3 has been reported in both human AD brains and AD mouse models. **C.** In a therapeutic perspective, pharmacological upregulation of mitophagy through NAD + precursors (NR and NMN), urolithin A (UA), and actinonin (AC) could decrease neuroinflammation and increase microglial phagocytosis, resulting in decreased proteinopathies (Aβ and pTau). Recently, it has been shown that the anti-inflammatory cytokine IL-10 induces mitophagy through inhibition of mTOR via phosphorylation of AMPK and activation of the Signal transducer and activator of transcription 3 (STAT3)- DNA Damage Inducible Transcript 4 (DDIT4) pathway. STAT3 is a key transcription factor downstream of IL-10R signaling, while DDIT4 is an mTOR inhibitor. See manuscript for details.

application. Subsequent direction towards a more complex model system, the transgenic mouse models of AD, reinforced the notion of reduced basal mitophagy and accumulation of damaged mitochondria in AD relative to wild-type (WT) controls, which upon UA or AC was rescued. Moreover, the neuronal mitophagy inducers were shown to protective against memory loss and ameliorated Aβ pathology (APP/PS1 mice) as well as inhibited phosphorylation at multiple tau sites (3 × Tg AD) in AD mice.

In addition to the proteinaceous characteristics, neuroinflammation, another hallmark shared by various neurodegenerative diseases, including AD, was suppressed upon UA and AC treatment in AD APP/PS1 mice. Microglia, one of the major players in inducing neuroinflammation, were shown to exhibit increased levels of pro-inflammatory cytokines including interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) coupled with a decrease of the anti-inflammatory cytokine interleukin-10 (IL-10), as previously reported (Heneka et al., 2013; Hickman et al., 2008). Furthermore, the activation of the NLRP3-inflammasome and the level of cleaved caspase-1 were increased in AD mice compared to control (Fang et al., 2019). In support of these findings, previous studies have shown that neuroinflammation in the form of greater frequency of activated microglia in close vicinity of Aβ plaques; thereby reinforcing the link between microglial activation and neuroinflammation in AD (Bamberger et al., 2003; Venegas et al., 2017). The close proximity of microglia to Aβ plaques has previously been interpreted as an attempt of the microglia to clear the pathological deposits of Aβ via a process known as phagocytosis; however, persistently activated microglia release pro-inflammotry cytokines which exacerbate the Aβ and/or tau pathology (Bamberger et al., 2003; Heneka et al., 2013). Upon UA treatment, transcriptome profiling revealed UA treatment mediated inhibition of pathways that promote inflammatory responses. Moreover, microglia were shown to display enhanced phagocytic activity accompanied by elevated levels of anti-inflammatory IL-10 coupled with reduced pro-inflammatory TNFα levels in a PINK1-dependent manner. Interestingly, IL-10 has previously been shown to induce mitophagy via activation of the mitophagy-related kinase AMPK and inhibition of mTORC1, leading to decreased inflammasome formation and inflammation in macrophages (Ip et al., 2017). The current study by Fang et al., confirms an association between mitophagy induction and decreased levels of the inflammasome components NLRP3 and cleaved caspase-1. Furthermore, treatment with another mitophagy inducer, the NAD^+ precursor nicotinamide riboside (NR), was previously shown to ameliorate several features of AD including dramatic reductions of neuroinflammation, and increased brain IL-10 levels in the 3xTgAD mouse model (Hou et al., 2018). The effect of NR/NMN treatment on inflammation has also been confirmed in relation to diabetes (Lee et al., 2015; Trammell et al., 2016).

The ability of mitophagy induction to reduce neuroinflammation might be explained by various mechanisms and benefits in microglia. Firstly, accumulated damaged mitochondria release DAMPs (damaged-associated molecular patterns), including increased release of reactive oxygen species (ROS), decreased levels of ATP among others (Green et al., 2011). Mitophagy/autophagy blockage has previously been shown to result in increased ROS levels (due to accumulation of ROS producing mitochondria), which in turn activates the NLRP3 inflammasome (Zhou et al., 2011). Additionally, the mitophagy inducer mitochonic acid 5 (MA-5), has previously been reported to reduce neuroinflammation. Treatment of microglia with MA-5 lead to increased mitochondrial quality dependent on the mitophagy activator BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3) (Lei et al., 2018). In connection, inflammation can induce a decrease in mitochondrial membrane potential, an increase in ROS production and contribute to the leakage of mitochondrial pro-apoptotic factors into the cytoplasm of microglia. Thus, the cellular energy metabolism diminishes resulting in elevated levels of oxidative stress (Lei et al., 2018). Secondly, induction of mitophagy could also inhibit the mitochondrial-associated antigen presentation via degradation of dysfunctional mitochondria and mitochondria-derived vesicles, resulting in inhibition of neuroinflammation (Matheoud et al., 2016). Collectively, these data suggest that mitophagy may inhibit neuroinflammation through multiple pathways; though further studies are required in order to improve our understanding of the mechanisms.

Mitophagy induction likely exhibits dual benefits in microglia. It not only inhibits neuroinflammation as just discussed, but also improves microglial ability to phagocytose. In fact, mitophagy induction increased levels of Aβ expression within microglia assessed in APP/PS1 mouse brain as well as isolated microglia (Fang et al., 2019). This finding suggests that increased mitophagy can activate or enhance microglial phagocytosis; thereby clearance of Aβ plaques in AD. Collectively, reinforcing the implication that inhibition of a pro-inflammatory response in AD, including loss of NLRP3 and caspase-1, beneficially reduce Aβ pathology (Heneka et al., 2013; Vom Berg et al., 2012). In addition, suppression of inflammatory cytokine production has previously been shown to reset microglial phagocytosis in APP/PS1 mice (Heneka et al., 2010). In line with this, microglia from NRLP3 and caspase-1 knock-out mice show increased phagocytotic activity (Heneka et al., 2013).

Diverse changes in mitophagy have been implicated in ageing and age-related diseases (Martin-Maestro et al., 2017; Scheibye-Knudsen et al., 2015), and mitophagy has been demonstrated to play a critical role in neuronal function and survival in premature aging models (Fang et al., 2014, 2016). The recent study expands our understanding of these inter-connections by showing that mitophagy is not only central to neuronal function but also to the function of microglia and their phagocytotic activity in the brain.

In summary, the results discussed here suggest that during chronic neuroinflammation and mitochondrial dysfunction, microglia become inefficient in clearance of Aβ plaques. Promoting mitophagy clears damaged/dysfunctional mitochondria, which in turn reduces the level of cellular stress. As a result, the microglial function is restored, activating phagocytosis and mitophagy. Therefore, the current study proves a causal link between defective mitophagy in inflammation and impaired phagocytosis in AD microglia thus contributing to our understanding of the underlining mechanism behind mitophagy and (neuro) inflammation. Several outstanding questions must be answered to fully comprehend the pathway (Fig. 1b and c). Firstly, can mitophagy markers be used as an early diagnostic/prognostic marker in AD? Secondly, how does mitophagy and other genetic susceptibility loci interact to shape individual risk or disease progression in AD? Third, what are the

detailed molecular mechanisms by which mitophagy induction improves microglial phagocytosis? One possibility might be by providing rapid production of ATP since phagocytosis is an energy-demanding process, or because of a functional overlap between mitophagy and phagocytosis? The current study sets the stage for the importance of further research into these outstanding questions to elucidate the intracellular cross-talk among neurons and glial cells in AD and neurodegenerative diseases.

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