Peer Review File

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Reviewer A

Thanks very much for your comments on our manuscript. As you pointed out, there is certainly plenty of room for the improvement of this work. Your comments were all valuable and very helpful for revising and improving our manuscript. We have carefully considered each one and made corrections, we hope to your satisfaction.

Comment 1: A major limitation of this study is that the authors use K562 cell line for all their studies. This cell line already shows high levels of fetal hemoglobin. Therefore, the authors would have to show the increase in fetal hemoglobin upon knockdown of the LncRNA in primary human erythroid cells derived from hematopoietic stem cells, which show very low basal fetal hemoglobin levels.

Reply 1: Thank you for your precious comments and advice. We agree with you that the K562 cell line already shows high levels of fetal hemoglobin. The K562 cell line is widely used in β-thalassemia related studies, screening of potential HbF inducers and drugs, etc., because it has the characteristics of erythroid cells (References: Induction of erythroid differentiation of human K562 cells by cisplatin analogs. Biochem Pharmacol. 2000, 60(1):31-40.; Tenofovir disoproxil fumarate induces fetal hemoglobin production in K562 cells and β-YAC transgenic mice: A therapeutic approach for γ-globin induction. Exp Cell Res. 2020, 394(2):112168). We found that many transcription factors as well as some lncRNAs were also established and successfully implemented in K562 cell model to regulate the expression of γ-globin gene (References: Human fetal globin gene expression is regulated by LYAR. Nucleic Acids Res, 2014, 42(15): 9740-9752; A long noncoding RNA from the HBS1L-MYB intergenic region on chr6q23 regulates human fetal hemoglobin expression. Blood Cells Mol Dis, 2018, 69: 1-9; Long noncoding RNA HBBP1 enhances γ-globin expression through the ETS transcription factor ELK1. Biochem Biophys Res Commun, 2021, 552: 157-163). K562 cells were also selected for this study, in combination with their low cost, rapid growth rate, and ease of survival. We agree with you that the result of this study would be best validated in other cell lines, especially those showing low basal fetal hemoglobin levels. So, we will further validate the results of this study in primary human erythroid cells derived from hematopoietic stem cells or even animal models in the future, if available. We have added this limitation in our text (see Page 11, line 342-347).

Changes in the text: Page 11, line 342-347.

Comment 2: In order to verify that the increase of fetal hemoglobin up on the LncRNA knockdown/knockout is due to the increase in S6K and RhoA, the authors will need to do

double knockdowns (ie knocking down S6K or RhoA along with LncRNA NR-120526). The lack of an increase in fetal hemoglobin upon double knockdown, will confirm the model that the authors have shown in figure.

Reply 2: Thank you for your advice. As you rightly pointed out, to verify that increase of fetal hemoglobin up on the lncRNA knockout is due to the increase in S6K and RhoA, it would be preferable to perform intervention experiments with S6K or RhoA in parallel with knockout of lncRNA NR-120526. However, due to time constraints, this study only describes the effect of lncRNA NR-120526 knockout on S6K, RhoA and γ-globin expression at present, and we expect to perform the validation of dual intervention in the future if available. We have added this limitation in our text (see Page 10, line 333-335).

Changes in the text: Page 10, line 333-335.

Comment 3: Without this experiment, the mechanism shown in the model is based on correlations.

Reply 3: Thank you for your advice. We agree with you that the mechanism shown in the model is based on correlations. Further study is needed to validate the mechanism. However, due to time constraints, we could not finish it in time. We will study it in the future work.

Changes in the text: None.

Reviewer B

We would like to express our sincere gratitude for your positive comments. Your sound comments and suggestions have helped profoundly improve our manuscript. We hope that this correction will meet your expectations.

Comment 1: The referee did not see Supplemental Data 1 about the ChIP-Seq, line 260.

Reply 1: Thank you for your advice. We have re-supplemented the data about ChIP-Seq in the attachment. Please kindly find the attached file.

Changes in the text: None.

Comment 2: Fig 2C, should the labeling of p-RhoA be RhoA?

Reply 2: Thanks for pointing this out. We have modified the labeling of p-RhoA to RhoA in Fig2C.

Changes in the text: Fig 2C (Page 16, line 486).

Comment 3: Fig 2D and F, since the protein expression was analyzed in triplicate, the authors need to include variation bars for the NC group as well.

Reply 3: Thank you for your suggestion. It is important to emphasize that protein expression in this study was indeed done in three replicates. The reason why there are no variation bars in the NC

group in Figure 2D and F is that we normalized the raw data. This study used NC to correct the relative quantification results of the target gene, and the values in the NC group are all 1. Many studies have used similar data processing. For example, Fig. 4C and E of the paper published in Environment International by Jia Yangyang et al (Reference: Circular RNA 406961 interacts with ILF2 to regulate PM2.5-induced inflammatory responses in human bronchial epithelial cells via activation of STAT3/JNK pathways. Environ Int. 2020, 141:105755). We can provide raw data for viewing.

Changes in the text: None.

Comment 4: Fig 3, the β-globin gene is wrongly labeled as δ-globin.

Reply 4: Thank you for pointing this out. We have modified the δ-globin gene to β-globin in Fig 3.

Changes in the text: Fig 3 (Page 17, line 496).

Comment 5: Though the manuscript is smoothly written, there are a few places that need to be corrected. line 37, repeated words; The sentence in line 252 could be deleted; line 105, has (been) intensified?

Reply 5: Thank you for your suggestion. We made corrections based on these suggestions. The repeated words have been deleted in line 39 (see Page 2, line 39); The sentence in line 252 has been deleted. We added another sentence to summarize this part of the results (see Page 8, line 251-252); We modify the sentence on line 105 to: “Recently, there has also been a research interest in long noncoding RNA (lncRNA)” (see Page 4, line 104).

Changes in the text: Page 2, line 39; Page 4, line 104; Page 8, line 251-252.