M U N I

COMMENTARY TO HABILITATION THESIS[1]

Characteristic of the Investigated Matter

The habilitation thesis investigates the role of specific genetic and molecular factors in the development and function of the human retina, particularly focusing on retinal organoids derived from human pluripotent stem cells (PSCs). These retinal organoids serve as *in vitro* models that closely mimic the complexity of human retinal development and offer valuable insights into photoreceptor specification, gene expression regulation, and retinal morphogenesis.

The primary objectives of the work are to:

- Investigate the regulatory role of the miR-183/96/182 cluster in human retinal development.
- Examine the impact of TMEM107 deficiency on retinal organoid development and function.
- Explore the role of light-regulated microRNAs (miRNAs) in the human retina
- Utilize advanced imaging techniques to analyze the structural and functional integrity of retinal organoids under various genetic manipulations.

Employed Methodologies

- Gene Editing: Using CRISPR/Cas9 to create gene knockouts and shRNA-mediated knockdowns in retinal organoids.
- Retinal Organoid Cultures: Differentiating human PSCs into retinal organoids to study retinal development.
- Micro-CT Scanning: Analyzing the three-dimensional structure of mouse embryonic eyes to investigate retinal morphogenesis.
- Scanning Electron Microscopy (SEM): Observing the ultrastructure of retinal organoids to assess photoreceptor formation.
- Basic molecular and cell biology methods including RT-qPCR, PCR, Western blot, Immunoprecipitation, Flow cytometry, Immuno-histo staining, Lentiviral transduction, Electrophysiological recordings, Next-generation RNA sequencing, *In situ* RNA hybridization.
- Photostimulation Experiments: Utilizing customized LED arrays to study the functional responses of retinal organoids to light.

Obtained Results

The significant findings of the thesis include:

- miR-183/96/182 Cluster: Inhibition of this cluster leads to increased neural retina expansion during early differentiation, indicating its crucial role in retinal morphogenesis.
- TMEM107 Deficiency: TMEM107-deficient retinal organoids fail to develop normal photoreceptor structures and exhibit significant downregulation of photoreceptor

[1] The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

markers and retinal ganglion cell markers, confirming the essential role of TMEM107 in retinal development.

- Light-regulated miRNAs: The research successfully identified 51 light-regulated miRNAs in human retinal organoids. A rapid turnover of light-responsive miRNAs was observed, indicating dynamic regulatory processes in response to light exposure. Different wavelengths of light elicited distinct miRNA responses, with red and green light strongly inducing the miR-183/182/96 cluster, and blue light significantly upregulating the miR-204 family. Functional analysis revealed that these miRNAs play crucial roles in retinal cell differentiation, photoreceptor development, and light adaptation processes.
- Functional Integrity: Retinal organoids exhibit functional characteristics similar to human retina, including phototransduction and synaptic signalling, validating their utility as a model system.

Applicant's Contribution

As the principal investigator and corresponding author of all manuscripts described below, my contributions to this work include both qualitative and quantitative aspects:

- Conceptualization and Design: Developed the research hypothesis and designed the experimental approaches.
- Methodological Execution: Performed gene editing, retinal organoid culture, and imaging techniques.
- Data Analysis: Analyzed the experimental data, including gene expression profiles and imaging results.
- Interpretation and Writing: Interpreted the findings and wrote the manuscript, highlighting the significance of the results in the context of retinal development and disease modelling.
- Supervision: Supervised a team of researchers and coordinated collaborative efforts to ensure the successful completion of the project.

[1][2] PESKOVA, Lucie, Denisa JURCIKOVA, Tereza VANOVA, Jan KRIVANEK, Michaela CAPANDOVA, Zuzana SRAMKOVA, Jana SEBESTIKOVA, Magdalena KOLOUSKOVA, Hana KOTASOVA, Libor STREIT and Tomas BARTA*(Corresponding Author)*. miR-183/96/182 cluster is an important morphogenetic factor targeting PAX6 expression in differentiating human retinal organoids. Stem Cells [online]. 2020, 38(12), 1557–1567. ISSN 1549-4918. Available at: doi:10.1002/stem.3272

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	100	70	90

[2] CELIKER, Canan, Kamila WEISSOVA, Katerina Amruz CERNA, Jan OPPELT, Birthe DORGAU, Francisco Molina GAMBIN, Jana SEBESTIKOVA, Majlinda LAKO, Evelyne SERNAGOR, Petra LISKOVA and Tomas BARTA*(Corresponding Author)*. Light-responsive microRNA molecules in human retinal organoids are differentially regulated by distinct wavelengths of light. Iscience [online]. 2023, 26(7, Article 107237). ISSN 2589-0042. Available at: doi:10.1016/j.isci.2023.107237

[2] Bibliographic record of a published scientific result, which is part of the habilitation thesis.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	95	80	100

[3] DUBAIC, Marija, Lucie PESKOVA, Marek HAMPL, Kamila WEISSOVA, Canan CELIKER, Natalia A. SHYLO, Eva HRUBA, Michaela KAVKOVA, Tomas ZIKMUND, Scott WEATHERBEE, Jozef KAISER, Tomas BARTA*(Corresponding Author)* and Marcela BUCHTOVA. Role of ciliopathy protein TMEM107 in eye development: insights from a mouse model and retinal organoid. Life Science Alliance [online]. 2023, 6(12, Article e202302073). ISSN 2575-1077. Available at: doi:10.26508/lsa.202302073

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	50	40	50