Regulation of mitophagy by the ubiquitin pathway in neurodegenerative diseases

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Impact statement

Neurodegenerative diseases place an enormous burden on patients and caregivers globally. Over six million people in the United States alone suffer from neurodegenerative diseases, all of which are chronic, incurable, and with causes unknown. Identifying a common molecular mechanism underpinning neurodegenerative disease pathology is urgently needed to aid in the design of effective therapies to ease suffering, reduce economic cost, and improve the quality of life for these patients. Although the development of neurodegeneration may vary between neurodegenerative diseases, they have common cellular hallmarks, including defects in the ubiquitin-proteasome system and mitophagy. In this review, we will provide a summary of our current understanding of the regulation of mitophagy by the ubiquitin pathway and discuss the potential of targeting mitophagy and ubiquitin pathways for the treatment of neurodegeneration.

Abstract

Mitophagy is a cellular process by which dysfunctional mitochondria are degraded *via* autophagy. Increasing empirical evidence proposes that this mitochondrial quality-control mechanism is defective in neurons of patients with various neurodegenerative diseases such as Ataxia Telangiectasia, Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis. Accumulation of defective mitochondria and the production of reactive oxygen species due to defective mitophagy have been identified as causes underlying neurodegenerative disease pathogenesis. However, the reason mitophagy is defective in most neurodegenerative diseases is unclear. Like mitophagy, defects in the ubiquitin/26S proteasome pathway have been linked to neurodegeneration, resulting in the characteristic protein aggregates often seen in neurons of affected patients. Although initiation of mitophagy requires a functional ubiquitin pathway, whether defects in the ubiquitin pathway are causally responsible for defective mitophagy is not known. In this mini-review, we introduce mitophagy and ubiquitin pathways and provide a summary of our current understanding of the regulation of mitophagy by the ubiquitin pathway. We will then briefly review empirical evidence supporting mitophagy defects in neurodegenerative diseases. The review will conclude with a discussion of the constitutively elevated expression of ubiquitin-like protein Interferon-Stimulated Gene 15 (ISG15), an antagonist of the ubiquitin pathway, as a potential cause of defective mitophagy in neurodegenerative diseases.

Keywords: Mitochondrial, neurodegeneration, pathology, post-translational, proteasome, proteolysis

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Introduction

The major functions of the mitophagy and ubiquitin pathways are to remove dysfunctional/old mitochondria from cells *via* autophagy,[1–6] and to eliminate dysfunctional/misfolded proteins *via* the 26S proteasome,[7–11] respectively. These specific functions enable the mitophagy pathway to regulate mitochondrial quality and the ubiquitin pathway to regulate protein quality in cells. Both healthy mitochondria and proteins are essential for cells to function normally. Therefore, defects in either the mitophagy or ubiquitin pathways are expected to disturb cellular homeostasis and are detrimental to cell survival. Indeed, defects in mitophagy and ubiquitin pathways lead to several human diseases, including cancer[12–14] and neurodegenerative diseases.[15–20] Although the mitophagy and ubiquitin pathways function to remove abnormal mitochondria and proteins, respectively, there is compelling evidence that the functional ubiquitin pathway is essential for the initiation of mitophagy,[1,5,6,21–23] and that both pathways are defective in various neurodegenerative diseases. However, whether mitophagy is defective in neurodegenerative diseases due to defects in the ubiquitin pathway is still unknown. In this mini-review, we summarize major studies regarding the involvement of the ubiquitin pathway in mitophagy

initiation and discuss the observed discrepancies and potential reasons for these discrepancies in these studies. Finally, we end this review with a discussion of the constitutively elevated expression of Interferon-Stimulated Gene 15 (ISG15), an antagonist of the ubiquitin pathway,[24–26] as a potential cause of defective mitophagy in neurodegenerative diseases.

