Dear Editors:

Thank you very much for your comments to our manuscript. I have read the reviewers' comments very carefully.

Regarding the reviewer's specific comments of increasing the detection time: When designing the experimental we considered it, so we collected and reserved part of the specimen at 48 h after recombinant adenovirus or physiological saline injection for subsequent research. This time we took out the 48 h specimens and tested some indicators, then added to the manuscript.

About the question of no information on the recovery of damaged liver in this study: In our study, attenuated alanine aminotransferase (ALT), Prothrombin time (PT), high-mobility group box 1 (HMGB1), endotoxin, tumour necrosis factor (TNF)-α and interferon (IFN)-γ of serum levels were observed in the Ad-HGF-, Ad-HIL-6- and Ad-HGF-HIL-6-treated rats with ACLF, likewise reduced hepatic damage and apoptotic activity, as well as reduced HMGB1 and Bax proteins, but raised expression of Ki67, Bcl-2 protein and Bcl-2 versus Bax (Bcl-2/Bax) at protein expression levels. More significant changes were observed in the Ad-HGF-HIL-6 treatment group without obvious side effects. Furthermore, caspase-3 at the protein level decreased in the Ad-HIL-6 and Ad-HGF-HIL-6 treatment groups, more predominantly in the latter group. These results of biochemistry, pathophysiology and molecular biology illustrated ACLF rats being in recovery mode. The title of my manuscript have been changed to "Recombinant adenovirus containing Hyper-IL-6 and HGF ameliorates acute-on-chronic liver failure in rats." I think this is more in line

with our research.

About “In Figure 1, serum levels of IL-6, HGF should be also tested to show whether the reduced serum levels of ALT, PT, endotoxin, and HMGB1 are correlated with increased serum levels of IL6, HGF.”: Hyper-IL-6 and HGF of adenovirus vectors containing in our study were derived from human. There are not ELISA kits simply for the genes of human origin while excluding rat genes interference, so the detections what you mentioned are unable to achieve the desired results.

About “Fig 2 and 3 should be combined together to show representative photos and quantitative analysis of the imaging data. The similar is true for Fig 5 and Figure 6.”: Fig 2 and 3 have been combined together, but it will be less attractive and rather unpleasant after Fig 5 and Fig 6 combined.

About “IL-6 is a strong pro-inflammatory cytokine, while ACLF is primarily caused by inflammatory damage; it is not known why IL-6 has a protective effect in this model, which should be discussed in the text.”: As shown in the **INTRODUCTION** of my manuscript, previous studies have reported that IL-6 plays a critical and unique role during the process of early-stage hepatic regeneration response[4]. Hyper-IL-6 (HIL-6) is an artificial fusion cytokine comprising IL-6 linked by an artificial linker with a soluble variant of gp80 (sIL-6R). HIL-6 is a stable protein displaying biological activity *in vitro* or *in vivo* 10–1000-fold higher than that of IL-6/sIL-6R soluble complex[5]. The coadministration of IL-6 and HGF most effectively increased both the wet weight of the unoccluded lobes and the hepatocellular DNA synthesis of the animals that were underwent portal branch

ligation (PBL) of the left lateral and median branches[9], suggesting a possible synergistic effect of these two factors.

About “Additional minor issues: 1. IL-6 is not a “transcription factor” (on page 5). 2. It is not clearly described how adenoviral vectors were administrated; via iv injection? 3. The control adenoviral vector is not empty, it still contains GFP. It is confusing to use Ad0. It is proposed to use Ad-GFP for the control adenoviruses.”: They have been amend in accordance with the requirements of reviewers.

About “the Authors should emphasize the importance to translate their findings in the human being and in the clinical practice.”: As shown in the **INTRODUCTION** of my manuscript, acute-on-chronic liver failure (ACLF) refers to the patients with chronic liver diseases that have the raised perils of multiple organ failure or death following one or a few precipitating incident, such as infection or bleeding. It still lacks effective treatment so far. Viral vectors have been engineered for gene therapy against various types of infectious diseases[1,2]. The adenovirus vector has become the ideal vehicle for liver diseases due to its hepatotropism[3]. Ad-HGF-HIL-6 is likely to be a feasible protective therapy for serious liver injury. As shown in the **DISCUSSION,** our experiment confirmed the functional synergistic effect of HIL-6 and HGF by combining them into an adenovirus vector. Moreover, the enhancement of treatment did not increase immunogenicity or toxicity. These have proved the importance to translate their findings in the human being and in the clinical practice.

About “RESULTS (no less than 120 words): You should present *P* values where appropriate. You must provide relevant data to illustrate how the statistical values

were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$." If I follow this requirement to modify, then the figures must be more than 120 words.

About the other minor comments, I have already corrected them, thank you for your referees, I will be more careful in the future.

I have highlight the changes to our manuscript within the document by using yellow colored text.

I greatly appreciate both your help and that of the referees concerning improvement to this paper. I hope that the revised manuscript is now suitable for publication.

Sincerely yours,

Dandan Gao

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