

Opinion

Rhes as a Potential Modulator of PINK1/Parkin-Dependent Mitophagy in Huntington's Disease

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Abstract

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by cytosine-adenine-guanine (CAG) repeat expansion in the huntingtin (*HTT*) gene, resulting in progressive motor, cognitive, and psychiatric impairment. Despite the widespread expression of mutant huntingtin (*mHTT*), disease progression is characterized by the selective degeneration of striatal neurons. Mitochondrial dysfunction and impaired mitophagy have emerged as important contributors to HD pathogenesis; however, the mechanisms underlying defective mitochondrial quality control remain incompletely understood. Ras homolog enriched in the striatum (Rhes), a small striatal-enriched guanosine triphosphatase (GTPase), has been implicated in mitophagy-related signalling. In this study, we examined the potential relationship between Rhes and PTEN-induced kinase 1 (PINK1)/Parkin-mediated mitophagy in HD. We propose that Rhes contributes to selective striatal vulnerability by promoting mitochondrial depolarization through BCL2 interacting protein 3-like (NIX/BNIP3L), facilitating activation of the PINK1/Parkin mitophagy pathway, while enhancing autophagosome biogenesis through Beclin-1 signalling. If validated, our proposed pathway would open new avenues for therapeutic development in HD.

Keywords: Huntington disease; huntingtin protein; mitophagy; mitochondria; ubiquitination; neurodegenerative diseases; autophagy; mitochondrial diseases; neurons; proteins

1. Selective Striatal Vulnerability in Huntington's Disease

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by the abnormal expansion of cytosine-adenine-guanine (CAG) repeats in the huntingtin gene (*HTT*), which results in the production of mutant HTT (*mHTT*) with an expanded polyglutamine tract [1]. Although *mHTT* accumulates and aggregates in neurons, the mechanisms of pathogenesis remain multifactorial and incompletely defined. In an effort to develop effective therapeutic strategies for HD, there is an increasing need to understand the deleterious cellular and molecular consequences of the abnormal expansion of *mHTT*. An emerging contributor to neurodegeneration is dysfunction of mitophagy, the autophagic process responsible for eliminating damaged or superfluous mitochondria by lysosomal degradation [1].

It is understood that HD is characterized by dysfunction and death of specific neurons within the brain [2]. While interneurons are relatively spared, neurons in the striatum are the most susceptible to apoptotic degeneration and atrophy, particularly medium spiny neurons [3,4]. The striatum functions as an integrative node within basal ganglia circuits, receiving dopaminergic and glutamatergic inputs and projecting inhibitory outputs to structures such as the globus pallidus and substantia nigra [5]. These con-

nections enable the striatum to regulate motor function and reward-related behaviours. Selective degeneration of these neurons appears to be a hallmark of disease progression, as their selective degeneration correlates closely with the onset and severity of motor and cognitive symptoms observed in HD [6,7]. Despite the ubiquitous expression of *mHTT* throughout the central nervous system and peripheral tissues, this region-specific vulnerability is thought to involve regionally enriched modulators [8].

2. The Complex Role of Rhes in Huntington's Disease

One such modulator is Ras homolog enriched in the striatum (Rhes), a striatum-enriched guanosine triphosphatase (GTPase) implicated in HD pathogenesis and potentially influencing quality-control pathways, including mitophagy. The Ras-related dexamethasone-induced 2 (*RASD2*) gene encodes Rhes and belongs to the Ras superfamily of proteins [9]. It is a small GTPase that is highly expressed in striatal neurons [9]; however, it is also expressed at lower levels in other brain regions [10]. Although Rhes has been implicated in the development of HD, it normally serves physiological functions in the striatum and is not inherently pathogenic. Under physiological conditions, Rhes has been shown to regulate motor signalling in the striatum [11].