Mechanisms for mitochondrial quality control

Mitochondria promote cell survival by generating bioenergy in the form of adenosine triphosphate (ATP), which is required for vital cellular functions and can also sense "danger signals" to promote apoptotic cell death.[27–29] Mitochondria are present in all human body cell types (except mature red blood cells) to perform these important cell fate-determining functions, and their abundance depends on the role of the cell. For example, mitochondrial content in endothelial cells is modest compared to hepatocytes, cardiac myocytes, and neurons that need constant energy supply for normal functioning. Mitochondria produce ATP in a process called oxidative phosphorylation.[30,31] In this process, electrons from NADH and $FADH_2$ formed during fatty acid oxidation, glycolysis, and the TCA cycle (cellular processes that break down proteins, carbohydrates, and fatty acids) are transferred to molecular oxygen (O_2) through electron transport carriers (Complexes I, II, III, IV, and V) located in the inner mitochondrial membrane. When electrons reduce molecular O_2 to water, a large amount of free energy is released which is then used to synthesize ATP. The transfer of electrons from NADH to O_2 through electron transport carriers is not perfect, however, and there is always leakage of electrons at Complexes I and III. This electron leakage results in a partial reduction of O_2 to generate a superoxide anion ($O_2^-\bullet$), which quickly dismutates to hydrogen peroxide (H_2O_2) *via* superoxide dismutase 2 (SOD2) in the mitochondrial matrix and superoxide dismutase 1 (SOD1) in the mitochondrial intermembrane space. H_2O_2 is then reduced to water or is partially reduced to a hydroxyl radical (OH•). All $O_2^-\bullet$, H_2O_2, and OH• generated in this process are collectively referred to as mitochondrial reactive oxygen species (ROS).[32] Low levels of ROS retain physiological functions.[33] However, when mitochondria are dysfunctional, levels of ROS increase, which in turn causes oxidative damage to cellular proteins and DNA. Consequently, cellular functions are impaired and cells undergo apoptosis.[34–36] Hence, ROS-generating dysfunctional mitochondria are lethal to cells when not adequately removed. Given that neurons have higher energy demands and are post-mitotic, improperly functioning mitochondria can have severe consequences on neuronal survival.

Several mitochondrial quality-control mechanisms that remove dysfunctional mitochondria in cells have been identified.[37] First, mitochondria maintain their quality by degrading unfolded, misfolded, or damaged membrane proteins (generated by environmental insults such as oxidative stress, viral infections, etc.) using their proteolytic system.[38] Second, during the fusion/fission process, when damage is mild and below a certain threshold, fusion of normal mitochondria and a mitochondrion with mutant/damaged DNA or proteins allows for sharing of components to compensate for these defects.[39] Third, a portion of mitochondria can bud off and form mitochondria-derived vesicles under conditions of oxidative stress, which further fuse with lysosomes to degrade oxidized mitochondrial proteins.[40] Fourth, damaged mitochondria can also form mitochondrial spheroids that acquire lysosomal markers, become acidic, and undergo limited degradation of mitochondrial proteins.[41] Lastly, damaged mitochondria can be degraded *via* mitophagy in conjunction with the ubiquitin pathway.[1,5,21,42–44] In this review, we shall summarize our current understanding of mitophagy regulation *via* the ubiquitin pathway.

The ubiquitin pathway

Ubiquitin is a highly conserved 8 kDa protein that is ubiquitously expressed in all eukaryotic cells. Ubiquitin and ubiquitin-conjugating enzymes, collectively known as the ubiquitin pathway, regulate many cellular functions including cell cycle progression, development, apoptosis, signal transduction, antigen presentation, among several others, by inducing the signaling or timely degradation of regulatory proteins involved in these processes.[7–11] The conjugation of ubiquitin to cellular proteins (ubiquitination) takes place in three steps: In the first step, the C-terminal glycine residues of ubiquitin are activated in an ATP-dependent manner to form a thioester linkage with a cysteine residue of an activating enzyme [E1]. In the second step, the activated ubiquitin is then transferred to one of the several (about a dozen) known carrier enzymes [E2s]. In the third step, ubiquitin is transferred either directly from the E2 carrier enzyme, or indirectly with the help of one of several (roughly 500–1000) known ubiquitin ligases [E3], that in turn, conjugates ubiquitin to target proteins. Although all three conjugating enzymes are necessary for ubiquitin conjugation, the E3 enzyme is key in the ubiquitination process, as this enzyme recognizes and covalently conjugates ubiquitin with high substrate specificity.[8,45] The E3 enzyme conjugates either a single ubiquitin (monoubiquitination) or multiple ubiquitins (polyubiquitination) onto its target.[11,46,47] In both cases, the first ubiquitin (Ub_1) is appended to a specific lysine (Lys) residue on the substrate, and following ubiquitins (Ub_2-Ub_n) are attached to one of seven Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, or Lys63) within Ub_1. Polyubiquitin chains conjugated through different lysines have distinct functions. For example, Lys48-linked polyubiquitin chains target proteins for degradation *via* the 26S proteasome.[48] Alternatively, Lys63-linked polyubiquitin chains retain a signaling function in various cellular processes such as DNA repair, apoptosis, and autophagy.[48] Although other polyubiquitin chains do exist, their functions are not well-defined in cells.[49] Details about the structure and function of ubiquitin, ubiquitin-conjugating enzymes, polyubiquitin chains, and the 26S proteasome are extensively reviewed in the literature.[48–53] Given the crucial role of the ubiquitin/26S proteasome in normal cell homeostasis, the contribution of defects in this system to the development of several

