Review Article

The Interrelation between Reactive Oxygen Species and Autophagy in Neurological Disorders

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Neurological function deficits due to cerebral ischemia or neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) have long been considered a thorny issue in clinical treatment. Recovery after neurologic impairment is fairly limited, which poses a major threat to health and quality of life. Accumulating evidences support that ROS and autophagy are both implicated in the onset and development of neurological disorders. Notably, oxidative stress triggered by excess of ROS not only puts the brain in a vulnerable state but also enhances the virulence of other pathogenic factors, just like mitochondrial dysfunction, which is described as the culprit of nerve cell damage. Nevertheless, autophagy is proposed as a subtle cellular defense mode against destructive stimulus by timely removal of damaged and cytotoxic substance. Emerging evidence suggests that the interplay of ROS and autophagy may establish a determinant role in the modulation of neuronal homeostasis. However, the underlying regulatory mechanisms are still largely unexplored. This review sets out to afford an overview of the crosstalk between ROS and autophagy and discusses relevant molecular mechanisms in cerebral ischemia, AD, and PD, so as to provide new insights into promising therapeutic targets for the abovementioned neurological conditions.

1. Introduction

Reactive oxygen species (ROS), an umbrella term for a category of active oxygen-containing compounds generated from aerobic metabolism [1], encompasses superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and free radical (superoxide and hydroxyl radicals). Each of these compounds can damage biomacromolecules essential for various cellular processes [2], while simultaneously playing an indispensable role in the redox signaling cascade required for critically important biological events [3]. ROS are likely to cause oxidative stress when the oxidation of ROS outweighs the antioxidation [4], which is believed to damage cells. Compared with other organs, the brain has the most active oxidative metabolism, with a high demand for oxygen. The brain's active oxidative metabolism combined with its deactivation of detoxification systems and severe deficiency of antioxidants jointly upsets the redox balance, causing immeasurable oxidative brain tissue injury. This process is closely correlated with the occurrence and development of cerebral ischemia and neurodegenerative diseases [5].

Autophagy is a precisely regulated biological process characteristic of eukaryotic cells during which the superfluous and damaged structures of cells are eliminated via lysosome degradation to maintain normal cellular physiological functions [6] for the purpose of adapting to all kinds of adverse stimuli. Existing studies suggest that autophagy is widely engaged in neuronal fate determination in diverse neurological conditions [7–9]. However, excessive autophagy can promote a programmed cell death, known as autophagic cell death or type II programmed cell death [10], which is morphologically distinct from apoptosis and necrosis. That is to say, moderate or optimal activation of autophagy is desirable, and neither excessive nor insufficient autophagy completely lacks toxicity to neurons [11].

ROS are associated with cell damage [12] and have traditionally been thought to function solely as unfriendly molecules, despite exposure to ROS being unavoidable for cells

in an aerobic environment [13]. However, an increasing number of researchers have found that ROS can participate in various physiological processes as a kind of signaling molecule, including the induction of autophagy that is considered to be an effective defense mechanism against cellular stress [14, 15]. More importantly, it is the mitochondrial ROS which is a master inducer of autophagy under conditions of nutrient starvation, ischemia, or hypoxia [16–18]. Conversely, activation of autophagy is a key part of the cellular response to oxidative stress because the process disposes defective components before further damage/aggregation occurs [19]. In summary, the interaction and the balance between ROS and autophagy can be a key part of regulating cellular homeostasis.

It is well established that both ROS and autophagy are strongly associated with neurological diseases, but clarifying the functional relationship of the two mechanisms seems to be difficult because of their dual role in many disease processes. This review is designed to state the role of ROS and autophagy in neurological disorders and their underlying molecular mechanisms so as to offer novel strategies for the treatment of nervous system diseases.

2. Reactive Oxygen Species (ROS)

2.1. Generation and Scavenging of ROS. It is now well documented that mitochondria are the main source of intracellular ROS; 90% of which are derived from the respiratory chain on the mitochondrial inner membrane. The generation of mitochondrial ROS is initiated by the formation of O_2^- via the combination of electrons leaking from the mitochondrial respiratory chain complexes (mainly complexes I and III) and O_2. Highly active O_2^- can then be transformed into more stable H_2O_2 in the presence of superoxide dismutase (SOD). The quick conversion of H_2O_2 into H_2O can be catalyzed by catalase (CAT) and glutathione peroxidase (GSH-Px) and serves as the source of OH^- as well [3, 20, 21].

Under normal circumstances, ROS emissions in mitochondria are rather low and render minimal damage because mitochondria have potent antioxidant defense systems that sufficiently scavenge unneeded ROS. Whereas, unbridled ROS ensue only if mitochondria are subjected to deleterious incidents while simultaneously experiencing a drop in transmembrane potential. There is a positive-feedback mechanism called "ROS-induced ROS release" (RIRR) that accounts for the interaction between ROS and mitochondria. During RIRR, a burst of mitochondrial ROS is evoked by ROS, reducing mitochondrial membrane potential (MMP) and causing a longer opening of mitochondrial permeability transition pores (mPTP) [22]. Generally, moderate activation of mPTP is required for healthy mitochondrial metabolism. Once mitochondria are attacked by an inappropriate release of ROS, mitochondrial membrane depolarization interferes with mitochondrial respiratory chain function and can create a vicious circle provoking further ROS accumulation [23].

As mentioned, abnormally high levels of ROS can be quickly neutralized to cellular levels by a complex network of various robust antioxidants, which is essential for sustaining the normal functions of cells [24]. There are two major

antioxidative systems that consist of enzymatic and nonenzymatic antioxidants. The former is represented by SOD, CAT, and GSH-Px, while the latter includes glutathione (GSH), vitamin C, vitamin E, and so forth. Nevertheless, when there exists any redox imbalance between the generation and the scavenging of ROS, oxidative stress occurs, leading to unpredictable oxidative damage to organelles, proteins, lipids, and DNA, as well as the disruption of cellular structures and functions and eventually cell death [25] (Figure 1).

2.2. Biomarkers of ROS/Oxidative Stress. Due to the nature of ROS, which are active for a relatively short lifespan, various complex and time-consuming detection means such as electron spin resonance and spin trapping technology are relatively difficult to practically implement, and the results are also often offset by the mixing of heterogeneous groups [26]. Therefore, ROS or oxidative stress level is usually measured by monitoring the activity of cellular antioxidant enzymes such as SOD and GSH-px, each of which can indirectly reflect the ability to remove ROS [27]. Concurrently, GSH and malondialdehyde (MDA) are used to mirror oxidative stress resistance and injury.