This striatum-enriched GTPase preferentially binds *mHTT* over wild-type *HTT*, supporting a role in HD pathogenesis. Specifically, it contributes to HD pathogenesis by enhancing cellular toxicity through SUMOylation of *mHTT* [11]. SUMOylation is a post-translational modification in which small ubiquitin-like modifier (SUMO) proteins are covalently attached to target proteins, altering their localization and function [12]. As a SUMO E3-like ligase, Rhes promotes the SUMOylation of *mHTT*, increasing the abundance of soluble *mHTT* species while reducing aggregate formation [13]. Soluble *mHTT* is considered more toxic than aggregated forms because it has more freedom to move around the cell and interact with its targets. This supports the findings of a pathogenic role for Rhes in HD [12,14].

On the other hand, subsequent work has suggested that Rhes may also exert protective effects in HD [15,16,17,18]. In HD mouse models, suppression of Rhes exacerbated striatal atrophy and behavioural abnormalities, whereas restoration of Rhes improved motor deficits and neuropathology [16,17]. Lee et al. [16] demonstrated that the neuroprotective effects of Rhes were dependent on its ability to activate the mechanistic target of rapamycin complex 1 (mTORC1) signalling. However, these findings do not exclude a pathogenic role for Rhes. The central conclusion that Rhes is neuroprotective is largely based on adeno-associated virus (AAV)-mediated overexpression of Rhes and constitutively active Rheb, which produces supraphysiological levels of these proteins. This approach does not replicate endogenous Rhes signalling in HD and may activate compensatory pathways that are not normally engaged in disease [16]. Consequently, the observed benefits may reflect experimental enhancement of mTORC1 signalling rather than the physiological function of Rhes itself [11,13].

In addition to its intracellular roles, Rhes has been implicated in the intercellular propagation of HD pathology. Rhes induces the formation of tunnelling nanotube-like protrusions, termed “Rhes tunnels”, through which it travels between neighbouring cells [19]. This mechanism facilitates the direct intercellular transport of *mHTT*, potentially amplifying its pathogenic footprint across the striatum [19].

The endogenous interaction of Rhes with *mHTT*, its promotion of toxic soluble *mHTT* species, and its facilitation of intercellular transport of *mHTT* support its pathogenic role in HD and its contribution to selective striatal vulnerability.

3. Mitochondrial Dysfunction in Huntington's Disease

Rhes-mediated toxicity is unlikely to operate in isolation in HD. HD pathogenesis is characterized by several converging cellular defects, among which mitochondrial dysfunction has emerged as one of the most consistent and biologically relevant mechanisms.

3.1 Impaired Mitochondrial Energy Production and Bioenergetics

Mitochondrial dysfunction in HD affects how neurons produce energy. Because neurons rely heavily on oxidative phosphorylation for adenosine triphosphate (ATP), even minor impairments in mitochondrial respiration can disrupt synaptic activity. There are also early signs of energy production defects in HD, with reduced glucose use in the basal ganglia, suggesting impaired oxidative metabolism and a shift toward less efficient energy production [20,21]. These defects are linked in part to the disruption of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α)/peroxisome proliferator-activated receptor (PPAR) signalling, a pathway that regulates mitochondrial biogenesis and oxidative respiration. In HD, *mHTT* can interfere with and reduce PGC-1 α transcription, which is required for mitochondrial oxidative phosphorylation [22]. Supporting the importance of this pathway, activation of this pathway improved respiratory capacity and oxidative respiration, ultimately improving mitochondrial function in HD models [23,24].

Bioenergetic impairment in HD extends beyond transcriptional regulation. In HD, reduced activity of respiratory chain complexes, specifically complexes II and III, lowers the mitochondrial transmembrane potential and compromises ATP production [25,26]. Enzymes involved in the tricarboxylic acid cycle and glucose metabolism are also affected [27,28,29]. This energetic vulnerability may be region-specific. In the cortex specifically, this reduction was associated with lower levels of aconitase, a tricarboxylic acid cycle enzyme, and increased protein carbonyls, a marker of oxidative protein damage [30].

