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HR EXCELLENCE IN RESEARCH

Review Report on the PhD thesis
submitted to the Medical University of Bialystok

Author: Mauro Galli MSc

Title: "Impact Of the expression modulation of Aquaporin 9 on proteomic profile and oxidative stress homeostasisin HepG2 model of hepatic lipid overload"

Scientific Supervisor: Assoc. Prof. Piotr Zabielski, MSc, PhD

PROJECT BACKGROUND

Obesity constitutes a worldwide epidemic with prevalence rates which are increasing in most Western societies and in the developing world. By 2025, if this trend continues, the global obesity prevalence will reach 18% in men and exceed 21% in women. Furthermore, it is now well-established that obesity (depending on the degree, duration, and distribution of the excess weight/adipose tissue) can progressively cause and/or exacerbate a wide spectrum of co-morbidities, including type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, non-alcoholic fatty liver disease, reproductive dysfunction, respiratory abnormalities, psychiatric conditions, and even increase the risk for certain types of cancer.

Growing evidence allows us to understand the critical role of adipose tissue in controlling the physic-pathological mechanisms of obesity and related comorbidities. Recently, adipose tissue, especially in the visceral compartment, has been considered not only as a simple energy depository tissue, but also as an active endocrine organ releasing a variety of biologically active molecules known as adipocytokines or adipokines. Based on the complex interplay between adipokines, obesity is also characterized by chronic low grade inflammation with

permanently increased oxidative stress (OS). Over-expression of oxidative stress damages cellular structures together with under-production of anti-oxidant mechanisms, leading to the development of obesity-related complications.

The role of aquaporins in glycerol metabolism facilitating glycerol release from the adipose tissue and distribution to various tissues and organs, unveils these membrane channels as important players in lipid balance and energy homeostasis and points to their involvement in a variety of pathophysiological mechanisms including insulin resistance, obesity and diabetes.

The PhD thesis of Mauro Galli is devoted to the investigations of the development of novel proteomic approach to the analysis of HepG2 cells and liver samples in the aspect of lipid overload and insulin resistance. Several important issues elucidating the impact of Aquaporin 9 (AQP9) modulation on cellular proteome HepG2-based model of lipid overload are addressed with the focus on the understanding the influence of AQP9 expression levels on oxidative stress homeostasis in the HepG2-based models of lipid overload.

Thus, this thesis addresses the highly relevant and vital areas of current proteomics research in the area of metabolic dysregulation.

GENERAL DESCRIPTION OF THE THESIS

The dissertation comprises 272 pages including the English and Polish abstracts, references list, and supplementary materials.

The front matter consists of the Title page, Acknowledgements, Contents, List of Tables, List of Figures, List of Abbreviations. The body mater is split into 12 chapters and follows a typical structure of a PhD thesis in science, including the Interlocutions, Aims, Materials and methods, Results, Discussion, and Conclusions. The dissertation contains four tables and 32 figures. The back matter includes the References, English and Polish abstracts, and the Supplementary material.

THE INTRODUCTION

It is 14 page-long and consists of the literature review on obesity and insulin resistance, reactive oxygen species (ROS) and redox regulation, integral membrane proteins, and aquaporins (AQPs), and the associations between AQPs, insulin resistance and type 2 diabetes mellitus. Furthermore, the PhD Candidate describes in detail the importance of high-throughput untargeted proteomics in the study of pleiotropic effects of AQP modulations. It provides an excellent background for understanding the following chapters.

The literature for this part is carefully referenced and the majority of references were published within the last decade in high-impact, international journals.

The introduction indicates a good substantive preparation of the PhD Candidate, his knowledge of the study subject and the ability to critically analyze the results of the literature, which leads in a logical way to the presentation of the assumptions and objectives of the dissertation.

STUDY AIMS AND SCOPE

The Candidate focuses on three areas of research as they were stated in the study aims:

1. The development of novel proteomic approach to the analysis of HepG2 cells and liver samples in the aspect of lipid overload and insulin resistance.
2. The elucidation of the impact of Aquaporin 9 (AQP9) modulation on cellular proteome HepG2-based model of lipid overload
3. The understanding the influence of AQP9 expression levels on oxidative stress homeostasis in the HepG2-based models of lipid overload.

The study aims are properly and concisely formed. The claims were relevant both at the times when the PhD project commenced and at the time when this review is being prepared.

MATERIALS AND METHODS

Cell culturing wee performed with a HepG2 cell line. Human liver samples were obtained from obese, non-diabetic, insulin resistant and type 2 diabetic patients during sleeve gastrectomy. The Candidate was granted the approval for carrying out the study by the Medical University of Bialystok Bioethics Committee (No. R-I-002/609/2018 and No. APK.002.107.2021).

