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Defective Mitophagy in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive, mental illness without cure. Several years of intense research on postmortem AD brains using cell and mouse models of AD have revealed that multiple cellular changes are involved in the disease process, including mitochondrial abnormalities, synaptic damage, and glial/astrocytic activation, in addition to age-dependent accumulation of amyloid beta (A β) and hyperphosphorylated tau (p-tau). Synaptic damage and mitochondrial dysfunction are early cellular changes in the disease process. Healthy and functionally active mitochondria are essential for cellular functioning. Dysfunctional mitochondria play a central role in aging and AD. Mitophagy is a cellular process whereby damaged mitochondria are selectively removed from cell and mitochondrial quality and biogenesis. Mitophagy impairments cause the progressive accumulation of defective organelle and damaged mitochondria in cells. In AD, increased levels of A β and p-tau can induce reactive oxygen species (ROS) production, causing excessive fragmentation of mitochondria and promoting defective mitophagy. The current article discusses the latest developments of mitochondrial research and also highlights multiple types of mitophagy, including A β and p-tau-induced mitophagy, stress-induced mitophagy, receptor-mediated mitophagy, ubiquitin mediated mitophagy and basal mitophagy. This article also discusses the physiological states of mitochondria, including fission-fusion balance, Ca²⁺ transport, and mitochondrial transport in normal and diseased conditions. Our

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article summarizes current therapeutic interventions, like chemical or natural mitophagy enhancers, that influence mitophagy in AD. Our article discusses whether a partial reduction of Drp1 can be a mitophagy enhancer and a therapeutic target for mitophagy in AD and other neurological diseases.

Keywords

Alzheimer's disease; amyloid beta; phosphorylated tau; mitochondrial dysfunction; mitophagy

1. Introduction

Alzheimer's disease (AD), a progressive and multifactorial neurological disease, is characterized by multiple cognitive impairments and memory loss (Selkoe, 2001; Swerdlow, 2009; Reddy et al 2010, Mao and Reddy 2011). AD occurs in 2 forms – early-onset familial and late-onset sporadic (Selkoe 2001). In early-onset AD, genetic mutations in the amyloid precursor protein (APP) and in presenilin 1 and presenilin 2 loci induce a cascade of cellular changes, including synaptic damage and loss, mitochondrial failure, oxidative stress, glial and astrocytic activation, and microRNA deregulation, primarily caused by amyloid beta (A β) and hyperphosphorylated tau (p-tau) (Selkoe, 2001; Swerdlow, 2009; Reddy et al., 2010; Mao and Reddy, 2011; Reddy et al., 2012; Reddy et al., 2017; Amakiri et al., 2019). Although causal factors are largely unknown for late-set AD, multiple factors are involved in the disease process of AD, including the apolipoprotein allele 4 genotype accumulation of reactive oxygen species (ROS), traumatic brain injury, lifestyle factors such as unhealthy diet, lack of physical activity, and chronic conditions like diabetes/obesity and hypertension (Reddy, 2019; George and Reddy, 2019; Mao and Reddy, 2011; Amakiri et al., 2019). Above all, aging is the major risk factor for developing both familial and sporadic AD. As mentioned above, pathological and morphological features are similar in the brains of both early-onset and late-onset AD patients, but cellular and pathological changes occur late in sporadic AD.

Synaptic failure and mitochondrial abnormalities are widely accepted as early cellular changes in the AD disease process (Reddy et al., 2012). In the brains of both early and late-onset AD patients, the following mitochondrial abnormalities occur: an age-dependent accumulation of mitochondrial DNA (mtDNA) changes (Santoro et al., 2006; Salvioli et al., 2006; Rose et al., 2017), a reduced mitochondrial membrane potential, increased mitochondrial ROS production, reduced axonal transport of mitochondria, reduced mitochondrial adenosine triphosphate (ATP), altered mitochondrial enzymatic activities, and, most importantly, increased mitochondrial fragmentation. These mitochondrial changes lead to defective mitophagy in AD patients.

Mitophagy is a cellular process by which damaged mitochondria are removed from the cell via autophagy (Reddy and Oliver 2019). This cellular event is used to confirm the elimination of damaged mitochondria, critically challenged in protein levels by proteasomal degradation pathways and alterations in biogenesis (Bragoszewski et al., 2017; Ross et al., 2005; Lou et al., 2020; Wu et al., 2019; Oliver and Reddy, 2019). Measurement of

mitochondrial content determines the markers of mitochondrial quality (Evans and Holzbaur, 2020; Calkins et al., 2011; Wang et al., 2009). Despite the significant recent advances in our knowledge regarding mitophagy, reliable quantitative assays still need to be developed. Interest on mitophagy has recently increased, mostly by our growing awareness that mutations in mitophagy proteins, like PTEN-induced kinase 1 (PINK1) or Parkin, occur in neurodegenerative diseases, such as Parkinson's and Alzheimer's, as well as A β and p-tau induced toxicities in mitochondria (Cai and Jeong, 2020; Liu et al., 2020; Kerr et al., 2017). Although there are several other relevant players in mitophagy other than PINK1 and Parkin, advances have been made in the mitophagy field (Chakravorty et al., 2019; Reddy et al., 2018; Koyano et al., 2014, but a better understanding of the pathological process still needs investigation.

Mitophagy is the selective degradation of mitochondria by autophagy. Mitophagy is a key process in keeping the cell healthy. It promotes turnover of mitochondria and prevents accumulation of dysfunctional mitochondria which can lead to cellular degeneration. Mitophagy is regulated by PINK1 and Parkin proteins. In addition to the selective removal of damaged mitochondria, mitophagy is also required to adjust mitochondrial numbers to changing cellular metabolic needs, for steady-state mitochondrial turnover (Fritsch et al., 2010; Fritsch et al., 2020; Wang et al., 2019; Wang et al., 2013; Zhang et al., 2009; Harper et al., 2018; Harper et al., 2006; Schulman et al., 2009; Sarraf et al., 2013; Ordureau et al., 2015; Cai and Jean, 2020).

Mitophagy is initially mediated by the several autophagy pathways, beginning with the formation of phagophores. It then expands through the acquisition of lipids, resulting in the autophagosomes (Roca-Agüetas et al., 2019; Seo et al., 2010; Weber and Reichert, 2010). In the cell, autophagosomes first engulf mitochondria via autophagosomes and their subsequent catabolism by lysosomes, which protein first binds with, what are the adaptor proteins, and who recruit the other autophagy proteins in terms of a phagophore formation around mitochondria are in debate.

The purpose of our article is to examine the status of mitophagy in healthy and disease states. We discussed 1) the tools and methods to study mitophagy; 2) the cellular events that occur in mitophagy pathways; 3) the abnormal interactions between A β and Drp1 and p-tau and Drp1, leading to defective mitophagy in AD and 4) the latest developments in mitophagy enhancers, using pharmacological and genetic approaches.

2. Tools and Methods to Study Mitophagy

Mitochondria are highly dynamic organelles. They undergo fusion and fission, and remove dead mitochondria by mitophagy. Mitophagy contributes to balancing mitochondrial morphology, the quantity of mitochondria, and their quality. Some of the important measures of mitophagy are: mitochondrial permeability transition, mitochondrial respiration, mitochondrial mass, mitochondrial axonal transport, mitochondrial fragmentation, mitochondrial functional parameters (free radical production, ATP levels, enzymatic activities), mitochondrial and nuclear DNA (mtDNA/nDNA) ratios, citrate synthase activity, mitochondria-autophagosome or mitochondria-lysosome colocalization and cellular and

mitochondrial calcium levels (Ding and Yin, 2012; Williams et al., 2017). Table 1 summarizes tools and methods to study mitophagy in AD and other human diseases. Details of tools and methods are given below:

2.1. qRT-PCR and immunoblotting techniques

Mitochondrial gene expressions and protein levels have been assessed by qRT-PCR and immunoblotting techniques (Reddy et al., 2018; Manczak et al., 2010; Manczak et al., 2018; Kandimalla et al., 2016; Kandimalla et al., 2018). Mitochondrial fission and fusion, matrix and biogenesis genes are important genes to assess mitophagy. It is also important to study neuronal, glial, and astrocytic markers of cell, because mitophagy occurs in all cell populations. Further, it is important to study genes responsible for subcellular compartments of a cell, such as synapses, endoplasmic reticulum (ER) and lysosomes.

2.2. Immunofluorescence analysis of mitochondrial genes

Advanced fluorescence techniques, such as immunofluorescence, and confocal microscopies can be used to assess the localization of proteins related to mitophagy and mitochondrial quality control (Ding and Yin, 2012; Williams, et al., 2017; Cossarizza et al., 2001). Mito-tracker, labels mitochondria within live cells utilizing the mitochondrial membrane potential. A mito-timer is a very useful tool for monitoring both mitophagy and mitochondrial biogenesis. Detection of mitophagy is keima-red is mito-keima and can be used in *in vitro* and *in vivo* studies. Mito-QC (*in vivo* - mouse model that can be used to study mitochondrial network) are some of the important reagents and markers that are extensively used to study mitophagy. These are pH-sensitive mitochondrial fluorescent probes and extensively used to assess mitophagy and mitochondrial quality control.

