

Manuscript NO: 78029

Zaiou M. Noncoding RNAs as additional mediators of epigenetic regulation in nonalcoholic fatty liver disease

Dear Editor,

I would like to thank and the Reviewers for your precious time and invaluable comments on the present manuscript. I am pleased to receive these encouraging comments along with the positive preliminary decision. I have carefully addressed all concerns especially those raised by the Reviewer #3. Please find summarized enclosed here my detailed answers as well as the original copy of the manuscript, which has already been checked for English by Filipodia.

I would like to mention that I did not bring any changes to the previous version of the manuscript and that based on the arguments below. I hope my responses will facilitate the decision to publish this work in your esteemed journal.

I am open to consideration of any further comment on my answers.

Sincerely,

Dr. Mohamed Zaiou

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Response to Reviewer # 1

Specific Comments to Authors: A concise, comprehensive, complex and well structured manuscript. There are no grammatical/spelling errors throughout the manuscript and the topic debated is of a great interest due to the fact that NAFLD prevalence is increasing and new data regarding the understanding of NAFLD pathogenesis are needed for a better management in the future.

Author response:

The author sincerely thanks the Reviewer for taking the necessary time and effort to review the manuscript and appreciates his/her encouraging and positive comments.

Response to Reviewer #2

Specific Comments to Authors: Thank you for the work you have done. I agree for publication of the review.

Author response:

The author highly appreciates the reviewer's comments and thanks him/her for accepting the manuscript.

Response to Reviewer #3

Specific Comments to Authors: Author should be congratulated for approaching an interesting topic, but to give readers a broader view of the issue, some points should be added. Recent findings link the mitochondrial sirtuin SIRT4 with cellular senescence, s kin aging, and mitochondrial dysfunction, one of the main mechanisms inducing and worsening

NAFLD. Authors observed that inhibition of miR-15b, in a SIRT4-dependent manner, increased generation of mitochondrial reactive oxygen species, decreased mitochondrial membrane potential, and modulated mRNA levels of nuclear encoded mitochondrial genes and components of the senescence-associated secretory phenotype ...as evident in....MicroRNA-15b regulates mitochondrial ROS production and the senescence-associated secretory phenotype through sirtuin 4/SIRT4. *Aging* (Albany NY). 2016 Mar;8(3):484-505. doi: 10.18632/aging.100905. PMID: 26959556; PMCID: PMC4833141. Recently, several human and animal studies have emphasized the involvement of senescence in the pathogenesis and development of liver steatosis including the progression to NASH as characterized by the additional emergence of inflammation, hepatocyte ballooning, and liver fibrosis, as evident in.....The Role of Senescence in the Development of Nonalcoholic Fatty Liver Disease and Progression to Nonalcoholic Steatohepatitis. *Hepatology*. 2020 Jan;71(1):363-374. doi: 10.1002/hep.30834. Epub 2019 Dec 18. PMID: 31230380. This aspect raises much interest in the light that sirtuin 4 plays a central role in the manic-morbidity of NAFLD. In fact, up-to- date study shows low circulating levels of SIRT4 in obese patients with NAFLD mirroring its reduced mitochondrial expression in an attempt to increase the fat oxidative capacity and then the mitochondrial function in liver and in muscle. SIRT4 modulates the metabolism of free fatty acids reducing their high circulating levels but, unfortunately, increasing ROS production. Great concentration of free fatty acids, released by adipose tissue, coupled with oxidative stress, directly results in endothelial dysfunction, early atherosclerosis, and coronary artery disease risk factor.

Author response:

The author would like to thank very much the reviewer for his/her congratulations and also for pointing out these very interesting thoughts and hypotheses with respect to SIRT4 and

miR-15b. The author definitely agrees that SIRT4 may be directly or indirectly involved in NAFLD, but feels that, although interesting, addressing the mechanistic role of this molecule along with senescence process are beyond the scope of this work, which aims to give an update on the role of most clinically relevant noncoding RNAs in NAFLD. However, addressing the crosstalk of SIRT4 with players involved in NAFLD in a separate note would constitute a hot topic.

As for miR-15b, the Reviewer has brought up a very nice study by Dr. Lang et al., showing that miR-15b regulates mitochondrial ROS production and the senescence-associated secretory phenotype through sirtuin 4/SIRT4 (Lang A et al. MicroRNA-15b regulates mitochondrial ROS production and the senescence-associated secretory phenotype through sirtuin 4/SIRT4. Aging (Albany NY). 2016 Mar;8(3):484-505). The author is also aware of a study (the only one as far as I know), published in 2013, suggesting that the expression profile of miR-15b is associated with NAFLD (Zhang Y, et al. Upregulation of miR-15b in NAFLD models and in the serum of patients with fatty liver disease. Diabetes Res Clin Pract. 2013;99(3):327-334). However, no further evidence was brought later on to support these preliminary findings. Thus, the author feels that it may not be necessary to address the potential involvement of miR-15b in NAFLD in this manuscript and that for the following reasons: due to the vast number of diverse types of ncRNAs that have been identified so far (miRNAs, lncRNAs, circRNAs, ...) and huge number of studies claiming an association between dysregulated ncRNA members and NAFLD, the aim of the review was to focus only the most relevant transcripts that have been experimentally confirmed by preclinical and clinical studies providing promising evidence regarding the potential use of these RNA species in future clinical practice. This was highlighted in the last sentences of the core tip and the introduction of this manuscript (*...only relevant studies shedding light on the roles played by ncRNAs' machinery will be reviewed next.*). Therefore, since there is only one study

linking miR-15b to NAFLD and because there are many biological aspects of miR-15b missing in the literature about its link with metabolic diseases, which remains entirely hypothetical, the author believes that this still uncharacterized miRNA may not be a good fit in this review.