Author's response to reviews

Title: Decreased PCSK9 expression in human Hepatocellular Carcinoma

Authors:

Mamatha Bhat (mamatha.bhat@mcgill.ca)
Marc Deschenes (marc.deschenes@muhc.mcgill.ca)
Victoria Marcus (victoria.marcus@muhc.mcgill.ca)
Nicolas Skill (nskill@iupui.edu)
Xianming Tan (xianming.tan@clinepi.mcgill.ca)
Jeanne Bouteaud (jeanne.bouteaud@mail.mcgill.ca)
Sarita Negi (sarita.negi@gmail.com)
Zuhier Awan (zuhier_awan@yahoo.com)
Reid Aikin (reid.aikin@mail.mcgill.ca)
Janet Kwan (janet.kwan@mail.mcgill.ca)
Ramila Amre (ramila.amre@muhc.mcgill.ca)
Sebastien Tabaries (sebastien.tabaries@mcgill.ca)
Mazen Hassanain (mazen.hassanain@mcgill.ca)
Nabil G. Seidah (nabil.seidah@ircm.qc.ca)
Mary Maluccio (mmaluccio@iupui.edu)
Peter Siegel (peter.siegel@mcgill.ca)
Peter Metrakos (peter.metrakos@mcgill.ca)

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Author's response to reviews: see over
Dear Dr. Zhang,

Thank you for your interest in our manuscript MS: 2284420631227453 entitled "Decreased PCSK9 expression in human Hepatocellular Carcinoma".

We addressed the comments brought forth by the reviewers as thoroughly as possible, as detailed below and in the enclosed revised manuscript. In particular, we performed the LDL-R PCR on the HCC tissues, and did find that LDL-R expression was increased by PCR in addition to increased expression at the protein level. We hope these adequately address their concerns, and thank you for considering our manuscript for publication.

Best regards,

Mamatha Bhat, MD, MSc, FRCPC
Assistant Professor of Medicine, Division of Gastroenterology
McGill University Health Centre
687 Pine Avenue West,
Montréal, Québec
Canada H3A 1A1

Reviewer 1:
The manuscript will gain by adding a representative picture of the LDLR staining quantification (Figure 3).

We thank the reviewer for the helpful comment. We have now added representative pictures of both hepatocellular carcinoma and background liver (cirrhosis) to Figure 3, as Figures 3E and 3F.

Reviewer 2:

1) The revised version has been improved by adding the expression data of LDL-R that correlates with those of PCSK9. However, some questions remain, to my opinion, unanswered. This is notably the case concerning the relationship between HCC, fibrosis and plasma PCSK9 concentrations.

We thank the reviewer for the comment. We in fact addressed this comment in the last revisions, and added a section to the Discussion on p. 9 as below so as to explain our theory behind the lack of correlation between tissue and serum levels of PCSK9.
Given that the serum PCSK9 levels are not correlated with tumor PCSK9 expression, there is no reason to say that tumors secrete PCSK9. We simply conclude that there is no correlation whatsoever between the tumor PCSK9 expression and serum levels. Hence, one can clearly say that serum PCSK9 levels are not reflective at all of the presence or absence of HCC. We have substituted a paragraph as follows to account for this:

"In our study, the systemic levels of PCSK9 did not correlate well with the presence or absence of tumor. In the literature, the reported mean values of human plasma PCSK9 concentrations are quite variable, ranging from a low of 80 ng/mL[30], 150 ng/mL[14] or 200 ng/mL[31] to a high of 4.1mg/mL[32] or 6.1 mg/mL[33]. This variability arises due to the different antibodies against PCSK9 used in the various assays. The assay used in our study has a mean of 77-80 ng/mL, meaning that the HCC patients had a ~12% higher value on average, which is not significantly different from normal values. The half-life of PCSK9 has been determined to be only 5 min in vivo[34], which implies that the liver is continuously producing high levels of PCSK9. However, at least based on our findings, the ongoing malignant process in the liver with modulation of PCSK9 levels locally has no impact systemically."

2) Additional comments:
- to validate the hypothesis that PCSK9 in HCC downregulates LDL-R expression at a post-transcriptional level, LDL-R mRNA should be measured in HCC.

We have performed this experiment now, demonstrating that PCSK9 in fact upregulates LDL-R expression at the transcriptional level, resulting in the increased LDL-R protein expression in HCC tumors. These findings have been added to the Results section on p. 8 and the discussion on p. 11, please see tracked changes.

3) - abstract (conclusions): conversely increased LDL-R expression rather than decreased...

We thank the reviewer for noting this typo, and have accordingly revised this sentence in the abstract.

4) - the discussion is too long.

We have accordingly shortened the discussion, please see the tracked changes.