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Review of Mehmet Onur Aksoy's doctoral dissertation entitled „Transcriptional and epigenetic regulation of in vitro adipogenic differentiation of porcine Mesenchymal Stem Cells (MSCs)."

***Legal basis*:** Act of 20 July 2018 - Law on Higher Education and Science (consolidated text: Journal of Laws of 2020, item 85, as amended)

Basic Information about the Candidate

Mr. Mehmet Onur Aksoy was awarded the degree of Master of Science in Biochemistry by Anadolu University, Türkiye, on 13 February 2020. He has not previously sought the award of the doctoral degree. Since 2021, he has been enrolled as a doctoral candidate at the Doctoral School of Poznań University of Life Sciences. According to the submitted documentation, the candidate has not held any other employment during this period.

Mr. Mehmet Onur Aksoy is the author of 12 publications, has participated in numerous conferences, and completed a five-month research internship under the Erasmus+ Internship Program 2020. The PhD candidate's achievements are above average for this stage of an academic career.

Assessment of the Structure of the Doctoral Dissertation

The doctoral dissertation entitled "Transcriptional and epigenetic regulation of in vitro adipogenic differentiation of porcine Mesenchymal Stem Cells (MSCs)" was prepared at the Department of Genetics and Animal Breeding, Faculty of Veterinary Medicine and Animal Sciences, Poznan University of Life Sciences, under the supervision of Prof. dr hab. Izabela

Szczerbal. The doctoral research was funded by the Polish National Science Centre (NCN) under the OPUS project led by Professor Szczerbal.

The doctoral dissertation is based on three scientific articles published in 2024 and 2026, for which the doctoral candidate is the first author. The dissertation is well organized and comprises the following sections: Table of Contents, List of Abbreviations, Abstract (in both Polish and English), Introduction, Research Problem and Hypothesis, Aim of the Study, Materials and Methods, Results, List of analyses performed by the doctoral candidate and by other contributors, Discussion, Conclusions, Bibliography, and List of Figures (19 figures). The dissertation cites 125 references, most of which are recent and appropriately selected.

The dissertation also includes a section presenting the candidate's scientific achievements. The appendices contain the three published articles together with the co-authors' statements specifying their individual contributions to each publication. My only minor comments concern the rather lengthy abstract (2.5 pages) and several section titles, which could be aligned with standard scientific writing conventions (e.g., Materials and Methods, Table of Contents, and Conclusions). The dissertation is very well written in terms of language, style, and editorial quality, and I did not notice any typographical errors (the only minor editorial issue is that the term HDR is explained twice).

The Introduction chapter (19 pages) provides an interesting and valuable overview of the research topic and covers all major aspects addressed in the doctoral dissertation. It presents comprehensive information on obesity, adipose tissue, mesenchymal stem cells, adipogenesis, the pig as a model organism, and CRISPR-Cas9 technology. The chapter is well illustrated, which enhances the clarity and accessibility of the presented information (although most figures are adapted from previously published ones rather than being original works). The information presented is supported by relevant scientific references. Minor comments concern Section 4.1, where a few examples from the more recent literature published after 2020 would have been more appropriate. In addition, statements beginning with phrases such as "Emerging evidence...", "Increasing evidence...", and "Recent studies..." (e.g., pages 14, 22, 25, and 29) are supported mainly by references published in 2014 or earlier. This is somewhat inconsistent with the terminology used. Despite these minor comments, the Introduction demonstrates the PhD candidate's broad theoretical knowledge and appropriate preparation for conducting the research presented in the dissertation.

Aim of the Study

The research presented in the doctoral dissertation focuses on elucidating the molecular mechanisms regulating adipogenesis, particularly the interactions between transcriptional and epigenetic pathways controlling adipocyte differentiation. Porcine MSCs were used as the experimental model throughout the study. A deeper understanding of the complex processes underlying adipose tissue development in pigs is important not only from a scientific perspective but also for its practical implications in animal production. Despite considerable progress in this field, our knowledge of the molecular regulation of adipogenesis remains incomplete. Although the key transcription factors governing adipocyte differentiation have been identified, the roles of epigenetic regulators and their dynamic interplay with transcriptional networks remain poorly understood. Moreover, the significance of the research presented in the doctoral dissertation extends beyond animal production. Obesity is a major global health challenge, and adipose tissue as a metabolically active endocrine organ plays key roles in energy homeostasis, lipid and glucose metabolism, and immune regulation. Therefore, elucidating the molecular mechanisms underlying adipose tissue development may also contribute to a better understanding of obesity and related metabolic disorders. The Author formulated research hypotheses and the following objectives of the study:

1. To determine the functional role of the SREBF1c isoform in the regulation of adipogenic differentiation and the expression of key adipogenic genes in porcine mesenchymal stem cells using CRISPR/Cas9-mediated gene editing.

2. To investigate the role of the CEBPB gene in the proliferation and adipogenic differentiation potential of porcine mesenchymal stem cells using CRISPR/Cas9-mediated gene editing.

3. To analyze changes in selected histone modifications (H3K9ac, H4K8ac, and H4K20me3) within promoter and exon regions of four adipogenic genes PPARG, GATA2, CEBPA, and CEBPB during adipocyte differentiation.

4. To evaluate the relationship between chromatin remodeling and transcriptional activity of key adipogenic genes during porcine adipogenesis.