human pathological conditions may come as no surprise. Indeed, the underlying pathology of diseases like cancer and neurodegenerative diseases has been linked to defects in the ubiquitin pathway.

Mitophagy and its regulation by ubiquitin

PTEN-induced putative kinase protein 1 (PINK1) and HECT/RING hybrid ubiquitin E3 ligase Parkin have been recognized as two important players of mitophagy.[2,42,54] When mitochondria are healthy (polarized), PINK1 activity is minimized through its degradation by the PARL (presenilin-associated rhomboid-like protein) protease present in the mitochondrial inner membrane space and the 26S proteasome,[55,56] and dephosphorylation and autoinhibition minimize Parkin activity. However, when mitochondria are damaged and depolarized, PINK1 is recruited onto the outer mitochondrial membrane and phosphorylates Ser65 of the ubiquitin moiety conjugated to outer mitochondrial membrane proteins. Binding of PINK1-generated phospho-ubiquitin and PINK1-mediated phosphorylation of the Ubl domain at Ser65 of Parkin releases Parkin from its auto-inhibited state.[57] Fully activated Parkin then appends more cytosolic ubiquitins on several mitochondrial proteins such as Mfn1/2, Miro1/2, VDAC, TOM, Fis1, and mitochondrial hexokinase. PINK1 and Parkin thus modify mitochondrial outer membrane proteins by phospho-ubiquitin polyubiquitin chains on depolarized mitochondria.[58–61] These polyubiquitin chains then recruit autophagy receptors NDP52 and optineurin (OPTN) onto outer membrane proteins of depolarized mitochondria, which further recruit autophagy factors Ulk1, Atg14, DFCP1, WIPI-1, and Atg16L1 to Parkin-bound and ubiquitin-labeled mitochondria for their degradation *via* the autophagy pathway.[62] In summary, PINK1 phosphorylates ubiquitin, Parkin amplifies this signal by appending more ubiquitins, these elongated phospho-ubiquitin chains then signal autophagy machinery, and damaged mitochondria are targeted for degradation *via* autophagy.[63]

Empirical evidence demonstrates that polyubiquitin chains appended onto multiple outer membrane proteins by Parkin is a prerequisite for mitophagy,[21,58–61] as deubiquitination of mitophagy substrates *via* deubiquitinating enzymes (DUBs) can suppress Parkin-mediated mitophagy.[64–67] However, the available information on the nature of polyubiquitin chains and their functions in the initiation and completion of mitophagy is puzzling. For instance, it has been reported that Parkin appends typical Lys48-linked polyubiquitin chains onto outer mitochondrial membrane proteins (e.g. Mfn1 and Mfn2) in response to the treatment of mitochondrial depolarizing agent CCCP.[21] Canonically, Lys48 polyubiquitin chains consisting of a minimum of four ubiquitin moieties target proteins for degradation *via* the proteasome.[48] Concurrently, outer mitochondrial proteins are degraded *via* the proteasome in response to CCCP treatment.[1,21] These observations thus led to the conclusion that degradation of mitochondrial outer membrane proteins on depolarizing mitochondria is a prerequisite for mitophagy.[1,21] On the other hand, it has also been observed that Mfn2 is not degraded but instead is deubiquitinated (evident by the disappearance of the Mfn2-modified bands on a Western blot) in response to CCCP treatment in conjunction with proteasome inhibitors.[22] In another study, Parkin mediates the robust ubiquitination of Mfn1, but not Mfn2, when Parkin is overexpressed in SH-SY5Y cells treated with a proteasome inhibitor.[43] Thus, whether outer mitochondrial proteins are indeed degraded *via* the proteasome before initiation of mitophagy is not clear. It is possible that the observed differences in the Parkin-mediated ubiquitination and degradation of mitochondrial substrates in distinct studies are simply due to variations in drug concentration, treatment time, cell lineages (cancer *vs.* normal), or the experimental design used in these studies. For example, in some cases, investigators have used a Parkin/PINK1-overexpression approach to investigate the "normal" mechanism of mitophagy in cancer cells. Certainly, utilizing protein overexpression to study cellular functions is feasible, but it has disadvantages. The abnormal expression of a protein can sometimes have a detrimental effect on cellular functions. Overexpression of protein-complex members could cause abnormal activation of a pathway leading to the excessive degradation or inhibition of degradation of intended substrates.[68] Notably, endogenous expression of Parkin is moderate (compared to Parkin-overexpressed cells) in primary fibroblasts and induced pluripotent stem-derived neurons.[69] It is possible that Parkin does not induce (or minimally induces) the degradation of outer mitochondrial proteins when mitochondria are depolarized. Robust degradation of outer mitochondrial membrane proteins seen in a non-physiological overexpression approach may be due to overactivation of the Parkin/PINK1 pathway in cells. A well-controlled study using proteasome inhibitors with/without depolarizing agents in normal cells may reveal whether Parkin indeed targets mitochondrial proteins on depolarized mitochondria for degradation before mitophagy.