Superoxide dismutase, a copper-containing protein isolated from bovine red blood cells by Mann et al. for the first time in 1938, was rediscovered and named as SOD by Fridovich and Mccord in 1969. SOD is able to scavenge ROS. The glutathione peroxide (GSH-Px) is extensively present in the cytoplasm. Mitochondria contain two kinds of GSH-Px, GSH-Px1 and GSH-Px4, by which lipid peroxide induced by OH^- can be decomposed into the corresponding alcohol or peroxide-induced injury can be reduced [28]. As is widely known, common ROS involved in cellular damage are mainly OH^-, H_2O_2, and O_2^- [29]. The dynamic conversion among the three parts depends upon SOD and GSH-Px, only by which can O_2^- be reduced into H_2O, thus mitigating oxidation damage [30]. In conclusion, the activity of these two antioxidant enzymes may be the reflex of the ability to eliminate ROS [31] (Figure 1).

Glutathione (GSH) is the most abundant nonprotein thiol and broad-spectrum antioxidant in mitochondria and contains two forms: reduced glutathione (GSH) and oxidized glutathione (GSSG). The former accounts for about 95% of GSH and, as the primary ROS scavenger, can effectively remove H_2O_2 and O_2^- and other free radicals, while concurrently being transformed into recyclable GSSG via glutathione reductase.

In the above processes, catalase (CAT) and glutathione reductase (GR) are typically used in combination with SOD, GSH-Px, and GSH as potential antioxidant biomarkers to evaluate oxidative stress.

There are also some other ROS measurement parameters based on oxidation of lipids, proteins, and DNA. Some examples of these parameters are MDA, 4-hydroxy-2-nonenal (4-HNE), 3-nitrotyrosine (3-NT), and 8-OHdG. Any accumulation of oxidation byproducts implies deterioration through oxidative damage, but different byproducts represent the different levels of cellular damage. Excess ROS inflict irreversible damage to nucleic acids, which has been reported to be an early event in oxidative damage.
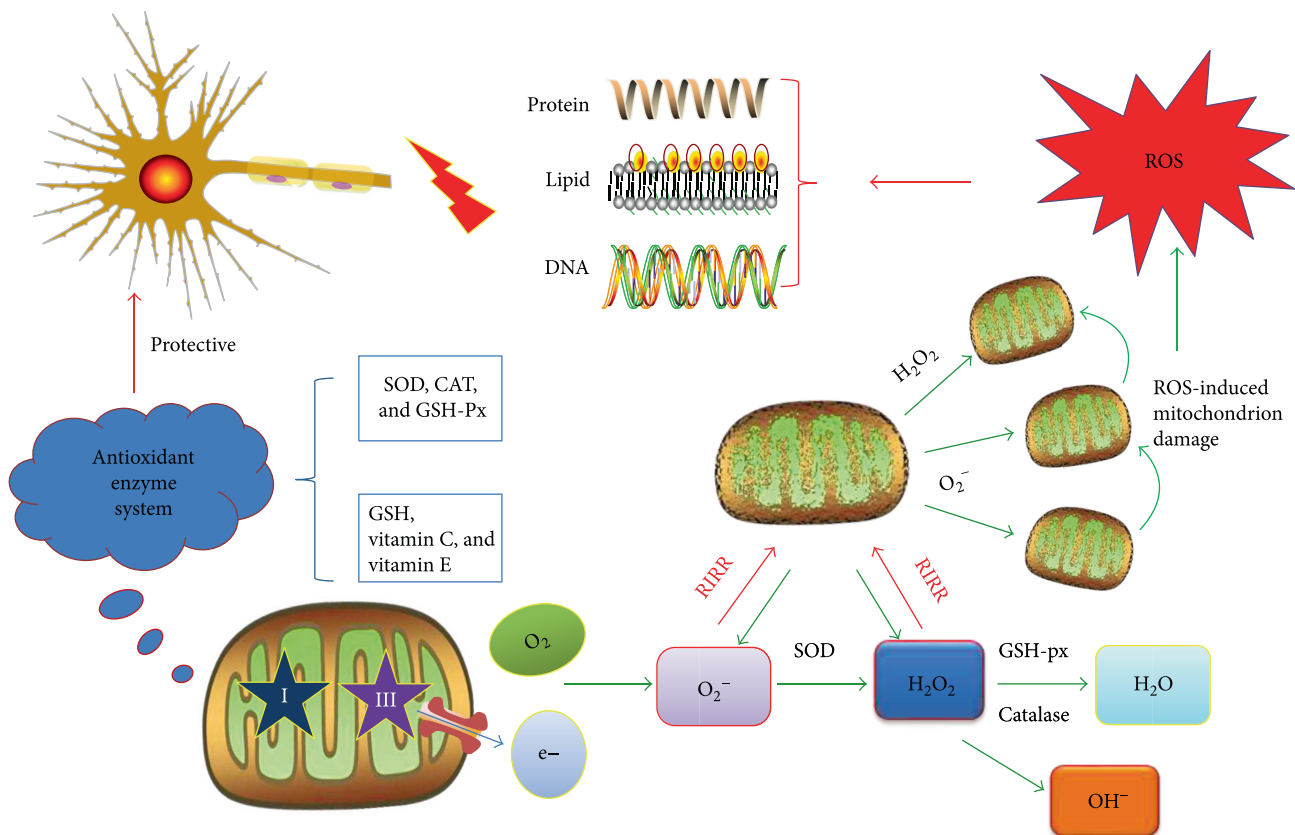
FIGURE 1: The generation and scavenging of reactive oxygen species (ROS) in mitochondria. The "→" refers to activation or induction, and the "⊢" refers to inhibition. Under normal or stress conditions, ROS is mainly born from the mitochondrial respiratory chain with the beginning of O_2^- production, followed by the conversion to H_2O_2 then OH^- under the catalysis of SOD and GSH-px. Defective mitochondria can instigate ROS accumulation with a "RIRR" positive-feedback mechanism. Excessive ROS can inflict severe damage on biomacromolecules, which can be counteracted by the antioxidant enzyme system to some degree.

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is a biological index generated by the oxidation of DNA along with the loss of its integrity. As a pivotal biomarker of endogenous oxidative DNA, 8-OHdG levels have been commonly assessed to estimate ROS-induced DNA lesions in multiple neurological disorders.

As for malondialdehyde (MDA), the ubiquitous final product of lipid peroxidation created by ROS attacking unsaturated fatty acids of biological membranes [32], its accumulation can incur cross-linking polymerization of macromolecules such as protein and nucleic acid, permeability and destruction of membrane structures, and eventually cell death. The degree of lipid peroxidation can be estimated by the quantity of MDA in the tissue, so MDA is proposed to be one of the indicators of intracellular oxidative stress [33]. Similarly, 4-hydroxy-2-nonenal (4-HNE), a specific clinical detection index of polyunsaturated fatty acid peroxidation, is also currently utilized to measure the extent of oxidative lipid damage. Isoprostane is the best available index of lipid peroxidation because of its stability. In summary, these indicators may better assess oxidative damage of brain tissue because various polyunsaturated fatty acids are susceptible to ROS during oxidative metabolism in the brain.

