Reviewer #1:

1. Background: Please add references about review article related to similar issue.

Answer: We have added the reviews (Ref. 3, 6-20) concerning the research background of HIF-1α.

2. Discussion: I am not familiar the hypothesis proposed in this study. The authors should clarify this concern for discussion.

Answer: By gene enrichment analysis, we found that the expression of IL-1ra increased after liver ischemia-reperfusion treatment. It was also found that IP-induced HIF-1α could promote the expression of IL-1ra and thus attenuated liver ischemia-reperfusion injury in the hypoxic stage. We have added a description of the hypothesis in the discussion section.

3. Illustrations and tables: I am not familiar in vivo and in vitro model. Please introduce commonly used illustrations and tables for mediation model.

Answer: (1) The *in vivo* ischemia-reperfusion injury model[1-3]: After anesthetizing mice, we made a ventral midline incision to fully expose the hilar structure, and clipped the left and middle lobe vessels for the corresponding time (3h, 6h, 12h, and 24h). (2) The *in vivo* ischemic preconditioning model[4-7]: After anesthetizing mice, we made a ventral midline incision to fully expose the hilar structure, clipped the left and middle lobe vessels for 10 minutes, and the removed the vessel clip for another 10 minutes. This is called a cycle. After the indicated IP cycles, mice were treated with ischemia-reperfusion. (3) The *in vitro* model[3, 8]: AML12 cells were cultured in a 37°C incubator with 95% N_2 and 5% CO_2 to induce a hypoxia condition. After a specific time period, cells were removed and placed back into the 37 °C incubators with 5% CO_2.

1 Qin JJ; Mao W; Wang X; Sun P; Cheng D; Tian S; Zhu XY; Yang L; Huang

Z; Li H. Caspase recruitment domain 6 protects against hepatic ischemia/reperfusion injury by suppressing ASK1. J Hepatol 2018, 69, 1110-1122, [PMID: 29958938. DOI: 10.1016/j.jhep.2018.06.014]

2 Kadono K; Kageyama S; Nakamura K; Hirao H; Ito T; Kojima H; Dery KJ; Li X; Kupiec-Weglinski JW. Myeloid Ikaros-SIRT1 signaling axis regulates hepatic inflammation and pyroptosis in ischemia-stressed mouse and human liver. J Hepatol 2022, 76, 896-909, [PMID: 34871625. DOI: 10.1016/j.jhep.2021.11.026]

3 Liu Y; Lu T; Zhang C; Xu J; Xue Z; Busuttil RW; Xu N; Xia Q; Kupiec-Weglinski JW; Ji H. Activation of YAP attenuates hepatic damage and fibrosis in liver ischemia-reperfusion injury. J Hepatol 2019, 71, 719-730, [PMID: 31201834. DOI: 10.1016/j.jhep.2019.05.029]

4 Motino O; Frances DE; Casanova N; Fuertes-Agudo M; Cucarella C; Flores JM; Vallejo-Cremades MT; Olmedilla L; Perez Pena J; Banares R; et al. Protective Role of Hepatocyte Cyclooxygenase-2 Expression Against Liver Ischemia-Reperfusion Injury in Mice. Hepatology 2019, 70, 650-665, [PMID: 30155948. DOI: 10.1002/hep.30241]

5 Rudiger HA; Graf R; Clavien PA. Sub-lethal oxidative stress triggers the protective effects of ischemic preconditioning in the mouse liver. J Hepatol 2003, 39, 972-977, [PMID: 14642614. DOI: 10.1016/s0168-8278(03)00415-x]

6 Teoh N; Field J; Farrell G. Interleukin-6 is a key mediator of the hepatoprotective and pro-proliferative effects of ischaemic preconditioning in mice. J Hepatol 2006, 45, 20-27, [PMID: 16600417. DOI: 10.1016/j.jhep.2006.01.039]

7 Banga NR; Homer-Vanniasinkam S; Graham A; Al-Mukhtar A; White SA; Prasad KR. Ischaemic preconditioning in transplantation and major resection of the liver. Br J Surg 2005, 92, 528-538, [PMID: 15852422. DOI: 10.1002/bjs.5004]

8 Zhang XJ; Cheng X; Yan ZZ; Fang J; Wang X; Wang W; Liu ZY; Shen LJ;

Zhang P; Wang PX; et al. An ALOX12-12-HETE-GPR31 signaling axis is a key mediator of hepatic ischemia-reperfusion injury. Nat Med 2018, 24, 73-83, [PMID: 29227475. DOI: 10.1038/nm.4451]

4. Biostatistics: Does the manuscript examined by experienced biostatistics? One-way analysis of variance (ANOVA) based on normal distribution assumption.

Answer: In our manuscript, the statistical analyses were performed using the GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA). All the data are presented as the means ± SEMs and represent three or five independent experiments. One-way analysis of variance (ANOVA) was used to compare means between treatment groups. Student's *t*-test for unpaired observations was applied. A P-value < 0.05 was considered significant.

5. Units: Does the manuscript meet the requirements of use of international system of units?

Answer: We examined the manuscript and made it conform to the requirements for the use of the international system of units.

6. References: Please cite appropriately the latest, important and authoritative references in the introduction and discussion sections.

Answer: We have updated the references in the introduction and discussion sections in the revised manuscript.

