

Unité de recherche 1193
Physiopathogénèse et Traitement
des Maladies du Foie
&
Dr Moniaux Nicolas

**Institut national
de la santé et de la recherche médicale**

Wednesday, 26 August 2020

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Dear Dr. Jia-Ping Yan,

We submit our revised manuscript entitled "The CDK inhibitors p21 and p27 function as critical regulators of early liver regeneration following 90% hepatectomy in the rat" for publication in World Journal of Hepatology.

We would like to thank the reviewer and yourself for your insightful and constructive comments and suggestions regarding several aspects of our original manuscript. As described in detail below, we have addressed your critical remarks and made the requested modifications. You will find a point-by-point reply to each comment and a list of the changes made accordingly below. We hope that we have met all the concerns expressed and that our manuscript is now publishable.

Sincerely,

Nicolas Moniaux

Reviewer #1

General Comments: A very interesting article was submitted for review. The subject of this study is fully consistent with the topics of the Journal, and the title reflects the content of the article. When considering the manuscript a few remarks arose.

Response: We thank the reviewer encouraging comment.

Concern 1: A number of studies have been devoted to the problem of liver regeneration after subtotal resection. These studies indicate the causes of impaired regeneration after subtotal liver resection. This reason is a block of the proliferation of hepatocytes. However, different authors point to different points of the mitotic cycle at which disturbances occur after subtotal resection of the liver. In addition, probably, different animal species have their own peculiarities of block of hepatocyte cell cycle after subtotal liver resection. In connection with this, it is recommended to review and discuss the data obtained in the indicated articles on the rat model in the discussion section. - Elchaninov AV, Fatkhudinov TK, Usman NY, Kananykhina EY, Arutyunyan IV, Makarov AV, Lokhonina AV, Eremina IZ, Surovtsev VV, Goldshtein DV, Bolshakova GB, Glinkina VV, Sukhikh GT (2018b) Dynamics of macrophage populations of the liver after subtotal hepatectomy in rats. BMC Immunol 19(1): 23

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<https://bmcimmunol.biomedcentral.com/articles/10.1186/s12865-018-0260-1> -
Inherent control of hepatocyte proliferation after subtotal liver resection. Elchaninov A, Fatkhudinov T, Makarov A, Vorobieva I, Lokhonina A, Usman N, Kananykhina E, Vishnyakova P, Nikitina M, Goldshtein D, Bolshakova G, Glinkina V, Sukhikh G. Cell Biol Int. 2019 Jul 12. doi: 10.1002/cbin.11203. PMID: 31297922
<https://onlinelibrary.wiley.com/doi/full/10.1002/cbin.11203>

Response: We are thankful to the reviewer's critical comments, which have helped us to improve the quality of the manuscript. The discussion section was modified accordingly to your wishes and now states: "This assumption is in line with findings of different studies, including those of Lehmann *et al.*, who showed an impairment of the regenerative capacity of the small remnant liver linked to a p21-dependent cell cycle block in a mouse model [18]. Inhibiting p21 in transgenic animals partially restored the regenerative capacity of the liver and improved the survival rate[18] and a treatment with a senescence-inhibiting drug improves liver regeneration after partial hepatectomy by disrupting aberrantly prolonged p21 expression in mice [34].

To further investigate the contribution of CKI in the failed liver regeneration, we examined the earliest events to occur in response to experimentally hepatic insufficiency induced by 90% hepatectomy in the rat. We showed that the delayed liver regeneration of the small remnant liver is associated with altered expression of p27 and p21, being detected as early as 3h and 12h postoperatively, respectively. The priming of quiescent hepatocytes occurred correctly, as depicted by STAT3 activation coincident with Rb phosphorylation as early as 3h post-resection, reflecting by entry into the cell cycle. But extended hepatectomy resulted in significant delay in S-phase progression and mitosis, which was compensated in surviving animals by increased DNA synthesis at later time points eventually leading to restore liver mass and functional activity. Our results therefore highlighted the critical importance of the cyclin-CDK inhibitors of the Cip/Kip family in regulating the liver regeneration timeline following 90% hepatectomy. To this is added a large number of molecular signals that were switched on or off to guarantee a timely hepatocyte entry and progression into the cell cycle[35,36]. However, the choice of the experimental conditions (hepatectomies ranging from 80 to 95% of total liver weight, glucose supplementation, species-specific features, housing conditions and diet) affects many of these signaling pathways accounting for the noticeable differences between the studies."

Concern 2: The authors indicate that the t test was used for statistical analysis. Did the authors check the conditions for using this criterion? What criteria was used for this?

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Response: We understand your concern about the statistical analysis. The Material and method chapter was modified to precise this point and now states: “Normal distribution of the data was analyzed by the Shapiro-wilk test and homogeneity of variances by the Levene test. All groups were normally distributed, and a two-tailed Student’s t-test was used to assess statistical differences between the groups. The statistics were performed with StatView 5.0 freeware (SAS Institute Inc., Cary, North Carolina, USA) and differences with $P < 0.05$ were considered significant. All data are presented as means over several independent experiments \pm standard error of the mean (SEM). Survival curve was constructed by the Kaplan-Meier method (Log-rank test). “

Editorial office’s comments

General Comments: Science Editor: 1 Scientific quality: The manuscript describes a basic study of the the CDK inhibitors p21 and p27 function as critical regulators of liver regeneration following 90% hepatectomy in the rat. The topic is within the scope of the WJH. (1) Classification : Grade C; (2) Summary of the Peer-Review Report: The manuscript is interesting, and the title reflects the content of the article.

Concern 1: However, some references should be updated. The questions raised by the reviewer should be answered;

Response: References were checked and updated, new references were added accordingly to reviewer concern, and the discussion section modified as aforementioned.

Concern 2: (3) Format: There are 5 figures. A total of 36 references are cited, including 5 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade B. The authors didn’t provide the language editing certificate.

Response: The language editing certificate was not provided as the manuscript was not check by an English native speaker. I have been living within the United States for up to 9 years, and able to speak and write English properly. In addition, several co-authors of the manuscript (e.g. Faivre and Samuel) present very good skill in English.

Concern 3: Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, the Institutional Animal Care and Use Committee Approval Form or

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Document, and the The ARRIVE Guidelines. The Institutional Review Board Approval Form was waived. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an invited manuscript. The study was supported by 4 grants. The topic has not previously been published in the WJH. 5 Issues raised: (1) The “Author Contributions” section is missing. Please provide the author contributions; (2)

Response: The “author contribution” section was present within the body of the manuscript, on the front page of the document.

Concern 4: The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s)

Response: All supports was mentioned within the manuscript body in the section called “Supported by:”. For each funding, the contract number was provided.

Concern 5: The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

Response: Original figures were uploaded with the revised form of the manuscript. Figures were prepared using the photoshop software and so provided in psd format.

Concern 6: PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout;

Response: PMID and DOI were added in the references section

Concern 7: The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text. 6 Re-Review: Required. 7 Recommendation: Conditional acceptance.

Response: The highlights section was added.