



CHARLES UNIVERSITY
Faculty of Pharmacy
in Hradec Králové

DISSERTATION THESIS REVIEW

submitted to the Faculty of Pharmacy in Hradec Králové, Charles University

TITLE: Modulation of cholesterol and bile acid metabolism via soluble endoglin and pharmacotherapy

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The dissertation thesis by Ivone Cristina Igreja e Sá, M.Sc. aims to investigate in a model the role of endoglin/soluble endoglin in the liver processing of bile acids, cholesterol, and triacylglycerol under healthy and pathological conditions and the effect of statins on levels of endoglins and liver disease progression.

The thesis is conceived as a set of **four articles** (three original articles and one review) published in the Q1-Q2 journals Life Sciences, International Journal of Molecular Sciences (2x), and Cellular and Molecular Life Sciences. It is structured into chapters according to the requirements for this type of scientific document. The candidate is the first author of two articles (in one case she shares the first authorship), once she is the second author, and once she is a member of a collective of authors. The candidate contributed extensively to all articles and her contributions have included conducting experiments, data analysis, data interpretation, and manuscript preparation.

Theoretical background reviews i) liver morphology and pathology with a special focus on non-alcoholic steatohepatitis and its treatment, and ii) endoglin and its signaling pathway and role in liver disease development. The introduction is appropriately supported by references and its legibility is further increased by self-explanatory figures. The hypothesis is logical and the aims are clearly defined. The thesis is written concisely without typos.

The results clearly show that soluble endoglin influences cholesterol and bile acids metabolism in healthy mice and that in the murine model of non-alcoholic steatohepatitis, endoglin and its soluble form are increased suggesting soluble endoglin as a potential biomarker of NASH development. In the next study, the effect of atorvastatin on soluble endoglin levels and liver fibrosis was tested, but the results of this study were not straightforward. Despite certain promising findings, the atorvastatin's effect on liver fibrosis, under the conditions used, was negligible and the author discusses that

treatment with atorvastatin likely needs to be prolonged to see a significant effect. In conclusion, the results of the presented studies fully addressed the objectives of the studies and significantly improved understanding of endoglin's participation in liver disease development.

Comments and questions:

1. Could you briefly describe the murine model overexpressing human soluble ENG? How are higher levels of human soluble ENG achieved in this model?
2. Could you mention some of the known molecular mechanisms of ENG regulation? Is it known whether the production of soluble endoglin is regulated?
3. Does the increased expression of ENG always result in higher levels of soluble ENG? Does the ENG level somehow correlate with the MMP14 expression? If so, is it possible that the protective effect of ENG is ruined by the increased activity of MMP14? Have increased levels of ENG/sENG and MMP14 been described in patients with liver disorders?
4. In the thesis, you have mentioned several markers that are used for the diagnosis of liver diseases, including NASH. Is there a need to improve the diagnostics of NASH? You suggested the soluble ENG as a potential biomarker of NASH. It takes a long time to confirm a molecule as a biomarker. But, based on your knowledge and published data, could you hypothesize whether soluble could function, for example, as a prognostic biomarker of NASH or in preventive medicine to identify patients at risk?

This thesis presents interesting and important results with potential clinical impact that have been obtained in the framework of consistent experimental studies. Ivone's publication record contains eight published papers (all in high-quality journals). Her work has been cited 16 times (without self-citations) and her H-index is three. She has proven herself as a full-fledged scientist who is able to design and conduct experiments and present the collected data attractively and clearly.

The thesis fulfills all criteria for a Ph.D. dissertation, and I recommend it for defense. After a successful defense, I recommend granting the academic title of "Ph.D." to Ivone Cristina Igreja e Sá, M.Sc.

In Hradec Kralove 25 August 25, 2022

Assoc. Prof. Lukáš Červený, Ph.D.