The therapeutic relevance of these defects is supported by strategies that attempt to restore mitochondrial energy capacity. Creatine targets the immediate ATP-buffering system by increasing phosphocreatine availability, which can temporarily support ATP regeneration when mitochondrial output is limited [31]. Other strategies target upstream by providing alternative carbon substrates that can replenish the tricarboxylic acid cycle, with evidence of improved brain bioenergetic profiles in early HD [32]. Olesoxime differs from these metabolic approaches by targeting mitochondrial membrane stability and permeability transition directly, in an attempt to preserve mitochondrial bioenergetics under stress [33,34]. However, there are limits to the efficacy of these therapies given they are downstream of *mHTT*.

3.2 Increased Oxidative Stress

Mitochondrial dysfunction in HD is closely linked to increased oxidative stress. Because mitochondria generate reactive oxygen species (ROS) as a by-product of electron transport, impaired respiratory-chain activity can increase electron leakage and ROS production. This creates a self-reinforcing cycle: ROS damages mitochondrial deoxyri-

bonucleic acid (DNA), which weakens mitochondrial function and increases oxidative injury. In HD, this is supported by evidence of reduced activity of ROS-sensitive enzymes [35,36].

mHTT may also prevent neurons from counteracting oxidative damage by disrupting antioxidant-response pathways. In particular, impaired nuclear factor erythroid 2-related factor 2 (Nrf2) and PGC-1 α signalling can reduce the expression of antioxidant defence genes, weakening mitochondrial resilience under stress [37,38]. Iron dysregulation may further amplify this process, as iron accumulation can promote hydroxyl radical formation and lipid peroxidation, linking oxidative stress to ferroptosis [39,40,41]. Oxidative stress in HD, therefore, is not simply a downstream consequence of mitochondrial dysfunction, but an active contributor to mitochondrial damage and neuronal vulnerability.

Therapeutically, this has motivated mitochondria-targeted antioxidant strategies. XJB-5-131 is a mitochondria-targeted antioxidant that accumulates in mitochondria and scavenges reactive oxygen species and *mHTT*-induced toxicity in HD models, supporting oxidative stress as a modifiable component of mitochondrial pathology [35,42,43].

3.3 Impaired Mitochondrial Calcium Handling

Oxidative stress and calcium dysregulation are closely connected in HD. Excess cytosolic calcium can overload mitochondria, while mitochondrial dysfunction can further impair calcium buffering and increase ROS production. Calcium signalling is critical for neuronal function because it regulates energy production, synaptic activity, gene transcription, and cell survival. However, *mHTT* has been shown to increase mitochondrial sensitivity to calcium, making mitochondria more likely to depolarize and undergo mitochondrial permeability transition pore opening [44]. This can impair ATP production, promote cytochrome c release, and contribute to neuronal apoptosis.

These defects become especially relevant during excitotoxic stress, when excessive glutamate receptor activation increases neuronal calcium influx [45,46]. In HD, calcium dyshomeostasis has also been linked to *mHTT*-induced neuronal death, partly through altered store-operated calcium entry and endoplasmic reticulum–mitochondrial calcium transfer [47,48]. Accordingly, calcium-targeted interventions aim to reduce calcium overload, limit mitochondrial depolarization, and protect neurons from excitotoxic injury.

However, the severity and direction of mitochondrial calcium defects appear to depend on the model used. Some studies have reported preserved or increased calcium uptake capacity in mitochondria [49,50,51]. Consistent with this complexity, genetically increasing mitochondrial calcium-buffering capacity by deleting cyclophilin D did not improve outcomes in HD [52].

3.4 Impaired Mitochondrial Trafficking, Dynamics, and Protein Import

Mitochondrial dysfunction in HD also affects mitochondrial structure and transport. Because neurons depend on mitochondrial trafficking to deliver ATP-producing and calcium-buffering capacity to synapses, disrupted transport can directly impair synaptic function. N-terminal *mHTT* disrupts microtubule-based mitochondrial movement, limiting mitochondrial delivery to neuronal processes [53]. This trafficking defect is closely linked to abnormal mitochondrial dynamics, as *mHTT* interacts with dynamin-related protein 1 (Drp1), a GTPase that drives mitochondrial fission. By enhancing Drp1 activity, *mHTT* promotes excessive mitochondrial fragmentation and impairs anterograde mitochondrial motility [54]. The functional relevance of this pathway is supported by fission-inhibition studies showing that P110-TAT and mitochondrial division inhibitor 1 (Mdivi-1) improve mitochondrial morphology and function in HD models [55,56].

