Peer Review File

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Responses to the reviewers' comments

Reviewer: 1

Comment 1: Some seminal articles are missing - please refer and discuss, Manczak et al 2011 HMG, this article described Drp1 interaction with amyloid beta and enhances GTPase Drp1 activity and increases mitochondrial fragmentation, and also refer Reddy and Oliver 2019, Morton et al 2021, Pradeepkiran and Reddy 2020 – these articles discussed how increased Drp1-Abeta interaction reduces mitophagy (PINK1 and Parkin) in Alzheimer's disease.

Reply 1: Thank the reviewer's critical comments.These studies are important to our article, and we have added related content.The references" 46.Manczak M, Calkins MJ, Reddy PH. Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. Hum Mol Genet. 2011 ;20(13):2495-2509; 61.Oliver D, Reddy PH. Dynamics of Dynamin-Related Protein 1 in Alzheimer's Disease and Other Neurodegenerative Diseases. Cells. 2019 ;8(9):961; 49.Morton H, Kshirsagar S, Orlov E, et al. Defective mitophagy and synaptic degeneration in Alzheimer's disease: Focus on aging, mitochondria and synapse. Free Radic Biol Med. 2021;172:652-667; 47.Pradeepkiran JA, Reddy PH. Defective mitophagy in Alzheimer's disease. Ageing Res Rev. 2020 ;64:101191. "have been added.

Changes in the text: We have modified our text as advised (see Page 13, line 278-288).

Comment 2: Please change the sentences that reasons unknown for Drp1-Tau and Drp1-Abeta interactions – Reddy Lab extensively reported that Drp1-Tau and Drp1-Abeta interactions reduce PINK and Parkin levels in AD.

Reply 2: Thank the reviewer's critical comments.We have deleted the sentence"the specific mechanism remains unanswered",and added related content.

Changes in the text: We have modified our text as advised (see Page 13, line 285-288).

Comment 3: It may be worth adding a subsection 'mitochondrial dynamics in Huntington's disease' – Huntington's disease is a pure genetics disease and mitochondrial dynamics is largely impaired – refer Sawant t al 2021, Shirendeb et al

2011, Shirendeb et al 2012.

Reply 3: Thank the reviewer's critical comments.These studies are important to our article, and we have added related content.The references" 60.Sawant N, Morton H, Kshirsagar S, et al. Mitochondrial Abnormalities and Synaptic Damage in Huntington's Disease: a Focus on Defective Mitophagy and Mitochondria-Targeted Therapeutics. Mol Neurobiol. 2021 ;58(12):6350-6377; 63.Shirendeb U, Reddy AP, Manczak M, et al.Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. Hum Mol Genet. 2011;20(7):1438-1455; 64.Shirendeb UP, Calkins MJ, Manczak M, et al.Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. Hum Mol Genet. 2012 ;21(2):406-420. "have been added.

Changes in the text: We have modified our text as advised (see Page 15-16, line 326-343).

Reviewer:2

Comment 1: The main point is stylistic. Ideally, review articles do more than recite facts from primary literature. They should also integrate that information into possible models and highlight specific gaps in knowledge that need to be solved by future studies.

As written, the manuscript covers many facts, but it does not go the extra step of highlighting specific future problems to be solved. One way to improve this would be to augment sections (e.g., fission or fusion) with current questions that are being addressed in the field and how the answers to those questions will open new levels of understanding that could be applied to diseases.

Reply 1: Thank the reviewer's critical comments.Alterations in Drp1 are necessary to mitochondrial dynamics and involved in the occurrence and development of various neurological diseases.We highlight the potential of DRP1 for the treatment of neurological diseases,and enumerate the main questions that remain to be elucidated in future studies.

Changes in the text: We have modified our text as advised (see Page 23-24, line 511-520).

Comment 2: Studies of mitochondrial dynamics include cell biological processes beyond fission and fusion. Mitochondrial movement and clearance of dysfunctional mitochondria are also important when considering mitochondrial dynamics or mitostasis.

The article could be improved if sections in the opening half also covered mitochondrial movement and clearance. Movement (via kinesin or dynein) is mentioned in places. So is mitophagy and autophagy (potential modes of clearance). But as currently written, those topics, which are highly relevant to mitochondrial dynamics, are only mentioned in passing.

