Peer Review File
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Reviewer A

In this paper we studied the effect of 85% liver resection on mitochondrial structure and expression of relevant mitochondria-related genes. A number of questions and comments arose while reading the work.

1. Judging by the data on gene expression obtained by PCR-RT, the authors took the level of expression of the corresponding gene in the control as 1. This should be reflected in the appropriate section of materials and methods.

Reply 1: We appreciate it this great comment from the referee. We supplement the experimental method section (see Page 9, line 228-229) and make the experimental method description more accurate.

2. It is not clear from the statistical analysis section what statistical criterion the authors used to detect statistically significant differences. This section should be supplemented.

Reply 2: Statistical analysis plays an important role in scientific research, and I apologize for not describing statistical methods in detail. We used the statistical analysis method of the t-test and supplemented the statistical methods section of the paper (see Page 9, line 236-237). Thanks.

3. The authors used chip analysis to find differentially expressed genes. Did they upload these data to a publicly available repository? This is a good tone for this type of study.

Reply 3: This is a very valuable comment, and I want to explain it in two ways. (1) Mitochondrial genes are not easy to detect in conventional second-generation sequencing, or they contain a small number of mitochondrial genes that do not meet our needs for studying respiratory chain complex genes. We chose SABiosciences custom PCR chip, but also contains less than 100 genes related to mitochondria, respiratory chain complex genes cannot be detected, in short, there are still some defects in the chip. At the same time, the SABiosciences PCR chip is used in a relatively small number of scientific research. (2) 85% PH model is a new model, it's been reported in rats before, but it hasn't been published in mice, and it's also an exploratory model, and we hope to share more sequencing data in the future when we build a stable and valuable model. We hope that the referee will be satisfied with our response and changes.

Changes in the text: None.

4. Resection of more than 80% of the parenchyma usually results in a temporary block of the mitotic cycle. Such data were obtained in rats (https://bmcimmunol.biomedcentral.com/articles/10.1186/s12865-018-0260-1, https://www.wjgnet.com/1948-5182/full/v12/i12/1198.htm). In addition, mice have a later onset of proliferation activity compared to other laboratory rodents (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3272022/). What explains the

discrepancy between the authors' data and the cited studies? This should be addressed in the Discussion section. Is it possible that PCNA is detected not only in proliferating cells, but also in cells in which DNA repair is in progress? In this regard, it would be interesting to detect not only PCNA but also Ki67 in the regenerating liver after 85% resection in mice.
Reply 4: This is a very interesting and valuable question. PCNA is a 36-kDa molecule that plays an essential role in nucleic acid metabolism as a component of the replication and repair machinery. It functions as the accessory protein for DNA polymerase d, required for processive chromosomal DNA synthesis in the S phase,and it interacts with cellular proteins involved in cell cycle regulation and checkpoint control.
Changes in the text: None.

5. The authors state that in regenerating liver cells from mice, the number of cristae is reduced in mitochondria. However, the authors do not provide any quantitative data, which needs to be done.
Reply 5: This is a very professional question, and I apologize for our inaccurate description. We observed the morphological changes of mitochondrial cristae by TEM. We did not count the number of cristae, we deleted the incorrect description (see Page10, line 252). Thanks.

Reviewer B

The article provides a comprehensive overview of the importance of mitochondrial function and energy metabolism in liver regeneration following PH. However, this article could be further improved by highlighting the following points:

1. Why did the author(s) choose 85% instead of 70% PH and the 24hrs time point.
Reply1: This is a very professional problem, 70% PH is currently very mature, and the relevant mechanisms have been studied by many teams. But split liver transplantation and larger hepatectomy require a higher proportion of resections. At present, more than 70% PH models have been successfully constructed in rat models, while more than 70% PH models have rarely been studied in mouse models. We aimed to explore the relationship between 85% PH models and energy metabolism in mice. Thanks!
Changes in the text: None

2. H&E images (Figs A & B) are not similar colour. x100 images are too small to be able to see the liver morphology and hepatic cell structure. Higher magnification images would be better so that the changes in liver morphology before and after PH can be appreciated.
Reply 2: Thank the reviewer for the kind and warm suggestion. We did a lot of HE to compare the two groups of models, and we found that 85% PH cell staining was difficult to be consistent with the Sham group. We reviewed a lot of papers and made a bold guess that the cells in the 85% PH model are highly edematous and fatty, containing many lipid droplets, and are not well stained by eosin. I hope you can be

satisfied with the above answer. Higher magnification images (x400) (see Page 23, Figure 1) were provided in the revised version of the manuscript. Thank you!

3. TEM analysis - a quantitative analysis of this part of experiment is crucial to highlight mitochondrial swelling, reduced numbers of cristae and cristae disintegration, as these are not so convincing by just looking at the images alone.
Reply 3: We are very sorry for our inaccurate descriptions and we have removed them. In this experiment, we only observed structural modification in mitochondrial cristae in our 85% PH model. Thanks.
Changes in the text: None.

4. What would be the significance of mitochondria structural changes in relation to the gene expression in this study?
Reply 4: The Mouse Mitochondrial Energy Metabolism RT² Profiler PCR Array profiles the expression of 84 key genes involved in mitochondrial respiration, including genes encoding components of the electron transport chain and oxidative phosphorylation complexes. Oxidation of NADH and FADH2, the metabolites from glycolysis and the TCA cycle, occurs via a series of four protein complexes embedded in the inner mitochondrial membrane: NADH-coenzyme Q reductase, succinate-coenzyme Q reductase, coenzyme Q-cytochrome c reductase, and cytochrome c oxidase. The free energy generated from these processes drives oxidative phosphorylation and ATP synthesis via a fifth protein complex (ATP Synthase). Dysregulation of these processes is a major pathological consequence of cancer progression. Many tumors contain decreased amounts of mitochondrial respiratory chain components, although the exact mechanism for this repression is unclear. However, recent studies demonstrate that the important tumor suppressor p53 induces the expression of COX2, an essential component for cytochrome c oxidase function. Mitochondrial dysfunction also contributes to metabolic syndrome and obesity, where excess b-oxidation overloads oxidative phosphorylation by generating excessive amounts of NADH. Using real-time PCR, you can easily and reliably analyze the expression of a focused panel of genes involved in mitochondrial energy metabolism with this array. We attempted to explore the relationship between energy metabolism and 85% PH by analyzing mitochondrial genes and mitochondrial cristae changes. Genes in the microarray constitute mitochondrial respiratory chain complexes, and changes in gene expression may lead to changes in mitochondrial cristae morphology. Thanks!
Changes in the text: None.