Peer Review File

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Reviewer A

In this manuscript, authors have presented a summary of the pathways involved in ferroptosis and the potential implication of ferroptosis in several human diseases. Overall, the content reviewed here may be of interest to the field of cell death. The article could be further improved by addressing the following points:

1. When describing the characteristics of ferroptosis as well as other cell death forms, the author should be clear about the definitive characteristics of them and use precise wording. For example, "ferroptosis is an iron-dependent form of non-apoptotic cell death, which is characterized by the accumulation of ROS." It should be specified as lipid ROS. In addition, ferroptosis is a type of necrosis, but not a different form of cell death in contrast to necrosis. Furthermore, "it lacks the morphological characteristics of necrosis including cytoplasm, organelle, and cell membrane rupture." This is incorrect, because ferroptosis also has ruptured cell membrane. Therefore, I would suggest the authors to use more precise words to describe these definitive features.

Re: Thanks for your suggestions. We examined the entire manuscript and used more precise words such as liquid ROS in the revised manuscript (see Page 3, line 50 and Page 6, line 131).

Ferroptosis is a new pattern of cell death and accompanied by a large amount of iron accumulation and lipid peroxidation. It does not have the formation of classical closed bilayer membrane structure. Ferroptosis is mainly characterized by obvious shrinkage of mitochondria with increased membrane density and reduction in or vanishing of mitochondrial cristae, which is a different process from other modes of cell death. We have added the above description in the revised manuscript (see Page 3, line 63).

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2. The manuscript has described several chemical inducers of ferroptosis, however, another class of ferroptosis inducers have not be mentioned in this manuscript. Recently, it was found that T cells and neutrophils can both induce ferroptosis in tumor cells (Wang, 2019 Nature; and Yee, 2020 Nature Communications). Therefore, these types of inducers should also be included in the manuscript.

Re: Thanks for your suggestions. We have added this class of ferroptosis inducers in our revised manuscript (see Table 1).

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3. There are many English writing issues need to be fixed.

Re: We have checked the whole manuscript and corrected the language errors by a

native English speaker.

Reviewer B

In addition to the anti-ferroptotic systems discussed in the review, the authors should include two additional systems:

First one is the tetrahydrobiopterin (BH4)-DHFR system. Activation of BH4 biosynthesis was found to protect from lipid peroxidation (Kraft et al. 2019, ACS Cent. Sci.). A recent study (Soula et al. 2020, Nat Chem Bio) described similar findings and identified DHFR as the enzyme regenerating BH4 (in a similar fashion to FSP1-CoQ10).

Re: Thanks for your suggestions. We have included these two anti-ferroptotic systems in our revised manuscript (see Page 5, line 101 and Figure 1).

Second system protecting from lipid peroxidation is that exerted by another mevalonate pathway intermediate, squalene (Garcia-Bermudez et al. 2019, Nature).

Re: Thanks for your suggestions. We have added it in our revised manuscript (see Page 12, line 252).

Finally, in the discussion of ferroptotic inhibitors in cancer, it would be worth mentioning the role of GPX4 in drug resistance and persister cell viability (Hangauer et al. 2017, Nature).

Re: Thanks for your suggestions. We have added it in our revised manuscript (see Page 16, line 329).

Reviewer C

The authors provide a general overview of the mechanism involved in ferroptotic cell death, the implication this has in pathology and the current evidence of pharmacological applications to modulate ferroptosis. While the review covers a broad range of pathways that are beginning to emerge as important in ferroptosis, the detail of the review is superficial and there are some clear discrepancies in the description that infer a lack of understanding to some of the pathways. There have been a strong number of reviews recently published in this field and for me to recommend publication of this review then I would need to see some novelty in what was being discussed. Unfortunately, I do not and therefore must reject the review outright. In general, I reached this decision as there was a lack of continuity in the sections of the manuscript and there was no effort to provide a balanced argument as to how each pathway is selective to ferroptosis over other cell death pathways. More specific points of concern are as follows:

1. In the introduction, there is a focus on only apoptosis, necrosis and autophagy as

alternative forms of cell death. This is rather an antiquated view of cell death and there have been a multitude of other forms reported in recent years. While it is relatively easy to distinguish ferroptosis from these more 'traditional' forms of cell death, a much more difficult task is characterising the novelty of ferroptosis from these newer forms of cell death and this needed to be addressed in the review.

Re: We appreciate your comments and have added the addresses in the revised manuscript. (see Page 3, line 60)".

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2. In the introduction, the authors also go into some detail on how autophagy is a distinguished form of cell death separate from ferroptosis but later explain the involvement of the ATG5-ATG7-NCOA4 axis as being a key mechanism for ferroptosis. This is clearly a contradiction and exemplifies my previous point that characterising ferroptosis is not so clear cut and that there are clear overlaps in the cell death pathways that need to be discussed.