2.3. Transmission electron microscopy (TEM) of neurons

TEM has been extensively used to assess the length, number, and structure of intact and damaged mitochondria. TEM is extremely useful in determining mitochondrial morphology and in providing a more accurate assessment of mitochondrial quality. We (Manczak et al., 2006; Manczak et al., 2010; Calkins et al., 2011; Manczak et al., 2011; Manczak et al., 2016; Manczak et al., 2018; Kandimalla et al., 2016; Kandimalla et al., 2018; Manczak and Reddy, 2015) and others (Wang et al., 2008; Wang et al., 2009, Han et al., 2020) have studied postmortem AD brains, brain tissues from AD mouse models, primary neurons and mammalian cells expressing A β and p-tau. These studies found altered mitochondrial structure and damaged mitochondria in AD specimens.

2.4. Mitochondrial membrane potential

The mitochondrial membrane potential is generated by proton pumps (in electron transport chain complexes I, III, and IV) and is an essential component in the process of energy storage during oxidative phosphorylation. Together with the proton gradient, membrane potential forms the transmembrane potential of hydrogen ions which is harnessed to make ATP (Zorova et al., 2018). The levels of mitochondrial membrane potential and ATP in the cell are kept relatively stable although there are limited fluctuations of both these factors that can occur reflecting normal physiological activity. We (Manczak et al., 2010) and others

(Cardosa et al., 2004) assessed mitochondrial membrane potential, in order to determine quality of mitochondria.

2.5. Mitochondrial function

To assess mitochondrial function, it is important to measure the levels of free radical production, ATP, and enzymatic activities. The outcome of these measures will determine quality of mitochondria in both healthy and disease states. We (Reddy et al., 2004; Manczak et al., 2006; Manczak et al., 2010; Manczak et al., 2011; Manczak and Reddy, 2012a; Manczak, et al., 2012b; Reddy et al., 2016; Reddy et al 2017; Reddy et al., 2018a; Reddy et al., 2018b; Manczak et al., 2016; Manczak et al., 2018; Manczak et al., 2019; Kandimalla et al., 2016; Kandimalla et al., 2018; Pradeepkiran et al., 2020) and others (Wang et al., 2008; Wang et al., 2009; Du et al., 2008; Du et al., 2010) extensively studied in AD cells, AD-affected and AD-unaffected tissues from AD mouse models and postmortem AD brains. The outcome of these studies is that mitochondrial function is defective and depends on the severity of disease progression.

Overall, the above-mentioned tools and procedures are important to assess quality of mitochondria and mitophagy in AD and other human diseases, but they have their own limitations in terms of accuracy. We need more research develop better and more reliable assays for assessing mitophagy.

3. Mitochondria

The mitochondrion is a self-autonomous double membrane organelle found in most eukaryotic organisms. The most promising role of mitochondria is the production of energy via oxidative phosphorylation in cells, ADP to ATP (Osellame et al., 2012; Bader and Winklhofer, 2020). The half-life period of mitochondria is about 2–3 weeks. Mitochondria are in a dynamic state in the cells (Reddy and Oliver, 2019). Mitochondria are synthesized in the cell body and travel along axons and dendrites to supply energy (ATP) to nerve terminals ‘synapses’ for normal neural communication. The transportation of mitochondria to synapses from axons is referred to as ‘axonal transport of mitochondria’ (Reddy et al., 2011; Kandimalla and Reddy, 2016; Oliver and Reddy, 2020; Zhu et al., 2013). In this process, mitochondria alter their shape and size, which facilitates their movement from the cell body to axons, dendrites, and synapses, and back to the cell body (Reddy, et al., 2011; Calkins, et al., 2012; Kandimalla and Reddy, 2016; Chen and Chan, 2009; Li, et al., 2004; Chan, 2006; Chen and Chan, 2009). Earlier transmission microscopy observations revealed the plasticity of mitochondria, which appear as slender filaments in which under normal conditions look like spaghetti (Nemani et al., 2012). In stressed or diseased conditions, mitochondria break up into shorter segments shaped like a torus (Ahmad et al., 2013). Mitochondria in a continual state of fission and fusion is a key flagging identity of mitochondrion is in a transient condition (Bui and Shaw, 2013; Scott and Youle, 2010). At any point in the cell cycle, a mitochondrion may undergo fission to give rise to two separate mitochondria (Lee and Yoon, 2016). Simultaneously mitochondria are undergoing fusion in which both the inner and outer membranes of the mitochondria break and rejoin to form a single intact mitochondrion. Fusion and fission events are governed by the mitochondrial shape transition

by unknown mechanisms during cellular activation (Sharma et al., 2019; Liu et al., 2020). Known proteins that are associated with mitochondrial dynamics (fission-fusion balance) are - Drp1, Fis1, Miro, Opa1, Mfn1, Mfn2, Mid49, Mid51 and Mff (Reddy et al., 2011; Kandimalla and Reddy, 2016; Oliver and Reddy, 2019).

3.1. Impaired Mitochondrial Dynamics in Alzheimer's Disease

Mitochondrial dynamics is a delicate balance between division and fusion. The shape and structure of mitochondria are maintained by fission and fusion balance (Reddy et al., 2011; Zhu et al., 2013). In healthy cells, fission and fusion events balance equally, which maintains mitochondria function. Mitochondrial fission is controlled by evolutionary conserved, dynamin-related large GTPases.

Fission is regulated by Drp1 and Fis1, the latter of which is localized in the outer membrane of the mitochondria (Reddy et al., 2011; Calkins et al., 2012; Kandimalla and Reddy, 2016; Chen and Chan, 2009; Li et al., 2004; Chan, 2006; Chen and Chan, 2009). When a mitochondrion signals to divide, Drp1 translocates from the cytosol to the outer membrane of the mitochondrion where it initiates the process of fragmentation.

In contrast, fusion is controlled by 3 GTPase proteins: Mfn1 and Mfn2, which are located in the outer mitochondrial membrane, and Opa1, which is located in the inner mitochondria membrane. The C-terminal portion of Mfn1 mediates oligomerization between Mfn molecules of adjacent mitochondria, facilitating mt fusion (Chan, 2006, Reddy et al 2011).

Recent studies from our lab (Manczak et al., 2010; Manczak et al., 2011; Manczak et al., 2011), and others (Wang et al., 2008; Wang et al., 2009; Silva et al., 2013) found increased mitochondria fission and reduced fusion, suggesting that impaired mitochondrial dynamics is present in AD-affected neurons. Recent studies of AD neurons from the lab revealed that A β interacts with fission protein Drp1, with a subsequent increase in free radical production, which in turn activates Drp1 and Fis1, causing excessive mitochondria fragmentation, defective transport of mitochondria to synapses, provides low synaptic ATP, and ultimately leads to synaptic dysfunction (Manczak et al., 2011). Further studies from our lab also revealed that p-tau interacts with Drp1 and enhances GTPase Drp1 enzymatic activity, leading to excessive fragmentation of mitochondria and mitochondrial dysfunction in AD (Manczak and Reddy, 2012).

Overall, abnormal mitochondrial dynamics (increased fission and reduced fusion) are present in AD and is an important biological phenomenon for defective mitophagy in AD.

3.2. Defective Mitochondrial Biogenesis and Alzheimer's Disease

Mitochondrial biogenesis is the process by which new mitochondria are synthesized in the cell and is activated by numerous different signals during times of cellular stress (Valero, 2014). It is affected by toxins and an accumulation of mtDNA mutations. There are four genes that are involved in mitochondrial biogenesis: PPAR - peroxisome proliferator-activated receptor- γ coactivator-1 α) (PGC1 α , nuclear respiratory factor 1 (NRF1), nuclear respiratory factor 2 (NRF2) and transcription factor A, mitochondrial (TFAM). In a diseased state, such as AD, mitochondrial biogenesis is reduced or defective, mainly because of the

association of mutant proteins such as A β and p-tau with mitochondria and increased free radical production (Sheng et al., 2012; Manczak et al., 2011; Manczak et al., 2018; Manczak and Reddy, 2012; Kandimalla et al., 2018), Therefore, mitochondrial biogenesis is an important parameter to assess mitochondrial function and mitophagy, particularly in a diseased state.

Several groups found reduced mitochondrial biogenesis in AD. In studies conducted to determine mitochondrial biogenesis in postmortem AD brains and AD cells, Sheng et al. (2012) found reduced mRNA and protein levels in the mitochondrial biogenesis genes PGC1 α , Nrf-1, Nrf-2, and TFAM in postmortem AD brains and AD cells. We also studied mitochondrial biogenesis in mouse models and AD cells. We found reduced levels of PGC1 α , Nrf1, Nrf2, and TFAM in 6- and 12-month-old APP transgenic mice (Manczak et al., 2016; Manczak et al., 2018) and tau transgenic mice (Kandimalla et al., 2016; Kandimalla et al., 2018), suggesting that mitochondrial biogenesis is defective in AD. More recently, using mutant APP mouse primary hippocampal neurons, Reddy and colleagues (2018) studied mRNA and protein levels of mitochondrial biogenesis proteins. They found reduced levels of mRNA and proteins of mitochondrial biogenesis genes PGC1 α , NRF1, NRF2 & TFAM. These observations strongly suggest that accumulation of mAPP and A β causes defective mitochondrial biogenesis in AD (Reddy et al., 2018).

Overall, defective mitochondrial biogenesis plays an important role in altered mitophagy in AD and other neurodegenerative diseases.