Likewise 3-nitrotyrosine (3-NT), an important metabolite of oxidative lesions in protein, has been measured in brain tissue, with increased levels in PD and AD populations. 3-NT is stable both in vitro and in vivo. There is a lot of value in the assessment of oxidative stress for clinical research [34].

2.3. ROS/Oxidative Stress-Related Signal Pathway. Keap1/Nrf2/ARE cascades have proven to be the most important antioxidant defense, and almost all protective antioxidant genes contain antioxidant response elements (ARE). When exposed to oxidative stress, Keap1 (kelch-like ECH-associated protein 1) can be separated from Nrf2 (nuclear factor erythroid 2-related factor 2) by uncoupling activity or reduction of ubiquitination and degradation, then translocation of Nrf2 into the nucleus targeting ARE. Nuclear translocation leads to the expression of antioxidants and phase II detoxifying enzymes, which is shown to greatly reduce ROS and ensuing oxidative damage.

In addition, several intracellular signaling pathways related to redox state, such as PI3K/Akt, JNK, MAPK, and ERK, can dissociate Nrf2 from Keap1 through phosphorylation of Nrf2. These signaling pathways also cause Nrf2 to translocate to the nuclease and activate the antioxidant

system, which is expected to augment the oxidative defense capacity [35, 36].

2.4. Reactive Oxygen Species (ROS) in Neurological Disorders. Loss of neurons is a key link in the pathophysiological process of nervous system diseases, which is mediated by oxidative stress, mitochondrial disturbances, abnormal protein aggregation, and so on [37]. One of the most important problems is oxidative stress, or ROS [38]. To our knowledge, the brain weighs just 2% of the body's weight, but its metabolic oxygen consumption accounts for 20% of total oxygen consumption of the organism under nonstress conditions. High oxygen demand is always accompanied by more ROS. The brain is rich in various polyunsaturated fatty acids sensitive to ROS, but is relatively devoid of antioxidant enzymes and GSH, adding that neurons are considered terminally differentiated cells [39], which make brain tissue more inclined to suffer damage from ROS [40, 41].

Robust evidence suggests that ROS display a recognized role in neuronal death after brain ischemia [42]. Either an initial burst of ROS induced by ATP consumption and mitochondria depolarization in the ischemic phase or the Ca+-dependent ROS generation at the reperfusion stage can pose a hazard for neurons [42]. As noted earlier, excessive production of ROS can not only damage cellular macromolecules but also impair antioxidant enzymes and nonenzymatic antioxidants during I/R insult, which is unfavorable for neurofunctional recovery. Sharma and Airao have shown that lipid oxidation byproducts such as MDA are markedly increased in ischemic tissues, but SOD, CAT, and GSH levels are reduced. Early administration of solasodine can ameliorate progressive ischemic injury through its potent antioxidant properties [43]. The Nrf2/ARE pathway is referred to as a potent defense mechanism against oxidative stress, which is expected to be a feasible direction of antioxidant treatment against ischemia-reperfusion (I/R) injury. In the Shah and Li study, they found that Nrf2 knockout mice in the I/R group present more obvious neurologic deficits than the wild type group with a significant increase in the area of infarction [44]. Enhancing the activation of Nrf2 by tBHQ, a natural Nrf2 inducer, can reduce and limit brain damage and is therefore possibly a practical prevention strategy for stroke-prone patients [45].

Studies have shown that the cellular damage in the early stages of AD is ascribed to oxidative stress [46], and notably, a large number of markers of oxidative stress are located in intracellular NFTs, a hallmark of the brains of AD patients [47]. A significant decrease in GPx and CAT activities and total GSH levels, which indicates a feeble antioxidant defense system in early AD, may facilitate the development of the disease. Meanwhile, extensive experiments collectively verify that antioxidants do delay the occurrence and progression of AD [48]. These oxidative stress indicators are used to characterize the earliest events of AD and are reliable tools for early diagnosis and prevention of AD [49].

Recent progress in PD has revealed that dopaminergic neurons are susceptible to oxidative stress because of inherent biological features. Clear evidences show that 4-HNE within such body fluids as CSF and serum is widely described as a clinical parameter of oxidative damage in PD individuals. Reactive (OH) and subsequent MDAs have been reported to be significantly increased in PD patients, which contribute to dopaminergic neuronal loss [50]. Nrf2 exists in the nigral dopaminergic neuron cytoplasm, but is located in the nucleus of age-matched PD patients, which strongly suggests that Nrf2 may contribute to combating oxidative brain damage via the transcription of genes encoding antioxidant enzymes [51]. Recent studies have claimed that upregulation of Nrf2 provides neuroprotection against oxidative stress-induced neurotoxicity in PD. Rb1 can enhance the transcriptional activation of Nrf2 and upregulate the expression of HO-1, an endogenous antioxidant enzyme and downstream effector of Nrf2, by modulating PI3K-mediated Nrf2-ARE signal pathway, which is shown to serve as a rational cytoprotective agent against oxidative insults of dopaminergic cells [52]. Taken together, ROS elevation initiates neuronal damage and we propose that Nrf2-related agents look set to offer an up-and-coming clinical therapy.

3. Autophagy

Autophagy was observed in mouse hepatocytes by Ashford and Porter for the first time in 1962 and visually described as cellular self-eating [53]. Nevertheless, it was De Duve that first came up with the concept of autophagy in 1967 [54]. Autophagy refers to macroautophagy in this review, the most common and well-studied form, which is distinguished from microautophagy and chaperone-mediated autophagy (CMA) by the different degradation pathways of substrates [55, 56]. Autophagy induction is a complicated and ordered multistep process, which mainly includes the following steps: the signal stimulus, then autophagosome formation and fusion with lysosomes, and finally the degradation and release of its contents.

It has also been copiously reported that autophagy can facilitate the renewal of cellular constituents to guarantee energy and materials of quality needed to sustain metabolic reactions, which orchestrates such biological processes as proliferation and differentiation of cells under various physiological or pathological conditions [57]. Typically, autophagy exists at a low level and a basal rate in most cells [58], but it can be activated rapidly in response to excessive release of ROS, abnormal aggregates of misfolded proteins, or a collapse of mitochondrial membrane potential (MMP) apart from infection, cancer, ATP, or nutrient deficiency [59, 60].

It is well established that only adequate autophagy is a kind of cellular self-defense mechanism in times of oxidative stress and other unfavorable conditions [61]. However, improper autophagy above or below a certain threshold is instead disadvantageous [11], likely accelerating the progression of all the related diseases such as neurodegenerative diseases, cerebral ischemia, and cancer [62, 63].

