Dear Prof. Jin-Zhou Tang, Manuscript Administration and Journal Staff:

Thank you for checking our manuscript. Our manuscript has been revised according to the feedback. We employed an English-language editing service AJE to polish our wording. Certification is attached.We also expanded part of the experiment providing details in current version.

On behalf of all co-authors, thank you very much for the comments and suggestions on our manuscript "GSDMD-mediated hepatocyte pyroptosis expands inflammatory responses that aggravate acute liver failure by upregulating MCP1/CCR2 to recruit macrophages "(Manuscript ID: 51464). We have carefully studied three reviewer's comments(Number ID: 02860897，03671246and03293832), double-checked our paper and gave the responses point-by-point. Once again, thank you very much for your kind comments and suggestions. If you have any other questions, please feel free to contact me.

Answers to reviewers:

Reviewer #l:

The manuscript describes the involvement of GSDMD on hepatocyte pyroptosis in acute liver failure using AML12 mouse lined hepatocytes, genetically engineered mice, and human samples. They showed that GSDMD positively regulates recruited macrophages to release inflammatory mediators and to lead expansion of the secondary inflammatory responses. They suggest that inhibition of GSDMD can reduce hepatocyte death and the secondary inflammatory responses of acute liver failure. I, reviewer suggest that the manuscript deserves publication in WJG if following items are improved;

1. The results section in abstract should be more concretely showing the real data (numerical value) of pivotal points.2. Introduction is too long. Make it neat and tidy. 3. Define 'healthy liver tissue'. Were they from part of deceased or living donor ? 4. Define 'Cell inhibition' shown in Figure 2. Describe in Materials and Methods. 5. Description in 'Statistical analysis' is

messy. Should be revised by a person who knows statistics well.

Response1:

1.We added data of pivotal points in the results section in abstract as follow:The level of GSDMD-N protein increased most obviously($P<0.001$). In vitro, downregulation of GSDMD by shRNA decreased the cell inhibition rate and the levels of proteins MCP1/CCR2（$P<0.01$）. In vivo, GSDMD knock out dramatically eliminated inflammatory damage in the liver and improved the survival of D-Galn/LPS-induced ALF mice（$P<0.001$）.

2.We have simplified Introduction again, reducing the number of words from 942 to 488 in order to make it neat and tidy.

3.In our study,'healthy liver tissue'was defined as the from deceased organ donors who died from intracranial bleeding or head injury and were free from chronic diseases.This part is added in Materials and Methods(Samples of human liver).

4.First,we should Correct 'Cell inhibition'as 'Cell inhibition rate'.According to the instructions of CCK8 kit, cell inhibition rate=
[(control- experimental)/(control- blank)]×100%.We described this part in Materials and Methods.

5.All the data in our study were re-analyzed and revised by another professional statistician,qualification certificate was uploaded.And we amended the description in 'Statistical analysis'.

どうもありがとうございます。

Reviewer #2: Humans express a complex array of chemokines and chemokine receptors that collectively orchestrate the trafficking of leukocytes, a central feature of the innate immune response. CC chemokine receptor 2 (CCR2) is the major chemokine receptor on monocytes and macrophages, cells that play central roles in the pathology of atherosclerosis, obesity, and type 2 diabetes. In atherosclerosis, CCR2 activation by the monocyte chemoattractant proteins MCP-1, MCP-2, and MCP-3 induces the recruitment of monocytes from the blood into the arterial walls, where they differentiate into macrophages and contribute to the development of atherosclerotic plaques. In acute liver failure, CCR2 activation by MCP1 is associated with macrophage infiltration. As an executor of pyroptosis, GSDMD plays a key role in the pathogenesis of acute liver failure. Major 1.This mechanism of inflammation has also been proposed for other liver disease such as steatohepatitis. Does pyroptosis play an important role only in the early stages of acute liver failure? 2.Please clarify the role of MCP-2 and MCP-3.

Response2:

1. Current research shows that Pyroptosis is associated with many liver diseases,such as drug-induced liver injury[1], hepatic ischemia reperfusion[2],alcoholic hepatitis injury[3],CLP-induced acute liver injury[4],et,al.Pyroptosis plays an important role in the process of liver inflammatory response.In our study,human liver tissue samples were collected from 7 patients with end-stage acute liver failure,Data showed that the protein expression levels of caspase 1/4, GSDMD full-length (GSDMD-FL) and GSDMD-N in liver tissues increased in ALF cases compared to the controls, and the expression of GSDMD-N increased significantly.This means that pyroptosis occurs not only in the early stages of acute liver failure,but throughout the whole process.It is a driving factor for the progression of acute liver failure[5,6].