mHTT may also compromise mitochondrial maintenance by disrupting protein import. Although mitochondria contain their own genome, most mitochondrial proteins are nuclear-encoded and must be imported after translation. In HD, *mHTT* binds the translocase of the inner mitochondrial membrane 23 complex (TIM23), a protein-import complex required for transporting many nuclear-encoded mitochondrial proteins into mitochondria, thereby inhibiting mitochondrial protein import [57]. Defects in mitochondrial trafficking and protein import may interfere with intracellular quality-control pathways, since damaged organelles must be properly recognized, and delivered for selective degradation [58].

It is important to recognize that mitochondrial dysfunction in HD is best characterized as a progressive loss of mitochondrial resilience rather than a single defect [59]. These abnormalities may not always produce clear mitochondrial failure at baseline, but likely reduce the capacity of striatal neurons to tolerate changes induced by excitotoxic, metabolic, or oxidative stress. This broader mitochondrial vulnerability provides the necessary foundation for examining whether Rhes may further influence mitochondrial quality-control pathways in HD.

4. Mitophagy Dysfunction and Related Pathways in Huntington's Disease

The pathogenic effects of Rhes may extend beyond its direct interactions with *mHTT*. Given the emerging role of mitochondrial dysfunction in HD, determining whether Rhes contributes to defects in mitophagy, the mitochondrial quality control pathway, represents an important gap in our understanding of the disease [38,60,61]. The most extensively characterized mitophagy mechanism involves the PTEN-induced kinase 1 (PINK1)-Parkin signalling axis [62]. PINK1 is a serine/threonine protein kinase that functions as a quality control sensor, detecting mitochondrial

damage through changes in membrane potential and initiating the mitophagic response [62]. In healthy mitochondria, PINK1 is continuously imported into the inner mitochondrial membrane and rapidly degraded by mitochondrial proteases, preventing its stabilization on the outer mitochondrial membrane [62].

In contrast, loss of the mitochondrial membrane potential can disrupt this import process, leading to the stabilization and accumulation of PINK1 on the outer mitochondrial membrane [62]. This stabilization serves as a molecular sensor of mitochondrial damage and initiates mitophagy by recruiting and activating the cytosolic E3 ubiquitin ligase Parkin [62]. Activated Parkin ubiquitinates outer mitochondrial membrane proteins, including mitofusins and anion channels, thereby labelling damaged mitochondria for degradation [62]. These ubiquitin chains are recognized by autophagy adaptor proteins such as optineurin (OPTN) and sequestosome 1 (p62/SQSTM1), which facilitate the recruitment of microtubule-associated protein 1 light chain 3 (LC3)-positive autophagosomal membranes, leading to the sequestration of mitochondria into autophagosomes and their degradation upon fusion with lysosomes [63].

In contrast to the PINK1/Parkin pathway, ubiquitin-independent mitophagy mechanisms have also been described [64]. These alternative pathways are mediated by receptors such as BCL2 interacting protein 3 (BNIP3), BCL2 interacting protein 3-like (NIX/BNIP3L), and FUN14 domain-containing protein 1 (FUNDC1), all of which contain LC3-interacting regions that enable the direct recruitment of autophagosomal membranes [64]. BNIP3 and NIX are transcriptionally upregulated during hypoxia and cellular stress, thereby promoting the lysosomal degradation of damaged mitochondria through selective autophagy via interactions with LC3-family proteins [64]. FUNDC1 also functions as a hypoxia-responsive mitophagy receptor that promotes mitochondrial clearance through interactions with LC3 [64]. These pathways can compensate when PINK1/Parkin signalling is impaired [64]. Given their critical roles in maintaining mitochondrial quality control, dysregulation within both the PINK1/Parkin-dependent and receptor-mediated pathways has recently been implicated in HD pathogenesis.