3.1. Autophagy-Relative Marker Proteins. As discussed previously, autophagic elimination is a highly sophisticated process during which unwanted or redundant organelles and bits of cytoplasm are enveloped then swept away in a lysosome-dependent manner. Each step is finely regulated

by relevant proteins that were first discovered in yeast but later verified in higher organisms [63, 64].

LC3 is a mammalian, homologous protein of Atg8 in yeast that has been identified to be the most widely used specific marker of autophagy initiation. LC3 is first synthesized as its precursor, then cut up into its cytosolic form, LC3-I, which can be processed into LC3-II [65]. LC3-II specifically binds to the newly formed autophagosome essential for the elongation stage of the phagophore membrane. The amount of autophagosome can be mirrored by the expression of LC3-II or LC3-II/LC3-I [66]. Mizushima et al. [67] were able to dynamically trace the formation of autophagosomes by using fluorescence characteristics of GFP in established GFPLC3 transgenic mice, which greatly facilitated the study of the molecular mechanisms of autophagy. Beclin1, the first mammalian autophagy-related gene to be identified, regulates the activity of autophagy particularly in the initiation phase by combining with different ligands [68]. Beclin1 can modulate autophagic flux by interacting with PINK1 [69].

In addition, there are observable changes of p62/SQSTM1 in the progression of canonical autophagy [70]. P62 is negatively correlated with autophagy activity, reflecting the degradative capability of autophagy and the intensity of autophagic flux [71]. The receptor protein p62 can be recruited to the autophagosome membrane when LC3-interacting region (LIR) motif targets a substrate (ubiquitinated protein aggregates, damaged mitochondria [72]) and initiates selective degradation in an autophagy-lysosome manner.

3.2. Autophagy-Relative Signal Pathway.

Prevailing studies indicate that signal transduction pathways associated with autophagy may be more complex than the following two: the mammalian target of rapamycin (mTOR) pathway and the class III phosphatidylinositol 3-kinase (PI3K-III) complex.

Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, is engaged in autophagy modulation as a dominant downstream negative regulator [73]. mTOR complexes exist in two types, namely mTORC1 and mTORC2, which are distinguished by different components. mTORC1, a regulatory associated protein composed of Rictor, has been demonstrated to terminate the autophagy progression as a critical signaling molecule that is susceptible to the strong inhibition of rapamycin [74, 75]. When cells suffer hypoxia, energy depletion, and other stimuli, mTORC1 activity is simultaneously restrained with the activation of autophagy. Suppressed mTORC1 plays a causal role in the activation of ULK1 complex by dephosphorylating the autophagy-related gene13 (Atg13) and mediating a tighter combination of ULK1, Atg13, and FIP200. ULK1 is homologous with Atg1 in yeast, which has been found to be involved in the induction of autophagy. The ULK1-Atg13-FIP200 complex is not only a direct target of mTOR but a key regulator of other autophagy-related signaling pathways.

The PI3K-III complex is composed of VPS34 (catalytic subunit), Beclin1, and Atg14. When activated by the ULK1 complex, the PI3K-III complex is positioned into the endoplasmic reticulum and further generates PI3P that binds to downstream effectors, playing an important role in the earlier period of autophagic vacuole formation [76, 77]. When discussing the PI3K-III complex, it is common to mention that the class I PI3K and its downstream target AKT, as with MAPK/ERK1/2 signaling, which can exert negative regulatory effects at any stage of induction of autophagy via activating mTOR [78].

Arguably, distinct signaling pathways involved in the autophagic process vary with different adverse stimuli. AMP-dependent protein kinase (AMPK), an upregulated modulator of autophagy, can sense subtle levels of ATP. On the one hand, AMPK can activate autophagy with a direct inhibitory effect on mTORC1 [79]. On the other hand, p-AMPK can activate TSC1-TSC2 complex, indirectly suppressing the activity of mTORC1 and concurrently initiating autophagy [80]. In addition, AMPK can also combine with ULK1 complex and phosphorylate ULK1, accelerating the progress of autophagic membrane formation [81] (Figure 2).

3.3. Mitophagy.

Past studies have argued that autophagy does not select which substrates are to be degraded [82]. However, a widely accepted view, proposed in 2005, is that there is a selective form of autophagy in which damaged or unnecessary mitochondria are eliminated [83]. This nonclassical autophagy was defined as mitophagy, and simultaneously or successively, other types of selective autophagy such as xenophagy, pexophagy, ribophagy, and reticulophagy were also identified [59, 84].

Mitochondria are a sensitive organelle ubiquitously found in eukaryotic cells. They are responsible not only for energy-generating processes, but also for producing a basic amount of ROS [85]. Mitochondria form a complicated network regulated by other cellular mechanisms, in which mitochondria are interconnected and interlocked in a perfectly coordinated order. Impaired mitochondria are a threat to proper cellular function because they result in a lack of energy generation and excessive release of ROS [86]. Therefore, it is urgent that dysfunctional mitochondria that interfere with the energy supply and provoke oxidative stress be quickly removed [87]. Fortunately, mitophagy can shoulder this responsibility as an effective cytoplasmic protection mechanism.

Mitophagy is a programmed mitochondrial elimination mechanism that fosters a balance of mitochondrial quantity and quality [59, 87]. It usually occurs in the case of an abnormal increase of ROS, poor nutrition, hypoxia [88], cells senescence, and such stress. These stimuli can cause mitochondrial membrane depolarization or a loss of MMP. Pathological opening of the mPTP may serve as the switch for mitophagy. Existing studies suggest that there are two relatively recognized mitophagy pathways involved in mitochondrial homeostasis. These two pathways are the PINK1/Parkin-mediated pathway and the Bnip3/Nix-mediated pathway. The PINK1/Parkin-mediated pathway is closely associated with Parkinson's disease and is a topic of current research [89].