Reference

1 Iorga A, Dara L.Cell death in drug-induced liver injury.Adv.Pharmacol 2019;85[PMID：31307591DOI：10.1016/bs.apha.2019.01.006]
2 Li Z , Zhao F , Cao Y , Zhang J , Shi P , Sun X , Zhang F , Tong L DHA attenuates hepatic ischemia reperfusion injury by inhibiting pyroptosis and activating PI3K/Akt pathway..Eur J Pharmacol 2018;9;835:1-10.[PMID: 30075219]
3 Khanova E, Wu R, Wang W, Yan R, Chen Y, French SW, Llorente C, Pan SQ, Yang Q, Li Y, Lazaro R, Ansong C, Smith RD, Bataller R, Morgan T, Schnabl B, Tsukamoto H.Pyroptosis by caspase11/4-gasdermin-D pathway in alcoholic hepatitis in mice and patients.Hepatology 2018; 05; 67 (5): 1737-1753.[PMID：29108122DOI：10.1002/hep.29645]
4 Chen YL, Xu G, Liang X, Wei J, Luo J, Chen GN, Yan XD, Wen XP, Zhong M, Lv X.Inhibition of hepatic cells pyroptosis attenuates CLP-induced acute liver injury.Am J Transl Res 2016;8(12): 5685-5695.[PMID：28078039]
5 Wu J, Lin S, Wan B, Velani B, Zhu Y.Pyroptosis in Liver Disease: New Insights into Disease Mechanisms.Aging Dis 2019,10(5):1094-1108.[PMID：31595205DOI：10.14336/AD.2019.0116]
6 Luan J, Ju D.Inflammasome: A Double-Edged Sword in Liver Diseases. Front Immunol 2018;9:2201[PMID：30319645 DOI：10.3389/fimmu.2018.02201]

2. Monocyte chemotactic proteins 1, 2, and 3 (MCP1, MCP2, and MCP3) are expressed in many cell lineages. By binding to their common receptor CCR2, MCPs play a role in amplifying inflammatory responses.Studies have found that among these, only MCP-1(CCL2) is expressed in hepatocytes and released to extracellular sites in response to hepatocyte injury. In contrast to MCP-1 expression, MCP-2(CCL8) and MCP-3(CCL7) were not expressed on hepatocyte and biliary epithelial cells.Therefore, most studies focused on the role of MCP2/CCR2 in liver disease.

どうもありがとうございます。

Reference

1 Vodovotz Y, Simmons RL, Barclay D, Yin J, Jefferson BS, Zamora R, Decoding the secreted inflammatory response of primary human hepatocytes to hypoxic stress.Ann Transl Med 2019;Aug;7(16):371[PMID：31555685DOI：10.21037/atm.2019.07.09]
2 Tsuneyama K, Harada K, Yasoshima M, Hiramatsu K, Mackay CR,

Mackay IR, Gershwin ME, Nakanuma Y.Monocyte chemotactic protein-1, -2, and -3 are distinctively expressed in portal tracts and granulomata in primary biliary cirrhosis: implications for pathogenesis.J Pathol 2001 Jan;193 (1): 102-9.[PMID：11169522
DOI：10.1002/1096-9896(2000)9999:9999<::AID-PATH725>3.0.CO;2-P]

Reviewer #3 Major comments: 1. Please clarify the cell sources of MCP1/CCR2 in the manuscript. The author should probe the involvement of MCP1/CCR2 in GSDMD-mediated pyroptosis through blocking CCL2/CCR2 axis. 2. Fig. 3A should include the sample without transfection. 3. In Fig. 4A, GSDMD accounted for about 55% survival while wt had only 25% survival beyond 12 h post-treatment. These numerical figures extracted from the graph are not in agreement with "12/15" and "1/15" written on the graph. A rescue of 30% (55%-25%) was accountable by GSDMD-mediated pyroptosis. What was responsible for the remaining 45% death? Were they caused by necrosis / apoptosis? 4. In Fig. 5A, GSDMD-N almost virtually disappeared. This is not in agreement with 70% efficiency of shRNA against GSDMD shown in Fig. 3A. Minor comments: 1. "GSDMD-medicated" in the title and throughout the manuscript should be corrected as "GSDMD-mediated". 2. How to calculate cell inhibition rate? 3. The labels in most bar graphs are too small and illegible.

Response 3:

1.We added the cell sources of MCP1/CCR2 in the manuscript.

2.We will go further to probe the involvement of MCP1/CCR2 in GSDMD-mediated pyroptosis through blocking CCL2/CCR2 axis in the following study.

3.Experiment and data include the sample without transfection in Fig. 3A were added.

4.The survial curve in Figure 4A were re-analyzed and revised.And we

amended the description in ‘Statistical analysis’.4.In Fig. 5A,we used GSDMD knock out mice,So the expression of protein GSDMD-N disappeared.

5.We corrected "GSDMD-medicated" as "GSDMD-mediated"throughout the manuscript.

6.The method of calculating cell inhibition rate by CCK8 was added in the part of “Materials and Methods”.

7.We amended all the bar graphs to make it clearer.