4. Mitophagy

Mitophagy is nothing but removal of dead mitochondria by autophagy. Mitophagy is initiated by the formation of spherical structure double membrane 'autophagosome' (Figure 1). An autophagosome is a spherical structure with double layer membranes. It is the key structure in macroautophagy, the intracellular degradation system for cytoplasmic contents. After formation, autophagosomes deliver cytoplasmic components to the lysosomes (Glick et al., 2010; Tan et al., 2014). The outer membrane of an autophagosome fuses with a lysosome to form an autolysosome. The lysosome's hydrolases degrade the autophagosome-delivered contents and its inner membrane. The formation of autophagosomes is controlled by Atg genes and LC3 complexes. The conjugate of Atg12-Atg5 also interacts with Atg16 to form larger complexes. Modification of Atg5 by Atg12 is essential for the elongation of the initial membrane. After the formation of the spherical structure, the complex of Atg12-Atg5:Atg16L1 dissociates from the autophagosome. LC3 is cleaved by ATG4 to generate LC3. LC3 cleavage is required for the terminal fusion of an autophagosome with its target membrane (Levine and Kroemer, 2008; Rajawat et al., 2009).

In recent years, much progress has been made on this issue, and studies have suggested that several different organelles and potential membrane sources are the key to this process initiation (Stamatakou et al., 2020). These include the plasma membrane, the Golgi apparatus, the ER and mitochondria. Many studies reported that autophagosomes are formed by the ER-mitochondria in mammalian cells. Nearly 30–41 autophagy-related genes (Atg) are involved in autophagosome formation.

Autophagy process involves recruitment of the protein complexes to regulate the autophagy which include (1) ULK kinase complex-ULK1-ATG13-FIP200; (2) the phosphatidylinositol 3-kinase (PtdIns3K) complex-Beclin1-ATG14-Vsp15-Vsp34-AMBRA1; (3) the Atg9-Atg2-Atg18 complex, and (4) Atg5-Atg12-Atg16 and Atg7/LC3 conjugation systems (Figure 1). This phagosome is ready to engulf the defective or damaged mitochondria with selective degradation through mitophagy (Reddy and Oliver, 2019).

5. Proteins Involved in Mitophagy

Recently, several groups extensively studied and discussed mitophagy types and proteins that are involved in mitophagy (Ding and Yin, 2012; Hirota, et al., 2012; Asrafi and Schwarz, 2013; Saita, et al., 2013; Pelligrino and Haynes, 2015; Cai and Thammineni, 2016; Burman et al., 2017; Lin et al., 2017; Pickles, et al., 2018; Safiulina, et al., 2018; Reddy, et al., 2018, Manczak, et al., 2018; Reddy and Oliver, 2019; Oliver Reddy, 2019; Cai and Jeong, 2020). These studies suggest that multiple pathways and several proteins are involved in mitophagy. The following proteins are important for mitophagy –Drp1, Fis1, Miro, Mfn1, Mfn2, Opa1, Ubiquitin, PINK1, Parkin, BNIP3, LC3, NIX, OPTN, FUNDC1, TBK1, SIAH1, and GP78. Among these, we describe important proteins for our article (Reddy and Oliver, 2019).

5.1. Drp1

Dynamin-related protein 1 (Drp1) is a fundamental component of mitochondrial fission and the key regulator of mitochondrial division in eukaryotic organisms. Drp1 is a dynamin-like GTPase, which mediates mitochondrial and peroxisomal division in a process dependent on self-assembly and coupled to GTP hydrolysis (Reddy et al., 2011; Ugarte-Urbe et al., 2014). In mitochondria fission, another GTPase called Drp1 is a mediator which facilitates mitochondrial fission, in apoptosis. Axonal transport of mitochondria allows the turnover of mitochondria in axons and axon terminals in neuron cells. Small GTPase proteins have been proposed recently as key regulators of mitochondrial motility (Kay et al., 2018). Mitochondrial Drp1 protein primarily, serves as a mitochondrial fission factor, inducing mitochondrial division, but when downregulated, indirectly promotes fusion (Osellame et al., 2012). Drp1 regulated fission may be used to excise depart damaged mitochondria for mitophagy, and reduction in Drp1 recruits parkin for excessive fission or fusion. Reports evidenced that Drp1 regulation will affect the mitochondrial axonal transport with fission or fusion exercise by promoting mitophagy mitochondrial dynamics.

5.1.2. Drp1 Structure—Drp1 protein is encoded by the Dnm1 gene, a member of the dynamin superfamily of GTPases, with 736 amino acids length with six different isoforms by peroxisome inhibition property. Drp1 has several splice variants: variant 1 consists of 736 amino acids with a calculated molecular mass of 81.6 kDa; in variant 2, exon 15 is spliced out and has a calculated molecular mass of 710 amino acids; in variant 3, exons 15 and 16 are spliced out and have a total of 699 amino acids; variant 4 has 725 amino acids; variant 5, 710 amino acids; and variant 6, 749 amino acids (Reddy et al., 2011). Similar to humans, mice have multiple variants, variant 1 consists of 712 amino acids, with a calculated molecular mass of 78.3 kDa; in variant 2, exon 3 is spliced out, and in variant 3, exons 15

and 16 are spliced out. It is possible that multiple splice variants are preset in mouse Drp1 (Reddy et al., 2011). The presence of a highly conserved GTPase domain in Drp1 indicates that Drp1 is important for several essential cellular functions.

5.1.3. Drp1 Function—Increasing evidence suggests that Drp1 is involved in several functions, including: 1) mitochondrial division, 2) mitochondrial distribution, 3) peroxisomal fragmentation, 4) phosphorylation, 5) ubiquitination, and 6) SUMOylation (Reddy et al 2011, Kandimalla and Reddy 2016).

Drp1 is primarily involved in mitochondrial division, a loss of Drp1 function increases mitochondrial fusion and mitochondrial connectivity. Drp1 interacts with Fis1 at the outer mitochondrial membrane, forms constriction and initiates mitochondrial division. Other than Drp1 receptors like Mff, Mid49 and Mid51 and Drp1 have been reported to be involved with fission process.

Drp1 activity is altered by its post-translational modifications like hyperphosphorylation activity. This affects the rate of mitochondrial fission and mitochondrial dynamics in cellular events. Drp1 has two major phosphorylated sites CDK S579, and PKA site is at S600 in Drp1 isoform 3. These sites are mostly responsible for hyperphosphorylation events of Drp1 (Kandimalla and Reddy, 2016; Oliver and Reddy, 2019).

As mentioned above, Drp1 is also involved in other modifications like S-nitrosylation, sumoylation, and ubiquitination, which alter the Drp1 activity. Drp1 has been shown to interact with toxic A β aggregates and p-tau intervening thought to play an important role in defective autophagy and mitophagy in AD. Drp1+A β and p-tau mediated autophagy and mitophagy exacerbating the disease and mitochondrial death promoted mitophagy in neurodegeneration with increased mitochondrial fission and reduced fusion in AD (Reddy and Oliver, 2019).

5.2. Fis1

Fission 1 (Fis1) is a mitochondrial division protein and plays a large role in mitochondrial dynamics and mitophagy. Fis1 encodes 150 amino acids with a molecular mass of 17 kDa (Reddy et al., 2011). Fis1 is a component of the mitochondrial complex that promotes mitochondrial fission. The fission protein Fis1 induced mitochondrial fragmentation and enhanced the formation of autophagosomes, which could enclose mitochondria (Figure 1). These changes correlated with mitochondrial dysfunction rather than with fragmentation, as substantiated by Fis1 mutants with different effects on organelle shape and function (Gomes and Scorrano, 2008). A recent study by Yu and colleagues revealed that human Fis1-mediated mitochondrial fragmentation occurs in the absence of Drp1 and Dyn2, suggesting that they are dispensable for human Fis1 function. Human Fis1 binds to Mfn1, Mfn2, and OPA1 and inhibits their GTPase activity, thus blocking the fusion machinery (Yu et al., 2019). They suggested a novel role for human Fis1 as an inhibitor of the fusion machinery, revealing an important functional evolutionary divergence between yeast and mammalian Fis1 proteins.

Overall, these studies indicate that Fis1 is involved in mitochondrial fission-fusion machinery and mitophagy activities.

5.3. Miro

Mitochondrial Rho GTPase protein miro is encoded by the Rhot1 gene on chromosome 17 plays a significant role in the mitochondrial transport. Miro protein is a member of the GTPase family and contains two isoforms Miro: Rhot1 (Miro1) and Rhot2 (Miro2) only expressed in the mitochondria where the mitochondrial transport is required (Tang 2015; Eberhardt, 2020; Fransson et al., 2006). Miro associates with Milton, forms a trimer complex and this complex contains multiple proteins, including trafficking kinesin protein 1/2 (TRAK1/2), motor protein kinesin and dynein to form the mitochondrial motor/adaptor complex. This complex helps to transport the mitochondrion via microtubules within cells by anterograde and retrograde transport (Cai and Thammineni, 2016).

Miro proteins are conserved one-way based transmembrane integral proteins with N-terminal regions exposed to the cytosolic side and consist of two GTPase domains separated by a pair of canonical EF hands (Kłosowiak et al., 2013). These miro proteins are sensitive to Ca^{2+} binding with the EF hands resulting in stopping the mobility of mitochondria on the microtubules structures. Ca^{2+} flows in and out in order to maintain a beneficial feedback cycle between Ca^{2+} buffering and mitochondrial dynamics that allows mitochondrial to the cellular events. Upon dysregulation of Ca^{2+} flow either side of the feedback mechanism causes signaling that leads both mitochondrial dysfunction and the activation of mitophagy events which can trap the cell by autophagosomes.