3.3.1. PINK1/Parkin-Mediated Pathway.

PINK1, a serine/threonine protein kinase, is located on the outer membrane of mitochondria and is the upstream regulator of Parkin [90]. Parkin is an E3 ubiquitin ligase, which is present in
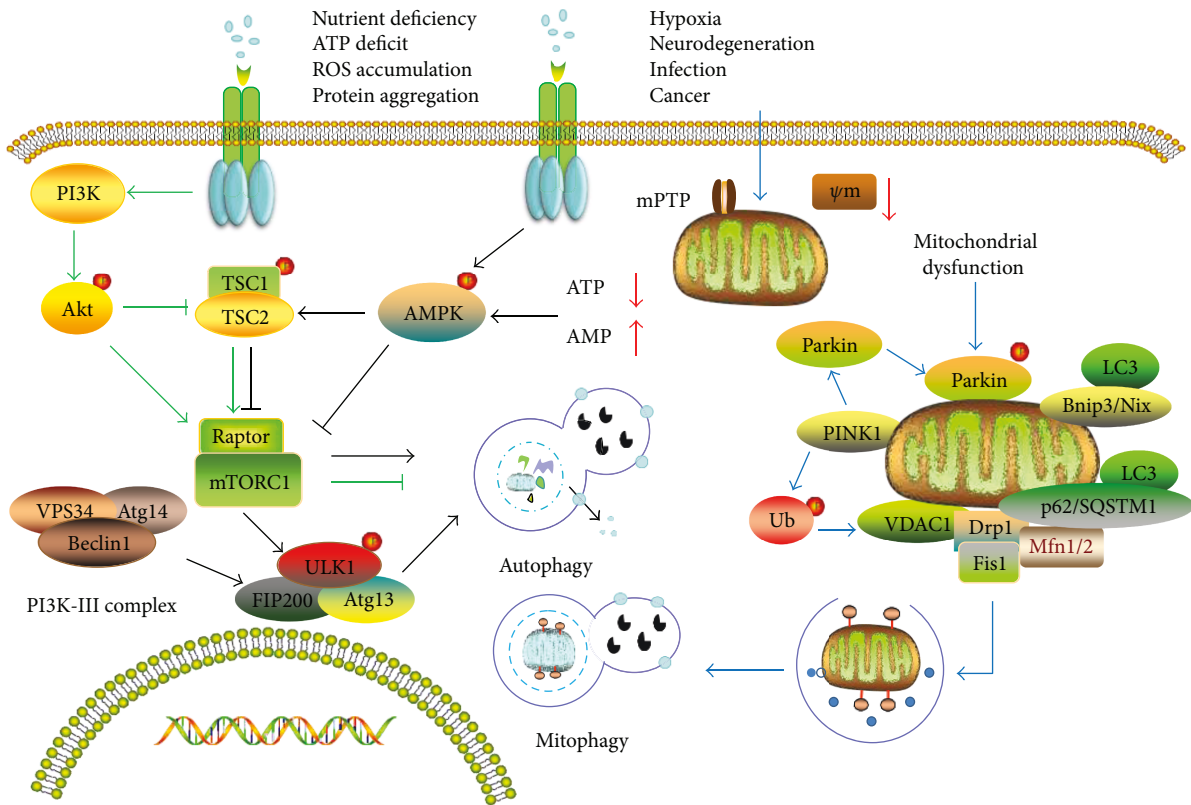
FIGURE 2: The generation and scavenging of reactive oxygen species (ROS) in mitochondria. The "→" refers to activation or induction, and the "⊢" refers to inhibition. Under normal or stress conditions, ROS is mainly born from the mitochondrial respiratory chain with the beginning of O_2^- production, followed by the conversion to H_2O_2 then OH^- under the catalysis of SOD and GSH-px. Defective mitochondria can instigate ROS accumulation with a "RIRR" positive-feedback mechanism. Excessive ROS can inflict severe damage on biomacromolecules, which can be counteracted by the antioxidant enzyme system to some degree.

the cell plasma [91] but has no mitochondrial targeting sequence (MTS) [92]. As a matter of fact, PINK1 can be degraded away quickly by proteolytic enzymes in healthy mitochondria. In the disturbed mitochondria, it will accumulate following depolarization of the membrane potential, phosphorylate Parkin, and then recruit Parkin from the cytoplasm [90]. Along with strengthening E3 ubiquitin ligase activity, Parkin can ubiquitinate the mitochondrial matrix proteins (voltage-dependent anion-selective channel protein 1, VDAC1), recruit p62/SQSTM1 to the surface of mitochondria, and then combine with LC3 to initiate mitophagy [93].

Emerging research indicates that RAD6A (Ube2a), a gene encoding ubiquitin binding enzyme (E2) that is required for the ubiquitination and subsequent clearance of defective mitochondria, can operate with Parkin to regulate mitophagy upon mitochondrial depolarization in mice cortical neurons. Whether the program is dependent on PINK1 needs further scrutiny [94].

3.3.2. Bnip3/Nix-Mediated Mitophagy. Bnip3, a proapoptotic protein, has some degree of homogeneity with BCL-2. Nix is 56% homologous with Bnip3. Both widely existed in mitochondria and are implicated with autophagy and mitophagy in particular [95]. Bnip3 induces autophagy after hypoxic damage and has been reported to have a protective effect by removing injured mitochondria [96]. Recent studies have

shown that Bnip3/Nix directly interacts with LC3 to activate the mitophagy pathway [97, 98]. Some researchers believe that though Bnip3 and Nix are involved in mitophagy upon the loss of mitochondrial membrane potential, they may execute mitochondrial clearance via independent but functionally related mechanisms [99, 100] (Figure 2).

Additionally, Mieap can also induce mitophagy after ROS and oxidative damage to restore a healthy pool of mitochondria [101]. Last but not least, mitochondrial fusion, division, and transportation are tightly linked to mitophagy [102].

3.4. Autophagy in Neurological Disorders. Not surprisingly, autophagy is extensively observed in nervous system disorders [61]. It has long been thought that autophagy is the primary means for the biodegradation of abnormal protein aggregation and dysfunctional organelles in CI, AD, and PD [103]. Defects in mitochondrial autophagy will aggravate ischemic tissue damage with irreversible neurologic deficit [104], render cognitive and memory defects in AD as a consequence of progressive aggregation of Aβ [105], and promote dopaminergic neuronal death and the occurrence of PD [106]. These results of defects in mitochondrial autophagy indicate that mitophagy acts as an endogenous protective mechanism in the process of neurological disorders. At present, although a growing number of studies have argued that autophagy is activated in various rat and mouse models of

cerebral ischemia or hypoxia-ischemia [9, 107–110], whether autophagy is protective or detrimental in the process of CI still remains unclear [111].

4. Reactive Oxygen Species (ROS) and Autophagy

A growing body of reports has demonstrated that most stressful events, such as nutrient deficit and hypoxia, which necessitate a greater energy supply and then aggravate mitochondrial burden along with increasing ROS, are related to the initiation of autophagy [16]. Intriguingly, an increasing amount of evidence suggests that ROS are seen as essential signals to activate autophagy under various stimulating conditions [112, 113]. Both moderate and increased ROS levels can specifically trigger mitophagy which is conducive to cell survival in a different manner, while only excessive ROS can activate general autophagy [114].

The molecular signaling pathways involved in both the initiation and execution of autophagy following exposure to ROS are sophisticated [16, 18]. The pathways mainly include transcriptional progress in the nucleus and posttranscriptional progress in the cytoplasm. These specific transcriptional regulatory mechanisms first involve the activation of HIF-1, p53, FOXO3, and NRF2; then, the corresponding proteins are produced and modulation of autophagy occurs where the cytoplasm was exposed to ROS. Take hypoxia-inducible factor (HIF) for example, it is involved in cell survival under hypoxic conditions and participates in the transcription of Bnip3 and NIX in response to ROS. These autophagy-associated protein products can constitutively stimulate autophagic clearance of damaged mitochondria and decrease ROS levels [115].