In addition to Lys48 polyubiquitin chains, Parkin also appends atypical polyubiquitin chains that are linked through Lys63[21,70] to several outer mitochondrial proteins in cells treated with CCCP in the absence of any mitochondrial stress.[43] In a distinct study, Parkin predominantly assembles Lys6 and Lys11 instead of Lys48 and Lys63 polyubiquitin chains *in vitro* and on depolarized mitochondria.[67] More recently, it has been demonstrated that Parkin assembles and USP30 (deubiquitinase) disassembles Lys6-linked polyubiquitin chains, and distally phosphorylated ubiquitin chains impair USP30 activity.[71] The overall abundance of Lys6 linkages does not increase with proteasome inhibition suggesting that Lys6 chains on mitochondrial proteins are not for their degradation *via* the proteasome.[72] In general, why Parkin conjugates different types of polyubiquitin chains onto mitochondrial proteins before mitophagy is an intriguing question. Notably, to test the types of Parkin-mediated ubiquitin-modification on outer mitochondrial substrates, some groups have used ubiquitin Lys mutants. However, as pointed out by Kulathu and Komander,[49] Lys mutation affects ubiquitin surfaces and influences ubiquitin structure. For example, a mutation on Lys6 prevents assembly of heterotypic

Lys11-Lys63 ubiquitin chains by Kaposi Sarcoma virus E3 ligase K5. Together, these results on Parkin-mediated mitophagy have raised some important questions: First, is ubiquitination of mitochondrial proteins indeed necessary for degradation *via* the proteasome? Second, what are the function of typical (Lys48) and atypical (Lys6, 11, 27, 63) polyubiquitin chains in mitophagy? Third, are polyubiquitin chains appended by Parkin homotypic (same ubiquitin linkages) or heterotypic (different ubiquitin linkages)? Fourth, does Parkin append multiple types of chains simultaneously or sequentially, and onto the same proteins? Finally, what are the cellular determinants that dictate the type of polyubiquitin chain that Parkin conjugates to its substrates? Answers to these questions will help clarify the role of the ubiquitin pathway in regulating normal mitophagy.