Reply 2: Thank the reviewer's critical comments.Mitochondrial movement and clearance of dysfunctional mitochondria are important. We added the mechanisms of mitochondrial transport and mitophagy,and mentioned the biological significance of mitochondrial transport and mitophagy.

Changes in the text: We have modified our text as advised (see Page 10-12, line 214-246).

Comment 3: There are many neurological diseases that can be caused by dysfunctional mitochondria. The article could clarify its focus up front by stating that it covers only the cases of disrupted mitochondrial dynamics and not the other cases that disrupt mitochondrial function in other ways.

Beyond disrupted dynamics, there are also a class of diseases caused by

disruptions of oxidative phosphorylation/bioenergetics. Additionally, it is also possible to disrupt an entire cellular process like axonal transport and result in secondary effects on mitochondrial movement or supply.

Reply 3: Thank the reviewer's critical comments.We clarify our focus on mitochondrial fission/fusion at the beginning of the section "Mitochondrial dynamics and neurological diseases".

Changes in the text: We have modified our text as advised (see Page 12, line 247-251).

Comment 4: (Related to point 1). Stylistically, the second half of the article is difficult to follow. The second half is organized by type of disease manifestation (e.g., neurodegenerative diseases), and there is a great deal of information included that comes at the reader in large chunks.

It would be easier to follow if the text were broken up into more subsections with distinct headers (e.g., Alzheimer's Disease, Parkinson's Disease) and then if the paragraphs below the subheadings were broken up by biological process. This way, the reader could think in general about a specific disease manifestation but then also consider the underlying biology in an easy-to-read context. As currently written, each disease section reads in a disjointed way.

Reply 4: Thank the reviewer's critical comments.The section "Mitochondrial dynamics and neurological diseases"has been broken up into more subsections with distinct headers,and the paragraphs have been broken up by biological process.

Changes in the text: We have modified our text as advised (see Page 12, line 252).

Comment 5: "Mitochondrial dynamics may be the future of the treatment of neurological diseases." This sentence seems overly broad, and it is not clear to the reader precisely what the authors mean.

Reply 5: Thank the reviewer's critical comments.This sentence has been changed to "Drp1,as a highly relevant molecular for mitochondrial dynamics， might be a potential target for treating neurological diseases in the future."

Changes in the text: We have modified our text as advised (see Page 4, line 69-70).

Comment 6: "Life activities are inseparable from mitochondria." This sentence is true for many types of eukaryotes, but it does not apply to all forms of life.

Reply 6: Thank the reviewer's critical comments.We have deleted this sentence.

Changes in the text: We have modified our text as advised (see Page 4, line 83).

Comment 7: "…as and when needed." Can delete, this is unclear.

Reply 7: Thank the reviewer's critical comments.We have deleted this sentence.

Changes in the text: We have modified our text as advised (see Page 4, line 85).

Comment 8: “highlights its role…” What does its refer to here? Roles of mitochondria? Mitochondrial dynamics?
Reply 8: Thank the reviewer’s critical comments.This sentence has been changed to "Our review reveals on the different molecular mechanisms of mitochondrial dynamics and highlights the role of mitochondrial dynamics in the event and development of various neurological disorders."
Changes in the text: We have modified our text as advised (see Page 5, line101-103).

Comment 9: Are the authors describing a promotion of fission or a prevention of fusion?
Reply 9: Thank the reviewer’s critical comments.We have changed the sentence to "The Fis1 protein was previously thought to be a Drp1 adaptor in mitochondrial fission but was later confirmed not to contribute directly to mitochondrial fission in normal cell homeostasis ".
Changes in the text: We have modified our text as advised (see Page 6, line126-128).