Miro, the mitochondrial outer membrane protein, involved in mitochondrial transport has recently gained great attention in mitochondrial biology. Studies reported that miro is involved in Ca^{2+} regulation at ER mitochondria encounter structures (ERMCS) and control of mitochondrial shape in response to Ca^{2+} (Csordás et al., 2018; Lee et al., 2018; Modi et al., 2019). Miro controls Ca^{2+} mitochondrial homeostasis at ERMCS by regulating the polo kinase-mediated phosphorylation of miro. Loss of Miro results in Ca^{2+} mitochondrial depletion and overexpression in mitochondrial Ca^{2+} burden to cell. The Polo/Miro based signaling pathway in the regulation of Ca^{2+} mitochondrial uptake in mitochondrial dynamics in this process PINK1 and leucine-rich repeat kinase 2 (LRRK2) are crucially involved. The increased mitochondrial Ca^{2+} levels, present in PINK1 and LRRK2 mutants, lead to mitochondrial swelling and eventually neuronal death observed in Parkinson diseases (Angelika and Hees, 2019). Miro-dependent mitochondrial shape transition maintains the Ca^{2+} homeostasis, this further validated by the mitochondrial quality control for autophagy and mitophagy by miro mediated Ca^{2+} sensing in mitochondrial dynamics (Nemani et al., 2018) (Figure 2).

5.4. OPA1

The optic atrophy 1 (Opa1) is a mitochondrial dynamin like GTPase class, inner membrane protein which is essential for mitochondrial fusion. Opa1 protein is associated with different functions, such as maintenance of the mitochondrial respiratory chain, cristae organization, apoptosis control and balanced membrane potentials (Lee and Yoon, 2018; Wu et al., 2019;

Ehse et al., 2018). Current studies support that mitochondrial inner membrane Opa1 associated with Mfn1 and Mfn2 mitochondrial outer membrane proteins is necessary for mitochondrial fusion and the process is regulated by proteolytic cleavage of Opa1 (Ali and McStay, 2018). Many different proteases are directly and indirectly involved in leading Opa1 processing associated with a number of cellular activities that includes matrix depended of inter-membrane ATPases. The proteases like presenilin-associated (PARL), high temperature requirement A2 protease (HTRA2) and overlapping activity of metalloendopeptidase 1(OMA1) (Jiang et al., 2014), are all associated with neurodegenerative diseases (Lebeau et al., 2018).

5.5. MFNs 1 and 2

Mitofusin1 and 2 proteins are encoded by the Mfn gene. These proteins are responsible for mitochondrial fusion. Mfn1 and Mfn2 have been implicated in regulating mitochondrial morphology (i.e., balancing fusion and fission events) and, when mutated, result in various diseases including AD (Filadi et al., 2018). Numerous studies suggest mitochondrial fusion is influenced by the pro-fission factors, such as Fis1 and the Mff interactions of Drp1 with A β and p-tau (Balog et al., 2016; Oliver and Reddy, 2019; Kandimalla et al., 2016). A number of papers state that dramatic remodeling of the mitochondrial network with a wide range of pathophysiological conditions, which include Ca²⁺ transfer inner and outer, apoptosis and autophagy leads to the progression of mitochondrial fission and fusion functions. A β and p-tau can also influence the regulation of mitochondrial dynamics through proteins such as Drp1, which are thought to alter processes of mitochondrial fission in AD and other neurological diseases (Qi et al., 2019). The activities of these GTPases proteins are highly regulated to control the mitochondrial morphology. These mechanisms include A β and p-tau induced defective autophagy and mitophagy in AD (Figure 3).

6. Mitochondrial Autophagy

Mitochondrial autophagy (mitophagy) plays an essential role in response to a variety of pathological stimuli, which includes A β , and p-tau in AD. There are a number of mitophagy pathways that have been identified mainly on - 1. A β and p-tau-induced mitophagy, 2. Stress induced mitophagy 3. Receptor mediated PINK1-Parkin-mediated mitophagy, 4. Ubiquitin mediated mitophagy and 5. Basal mitophagy. Among all, receptor mediated PINK1-Parkin-mediated mitophagy is well studied and the best-understood mitophagy pathway (Cai and Jeong, 2020). We briefly describe these pathways below:

6.1. A β and p-Tau-induced Mitophagy

Major pathological hallmarks of AD include intracellular deposition of neurofibrillary tangles (NFTs), which were associated with paired helical filaments (PHF), p-tau, and extracellular accumulation of A β peptide in the senile plaques (Pradeepkiran et al., 2019a; Pradeepkiran et al., 2019b; Oliver and Reddy, 2019). A β is generated predominantly in a form containing 40–42 amino acids from APP on sequential cleavage by β - and γ - secretases. Indeed, A β is a neurotoxic peptide and intra- and extra-cellular accumulation of this peptide characterized by aggregation, insolubility, protease resistance and delayed turnover (Reddy and Beal, 2008).

The sequential functional modifications of the mitochondrial dysfunction, including inflammation, impaired energy metabolism, overwhelmed oxidative stress and synaptic dysfunction leads to cognitive decline (Reddy and Oliver, 2019). Accumulation of A β within mitochondria by interacting with mitochondrial proteins cause mitochondrial damage through ROS formation and mitochondrial dysfunction (Pagani and Eckert, 2011).

Recently, Reddy group found mitochondrial fission Drp1 interacts with A β monomers and oligomers in brain tissues from AD patients and APP mice, and these abnormal interactions are increased with disease progression. Their findings suggest that increased production of A β and the interaction of A β with Drp1 are crucial factors in mitochondrial fragmentation, abnormal mitochondrial dynamics, and synaptic damage in AD (Manczak et al., 2011). In another study, the Reddy lab (Manczak and Reddy, 2012) found P-tau interacts with Drp1 and p-tau brain tissues from three different lines of transgenic APP, APP/PS1, and 3XTg.AD mice and postmortem AD brains. Mitochondrial fission-linked GTPase Drp1 activity was significantly elevated. Based on these findings, they concluded that Drp1 interacts with A β and P-tau, likely leading to excessive mitochondrial fragmentation and mitochondrial and synaptic deficiencies.

Despite tremendous progress has been made in mitochondrial biology in AD, the precise pathophysiology of AD neurons in relation to the mitochondria-inflammatory responses remains elusive.

Overall, these observations strongly suggest that A β and p-tau-induced mitophagy is a major component in AD.

6.2. Stress Induced Mitophagy

Mitophagy that is induced by mild and transient stress is referred to as 'stress induced mitophagy'. It is caused by several ways – ROS is the major factor that induces stress related mitophagy. Several recent findings reported that advanced glycation end products (AGEs) are largely involved in many human diseases, including diabetes mellitus, inflammation, renal failure, atherosclerosis, and neurodegenerative diseases (Glass et al., 2010; Pinkas and Aschner, 2016; Min et al., 2020). AGEs produce ROS that induce stress induced mitophagy. Both AGE and its receptor, advanced glycation end product receptor (RAGE) were expressed in astrocytes, microglia and neurons, a high level of RAGE expression was reported in AD brain samples. They induce ROS production; contribute significantly to stress induced mitophagy.

AGEs can influence mitochondrial dynamics by impairing fusion-fission balance, with a significant increment of mitochondrial fission in AD. This effect of AGEs may be attributable to their role in upregulating the expression of fission proteins Drp1 and Fis1 and down-regulating fusion proteins Mfn1, Mfn2, and Opa1. AGEs are also involved in abnormal APP processing and A β production via ROS.

AGEs are the end products of post-translational modifications of proteins, lipids and nucleic acids that should be facilitated by non-enzymatic reaction of reducing sugars and protein amino groups (Sharma et al., 2015). AGEs formation is accelerated under the oxidative

stress, hypoxia, hyperglycemia and inflammation. The pathophysiological consequences of AGEs have been mediated by ROS, especially excessive superoxide and hydrogen peroxide production within the mitochondria. These pathological actions are mediated by cascade events of inflammation (Cepas et al., 2020), which induce lowered glucose consumption, lowered ATP levels, and down regulated mitochondrial activity that leads to neuronal cell death (Sivitz and Yorek, 2010).

AGEs have been reported to impair the neuronal cells by cross-linking with the elevated kinases, GTPases and abnormal APP processing resulting increased A β deposition in AD (Li et al., 2013). Oxidative stress and neuronal inflammation caused by AGE-RAGE and/or RAGE-A β interactions lead to defective autophagy and mitophagy (Srikanth et al., 2011). Increased evidences of RAGE actions perturbation with A β interactions in neurons causes neuroinflammation and levies to increased A β levels in the brain by enhancing the rate of A β influx (Figure 4) (Stock et al., 2016). Additional aspects of AD via RAGE-A β interaction driven by overwhelmed A β and p-tau interactions and increased ROS production leads the stress-induced mitophagy in AD (Figure 5C).

Overall, these observations indicate that stress induced mitophagy is common in aging, AD and other neurological diseases. The reduction of stress is a suggested therapeutic approach for defective mitophagy.

6.3. Receptor Mediated PINK1/Parkin Mitophagy

Receptor based putative kinase protein 1 (PINK1)-parkin-mediated mitophagy comes under ubiquitin-guided mitophagy. The PINK1/Parkin mitophagy is well investigated and a popular mitophagy in human diseases (Cai and Jeong 2020). In receptor mediated mitophagy, PINK1 protein stabilizes and promotes parkin recruitment to mitochondria to trigger the mitophagy actions (Vives-Bauza et al., 2010). The dysfunctional mitochondria tend to have depolarized membranes due to accumulated PINK1 on the outer membrane of mitochondria (OMM) and generates proton gradients across the inner mitochondrial membranes (IMM) (Sekine and Youle, 2018). This depolarization allows Pink1 kinase to accumulate at the OMM inability of the ETC to phosphorylate mitochondrial surface proteins, and activate parkin, an E3 ubiquitin ligase, through phosphorylation of ubiquitin. E3 ubiquitin ligase is activated by PINK1 and ubiquitinates several substrates in the outer mitochondrial membrane. Ubiquitination leads to the recruitment of several autophagy receptors to interact with ubiquitin chain, such as adaptor proteins (p62, OPTN, NDP52) and recognize the phosphorylated poly-ubiquitinated chains on mitochondria that will drive mitophagy by binding with LC3 (Figure 5A) (Chen et al., 2019). PINK1 mediated mitophagy triggered by proteins other than PINK1 and Parkin are NIX /BNIP3L, LC3 FUNDC1 to LC3 (Ye et al., 2015; Cai et al., 2012). These proteins mediate engulfment of damaged mitochondria by the autophagosome in PINK1/parkin-independent mitophagy in hypoxic condition (Yoo and Jung, 2018; Palikaras et al., 2018).