The Candidate describes in detail a wide variety of research methods and techniques, including reagents and treatments, FFAs-BSA conjugation protocol, SCD-assisted lysis method, MCX zip-tipping for peptides desalting/cleaning, MS acquisition, Data-dependent analysis (DDA), Data-independent analysis (DIA), ion chromatogram libraries, bioinformatics analysis, lipid droplets staining with Oil red O, lipid droplets staining with Bodipy 493/503, apoptosis assay, microscope short time-lapse experiments, microscope long time-lapse experiments, and digitonin treatment.

Statistical analysis was done with one-way ANOVA with a *post hoc* test. Proteomics data have been analyzed in Biognosys Spectronaut, whereas pathway analysis was performed with Ingenuity Pathway Analysis software.

RESULTS

The results are meticulously described, in the majority of cases presented in the tables and figures. This chapter is divided into 15 sections (and subsections in several instances), in which the Candidate describes in detail the results obtained for every method and technique that was used during the study.

DISCUSSION

It is 21-page long. The Candidate restates research problem and underlines the necessity to perform omics studies to better understand the molecular aspects of AQPs modulation in the model of hepatic lipid overload and highlights the need of improving the current methodologies and techniques which could be applied on a broader range of sample also in clinical settings. Furthermore, the Candidate evaluated how the results reflect the existing literature.

The Candidate clearly demonstrates how does his research contribute to the field of proteomics in metabolic dysregulation. During the course of his PhD research, the Candidate developed two valid and efficient approaches for the untargeted proteomics analysis by combining the most recent advances in proteomics world.

In summary, the doctoral dissertation, and in particular the discussion, indicate good synthetic and analytical skills of the PhD Candidate. The discussion is well written and adequately addressed. The author provides key points and highlights the contribution of the current study to literature.

CONCLUSIONS

The conclusions are entirely supported by the results presented and all of the research objectives set at the commencement of the project are answered.

SPECIFIC COMMENTS

Of note, the reviewed doctoral dissertation has no significant substantive flaws. Notwithstanding, there remain several issues that require to be addressed.

1. Materials and methods chapter lacks the description of the study population from which liver samples were obtained. Please provide the number of patients in each group. In many of the figures the

Candidate states N=3 – does that denote that 3 patients were in each of the group (ND, IGT, T2D)? Or was there only one patient per each group?

2. Speaking of study groups, in the Materials and methods section the Candidate states that liver samples were obtained from obese non diabetic, insulin resistant or type 2 diabetic patients. Meanwhile in several figures (9, 10, 11, 12, 13) the Candidate divide study population in ND, IGT, T2D. By stating insulin resistant did the Candidate mean impaired glucose tolerance?
3. It would seem that the Candidate only presents *post hoc* test in multiple group comparisons (Fig. 10-13). Please provide the values of one-way ANOVA test. Also, please add the information on which *post hoc* test were used. Given the very small number of study participants wouldn't it be more appropriate to use Kruskal-Wallis ANOVA?
4. Some portions of the Discussion remain unreferenced (pp. 74-76, 78, 82, 86-89, 91-94). Upon preparing the manuscript for publication please amend corresponding references.
5. Both the English and Polish abstracts are unstructured.

The afore-mentioned comments for modification partly arise from the reviewer's scientific curiosity and mostly are technical and editorial in nature. They have no influence on the positive merit evaluation of the dissertation.

FINAL REMARKS and CONCLUSION

The thesis is prepared in good editing standard. All the figures are carefully prepared and clearly presented. The language is comprehensive and coherent while errors and inaccuracies are relatively rare. In my opinion, the dissertation by Mr. Mauro Galli fully complies both with the Polish and international standards for PhD dissertations in the field of biomedical research. It meets the requirements of the art. 187 of the Polish Bill on The Law on Higher Education and Science of July 20, 2018 with subsequent amendments (Ustawa Prawo o szkolnictwie wyższym i nauce [Dz. U. z 2021 r., poz. 478]). Specifically, it is clear that Mr. Galli has presented original solutions to a scientific problem. He has

demonstrated general theoretical knowledge in the area of proteomics in metabolic dysregulation. The PhD Candidate clearly has the ability to independently conduct scientific research.

Therefore, I strongly recommend the Senate of the Medical University of Bialystok that the dissertation move to further stages of the doctorate process and may be subjected to a public defense. Furthermore, I strongly propose the dissertation be distinguished.

Bytom, May 31, 2022

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