In conclusion, the choice of research topic and experimental model is well justified and aligns well with the strong scientific expertise of Prof. Szczerbal's group in this field. The goals set by Mr. Mehmet Onur Aksoy are ambitious and clearly defined. The presented research objectives allow for a better understanding of the studied mechanism of adipogenesis and have both scientific and potential practical value.

Evaluation of the Methodology

The thesis provides general information about the materials and methods used, while details are described in the related publications. The study was performed using porcine mesenchymal stem cells (MSCs) differentiated into adipocytes. Fluorescence microscopy and BODIPY staining were used to analyze lipid accumulation in cells. The CRISPR-Cas9 method was used to inactivate (knockout) the *SREBF1* and *CEBPB* genes. The experimental strategy employed paired gRNAs to generate targeted deletions within the regulatory regions of these genes. The obtained monoclonal cell lines were characterized by PCR and Sanger sequencing. The study also included RT-qPCR and ChIP-qPCR analyses. The experiments were performed with an appropriate number of replicates, and suitable statistical analyses were applied. The methods used are appropriate for achieving the project's aims. Although the description of the co-authors' contributions is relatively general, it indicates that the PhD candidate played a significant role in the design and implementation of the research.

Scientific Assessment of the Doctoral Thesis

The first publication, entitled "*Deciphering the role of the SREBF1 gene in the transcriptional regulation of Porcine adipogenesis using CRISPR/Cas9 editing,*" was published in 2024 in the International Journal of Molecular Sciences. The PhD candidate is one of the two co-first authors of this publication. To investigate the role of the SREBF1c isoform in the regulation of adipogenesis, the PhD candidate selectively suppressed its expression by deleting a regulatory element of the gene using the CRISPR-Cas9 system. Successful genome editing in MSCs was confirmed using standard validation methods. Inactivation of the SREBF1c isoform resulted in complete inhibition of adipocyte differentiation and altered the expression profiles of key adipogenic genes. The author observed a concomitant increase in the expression of the second *SREBF1* gene isoform, SREBF1a, which was interpreted as a potential compensatory response that, however, did not improve adipocyte differentiation. Another interesting finding was increased *CEBPB* expression in the edited cells, suggesting coordinated regulation of these two genes during adipocyte differentiation. This observation

was further investigated in the second paper entitled "*The role of the CEBPB gene in porcine adipogenesis: a study using CRISPR/Cas9-edited mesenchymal stem cells*" by Aksoy, M.O. et al., published in Journal of Applied Genetics in 2026. Using the same experimental strategy, the author inactivated the *CEBPB* gene and investigated the course of adipocyte differentiation, as well as the expression levels of key transcription factors and differentiation and proliferation markers. The results demonstrated that *CEBPB* plays an important role in adipogenesis and MSCs proliferation. Its inactivation led to reduced expression of multiple genes, including *GATA2*, a well-established negative regulator of adipocyte differentiation. Was this result expected? How does the author explain the similarly low CEBPB transcript levels in the cell lines with homozygous and heterozygous promoter deletions relative to the wild-type cells (Fig. 3)? Was any CEBPB protein produced from these transcripts? How many monoclonal cell lines were tested? What are the potential risks associated with using only a single edited cell line?

In the final study, "*Dynamic Histone Modification Patterns in Key Transcription Factor Genes During Porcine Adipogenesis*" by Aksoy, M.O., et al., Genes, 2026, the authors investigated epigenetic regulation of adipogenesis. Histone modification patterns at key adipogenic genes during the differentiation of porcine MSCs were analyzed using ChIP–qPCR. The study demonstrated that histone modifications occurring during adipogenesis are both gene- and region-specific. Histone marks associated with transcriptional activation were enriched at key adipogenic regulators, including *CEBPB* and *PPARG*. Notably, genes located on the same chromosome exhibited distinct histone modification profiles, highlighting the locus-specific nature of epigenetic regulation during adipogenesis.

The Author summarizes the results in the Discussion chapter, which is written in an engaging, scientifically mature manner. However, certain aspects could be presented more concisely, as they are discussed repeatedly throughout the chapter. Importantly, the Author critically addresses the study's limitations, including the choice of the in vitro model, the lack of protein-level analyses, and the absence of comprehensive transcriptomic and epigenetic profiling. This critical discussion facilitates a balanced interpretation of the findings and demonstrates the Author's critical approach to the research. During the doctoral defense, I would like the PhD candidate to discuss the most promising directions for future research to translate the findings of his study into practical applications.

Summary

The doctoral dissertation of Mr. Mehmet Onur Aksoy is a complete work that presents theoretical knowledge in the discipline and offers an original solution to a scientific problem. This PhD project provides significant new insights into the transcriptional and epigenetic regulation of porcine adipogenesis. The findings have been published in three scientific articles, to which the PhD candidate made a leading contribution. Taken together, these achievements demonstrate that Mr. Mehmet Onur Aksoy is fully capable of conducting independent scientific research.

In conclusion, the doctoral dissertation submitted for review meets the requirements specified in the Act of July 20, 2018, Law on Higher Education and Science (Journal of Laws of 2020, item 85, as amended), and I request the Scientific Council of the Discipline of Biological Sciences of the Faculty of Veterinary Medicine and Animal Science at the Poznań University of Life Sciences to admit Mr. Mehmet Onur Aksoy to the further stages of the proceedings for the award of the doctoral degree. Considering the novelty and significance of the results presented in the dissertation, as well as their publication in three peer-reviewed papers, I propose that the dissertation be awarded a distinction.

Marta Olejniczak