6.4. Ubiquitin Mediated Mitophagy

In ubiquitin mediated mitophagy, the protein kinase PINK1 and E3 ligase Parkin play key roles in building the ubiquitin chains on the outer surface of damaged mitochondria. The

outer membrane proteins of mitochondria BNIP, NIX, initiate receptor dependent mitophagy and FUNDC1 are the receptor proteins that localize the autophagy machinery to the surface of damaged mitochondria (Hamacher-Brady and Brady, 2018; Han et al., 2020a; Han et al., 2020b). These receptor proteins bind directly to LC3 and mediate the mitochondrial elimination process. NIX protein plays an important role in mitochondrial differentiation in mitophagy by flagging that deficient NIX on outer membrane cells, leading to signaling of apoptosis process (Thomas and Ashcroft, 2019). Several studies reported that NIX associated with LC3 in stress conditions and BNIP3 involving in mitophagy through Opa1 release and Drp1 recruitment on mitochondrial surface (Zhang et al., 2016). BNIP3 and PINK1 stabilize the mitochondrial homeostasis by recruiting the parkin by interacting with PINK1-parkin mediated mitophagy. FUNDC1 receptor promotes the mitochondrial clearances during hypoxic condition; it interacts with ER and recruits the Drp1 during mitochondrial fragmentation. FUNDC1 is a good marker protein to identify the stress-mediated mitochondrial mitophagy (Figure 5B) (Wu et al., 2016).

6.5. Basal Mitophagy

Basal mitophagy is referred to as artificially induced mitophagy. It can be induced with chemicals and/or supplements. It is mostly studied in cell cultures and is characterized for altered components and proteins in cells. As reported, mitophagy is a process integral to normal mitochondrial network homeostasis maintained by the various factors (Reddy and Oliver, 2019). Rate of mitophagy varies by tissue type, suggesting a reasonable independent control of basal mitophagy. Basal mitophagy proceeds in the mammalian dopaminergic system *in vivo* in the absence of PINK1. Neural tissue has a high-energy demand and maintenance of mitochondrial homeostasis is essential to sustain this. Currently, the role of PINK1 in basal mitophagy is not completely understood. Therefore, it is important to study PINK1 knockout (KO) mice to understand the involvement PINK1 in basal mitophagy (McWilliams, et al., 2018).

Various human cell culture studies of heart, skeletal muscle, neuron and hepatic cells reported that PINK1-independent in chemically induced basal mitophagy. Tissue specific mitophagy activation and KO studies are required for further investigation of PINK1 and PINK1/Parkin aspects of basal mitophagy (Lee et al., 2018).

7. Reduced Drp1 as Mitophagy Therapeutic Target for Alzheimer's disease

Impaired mitochondrial dynamics (increased fission-reduced fusion or vice versa) and defective mitophagy conditions contributes to many pathological states including the synaptic dysfunction and cognitive deficits by triggering A β and p-tau accumulation in disease process of AD (Wang et al., 2008; Wang et al., 2009; Manczak et al., 2010; Manczak et al., 2011; Calkins et al., 2011; Reddy et al., 2017; Reddy et al., 2018; Cai and Thammineni, 2016). Several others also extensively reported impaired mitochondrial dynamics and defective mitophagy in other neurodegenerative diseases such as Huntington's (Song et al., 2011; Shirendeb et al., 2011; Shirendeb et al., 2012), Parkinson's (Bueler 2009; Krebiehl et al., 2010; Wang et al., 2010) and ALS (Joshi et al., 2018; Kodavati et al., 2020). These studies strongly suggested reduced excessively fragmented mitochondria and

enhanced mitophagy as therapeutic targets for these diseases. Therefore, a partial reduction of Drp1 is important and can be used to reduce excessively fragmented mitochondria and maintain and/or enhance mitophagy and autophagy events in AD and other neurodegenerative diseases. Figure 6 illustrates the Drp1 involvement in mitophagy in AD and other human diseases. In AD, we extensively studied the protective effects of reduced Drp1 as therapeutic target by 2 approaches – 1) genetic and 2) pharmacological.

7.1. Genetics approach

Using genetic crossings of Drp1 heterozygote knockout (Drp1^{+/-}) mice with APP tg and Tau tg mice, we studied mitochondrial dynamics, biogenesis and synaptic alterations (Manczak et al., 2016; Kandimalla et al., 2016).

7.1.2. Drp1^{+/-} X APP mice—We found reduced mRNA expressions and protein levels of Drp1, Fis1 (fission), CypD (matrix) genes, increased levels of Mfn1, Mfn2 and Opa1 (fusion), Nrf1, Nrf2, PGC1 α , TFAM (biogenesis) and synaptophysin, PSD95, synapsin 1, synaptobrevin 1, neurogranin, GAP43 and synaptopodin (synaptic) were found in 6-month-old APP X Drp1^{+/-} mice relative to APP mice (Manczak et al., 2016). Mitochondrial functional assays revealed that mitochondrial dysfunction is reduced in Drp1^{+/-} X APP mice relative to APP mice, suggesting that reduced Drp1 reduces mitochondrial fragmentation, enhances mitochondrial fusion, mitochondrial function and synaptic activity in AD neurons. Sandwich ELISA assay revealed that soluble A β levels were significantly reduced in Drp1^{+/-} X APP mice relative to APP mice, indicating that reduced Drp1 decreases soluble A β production in AD progression. These findings may have implications for the development of Drp1 based therapeutics for AD patients.

7.1.3. Drp1^{+/-} X Tau Mice—Similar to APP mice with reduce Drp1, decreased mRNA and protein levels of fission (Drp1 and Fis1), matrix (CypD) and increased levels of fusion (Mfn1, Mfn2 and Opa1), mitochondrial biogenesis (Nrf1, Nrf2, PGC1 α , TFAM), and synaptic genes (Nrf1, Nrf2, PGC1 α , TFAM) were found in 6-month-old Drp1^{+/-} X Tau mice relative to age-matched Tau mice (Kandimalla et al., 2016). Mitochondrial dysfunction was reduced in Drp1^{+/-} X Tau mice. Phosphorylated Tau found to be reduced in Drp1^{+/-} X Tau mice relative to Tau mice. These findings suggest that a partial reduction of Drp1 decreases the production of p-tau, reduces mitochondrial dysfunction, maintains mitochondrial dynamics, and enhances mitochondrial biogenesis and synaptic activity in Tau mice.

7.2. Pharmacological approach

Studies on AD—We sought to determine the protective effects of mitochondrial division inhibitor 1 (Mdivi-1) against A β - and mitochondrial fission protein, Drp1-induced excessive fragmentation of mitochondria in AD progression using cell cultures (Reddy et al., 2017). We also studied preventive (Mdivi1+A β 42) and intervention (A β 42+Mdivi1) effects against A β 42 in N2a cells. Using real-time RT-PCR and immunoblotting analysis, mRNA and protein levels of mitochondrial dynamics, mitochondrial biogenesis, and synaptic genes were measured. Mitochondrial function was measured by H₂O₂, lipid peroxidation, cytochrome oxidase activity, and mitochondrial ATP. The cell viability was assessed using

MTT assay. A β 42 was found to impair mitochondrial dynamics, lower mitochondrial biogenesis, lower synaptic activity, and lower mitochondrial function. On the contrary, Mdivi-1 enhanced mitochondrial fusion activity, lowered fission machinery, and increased biogenesis and synaptic proteins. Mitochondrial function and cell viability were elevated in Mdivi-1-treated cells. Interestingly, Mdivi-1 pre- and post-treated cells treated with A β showed reduced mitochondrial dysfunction, and maintained cell viability, mitochondrial dynamics, mitochondrial biogenesis, and synaptic activity. The protective effects of Mdivi-1 were stronger in N2a+A β 42 pre-treated with Mdivi-1, than in N2a+A β 42 cells than Mdivi-1 post-treated cells, indicating that Mdivi-1 works better in prevention than treatment in AD like neurons.

Similar observations were found in a synergetic study of both Mdivi-1 and SS31 treated in N2a cells (Reddy et al., 2018). Significantly reduced levels of A β 40 and A β 42 were found in mutant A β PP cells treated with SS31, Mdivi-1, and SS31+Mdivi-1, and the reduction of A β 42 levels were much higher in SS31+Mdivi-1 treated cells than individual treatments of SS31 and Mdivi-1. The levels of mtDNA copy number and cell survival were significantly increased in SS31, Mdivi1, and SS31+Mdivi-1 treated mutant A β PP cells; however, the increased levels of mtDNA copy number and cell survival were much higher in SS31+Mdivi-1 treated cells than individual treatments of SS31 and Mdivi-1. Mitochondrial dysfunction is significantly reduced in SS31, Mdivi-1, and SS31+Mdivi-1 treated mutant A β PP cells; however, the reduction is much higher in cells treated with both SS31+Mdivi-1.