Comment 10: “…presumably by interacting with other proteins.” What does this mean? Does it mean that P-S616 promotes Drp1 to interact with other proteins and those interactions result in fission?
Reply 10: Thank the reviewer’s critical comments.
Taguchi N found that the phosphorylation of Drp1 at ser616 does not directly affect GTPase activity.The endophilin family protein endophilin B1 acting downstream of Drp1 is targeted from the cytoplasm to the mitochondrial fission foci to be involved in the fission reaction. Taguchi N presumed that the Drp1 phosphorylation stimulates the interaction with endophilin B1 or other unidentified partner protein(s). (Taguchi N, Ishihara N, Jofuku A, et al. Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. J Biol Chem. 2007;282(15):11521-9.)
Stefan Strack found that CDK5 can also phosphorylate Drp1S616, which induces mitochondrial fragmentation by the mobilization of Drp1 to mitochondria from microtubule.(Strack S, Wilson TJ, Cribbs JT. Cyclin-dependent kinases regulate splice-specific targeting of dynamin-related protein 1 to microtubules. J Cell Biol. 2013 ;201(7):1037-51.)
Changes in the text: We have removed relevant content to avoid misunderstandings. (see Page 7, line134).

Comment 11: high levels of calcium where exactly?
Reply 11: Thank the reviewer’s critical comments.The study found the high levels of calcium ions in the mitochondrial compartment.
Changes in the text: We have modified our text as advised (see Page 8, line162).

Comment 12: “devoid of these changes” Devoid compared to what? Compared to the

case of peripheral fission? (Meaning that central fission is the baseline case?

Reply 12: Thank the reviewer's critical comments.The Study found that peripheral fission was associated with changes in ROS,MMP and calcium compared to midzone(central) fission.Peripheral mitochondrial fission is asymmetric fission, where the healthy daughter can continue to function, but the smaller daughter is degraded and reused. On the other hand, midzone fission is symmetrical fission, which is essentially a mitochondrial copy. The daughter after midzone fission can exist independently or function by fusion into the mitochondrial network.Mitochondrial peripheral fission is mainly for the degradation and reuse of damaged mitochondria, while midzone fission is for the biogenesis of mitochondria.

Changes in the text: We have modified our text as advised (see Page 8, line162).

Comment 13: Say more to explain this conclusion (challenging classical topology). As written, it is unclear.

Reply 13: Thank the reviewer's critical comments.It was considerd that MFNs were composed of 2 TM domains.However,recent studies have found that the human sapiens MFNs' C-terminus is exposed to the mitochondrial inter membrane space (IMS),suggesting that MFNs carry a single transmembrane domain(TM) with conserved redox-regulated cysteine resides and the HR2 domain exposed to the IMS.Thus, further research is necessary for the topology of TM domain in MFNs.

Changes in the text: We have modified our text as advised (see Page 9, line190-194).

Comment 14: I assume the authors define "ALS causing gene" as actually meaning dysfunction in one or more of the genes listed.

Reply 14: Thank the reviewer's critical comments.Studies have found that mitochondrial fission was highly enhanced in muscles and motor neurons of TDP-43, FUS, and TAF15-induced fly models of ALS, and overexpression of OPA1 or knockdown of Drp1 restored mitochondrial morphology.

Changes in the text: We have modified our text to avoid misunderstandings (see Page 15, line316-319).

Comment 15: An inhibitory effect on the cancer? Or do the authors mean that fission promotes more cancer, and that fission needs to be counteracted by leflunomide to slow the cancer progression?

Reply 15: Thank the reviewer's critical comments. Enhanced mitochondrial fission inhibits metastasis in triple-negative breast cancer, and leflunomide has been shown to counteract this inhibitory effect. Leflunomide has an inhibitory effect on mitochondrial fission not on the cancer.

Changes in the text: We have modified our text to avoid misunderstandings (see Page 21, line455-456).

Comment 16: There is a sentence fragment in line 410, and the idea about “enhanced mitochondrial dynamics” is vague.

Reply 16: Thank the reviewer’s critical comments.This sentence has been changed to "several compounds have restored proper mitochondrial dynamics and nerve function in preclinical experiments"

Changes in the text: We have modified our text as advised (see Page 23, line506-507).

Comment 17: Do the authors mean restoring proper mitochondrial dynamics?

Reply 17: Thank the reviewer’s critical comments.Our review focuses on a few drugs with fewer side effects of these compounds that have passed clinically trials.Restoration of proper mitochondrial dynamics by these drugs might be a promising therapeutic strategy for neurological diseases in the future.

Changes in the text: We have modified our text to avoid misunderstandings (see Page 23, line507-510).