Evidence from HD models suggests that mitophagy impairment does not arise from a complete failure of PINK1/Parkin signalling [65,66]. Khalil et al. [65] demonstrated that Parkin translocation to depolarized mitochondria and subsequent mitochondrial ubiquitination remain largely preserved in HD models. Instead, the pathology is characterized by defective downstream clearance, resulting in the accumulation of dysfunctional mitochondria [65]. Franco-Iborra et al. [66] supported this finding by demonstrating that the major defect in mitophagy occurs downstream at the level of autophagosome formation and mitochondrial sequestration.

5. Mitophagy and Rhes in Huntington's Disease

Building on these observations, evidence linking Rhes to mitophagy in HD was provided by Sharma et al. [67], who identified Rhes as a regulator of mitophagy through its interaction with the mitophagy receptor NIX. Under mitochondrial stress induced by 3-nitropropionic acid, a mitochondrial Complex II inhibitor commonly used to model HD-like striatal degeneration, Rhes expression promoted mitochondrial depolarization and increased mitophagy [67]. Loss of NIX abolished the effects of Rhes, indicating that Rhes regulates mitochondrial clearance through an NIX-dependent mechanism [67]. They also demonstrated a physical association and interaction between Rhes and NIX through co-immunoprecipitation experiments [67]. This supports a regulatory role for Rhes during mitophagy through the NIX pathway in HD [67,68].

This process may also be influenced by the interaction of Rhes with Beclin-1, a key regulator of autophagosome formation [69]. Rhes has been shown to bind Beclin-1 and reduce its inhibitory association with B-cell lymphoma 2 (Bcl-2), promoting autophagy activation. If a similar interaction occurs during mitophagy, Rhes may facilitate the lysosomal degradation of damaged mitochondria through mitophagy by releasing Beclin-1 from Bcl-2-mediated suppression [69].

Additionally, beyond Rhes' direct interactions with mitophagy, Rhes-mediated SUMOylation of *mHTT* may indirectly influence this process. By increasing the abundance of soluble *mHTT* species that promote mitochondrial damage, Rhes may indirectly exacerbate the mitochondrial dysfunction and autophagic defects that ultimately characterize HD.

To our knowledge, direct regulation of PINK1/Parkin-dependent mitophagy by Rhes in *mHTT*-expressing striatal cells has not yet been demonstrated. This represents a significant research gap in the HD literature.

6. Proposed Role of Rhes in PINK1/Parkin-Dependent Mitophagy

Our central hypothesis, based on the available evidence and summarized in Fig. 1, is that Rhes modulates different stages of mitophagy in HD. Rhes likely interacts with the mitophagy receptor NIX at the mitochondrial outer membrane in the presence of *mHTT*, given its established role in HD pathogenesis [68]. This interaction promotes mitochondrial depolarization, and we propose that this promotes PINK1 stabilization. This may enhance Parkin recruitment and the subsequent ubiquitination of outer mitochondrial membrane proteins. These ubiquitin chains are then recognized by adaptor proteins such as p62/SQSTM1 and optineurin, which recruit LC3-positive autophagosomal membranes and initiate mitochondrial sequestration (Fig. 1).

