Peer Review File

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Reviewer A

This study sought to use existing GEO data to develop a network of lncRNA, miRNA, and mRNA to explore the potential relationship between ferroptosis and neonatal HIBD. This relationship is a fairly new and relevant field of investigation within neonatal HIBD, and the approach appears to be mostly appropriate, but there are several aspects of the study and manuscript that the authors should address.

The first, and most significant surrounds the data that the authors chose to use. GSE121178 is an appropriate data set to meet the proposed needs of the investigators. The RNA expression, however, changes significantly over the first 72 hours after injury, so the authors must note the timing of blood samples and take those into consideration with regard to the typical timing of the tri-phasic injury. While GSE121178 is appropriate for this question, GSE112137 is not. These data were derived from a cell culture organoid model of preterm brain injury (not term HIBI which is what the first data set was derived from and presumably what the authors' animal study models) so cannot be combined with the first data set in any meaningful way. The authors should seek out a more appropriate source for their non-lncRNA data in order for the results of their study to be interpretable.

This reviewer has other more minor concerns as well (including a lack of adequate description of the animal model methodology) but the above concern would need to be addressed before the remaining manuscript can be evaluated in detail.

Reply: We thank the reviewer's constructive comments. We understand the reviewers' concern that different tissues and cell types may differ in gene expression and regulatory mechanisms. LncRNAs in blood may differ from mRNA expression in cultured cells in terms of tissue or cell type specificity. In addition, there are few datasets of neonatal HIBD. Therefore, after identifying key mRNAs, we used GSE112137 as an external dataset to verify the expression pattern of the key genes. mRNA with statistical significance and consistent expression trend were defined as key genes, which may reveal that key genes are not only expressed in peripheral blood, but also biomarkers for tissues.

More detailed description of the method in the induction of the animal model has been added. Changes in the text: Line 250-274.

Reviewer B

This is an extensively written article on a novel topics which can generate new insights for diagnosis, prognosis and management of Hypoxic brain injury.

Few grammar mistakes to be corrected.

Drug prediction is not clearly explained, so need little clear approach.

Reply: We thank the reviewer' comments and have added further explanations about the details in drug prediction. In addition, the manuscript has been edited by a native English speaker to remove all the possible grammatical and spelling mistakes.

Changes in the text: Line 236-247.

Reviewer C

The manuscript entitled "A ferroptosis-related ceRNA network for investigating the molecular mechanisms and the treatment of neonatal hypoxic-ischemic encephalopathy" describes data mining through the Gene Expression Omnibus to determine specific pathways that might be disrupted in HI and therefore contribute to brain injury. I have some general comments. My comments are general for two reasons: 1. There are so many levels of analysis, this reviewer cannot possibly sincerely evaluate the rigor of the analyses. There are few details about the human samples collected and the number of humans samples is very low. I understand these samples were already in an existing database. Still, it is difficult to judge the rigor of the results.

Reply: We thank the reviewer's comments. The aim of this study is to construct a ceRNA regulatory network for disease-related ferroptosis based on the differentially expressed lncRNA, miRNA, and mRNA in the disease. Subsequently, GSE112137 was used as an external validation set to verify the expression of the key genes. By analyzing the co-expression of the key genes in infiltrated immune cells, the proportion of different immune cells, and the correlation between the key genes and immune factors in neonatal HIBD, the study revealed the role and the mechanism of the immune system in the development of the disease, and provided new ideas and targets for the treatment of this disease. In addition, we also analyzed the signaling pathways regulated by the key genes in disease, which improved our understanding of the potential molecular mechanisms of key genes in disease progression, and the transcriptional regulatory networks of the key genes.

The datasets used in this study were downloaded from GEO database (GENE EXPRESSION OMNIBUS, https://www.ncbi.nlm.nih.gov/geo/info/datasets.html, created and maintained byUS NCBI) using the keyword "neonatal hypoxic ischemic encephalopathy"., The species was set as "Homo sapiens", and the data sets of less than 6 samples were filtered out. Finally, GSE121178 and GSE112137 were retained and included in this study. The Series Matrix File data file of GSE121178 was downloaded and the annotation platform was GPL22120. In this dataset, 6 whole human blood samples were collected for lncRNA and mRNA chip analysis, including 3 HIE samples and 3 non-HIE samples. The Series Matrix File data file of

GSE112137 was also downloaded from GEO database, and the annotation platform was GPL20301. This dataset was derived from 16 samples in total, including non-HIE samples (n=8) and HIE samples (n=8). The 431 iron death genes in this study were obtained from the FerrDb V2 database.