In 2017, two groups independently studied the protective effects of Mdivi-1 in 2 different mouse models of AD. Baek and colleagues (2017) treated APP/PS1 mice with Mdivi-1 and studied cognitive behavior, mitochondrial fragmentation, mitochondrial transport and synaptic activities. They found that mice untreated with Mdivi-1 improved cognitive behavior and prevented mitochondrial fragmentation. They also noticed that the levels of lipid peroxidation, BACE1 expression, and A β deposition were reduced in APP/PS1 mice treated with Mdivi-1 (Baek et al., 2017). In another study, Wang and colleagues (2017) also studied the effect of Mdivi-1 on mitochondrial dynamics, particularly fission in CRND8 mice (tg APP strain). In Mdivi-1 treated 3-months-old CRND8 mice, they found rescued mitochondrial fragmentation and distribution deficits and improved mitochondrial function. The mitochondrial dynamic deficits, A β 1–42/A β 1–40 ratio and A β levels were reduced by Mdivi-1 treatment. Further, cognitive deficits were reduced in Mdivi-1 treated CRND8 mice relative to Mdivi-1-untreated CRND8 mice (Wang et al., 2017).

Overall, these studies indicate that Mdivi-1 reduced mitochondrial fragmentation, A β levels, improved synaptic function and ameliorated cognitive decline in disease process of AD mice.

Studies on HD and PD—Several others found reduced mitochondrial fragmentation and enhanced mitochondrial fusion, biogenesis and mitochondrial function in striatal progenitor cells (STHDhQ111/Q111) of HD knockin mice in HD and dopaminergic neuronal cells in PD treated with Mdivi-1 (Yin et al., 2016; Cui et al., 2010; Rapplod et al., 2014; Fan et al., 2019).

Overall, findings from cell culture and mouse model studies of AD, PD and HD revealed that Mdivi-1 reduces excessive fragmentation of mitochondria, enhances mitochondrial fusion, mitochondrial biogenesis, mitochondrial function and mitophagy.

8. Mitophagy Enhancers as new Therapeutic Targets for Alzheimer's disease

Increasing evidence suggests that accumulation of defective mitochondria, primarily due to impaired mitophagy is involved in AD and other neurodegenerative diseases (Xie et al., 2019; Reddy and Oliver 2019). Recent evidence from our lab suggests that defective mitophagy is an early event in the disease process of AD (Reddy et al., 2004; Manczak et al., 2006 and Manczak et al., 2018). Age-dependent increased production and accumulation of A β and p-tau and their abnormal interactions with Drp1, VDAC, CypD and ABAD, cause excessive fragmentation of mitochondria, impaired mitochondrial dynamics and defective mitophagy, ultimately leading to synaptic and cognitive dysfunction in AD (Lustbader et al., 2004; Du et al., 2008; Manczak et al., 2011; Manczak and Reddy 2012). These studies also suggest that reduced levels of Drp1, VDAC, CypD and ABAD may inhibit abnormal interactions with A β and p-tau and maintain quality of mitochondria and mitophagy in AD.

The other important factor is that increase and/or restoration of mitophagy with pharmacological approach, in other words, treat AD cells, AD mice and AD patients mitophagy enhancers. Currently, several mitophagy enhancers, urolithin, tomatidine, NAD⁺ riboside and others are being investigated in cell cultures and AD mouse models. The outcome of these studies will provide new information. Several other drugs/molecules like rapamycin, torin1, perhexiline, niclosamide, rottlerin, targeting by attenuate mTORC1 (AMP-activated protein) helped to enhance mitochondrial biogenesis in disease states (Balgı et al., 2009; Fang et al., 2019; Lou et al., 2020). Natural compounds such as resveratrol and quercetin have beneficial effects by increasing mitophagy transcriptome in cardiac and hepatic cells (Yu et al., 2016). A recent study reported that resveratrol reduces defective mitophagy by activating mitochondrial biogenesis (Malek et al., 2018). Rosmarinic acid was also reported to attenuate insulin resistance in rat skeletal muscle (Naoi et al., 2019). However, further research on identification of mitophagy enhancers is still needed in order to develop potential mitophagy enhancers.

9. Conclusions and Future Directions

The molecular and cellular mechanisms of mitophagy have been extensively studied in the past. However, mitophagy deficit has only been recognized recently as a key player involved in aging, AD and other neurodegenerative diseases. Several studies revealed that in both early- and late-onset AD, defective mitophagy contributes to synaptic dysfunction and cognitive deficits by triggering A β and p-tau accumulations. It is therefore imperative to understand the molecular basis of A β and p-tau-induced defective mitophagy in AD and other neurodegenerative diseases. Our lab has been focusing on mitochondrial biology and/or pathological aspects of AD for the last 20 years, including (1) the defective mitochondrial biogenesis and impaired mitochondrial dynamics; (2) interactions between A β and Drp1, p-tau, Drp1 and defective mitophagy and (3) small molecule therapeutic

interventions and genetic crossing studies on rescue and/or prevention of defective mitophagy in AD. In terms of therapeutics of mitophagy in AD, both genetic and pharmacological approaches are possible options: 1) genetic crossings of Drp^{+/-} mice with A β and tau mice; 2) treating A β and tau mice with small molecules such as Mdivi-1, SS31, curcumin, DDQ and other mitophagy enhancers (Olive and Reddy, 2019; Fang et al., 2019, Kuruva et al., 2017) and validating the defective mitophagy and cellular events in both disease and normal state. We published several articles on abnormal mitochondrial interactions between A β and Drp1, p-tau and Drp1 leading to defective autophagy and mitophagy proteins in AD. These abnormal interactions lead to increased mitochondrial fragmentation in mitophagy (PINK1, parkin, P62, BNIP3, FUNDC1, LC3-1, OPTN, TBK1), autophagy (ATG proteins, LC3-I and II), and ubiquitination (SIAH1, Gp78, MUL1, ARIH1, SMURF1) fusion and fission process involving important genes such as Drp1, Fis1, Miro, Opa1, Mfn1, and Mfn2. Currently, we and others are focusing on identification of mitophagy modulators, lysosomal activators, mitochondrial protectors, small molecules such as SS31, Mdivi-1 and DDQ to protect mitochondria by enhancing mitochondrial quality, mitochondrial dynamics and mitophagy and autophagy in AD and other neurological diseases. However, further research is still needed for 1) better understanding the biology of mitophagy/autophagy both in normal and disease states and 2) to test the currently available mitophagy/autophagy enhancers using preclinical rodent models.

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List of Abbreviations

AD	Alzheimer's disease
ROS	Reactive Oxygen Species
mtDNA	Mitochondrial DNA
Aβ	Amyloid beta
Drp1	Dynamin-1-like protein
ER	Endoplasmic reticulum
TEM	Transmission electron microscopy
Fis1	Fission 1 protein
Miro	Mitochondrial Rho GTPase
Opa1	Optic atrophy 1
Mfn1	Mitofusin-1
Mfn2	Mitofusin-2

Mid49	Mitochondrial dynamics protein MID49
Mid51	Mitochondrial dynamics protein MID51
Mff	Mitochondrial fission factor
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
NRF1	Nuclear respiratory factor 1
NRF2	Nuclear respiratory factor 2
TFAM	Transcription factor A, mitochondrial
mAPP	Mutant amyloid beta precursor protein
PINK1	PTEN-induced kinase 1
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3
LC3	Microtubule-associated protein 1A/1B-light chain 3
OPTN	Optineurin
FUNDC1	FUN14 domain containing 1
TBK1	TANK-binding kinase 1
SIAH1	E3 ubiquitin-protein ligase SIAH1
GSK3β	Glycogen synthase kinase 3 beta
CDK	Cyclin-dependent kinases
PKA	Protein kinase A
EF	Helix-loop-helix structural domain
ERMCS	ER mitochondria encounter structures
LRRK2	Leucine-rich repeat kinase 2
PARL	Proteases like presenilin-associated
OMA1	Metalloendopeptidase 1
AGEs	Advanced glycation end products
RAGE	Receptor for advanced glycation end products
OMM	Outer membrane of mitochondria
ETC	Electron transport chain
SIRT1	Sirtuin 1

SS-31	Szeto Schiller peptide 31
Mdivi-1	Mitochondrial division inhibitor 1

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Highlights

- Healthy mitochondria are essential for functional bioenergetics, cellular functions and balanced homeostasis.
- Mitophagy is a cellular process whereby damaged mitochondria are selectively removed.
- In Alzheimer's disease, toxic amyloid beta and hyperphosphorylated tau formation and accumulation, mitochondrial damage, are the early cellular events.
- Abnormal interactions between A β and Drp1 and p-Tau and Drp1, causes defective mitophagy and autophagy in AD,
- Reduced Drp1 enhances mitophagy and autophagy in AD.