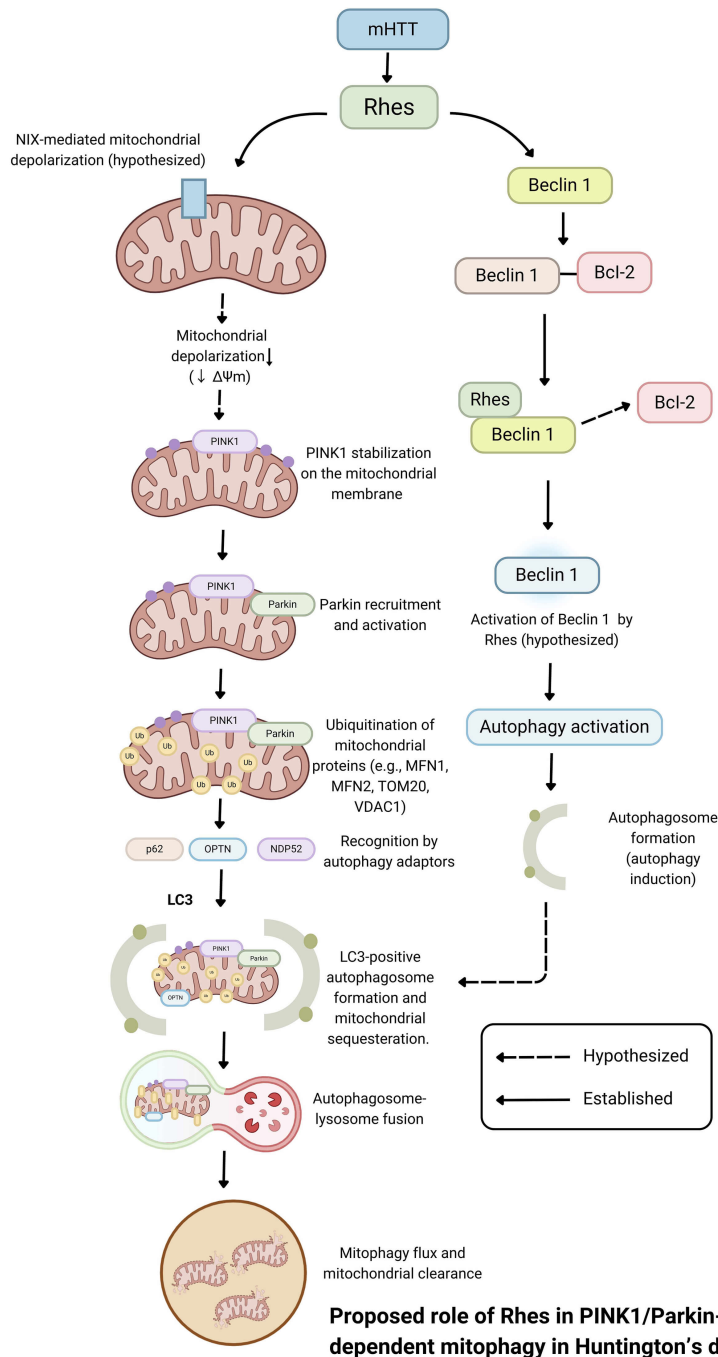


Fig. 1. Proposed role of Rhes in PINK1/Parkin-dependent mitophagy in Huntington's disease. Rhes interacts with NIX/BNIP3L and has been shown to promote mitochondrial depolarization. We hypothesize that Rhes-mediated loss of mitochondrial membrane potential facilitates PINK1 stabilization on the outer mitochondrial membrane, leading to Parkin recruitment, ubiquitination of outer mitochondrial membrane proteins, recognition by autophagy adaptors, and subsequent mitochondrial sequestration within LC3-positive autophagosomes. We further propose that Rhes-mediated activation of Beclin-1, potentially through disruption of the inhibitory Beclin-1–Bcl-2 complex, promotes autophagosome biogenesis. These pathways may converge to enhance mitophagic flux and lysosomal degradation of damaged mitochondria. Solid arrows indicate previously reported molecular interactions or established pathway components, whereas dashed arrows indicate proposed mechanistic links within this model that require further experimental validation. mHTT, mutant huntingtin; PINK1, PTEN-induced kinase 1; Rhes, Ras homolog enriched in the striatum; NIX/BNIP3L, BCL2 interacting protein 3-like; p62/SQSTM1, sequestosome 1; OPTN, optineurin; NDP52, nuclear dot protein 52; MFN1, mitofusin 1; MFN2, mitofusin 2; TOM20, translocase of outer mitochondrial membrane 20; VDAC1, voltage-dependent anion channel 1; LC3, microtubule-associated protein 1 light chain 3; BCL-2, B-cell lymphoma 2. Figure created in Canva.

We also hypothesize that Rhes influences downstream stages of mitophagy. Based on earlier evidence, we propose that Rhes binds to the Beclin-1–Bcl-2 complex and dissociates Beclin-1 from Bcl-2 [69]. As a result, this would activate Beclin-1, enhancing autophagosome biogenesis and autophagosome–lysosome fusion, facilitating mitophagic degradation of damaged mitochondria.