Another observation that warrants further exploration in the ubiquitin field is the novel, non-canonical finding that PINK1 phosphorylates ubiquitins on mitochondrial proteins of depolarized mitochondria. One schematic model in the literature shows that PINK1 phosphorylates free ubiquitins.[57] Other models show that conjugated ubiquitins are phosphorylated by PINK1.[73] Thus, it is not clear whether free ubiquitin, conjugated ubiquitin, or both are substrates of PINK1. Although cellular protein substrates are canonically phosphorylated prior to ubiquitination and degradation, ubiquitins conjugated to these proteins for degradation are not phosphorylated. Moreover, disassembly of ubiquitin chains by DUBs is a prerequisite for the proteasome-mediated degradation of proteins,[74] and phospho-ubiquitin chains are resistant to DUBs.[75] Therefore, whether these phospho-ubiquitin chains added onto mitochondrial proteins are for degradation, and whether these phospho-ubiquitin chains are disassembled before their degradation *via* the proteasome, is not clear. Notably, PGAM5, a mitochondrial phosphatase that associates with PINK1,[76] may dephosphorylate phospho-ubiquitin before degradation. Evidence that PGAM5 knockout mice exhibit a Parkinson's disease phenotype supports such a possibility.[77] However, PINK1 is stabilized and PGAM5 is degraded when mitochondria are depolarized,[78] thus ruling out the possibility of PGAM5 involvement in ubiquitin dephosphorylation on mitochondrial proteins before their degradation. Whether any other unknown specific cellular phosphatase dephosphorylates ubiquitins on substrates before degradation *via* the 26S proteasome is not known. It is possible that phospho-ubiquitin chains are not degraded, but phosphate groups are added onto ubiquitin chains to protect these substrates from proteasomal degradation, and that the purpose of these degradation-resistant phospho-ubiquitin signals on depolarized mitochondria is to target the damaged mitochondria to undergo autophagy. Indeed, Benischke *et al.*[79] reported that Mfn2 is degraded and that inhibition of autophagy rescues this degradation. Furthermore, Richter *et al.*[80] demonstrated that phospho-ubiquitin chains interact with phosphorylated OPTN to promote selective autophagy of damaged mitochondria.

Finally, although empirical evidence suggests that binding of ubiquitin to Parkin is necessary for its activation, Parkin also interacts with ubiquitin-like proteins (Ubl) SUMO1,[81] NEDD8,[82] and ISG15.[83] SUMOylation increases Parkin's nuclear localization and auto-ubiquitination,[81] and NEDDylation and ISGylation increase Parkin's E3 ligase activity.[82,83] Notably, ISG15 is minimally expressed in normal cells.[26] Therefore, whether ISG15 indeed activates Parkin (without its overexpression), and the precise mechanism by which ISG15 and other Ubls (SUMO1 and Nedd8) regulate Parkin activity for mitophagy in normal cells requires further experimentation.

Defects in mitophagy and ubiquitin pathways in neurodegenerative diseases

Defective mitophagy has been linked to several neurodegenerative diseases.[84–86] Mutations in PINK1 and Parkin cause familial Parkinson's disease, and the impairment of mitochondrial motility and mitophagy has also been reported in sporadic Parkinson's disease.[87,88] In Huntington's disease, defects in autophagic cargo recognition lead to accumulation of damaged mitochondria in the cytoplasm.[89] Additionally, mutations in Mfn2 cause Charcot-Marie-Tooth type 2A neurodegenerative disease,[90] and mutation of ataxin 3 (SCA3/MJD1) impairs Parkin ubiquitination and mitophagy in spinocerebellar ataxia (SCA) type 3 (Machado-Joseph disease).[91] Aberrant accumulation of dysfunctional mitochondria due to inadequate mitophagic capacity has also been demonstrated in neurons and brains of patients with Alzheimer's disease. Notably, accumulation of misfolded protein deposits in affected brain regions has been reported in Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jakob, Huntington's disease, A-T, and ALS.[17] In most cases, proteinaceous deposits were composed of ubiquitin conjugates, suggesting a failure in their degradation *via* the ubiquitin/26S proteasome. Extensive evidence of mitochondrial dysfunction and defective mitophagy in ALS exists in the literature. For example, in mitochondrial-associated mutant SOD1 (G93A) mice, a mouse model most commonly used to study familial ALS, severe mitochondrial swelling is seen around postnatal day 30 (P30) with motor axon degeneration evidenced around P50, and clinical phenotypes at P60.[92,93] Mutant SOD1 mice also exhibited impaired ATP production and an accumulation of damaged mitochondria, suggesting a failure of mitophagy.[94] Large vacuoles were also seen *via* transmission electron microscopy analysis of motor neurons obtained from the spinal cords of mutant SOD1 mice. These vacuoles contained autophagy markers LC3 and beclin 1, further suggesting the presence of defective mitophagy.[92] The link between defective mitophagy and ALS is further strengthened by localization of misfolded SOD1 proteins to motor neurons[95] and mitochondrial fragmentation[96] *in vivo*. Furthermore, transgenic mice harboring deletions of genes essential for autophagy (e.g. atg5 and atg7) exhibited neurodegenerative phenotypes as well as accumulation of polyubiquitinated proteins.[97,98] Interestingly, the same mitochondrial defects were found in skeletal muscle of mutant SOD1 G93A mice,[99] and these defects preceded disease onset, suggesting defective mitophagy to be critical