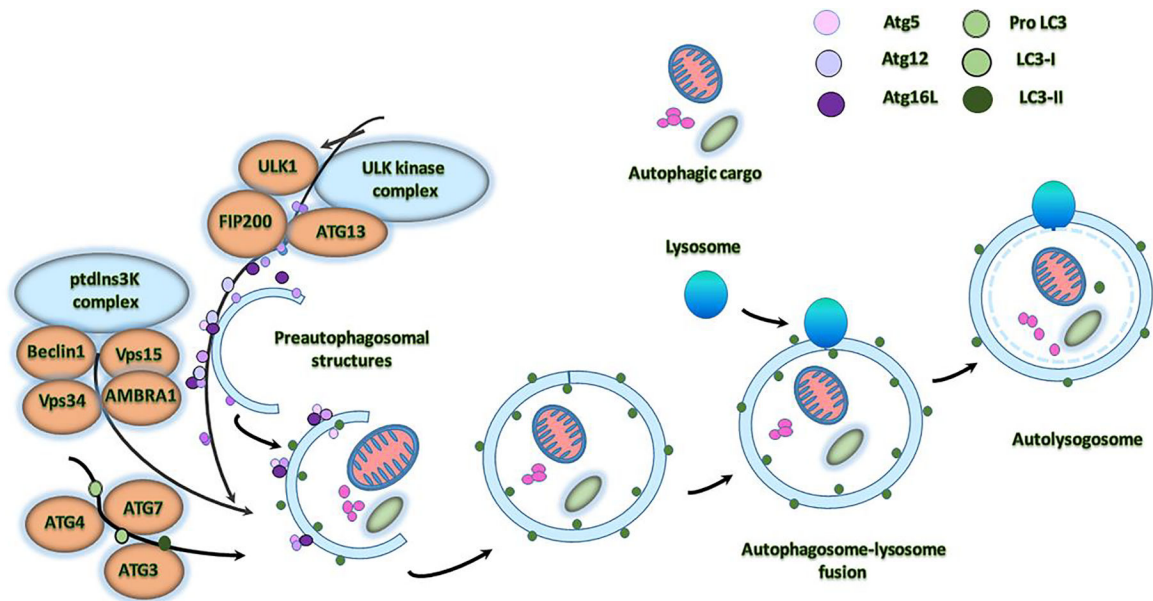


Figure 1. Molecular mechanism underlying autophagosome formation in normal cells.

The ULK complex consisting of ULK1/2–Atg13–FIP200–Atg101 is responsible for first initiation of autophagy, in response to certain signals. Formation of double-layered, membrane within the cytosol requires the PI3KC3 complex with contains Vps15–Vps34, Beclin-1, AMBRA1, and NBRF2. Next conjugation system is Atg, which add to the Atg5–Atg12–Atg16L complex, and LC3 conjugate system, which contains the Atg4, Atg7, Atg 3 to the elongating membrane along with the Pro LC3, LC3–1 and LC3–2. The membrane grows to enwrap a portion of the cytosol, forming an autophagosome. In the final step of the process, lysosomes fuse with the autophagosome, releasing lysosomal hydrolases into the interior, resulting in degradation of the vesicle contents.

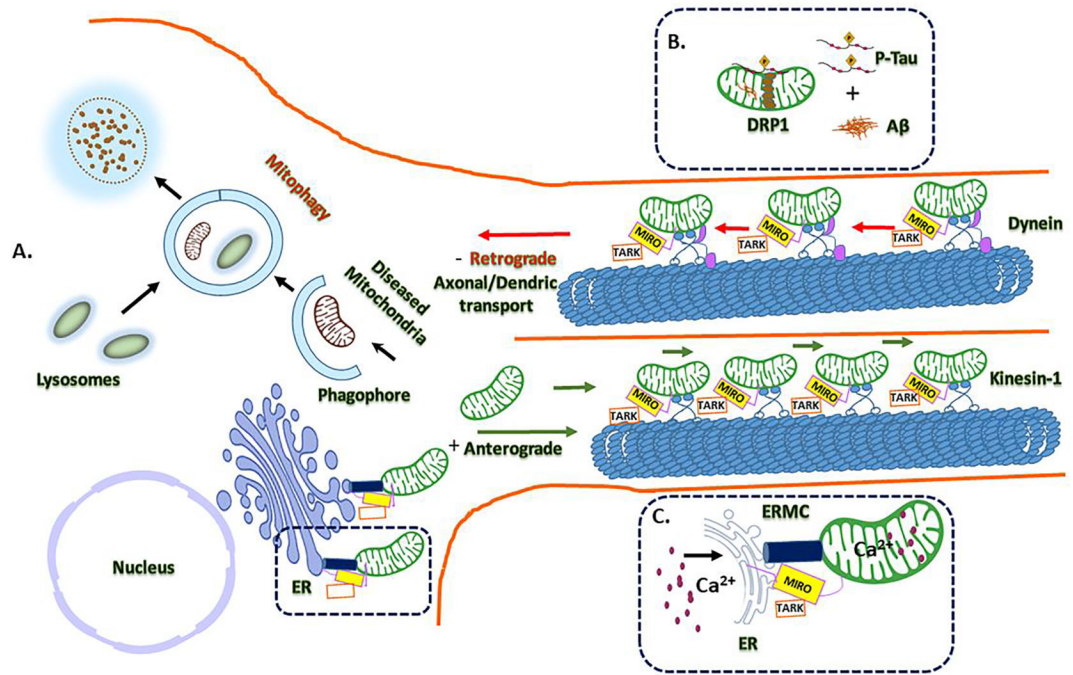


Figure 2. A schematic illustrating of mitochondrial mitophagy events and mitochondria axonal transport.

(A) Microtubule-based transport of healthy mitochondria in neurons transfer from nucleus to cell soma (+) anterograde transportation through highlight interacting partners of kinesin-1-MIRO/TARK in three functional aspects. MIRO is the mediated by PINK-1/parkin and could be Ca²⁺ efflux is key for the transportation, which is mediated by the mitochondrial encounter structure (ERMES). (B) This attenuates mitochondrial mobility with Aβ and p-tau interactions forward to the mitophagy of damaged mitochondria with abnormal mitochondrial dynamics. (C) Milton/TRAK and dynein mediates the retrograde axonal and dendritic transport. MIRO/TARK complex found to shown to be part of the ER- found at ERMES mitochondrial contact sites and showing Ca²⁺ exchange regulation in mitochondrial transport mechanism.

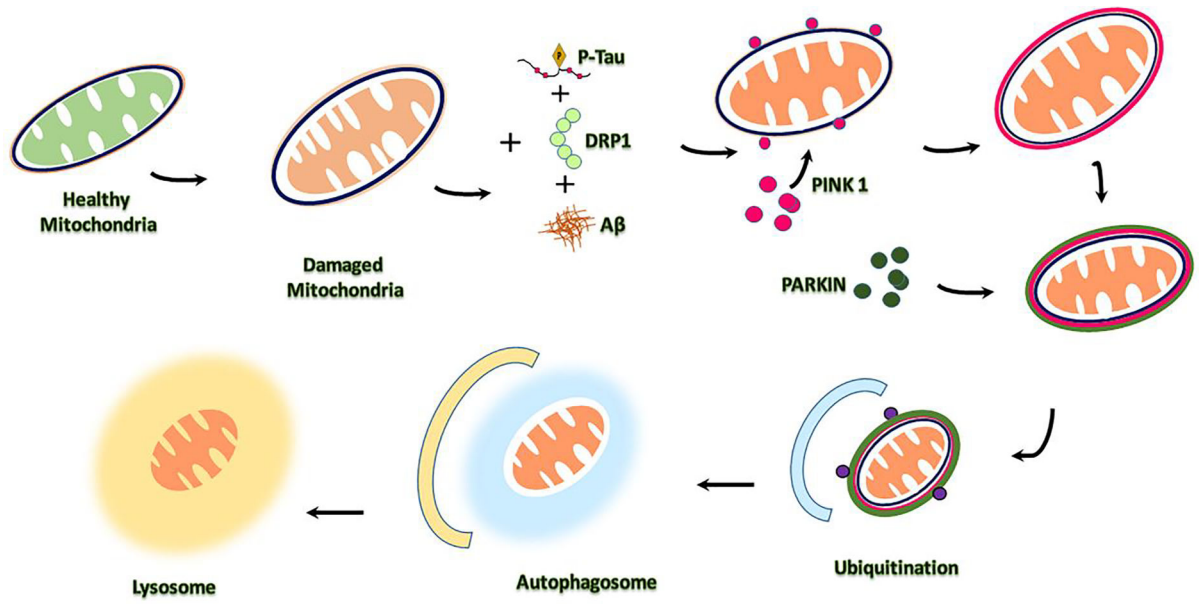


Figure 3. A brief autophagy and mitophagy events in Alzheimer's disease with key toxic players like A β and p-tau and Drp1 inducers that progress the activation of PINK1/parkin mediated abnormal mitochondrial dynamics.