7. Future Directions and Therapeutic Implications

Future studies should determine whether Rhes regulates PINK1/Parkin mitophagy initiation, downstream mitophagic flux, or both in HD. This could be achieved using gain-of-function and loss-of-function approaches to Rhes in wild-type and *mHTT*-expressing striatal cell models, followed by assessment of established mitophagy markers. Early mitophagy signalling can be evaluated by measuring PINK1 stabilization at the outer mitochondrial membrane, a primary indicator of mitochondrial damage and the initiation of PINK1/Parkin-dependent mitophagy [70]. Parkin recruitment and translocation to depolarized mitochondria can be assessed by immunofluorescence microscopy or mitochondrial fractionation to measure activation of the mitochondrial ubiquitination machinery [71]. Subsequent ubiquitination of outer mitochondrial membrane proteins, including the mitofusins mitofusin 1 (MFN1) and mitofusin 2 (MFN2), can be assessed by immunoblotting for ubiquitin, providing evidence that damaged mitochondria have been targeted for mitophagic degradation [72]. Downstream stages of mitophagy can be examined through markers of autophagosome formation and cargo recognition. The conversion of LC3-I to LC3-II and the accumulation of LC3-positive puncta are widely used as indicators of autophagosome biogenesis [73]. The autophagy adaptor p62/SQSTM1 links ubiquitinated mitochondria to LC3-positive autophagosomes, making changes in its abundance or localization useful indicators of mitochondrial cargo recruitment and mitophagic flux [74]. An important consideration in these experiments is the established role of Rhes as a SUMO E3-like ligase. Changes in mitophagy following varying Rhes expression may reflect alterations in *mHTT* SUMOylation rather than direct regulation of mitophagic signalling pathways. Future studies should control for SUMOylation effects, for example, by using SUMOylation-deficient Rhes mutants.

To determine whether mitochondria are successfully degraded, mitochondrial proteins can be quantified following mitophagy induction [34]. A reduction in these markers is commonly interpreted as evidence of mitochondrial clearance, defined as the lysosomal degradation of damaged mitochondria through selective autophagy (mitophagy) [34]. However, measurements of mitochondrial protein abundance provide only a static snapshot of mitochondrial content and cannot determine whether mitochondria are actively progressing through the degradation path-

way. Therefore, these measurements should be complemented by assessments of mitophagic flux, which evaluate the rate and completion of mitochondrial clearance [37]. In particular, the mitochondrial Keima (mt-Keima) reporter system is useful because it exploits the pH-sensitive fluorescence of the Keima protein to distinguish mitochondria residing in the cytosol from those delivered to acidic lysosomes [37]. As a result, mt-Keima provides a measure of mitochondrial trafficking through the mitophagy pathway and completion of mitochondrial turnover.

From a therapeutic perspective, clarifying the relationship between Rhes and dysregulated mitophagy via the PINK1/Parkin pathway may identify novel opportunities to modulate mitochondrial quality control in HD. Rhes is already implicated in other pathogenic processes relevant to HD; therefore, therapeutic strategies targeting Rhes may offer the dual benefit of reducing *mHTT*-associated toxicity while restoring mitochondrial quality control. If our proposed model is validated, therapeutic strategies could be developed to target specific interactions between Rhes and the mitophagy machinery. These may include: (1) modulation of Rhes-NIX interactions involved in mitochondrial depolarization to further recruit PINK1 or (2) modulation of Rhes-Beclin-1 signalling to regulate autophagosome biogenesis. However, the development of therapies targeting this pathway will require overcoming issues associated with efficient drug delivery across the blood-brain barrier. Further advances in brain-penetrant therapeutics and delivery systems may help address this barrier. If validated, our proposed pathway would open new avenues for therapeutic development in HD.

Author Contributions

MS conceived the opinion and drafted the manuscript. HAK and HA contributed to the conceptual development, critical revision, and editing of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflicts of Interest

The authors declare no conflicts of interest.

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