in the progression of ALS.[92] In ALS patients, mitochondrial damage is evidenced by cristae breakage, matrix dilution, and increases in mitochondrial mass and number.[92,100] Importantly, this damage is explicitly seen in severely damaged and dying motor neurons, the cell type principally affected in ALS.[92] Accumulation of autophagosomes and autophagolysosomes containing swollen and damaged mitochondria in spinal cords of ALS patients has also been reported,[92,100] reflecting defects in mitophagic flux. These autophagosomes and autophagolysosomes have been shown to accumulate throughout the entire neuronal soma, subsequently initiating slow necrosis.[100] It is worth noting that, as seen in mutant SOD1 mice, these vesicles also stained positive for autophagic markers, further corroborating the incidence of defective mitophagy in ALS. Two independent groups using human and mouse models have also demonstrated that mitophagy is defective in A-T, a neurodegenerative disease in children similar in pathology to ALS.[101,102] Results from our group using human fibroblasts obtained from distinct A-T patients corroborate these results (Desai Lab, unpublished results). Mitochondrial damage manifests in A-T as swollen mitochondria, compromised respiration/ATP synthesis, and increases in mitochondrial mass and number. Interestingly, basal autophagy is increased but mitophagy is decreased in A-T cells.[102,103] Together, these results suggest that mitophagy defects are common in neurodegenerative diseases. Unlike Parkinson's, Charcot-Marie-Tooth type 2A, Machado-Joseph, and Alzheimer's diseases where causes of defective mitophagy are known (mutations in Parkin/PINK1, Mfn2, ataxin 3, and depletion of Parkin, respectively), the causes underlying defective mitophagy in A-T and ALS are largely unknown. Knowing that the protein and mitochondrial turnover pathways are defective in A-T and ALS and that mitophagy requires a functional ubiquitin pathway, it is plausible that inhibition of the ubiquitin pathway may lead to defective mitophagy in these diseases. Recent studies from our group on ISG15 (Interferon-Stimulated Gene 15), a cellular antagonist of the ubiquitin pathway, support the idea that mitophagy may be defective due to ISG15-mediated impairment of the ubiquitin pathway in A-T and ALS (Desai Lab, unpublished data).

ISG15, a potential modulator of mitophagy in neurodegenerative diseases

ISG15 is an interferon-inducible ubiquitin-like (Ubl) protein. ISG15 is synthesized from the *ISG15* gene, and either remains in an intracellular free form, appended to proteins in cells (conjugated form), or secreted from cells (extracellular form) by an unknown mechanism.[104–106] ISG15 conjugates to intracellular targets through a multi-enzyme pathway parallel to that of ubiquitin and other Ubl proteins (reviewed in literature[104,107–109]). ISG15-specific enzymes E1 (UbE1L), E2 (UbcH8), and E3 (HERC5, EFP, and several others) are also IFN-stimulated proteins that conjugate intracellular free ISG15 to cellular proteins, a mechanism referred to as ISGylation.[108,110–112] The ISG15 pathway (free ISG15 and its conjugates) is minimally expressed in normal cells. [24,106,113–115] However, the ISG15 pathway is elevated in pathogen-infected cells,[116–118] malignancies,[25,26] A-T,[26] and ALS[93] cells. Notably, although ISG15 is a Ubl protein, unlike ubiquitin, which targets proteins for degradation, ISG15 inhibits polyubiquitylation and consequently the ubiquitin-mediated degradation of proteins in cancer[25,26] and A-T[27] cells. These findings led to our hypothesis that constitutively elevated ISG15 may inhibit ubiquitin-conjugating enzymes (E2 and E3s) and thus polyubiquitylation (a signal for proteasome-mediated degradation) in cells. Literature reports demonstrating decreased polyubiquitylation of cellular proteins in cells expressing ISG15[24,26] and ISG15 inhibition of ubiquitin ligase activity[119,120] support this hypothesis. Notably, recent studies have demonstrated that ISG15 is conjugated to an artificial proteasome substrate and that endogenous substrates are conjugated to ubiquitin-ISG15 mixed chains in cells.[121] Authors have provided empirical evidence that these ubiquitin-ISG15 chains do not serve as a signal for protein degradation. Together, these findings suggest that ISG15 may potentially inhibit the ubiquitin pathway by inhibiting the function of ubiquitin-conjugating enzymes, thus decreasing polyubiquitylation by directly conjugating to cellular substrates and/or by modifying the degradation signal (ubiquitylation) for protein degradation *via* the 26S proteasome.