In addition, numerous studies have supported that ROS may regulate autophagy via mTOR-dependent pathways in the cytoplasm [116–118]. Nevertheless, most of the literature maintains that ROS available to elicit autophagy are mainly H_2O_2 and O_2^- produced by mitochondria [14, 112]. When there is an elevated level of H_2O_2, a relatively stable and prolonged stimuli, suppressed autophagy via the PI3K-Akt pathway, can be reactivated by blocking PTEN as well as inhibiting the activity of Akt or mTORC1 [119]. Similarly, H_2O_2 in excess can induce autophagy in an AMPK-dependent manner and is accompanied by the decline of mTORC1 activity [18]. Beyond that, a wide range of stress response proteins such as p38MAPK, extracellular regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) is also involved with autophagy induction in the presence of abundant ROS [120]. Taken together, it is an indisputable fact that ROS is an available regulator despite autophagy making a difference in both cell survival and death as a double-edged sword.

From another perspective, autophagy has been proposed as a potential survival mechanism in the face of ROS production by removing damaged or redundant components to prevent unnecessary oxidative damage [19]. Furthermore, there are increased intracellular ROS levels in cells with defective autophagy protein Atg7 [121]. Specially, the selective elimination of dysfunctional mitochondria via autophagy also

serves as a cytoprotective process to limit the production of ROS and avoid potential oxidant injury [122].

It is also believed that a number of signal transduction pathways related to autophagy are available to modulate ROS. The Keap1-Nrf2 system is now considered a defense mechanism upon exposure to oxidative stress [123, 124]. As mentioned earlier, the p62/SQSTM1 protein, or p62 for short, may contribute to autophagosome formation as an autophagic adaptor and/or receptor [125]. Phosphorylation of P62 in the mTORC1-dependent autophagy pathway can promote the integration of ubiquitinated cargos and phosphorylated Keap1, which is necessary for the degradation of Nrf2 [126, 127]. Released Nrf2 is reactivated, translocated into the nucleus while binding to ARE, and eventually stimulates transcription of antioxidant genes. Beyond that, mitochondrial hexokinase II (HKII) shares a deep relationship with autophagy and redox homeostasis. HKII induces the inactivation of mTORC1, further opens mPTP, and creates a preventive antioxidant defense by decreasing release of ROS [128, 129].

In conclusion, there is little doubt that ROS play a positive role in the activation of autophagy under various stimulating conditions [112, 113]. By coincidence, autophagy plays a crucial role in maintaining redox homeostasis [6]. ROS can induce autophagy, and autophagy serves as a buffer system to control the level of ROS in cells and reduce their toxic effects [130]. The interplay of autophagy and redox response via various signaling pathways may be involved with the modulation of cellular homeostasis [127] (Figure 3).

4.1. Reactive Oxygen Species (ROS) and Mitophagy. As stated earlier, mitochondria are believed to be the primary source of ROS. Coincidentally but unfortunately, they are also the major target of oxidative stress triggered by ROS, which may result from the fact that mitochondria are an important site for nucleic acid, lipid, and amino acid production. Excessive ROS stimuli can inflict peroxidation damage on these biomacromolecule precursors and create toxic byproducts [131]. Note that mtDNA lacks the protection of histones, and its repair capacity is rather poor. It is therefore more vulnerable to ROS than nuclear DNA [132] and is bound to leave mitochondria heavily damaged by ROS.

Mitochondrial dysfunction caused by a high concentration of ROS not only can activate and regulate nonselective autophagy, but also can be involved in mitophagy which selectively removes damaged mitochondria. ROS and oxidative stress have been shown to be involved in the recruitment and localization of Parkin and DJ-1, specific proteins that are closely tied to the activation of mitophagy [133].

Selective autophagy is a protective mechanism that reduces ROS production by means of removing unneeded mitochondria, thereby alleviating oxidative damage [16, 122]. More importantly, defects in mitophagy can aggravate lipotoxicity, hinder selective degradation of defective mitochondria caused by ROS, and thus cause subsequent damage to the cells [134].

Haddad et al. [94] discovered that RAD6A can cooperate with Parkin to ubiquitinate mitochondrial proteins associated with the initiation of mitophagy for clearing dysfunctional
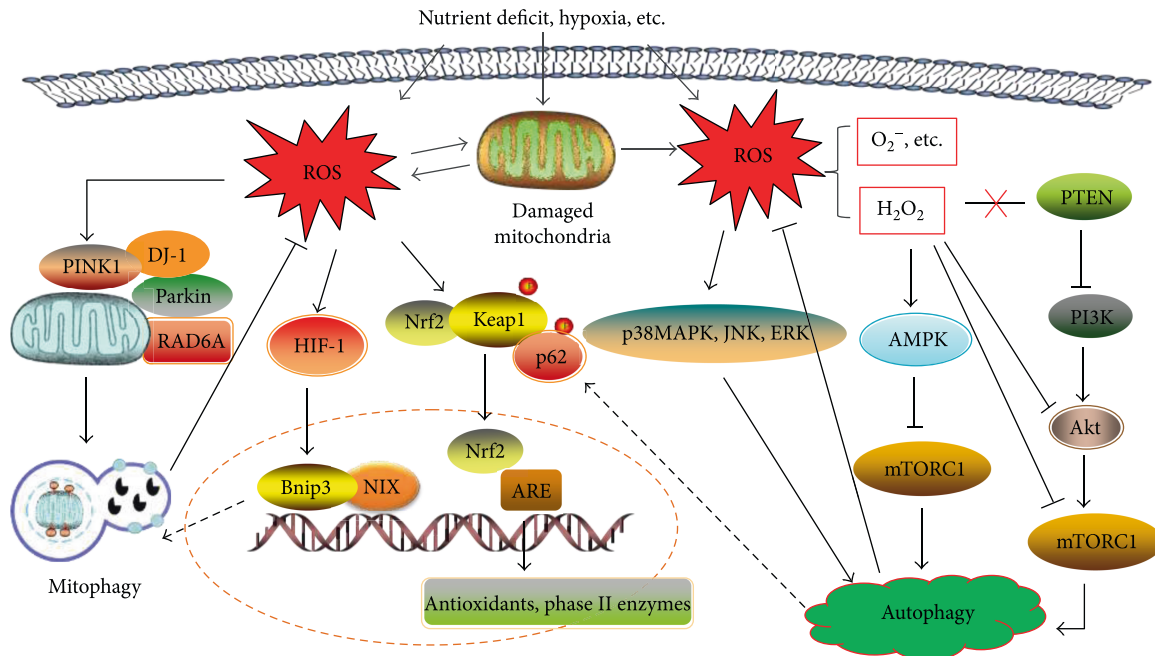
FIGURE 3: The interrelation of ROS and autophagy/mitophagy, coupled with the relevant signal transduction pathways. ROS available to induce autophagy is mainly mitochondrial H_2O_2 and O_2^-, which may modulate autophagy via mTOR-dependent pathways. ROS-induced autophagy and mitophagy both can abort ROS for redox homeostasis. In response to abundant ROS, the Keap1/Nrf2/ARE cascade is activated as a potent antioxidant mechanism. Phosphorylation of P62 by autophagy can promote the integration of phosphorylated Keap1 and ubiquitinated Nrf2, then negative regulation of Keap1 frees Nrf2 from degradation, and reactivated Nrf2 is translocated into the nucleus to bind to ARE for the transcription of antioxidant genes and phase II enzymes.