7. Quality of manuscript organization and presentation: Please provide English editing certificate.

Answer: Our revised manuscript has been polished by MedE editing service.

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EDITORIAL CERTIFICATE
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Manuscript title
Hypoxia-inducible factor 1α promotes expression of interleukin-1 receptor antagonist during hepatic ischemia-reperfusion injury

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Date issued
August 16, 2022
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8. Research methods and reporting: Please provide appropriate research methods and reporting. Authors should have prepared their manuscripts according to manuscript type and the appropriate categories, as follows: (1) CARE Checklist (2013) - Case report; (2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial; (3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis; (4) STROBE Statement - Case Control study, Observational study, Retrospective Cohort study; and (5) The ARRIVE Guidelines - Basic study.

Answer: We have provided the ARRIVE Guidelines statement in the revised manuscript.

9. Ethics statements: Please provide appropriate ethics approval.

Answer: We have provided the appropriate ethics approval in the revised manuscript.

Reviewer #2:

1. The authors should express the abbreviations of the words (e.g. IL-1ra, and IL1rn) in the abstract.

Answer: We have added the abbreviations in the revised manuscript.

2. Figure 2B shows the protein levels of IL-1ra in the liver after IRI. How about the quantitative analysis?

Answer: We have added the quantitative analysis of Figure 2C in the revised manuscript.

3. Figure 2D shows the statistical analysis of immunohistochemial scores. In general, the score should be shown as a dot plot. Also, a specific statistical analysis should be considered.

Answer: We have revised Figure 2F as a dot plot in the revised manuscript.

4. Cells were subjected to hypoxia-reoxygenation (HR) in the study. The authors should express the detail condition of hypoxia. Did the authors confirm the hypoxia by measuring the concentration of oxygen level in the medium?

Answer: For the *in vitro* model[1, 2], AML12 cells were cultured in a 37°C incubator with 95% N_2 and 5% CO_2 during the hypoxia. An oxygen concentration indicator was used to measure the oxygen concentration in the incubator to ensure that it had been emptied of oxygen. After a specific time period, cells were removed and placed back into a 37°C incubator with 5% CO_2.

1 Liu Y; Lu T; Zhang C; Xu J; Xue Z; Busuttil RW; Xu N; Xia Q; Kupiec-Weglinski JW; Ji H. Activation of YAP attenuates hepatic damage and fibrosis in liver ischemia-reperfusion injury. J Hepatol 2019, 71, 719-730, [PMID:

31201834. DOI: 10.1016/j.jhep.2019.05.029]

2 Zhang XJ; Cheng X; Yan ZZ; Fang J; Wang X; Wang W; Liu ZY; Shen LJ; Zhang P; Wang PX; et al. An ALOX12-12-HETE-GPR31 signaling axis is a key mediator of hepatic ischemia-reperfusion injury. *Nat Med* 2018, ***24***, 73-83, [PMID: 29227475. DOI: 10.1038/nm.4451]

5. Figure 2D shows the protein levels of IL-1ra in AML12 cells after HR. How about the quantitative analysis?

Answer: We have added the quantitative analysis of Figure 3F in the revised manuscript.

6. Figure 4B and 4E show the protein levels of IL-1ra in the liver after ischemia and the protein levels of IL-1ra in AML12 cells after hypoxia. How about the quantitative analysis in both experiments?

Answer: We have supplemented the results of quantitative analysis in Figure 4C and 4G.

7. Figure 5A, 5b and 5E also needs the quantitative analysis to confirm the results.

Answer: We have supplemented the results of quantitative analysis in Figure 5B, 5D, 5H and 5I.

8. Figure 7I and 7J need much better images for the IL-1ra expression or the quantitative analysis for better understanding.

Answer: We have repeated the experiments and supplemented the updated results in Figure 7L, 7M, 7O and 7P.

9. The authors analyzed the gene enrichment analysis for IR3h and IS 1.5h and IL1rn was focused. How about the gene enrichment analysis in different time point (e.g. IR6h)?

Answer: Our gene enrichment analysis was performed in three groups: the control group, IS 1.5h group and IR 3h group. Based on the current studies, more severe liver damage was expected at IR 6h. Since we aimed to look for the transcriptomic changes at early stage of IRI, we did not prepare for the sample of IR 6h treatment group.

10. The authors showed that IP protected the liver by promoting IL-1ra expression via HIF-1alpha. The results appear to be already known?

Answer: According to the previous reports, IP has been indicated a protective effect on liver IRI. However, the molecular mechanism remains elusive. Here we extended the study showing that IP protects against liver injury by promoting HIF-1α accumulation and consequently lead to the increased expression of IL-1ra.

Revision reviewer:

The manuscript was re-reviewed for publication in the journal. The manuscript was designed to evaluate a mechanism that could protect the liver in the early stage of ischemia-reperfusion injury (IRI). The results obtained show that ischemia or hypoxia leads to increased expression of interleukin-1 receptor antagonist (IL-1ra) regulated by HIF-1alpha and that ischemic preconditioning (IP) protects the liver from IRI via the HIF-1alpha-IL-1ra pathway. It is the reviewer's opinion that the manuscript is interesting and easy to follow. The authors promptly explained/discussed all issues/points. I have no more concern in the manuscript.

Answer: Thanks for your comments.