ISG15 is elevated in human A-T cells and mice.[26,122] Similarly, interferon signaling is also elevated in mutant SOD1 mice,[93,123] and specifically upregulated in their

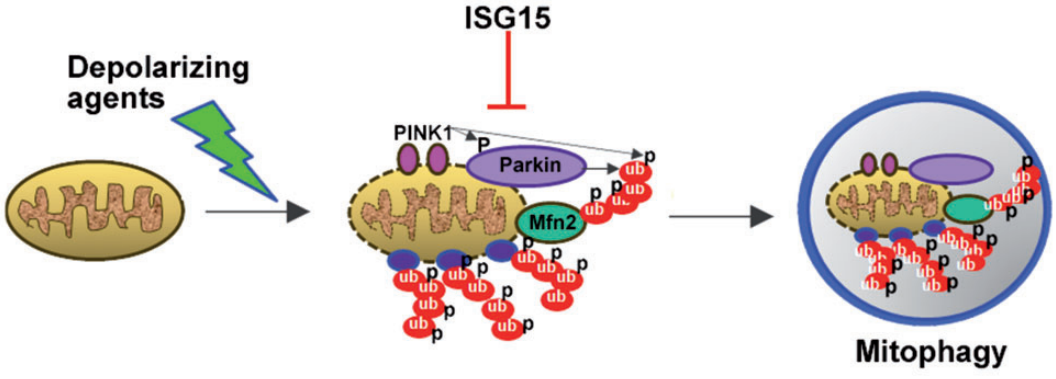

Figure 1. A proposed model for defective mitophagy in neurodegenerative diseases. Schematic shows that the constitutively elevated ISG15 pathway may inhibit the ubiquitin pathway (polyubiquitination) and consequently ubiquitin-dependent mitophagy in neurodegenerative diseases.

spinal cords approximately 30 days before the presentation of clinical phenotypes. Phosphorylated transcription factors STAT1 and STAT2, downstream targets in Type I Interferon signaling, were also found in the spinal cords of the same transgenic mice,[93] further reinforcing the elevation of interferon-stimulated genes in ALS pathology. Wang *et al*.[123] have demonstrated that elevation of ISG15 occurs in inflammatory neuronal injury (e.g. traumatic brain injury). Additionally, ISG15 levels have been shown to be elevated precisely where motor neurons are damaged (in the ventral horn of the spinal cord, where motor neurons reside) suggesting the possibility of ISG15 as a biomarker for neuronal injury and consequently ALS pathology.[123] ISG15 was also shown to colocalize with GFAP, an astrocyte marker, suggesting a dialogue between neurons and astrocytes in interferon-stimulated disease processes.[93,123] More recent studies from our group have revealed that ISG15 is also constitutively elevated in Parkinson's and Alzheimer's cells (Desai Lab, unpublished data). Because the ubiquitin pathway regulates mitophagy, and ISG15 inhibits the ubiquitin pathway, we propose that the constitutively elevated ISG15 pathway inhibits the ubiquitin pathway (polyubiquitination[25–27]) and consequently ubiquitin-dependent mitophagy, which contributes to neurodegeneration in neurodegenerative diseases (see schematic Figure 1). Recent studies demonstrating the presence of mixed ubiquitin-ISG15 chains on cellular proteins, subsequently inhibiting their degradation *via* the proteasome, support this model.[121] Further studies are needed to confirm this hypothesis, however.

Summary

Neurodegeneration affects millions of people worldwide, especially in the aging population, and the number of affected individuals over the age of 60 is projected to double by 2050. The estimated economic burden in the United States is $1.5 trillion per year, highlighting the necessity for understanding the common mechanisms that underly neurodegeneration. Defects in mitophagy and the ubiquitin pathway have been identified as leading causes of neurodegeneration in several neurodegenerative diseases, rendering pharmacological modulation of the mitophagy and ubiquitin pathways a promising future therapeutic strategy. However, additional understanding of the cellular processes highlighted in this mini-review is required before this becomes a possibility.

Authors' contributions: SD: Wrote the review.
MJ: Assisted SD to write and proofread the review.
CK: Assisted SD to write the review.

DECLARATION OF CONFLICTING INTERESTS

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