mitochondria and dampening oxidative stress. Particularly, RAD6A mutations cause neuronal function defects primarily by disrupting mitophagy (Figure 3).

4.2. Reactive Oxygen Species (ROS) and Autophagy (Mitophagy) in Neurological Disorders. ROS are described as the culprit of almost all neurological conditions [135]. Mounting evidence has indicated that ROS participate actively in autophagy in many cells, including neurons [131, 136]. Autophagy removes or degrades nonfunctional cytoplasmic content as an intracellular self-purification mechanism. Neurons are highly sensitive to autophagic degradation, and the integrity of mature neurons depends on the high level of autophagy because of their postmitotic nature [14, 137]. Also, autophagy can reduce ROS damage by eliminating unnecessary or damaged organelles and abnormal protein aggregates, as well as inhibiting the excessive activation of ROS in response to neuronal damage, which is conducive to the survival of nerve cells [138]. Emerging evidence indicates that autophagy may exhibit an antioxidant defense system, which has been proposed to provide a remarkable impact on neuronal bioenergetic health [139].

4.2.1. Reactive Oxygen Species (ROS) and Autophagy (Mitophagy) in CI. Ischemic cerebrovascular disease (CI) is a leading cause of death and disability worldwide [140]. Currently, endovascular intervention and venous thrombolysis are conventional therapies for restoring the blood supply required for the recovery of nervous function. However, both animal studies and clinical findings have revealed that

reperfusion following ischemia results in a more serious brain damage [141]. The ischemia-reperfusion injury is a complicated pathological process involving multiple factors, among which oxidative stress stands out [5, 142]. There is an extensive damage of mitochondria, including irregular mitochondria swelling and their crista fragmenting, in CI especially during the acute phase. This damage stimulates mPTP to open continuously, leading to a change in membrane potential, energy deficit, and ROS generation, thus inducing autophagy.

It has been observed that autophagy occurs dramatically in the mouse striatum and cortex following cerebral hypoxic-ischemic injury and can then be strongly amplified by an ensuing overproduction of ROS. Autophagy in this context can substantially rescue neurons in the ischemic penumbra by preventing necrosis and apoptosis via eliminating impaired mitochondria [143].

It has been reported that FA deficiency dramatically alters ischemia-induced activation of autophagy. This is reflected by the elevated levels of LC3 and Beclin1 expression, which are accompanied by a remarkable increase in 8-OHdG, indicating that FA deficiency may enhance autophagy levels by triggering oxidative damage [144]. One study has shown that both ROS and autophagy are engaged in reperfusion injury after cerebral ischemia and that autophagy can be activated by antioxidants. The application of antioxidants or autophagy revulsive can reduce neuronal damage and significantly decrease the infarction area [145]. Thus, we can speculate that antioxidants might play a protective role in ischemic injury by inducing autophagy. There may

TABLE 1: The interplay between ROS and autophagy/mitophagy in neurological diseases.

Author	Year	Model (animal/cell)	Main idea	Effect of autophagy
Zhao et al. [144]	2016	MCAO/SD rats	FA deficiency simultaneously enhanced the activity of autophagy and induced the generation of oxidative stress following the MCAO model; oxidative injury seems to be involved in excessive activation of autophagy caused by FA deficiency.	Detrimental
Wenjing et al. [145]	2013	Mouse & neural cells	Autophagy is upregulated, and the level of ROS is elevated in the central nervous system after ischemia-reperfusion; Antioxidants can protect neural cells and decrease infarct volume possibly by activating the autophagic pathway of cells.	Protective
Kubota et al. [146]	2010	MCAO/SD rats	Chemical inhibitors of autophagy or lysosomes can delay the release of mitochondrial ROS to prolong the therapeutic time window. Ischemic insults will immediately initiate autophagy induction with undefined mechanisms, which significantly will impact ROS production and oxidative damage in vivo.	Detrimental
Dai et al. [147]	2017	OGD/cortical neurons	Sirt3 showed a protective role in eliminating intracellular H_2O_2, attenuating mitochondrial O_2^-, and promoting autophagy through the AMPK-mTOR pathway in neuronal ischemia.	Protective
Shao et al. [149]	2016	SH-SY5Y/neuronal cells	SIRT6-mediated autophagy contributes to oxidative stress-induced neuronal injury since inhibition of autophagy could prevent the detrimental effect of SIRT6 on cell survival, which could be attributed to attenuation of AKT signaling closely related to oxidative stress.	Detrimental
Khandelwal et al. [105]	2011	3xTg-AD mice	The autophagic removal of Aβ mediated by Parkin can attenuate oxidative stress and mitochondrial dysfunction to restore energy supply for a better modulation of autophagy in AD transgenic mice.	Protective
Giordano et al. [154]	2014	PD mouse model	Autophagy is proposed as an antioxidant protective pathway that can clear cumulative ROS and reverse established ROS-induced protein, DNA, and lipid damage independent of the disposal of radical scavengers.	Protective
Underwood et al. [155]	2010	Mouse cortical neurons	Autophagy can scavenge aggregate-prone proteins and increased ROS, while antioxidants can block autophagy and thereby counterbalance the benefits of autophagy and exacerbate neurodegeneration.	Protective
Dagda et al. [159]	2009	PD cell model/SH-SY5Y	Loss of PINK1 function can stir oxidative stress, which can then elicit coordinated autophagy and mitophagy for mitochondrial turnover by a removal of dysfunctional mitochondria.	Protective

be some more complicated mechanisms of crosstalk between autophagy and oxidative stress in need of further research. One study pointed to the finding that ischemic insults could immediately activate autophagy as a neuroprotective mechanism, which significantly affects ROS generation and oxidative toxicity. As well, pharmacological inhibition of autophagy or lysosomes can delay the mitochondrial ROS burst [146]. Scherz-Shouval and Elazar [15] have argued that ROS can upregulate autophagy through multiple signaling pathways. Sirt3 is a conserved deacetylase associated with biological functions such as energy metabolism, stress resistance, and mitochondrial redox homeostasis. Furthermore, it can positively regulate autophagy through the AMPK-mTOR pathway [147], which promotes neuronal survival within an in vitro oxygen and glucose (OGD) deprivation model of cerebral ischemia created by attenuating H_2O_2 and O_2^- [148]. Pharmacological or genetic inhibition of autophagy can ameliorate SIRT6-mediated neuronal injury, probably via attenuating AKT signaling closely related to oxidative stress in the OGD model of SH-SY5Y neurons [149]. Further,

in vivo mechanistic studies are needed to verify the interplay of oxidative stress and autophagy. Furthermore, moderate activation of ROS can promote the translocation of Parkin to injured mitochondria and then incur Parkin-mediated mitophagy and ensure the integrity of mitochondria in ischemic brain injury [150] (Table 1).