2. There is little detail regarding the animal samples collected. See below.

Reply: We thank the reviewer's comment, and have included detailed information regarding the method of the animal model.

Changes in the text: Line 250-274.

Overall, this paper is difficult to follow. There are many statements that do not make a point and merely obscure the important things, like methods and veer from reasonable interpretation. An example is in the introduction: "According to recent data, the development of HIBD can be promoted by various factors, such as energy consumption, mitochondrial damage, and delayed inflammatory response." I think the authors mean disrupted or dysregulated metabolism and a protracted inflammatory response. As written it doesn't make sense. Energy consumption isn't inherently bad, and would it be better if there were an acute inflammatory response (which there is)?

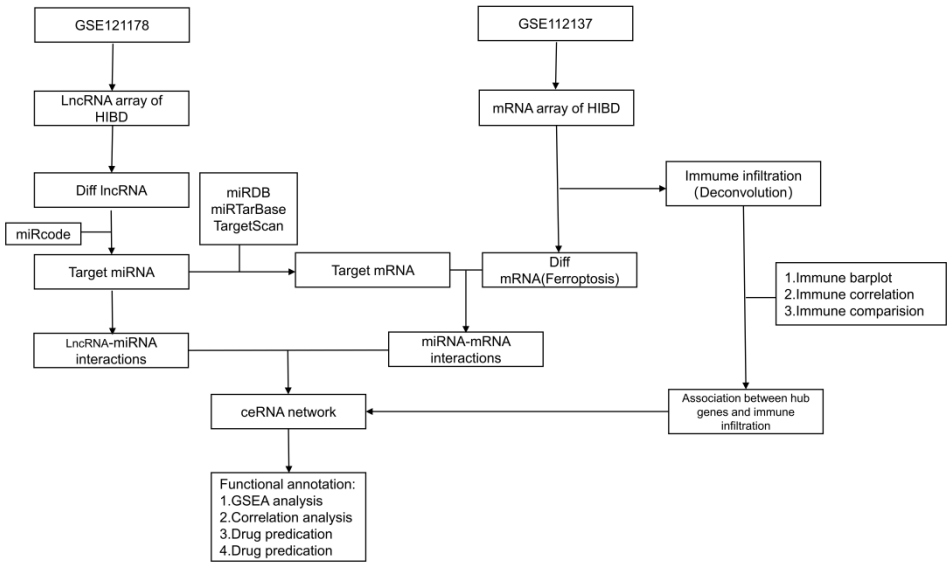
Reply: We thank the reviewer's suggestion**.** We have revised the corresponding part of the article.

Changes in the text: Line 115-117.

I appreciate the intellectual drive behind the approach presented in this manuscript. I think the writing needs to be tightened and more description of the many many analyses would greatly improve the reader's ability to understand the way the data were derived. A flowchart or some kind of schematic to demonstrate the ultimate goal would help. The introduction touches on mRNA, miRNA and competitive interactions between many types of RNA. The main point gets lost.

Reply: We appreciate the reviewer's constructive suggestion and have provided a flow chart of our work to facilitate the understanding of the readers.

Changes in the text: Line 570.



The number of samples represented is very low. It is worth presenting any demographic variables in the HI infants samples that might affect the data. For instance, were infants term equivalent? Was hypothermia used? Were control samples age and sex matched?

Reply: We understand your concern. First of all, this is a rare disease and there is limited data available. Secondly, GSE121178 currently has been used in two publications (shown below), and the quality and reliability of the data in both studies are relatively high.

Dong X, Zhao Y, Huang Y, Yu L, Yang X, Gao F. Analysis of long noncoding RNA expression profiles in the whole blood of neonates with hypoxic-ischemic encephalopathy. J Cell Biochem. 2019 May;120(5):8499-8509. doi: 10.1002/jcb.28138.

Dong X, Zhuang S, Huang Y, Yang X, Fu Y, Yu L, Zhao Y. Expression profile of circular RNAs in the peripheral blood of neonates with hypoxic-ischemic encephalopathy. Mol Med Rep. 2020 Jul;22(1):87-96. doi: 10.3892/mmr.2020.11091.

The figures—the text in the figures—is too small.

Reply: We thank the reviewer's comments and have provided figures with higher resolution.