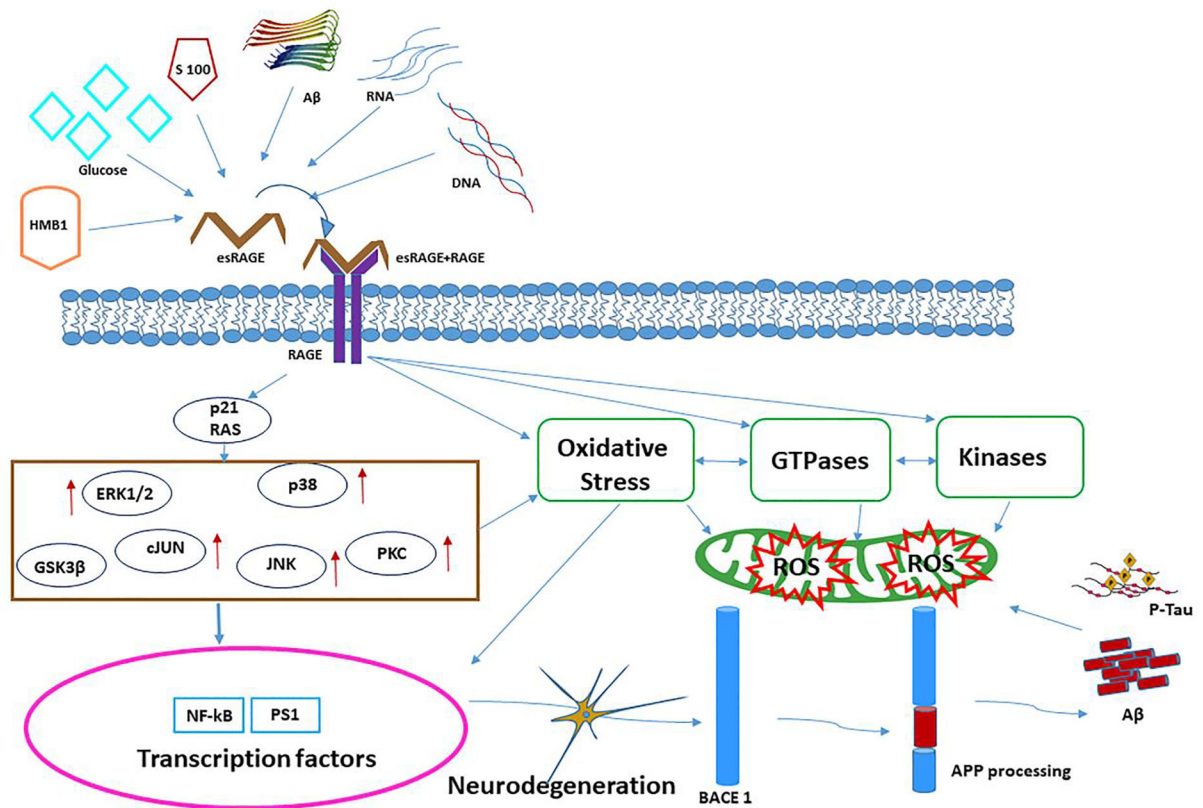


Figure 4. The extracellular and intracellular effects of AGEs induced ROS mechanism in AD. The receptor for advanced glycation end products (RAGE) is a transmembrane, immunoglobulin-like receptor that exists and binds to ligands like HMB1, Glucose, S100, A β peptide, RNA, DNA. Ligand molecules binding at the extracellular domain of eRAGE initiates a complex with RAGE to form complex with esRAGE-RAGE and intracellular signaling cascade p21 RAS, stimulating NAD(P)H oxidase resulting in the production of reactive oxygen species (ROS). Up-regulation followed by ERK1/2, c-JUN, p38, JNK, PKC, GSK3 β cellular events, and/or apoptosis with concomitant up regulation of and activation of NF-kB and PS1. The processing of APP has mainly focused on the correlation between RAGE activity and pathological conditions, such as BACE1 processing and A β accumulation and p-tau interaction in neuronal cells. The ROS formation influenced oxidative damage NADPH leads increased GTPases and kinases regulation to mitochondria mitophagy events leads neurodegeneration in RAGE mediated ROS signaling.

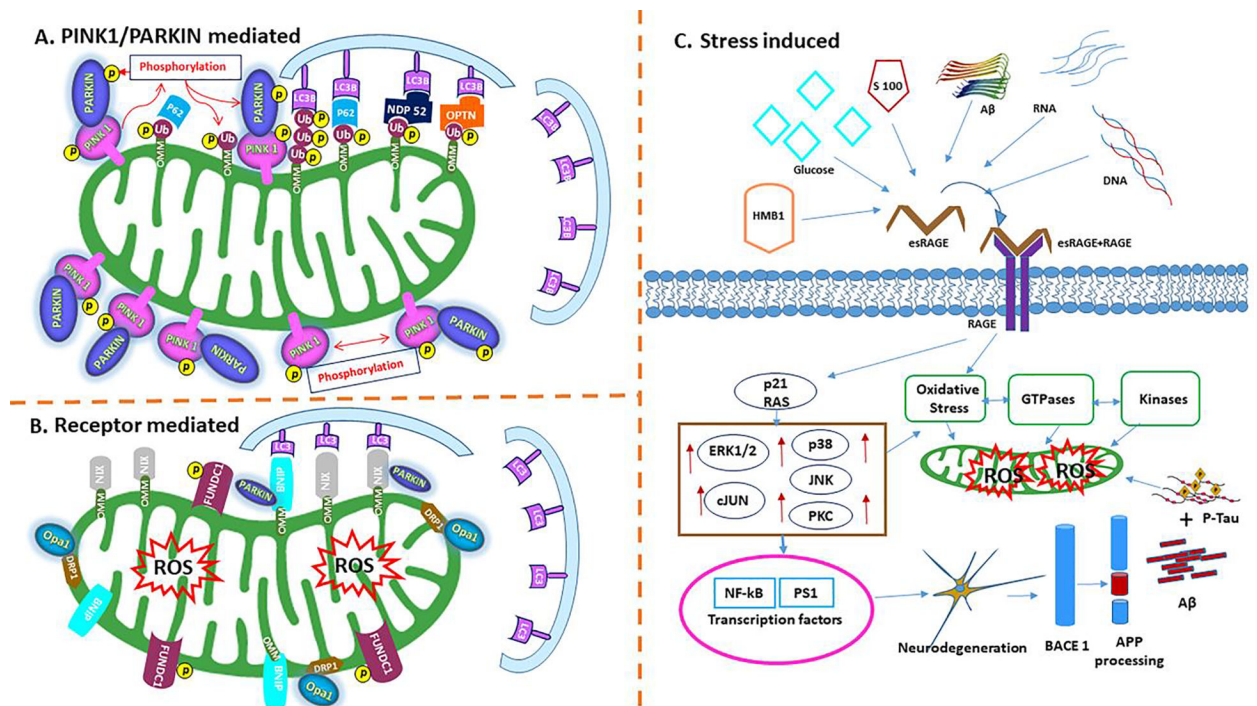


Figure 5.

(A) Mechanism of mitophagy with PINK1–parkin based. PINK1 recruited on outer mitochondria membrane (OMM) and promoting parkin recruitment. PINK1 phosphorylates and recruiting parkin and ubiquitinated several outer membrane components with activated phospho mechanism. Ubiquitinated chains are attached to the OMM and polymerized subsequently phosphorylated by PINK1 serving as signal for the autophagic machinery. Adaptor proteins (p62, OPTN, NDP52) recognize phosphorylated poly-Ub chains on mitochondrial proteins and initiate autophagosome formation through binding with LC3B.

(B). Receptor-mediated mitophagy. Specialized receptors, like NIX, BNIP3 and FUNDC1, expressed on the OMM in response to different stimuli. These receptors directly interact with LC3 to mediate mitochondrial elimination. NIX and BNIP3 phosphorylation enhances their association with LC3. FUNDC1 phosphorylation status, regulating mitochondrial dynamics during hypoxia. Mitophagy receptors promote fission of damaged organelles through the recruitment of DRP1, Opa1 on the mitochondrial surface. Only parkin-dependent ubiquitination of NIX and BNIP3 mediated mitophagy in hypoxic condition.

(C). Stress-mediated mitophagy. Ligand molecules binding at the extracellular domain of receptor for advanced glycation end products (eRAGE) initiates a complex with RAGE to form complex with esRAGE-RAGE and intracellular signaling cascade p21 RAS, stimulating NAD(P)H oxidase resulting the production of reactive oxygen species (ROS). Upregulated ERK1/2, c-JUN, p38, JNK, PCK, GSK3 β cellular events, and/or apoptosis with concomitant up regulation of and activation of NF- κ B and PS1. Processing of APP, RAGE activity and BACE1 processing leads A β accumulation and p-tau interaction resulting increased oxidative stress on mitochondria leads abnormal mitochondrial defects in AD.

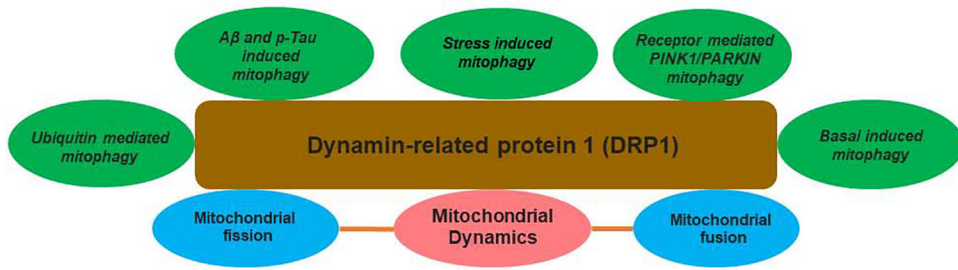


Figure 6. Factors contributing mitophagy players in response to a variety of pathological stimuli connected to centric protein, dynamin related protein 1 of neuronal cellular changes in AD.

Table 1.

Summary of tools and methods to study mitophagy in Alzheimer's disease and other human diseases

	Methods	Comments
1.	qRT-PCR	Measuring mRNA expressions in mitochondrial dynamics and mitochondrial biogenesis, synaptic, autophagy and mitophagy genes.
2.	Western Blot	Measuring the protein levels in mitochondrial dynamics and mitochondrial biogenesis, synaptic, autophagy and mitophagy genes.
3.	Localization	Immunofluorescence analysis
4.	Transmission electron microscopy	Transmission electron microscopy of cellular organelles - Mitochondria Neurons Nucleus Cytoplasm Golgi body Lysosome Autophagosomes Chromatin structure Number of mitochondria Length of mitochondria Structure of the neuronal soma Synapse density
5.	Mitochondrial functional assays	H ₂ O ₂ production Lipid peroxidation Cytochrome c oxidase activity ATP levels
6.	Mitochondrial membrane potential	Generated by proton pumps in electron transport chain (ETC) (Complexes I, III and IV).