4.2.2. Reactive Oxygen Species (ROS) and Autophagy (Mitophagy) in AD. Alzheimer's disease (AD) is one of the most common types of late-onset neurodegenerative diseases, hallmarked by a progressive loss of memory and cognition coupled with typical pathological features including neuritic plaques (NPs) and neurofibrillary tangles (NFTs) [46, 151]. Enhanced ROS and oxidative damage have been proven to be implicated in the evolution of neuronal dysfunction during the early events of AD [152].

Growing evidence suggests that spatial learning and memory deficits in AD may be tightly correlated with the impairment of the Nrf2-ARE pathway since Nrf2 knockout confers AD model mice with more sensitivity to neuronal

damage. Strikingly, some scholars have proposed that the interaction between oxidative stress and mitochondrial dysfunction may be involved in the process of AD because of the influence oxidative stress has on mitochondrial transport [153]. It has been observed that autophagic vacuoles with engulfed, defective mitochondria increased in the pyramidal neurons of AD patients [7]. The autophagic removal of damaged mitochondria and Aβ mediated by Parkin can attenuate oxidative stress and restore the energy supply so as to delay or prevent neurodegeneration in AD transgenic mice [105] (Table 1).

4.2.3. Reactive Oxygen Species (ROS) and Autophagy (Mitophagy) in PD. Parkinson's disease (PD) is a movement disorder with three outstanding clinical characteristics: bradykinesia, resting tremor, rigidity, and postural instability. Although the underlying etiology of PD is still far from clear, oxidative stress and mitophagy deficiency have been proposed as the principal elements in the development of dopaminergic neuronal death in the substantia nigra of PD patients.

Neurodegenerative diseases such as PD are often accompanied by increased oxidative brain damage coinciding with a reduction in antioxidants. This leads to dysfunctional mitochondria or protein aggregates that can be rescued to some extent by radical scavengers. Autophagy has been proposed as an endogenous, antioxidant, protective pathway that can clear accumulated ROS and reverse established ROS-induced protein, DNA, and lipid damage independent of the disposal of radical scavengers [154]. Protein accumulation and oxidative stress are pathologically pronounced in neurodegenerative diseases. Enhancing autophagy could scavenge aggregate-prone proteins and increased ROS, while antioxidants could block the benefits of autophagy and exacerbate neurodegeneration [155].

Mitochondria have a central role in redox regulation of autophagy as the generator and scavenger of ROS [156], but can be attacked when ROS exceed the scavenging activity. The dysfunction of mitochondria is a prominent initiating factor of nervous system diseases [157]. This dysfunction then amplifies oxidative damage, with the underlying assumption that the quality and quantity of mitochondria significantly affects neuronal function. Mitophagy was originally proposed to clear disturbed mitochondria after pathological stress in an attempt to restore homeostasis [158].

Dagda et al. discovered that knockdown of PINK1 in a recessive PD model can result in the accumulation of mitochondrial ROS, accompanied by clustered fragmented mitochondria and depolarized mitochondria which correlate with autophagy. More importantly, autophagy does play an essential role in limiting dopaminergic neuronal death in this genetic model and RNAi knockdown of genes necessary for inducing autophagy exacerbates the occurrence of PD [159] (Table 1).

Several studies have claimed that mitochondrial dysfunction and the existence of mitochondrial complex I defects also contribute immeasurably to the disease by playing a causative or consequential role in the exacerbation of oxidative stress in dopaminergic neurons. DJ-1, a causative protein of familial PD, is essential for modulating PINK/Parkin-mediated mitophagy [160]. Both DJ-1 and DJ-1-binding compounds have been identified as neuroprotective against oxidative stress in PD rats [161].

5. Conclusion and Perspective

Plenty of studies have repeatedly shown that ROS accumulation displays detrimental implications for the basic function and survival of neurons. ROS or oxidative stress can provoke autophagy, and autophagy can take part in the removal and repair of ROS-induced oxidative lesions through a variety of signaling pathways. But autophagic neuronal death will still result if cumulative ROS go beyond the scavenging activity of autophagy. At present, it appears to be contradictory that autophagy serves as a cellular self-purification mechanism, but hyperactivity or hypoactivity of autophagy is unfavorable for the normal functionality of neurons [162, 163]. After all, the predetermined threshold level of perfect autophagy is often blurred, particularly under a variety of disease courses. So, more relevant, constructive research should be undertaken without delay.

Mitochondria are thought to be crucial for neuronal function and fate by supplying energy and modulating redox status. It is well established that brain mitochondrial dysfunction or mitophagy defects are strongly associated with the initiation and progression of CI, AD, and PD. Neuronal mitochondrial impairments exhibit pronounced effects on mitochondrial membrane potential, leading to the prolonged opening of mPTP and an elevated production of ROS which can be rescued by mitophagy and ensuing mitochondrial turnover. Concurrently, treatment with mitochondria-targeted antioxidants substantially mitigates neuronal mitochondrial disturbance and oxidative damage [164, 165].

In summary, we provided a basic knowledge of ROS and autophagy/mitophagy and then expatiated specifically on the interrelation between ROS and autophagy as well as on their molecular regulatory mechanisms. Finally, we discussed the interplay of ROS and autophagy in CI, AD, and PD. Nonetheless, a lot of current work only focuses on the close interplay between ROS and autophagy/mitophagy in CI and PD, while there are few studies on how they are involved in AD and the underlying, precise, regulatory mechanisms are not well investigated. In the future, more basic research is needed to further excavate the correlation between autophagy/mitophagy and ROS together with their possible mechanisms in neurological disorders. Such research will lay a good foundation for pinpointing late-model drug targets and exploring aggressive therapeutic tactics that are applicable for the clinical treatment of such life-threatening neurological diseases.

Conflicts of Interest

The authors have no conflicts of interest.

Authors' Contributions

Congcong Fang and Lijuan Gu equally contributed to this work.

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