Re: Ferroptosis and autophagy are two different forms of cell death. Ferroptosis is a new pattern of cell death that has been discovered in recent years. Ferroptosis is usually accompanied by a large amount of iron accumulation and lipid peroxidation. Autophagy is a lysosomal self-protective cellular catabolism pathway. Generally speaking, autophagy promotes cell survival, but excessive autophagy, especially selective autophagy, promotes ferroptosis by promoting iron accumulation and lipid peroxidation, which is one of the important pathways in the occurrence of ferroptosis, called ferritinophagy. Thus, ferritinophagy is a pathway in ferroptosis.The occurrence of ferritinophagy is mainly through the autophagy degradation of ferritin in lysosome mediated by NCOA4 and the release of free iron to promote ferroptosis.

3. There are a number of points in the review where the authors have formed unfounded opinions as to the original discoverers of novel mechanism and targets related to ferroptosis. An example of this is when describing the identification of FSP1 and describing a chronological discovery in which Bersuker et al was first and then followed by Doll et al. As both were published in the same journal and same edition I would find it hard not to describe this as a joint discovery between the groups. Independent of the authors opinion, the review should be written in such a way as to be impartial and just provide both sides of an argument.

Re: We appreciate your comments and apologize for the mistakes. We have corrected it in the revised manuscript (see Page 5, line 87). We have examined throughout to eliminate such errors.

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4. There are occasions in the review where the descriptions to proteins or drugs are out of order or context. For example, line 115 is the first time that ferrostatin-1 is mentioned

but with no reference to what it actually targets and this being an indirect modulator to the pathway in which the rest of the section is describing. In the following sentence it is stated ‘P53 could inhibit cytine absorption by cystine-glutamate transporter receptor’, but how is this done? If this is to be included in a mechanism then a greater explanation is required.

Re: We appreciate your comments. We have modified our text as advised (see Page 6, line 124)".

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5. The whole section on iron metabolism pathway is littered with mistakes and oversimplifications of iron homeostasis that indicates a severe lack of understanding in the binding affinities of each protein to the di- or tri-valent iron. On top of this, there are some major omissions in referencing recent studies related to iron and ferroptosis. These include the multiple publications on the importance of FPN in ferroptosis as well as Tf being a new biomarker for ferroptosis.

Re: We have added the description in our revised manuscript as your suggestion (see Page 9, line 181).

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6. If the authors are going to refer to the p62-Keap1-NRF2 pathway as of interest in cancer (line 251) then this needs to be explained in more detail in the previous section (line 233). Indeed, I would have thought that both the glutamine metabolic pathway and the p62-Keap1-NRF2 pathway warrant a more detailed description than the one sentence they received.

Re: We appreciate your comments. The p62-keap1-nrf2 pathway and glutamine metabolism pathway are explained in detail in the revised manuscript (see Page 12, line 246).

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7. In the second half of the review, the authors state their desire to focus on ‘pharmacological applications of ferroptosis with disease’ but there are multiple times when the text wanders from this point and just provides information of mild relevance. An example of this would be from lines 291-296; what is the pharmacological application of this work and how does it have relevance to neurodegeneration. Please provide continuity to the information you present and better section structure.

Re: We appreciate your comments. The pharmacological application of this work and how does it have relevance to neurodegeneration have been explained in detail in the revised manuscript

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8. There are several points throughout the review where the sentence structure needs to be improved. On occasions I found it hard to follow a point the authors were trying to

make in these sections. Furthermore, there are statements (particularly at the end of paragraphs) were statements are made that do not summarise the points in the preceding sentences of the paragraph and there is no data referenced to support this point. These issues need to addressed with significant improvements.

Re: Thanks for your suggestions. A summary is added to each chapter at the end of the paragraph in the revised manuscript.

9. Lastly, the conclusion is mostly in the form of a list of points that the authors feel the community need to resolve and there is little description in how the authors propose this will be done. It is also not clear what the authors see as the 'classical pathways' of ferroptosis. Would these be the ones described earlier in the review? If so then I would find these as far from classical when some have only been discovered in the last 18 months and it is still not clear how these interlink.

Re: We appreciate your comments. We have modified our text as advised (see Page 16, line 348)".

Minor comments

1. Why is AIFM2 an 'anti-ferritin gene'? I don't know what this is.

Re: Apoptosis-inducing factor mitochondria-associated 2 (AIFM2,also known as FSP1), a traditional apoptosis inducer in mitochondria, has been reported to play an important role in ferroptosis. And FSP1 catalyzed CoQ10 regeneration through NAD(P)H to inhibit ferroptosis, so FSP1 can inhibit the occurrence of ferroptosis.

2. Line 161 and 162; I think you are mixing up cysteine with cysteine.

Re: we have corrected it in the revised manuscript (see Page 8, line 169).

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3. The sentence starting of line 166 needs to be made a lot clearer. I think there is a mix up in the sentence structure and so it should start with what inhibiting system Xc- is doing to downstream components and not the other way around.

Re: We appreciate your suggestion! We have revised it as your suggestions (see Page 8, line 174)".
