UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

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Chair: prof. dr hab. Robert Śmigiel

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The review of the doctoral dissertation

„Molecular Therapies for Mucopolysaccharidosis in Mouse Models"

authored by Estera Rintz

ordered by Scientific Council of the Biological Sciences Discipline

at the University of Gdańsk

The dissertation presents the results of a study on the molecular basis of the treatment of mucopolysaccharidosis in mouse models, based on five highly cited scientific articles published in internationally recognized journals indexed by JCR. The first author of all five cited articles in the dissertation is MSc. Estera Rintz, the PhD degree applicant.

The reviewed dissertation have two doctoral supervisors: Professor Grzegorz Węgrzyn, PhD, DSc from Department of Molecular Biology, Faculty of Biology, University of Gdańsk and Professor Shunji Tomatsu, MD, PhD from Nemours Children's Hospital, Wilmington, Delaware, United States.

Background of dissertation subject:

Mucopolysaccharidoses (MPS) belong to the group of hereditary metabolic diseases where, as a result of a mutation in a gene encoding an enzyme responsible for the breakdown of

compounds from the mucopolysaccharide group (GAG), these compounds accumulate in the cell. The process of their degradation in a healthy organism is a sequence reaction of several enzymes, however, when one of them does not function properly, the reaction stops and GAGs accumulate in the cells. Depending on which enzyme is inactive, there are 13 types and subtypes of MPS. Many symptoms are common to all or most types and subtypes of MPS, but the most severe are those related to the central nervous system and the skeletal system. In both cases, the currently available therapies are not able to treat the symptoms of above systems.

The most commonly used MPS therapy is enzyme replacement therapy (ERT), which uses the active form of the missing enzyme. However, the supply of the missing enzyme is not sufficient in the case of MPS types whose symptoms are expressed in central nervous system, because the enzyme does not cross the blood-brain barrier. One alternative strategy is accelerating the degradation of GAG(s) through autophagy. It is supposed that resveratrol, the polyphenol, which has multiple biological functions, such as anti-inflammatory, antioxidant and neuroprotective effects, by its pleiotropic mechanism of autophagy induction, ability to cross the blood-brain barrier, and safety profile, is a promising candidate for treating neuronopathic forms of MPS. Moreover, a promising alternative therapy for MPS IVA patients is gene therapy with using C-type natriuretic peptide which can induce the bone growth by activating the natriuretic peptide receptor on chondrocytes.

The above preliminary remarks indicate the relevance of the scientific issues addressed by the doctoral candidate. Research into alternative therapeutic approaches for these types of MPS is highly desirable.

Scientific Achievements of the Doctoral Candidate:

The doctoral candidate is a highly qualified individual who undertakes further scientific challenges, as evidenced by her publications available on PubMed. She is the author and co-author of 33 publications listed in the PubMed database, including 8 works where she is the first author and corresponding author. The doctoral candidate has an H-index of 13, and her works have been cited 320 times without self-citations (Scopus, Web of Science). The candidate for the doctoral degree wrote three chapters in scientific books and was an active participant in many congresses, presenting her scientific achievements. In my assessment, the achievements presented by the doctoral candidate are significant and promising for her academic career.

Moreover the candidate of PhD degree was a primary investigators in three research projects titled as:

1. “Mechanizm degradacji glikozoaminoglikanów pod wpływem resweratrolu w mysim modelu neuronopatycznej choroby z grupy mukopolisacharydoz” (2019/35/N/NZ2/00505) financed by National Science Center in Poland in PRELUDIUM 18 program

2. “Kombinowana terapia enzymatyczna z resweratrolem w komórkowym modelu MPS I” (533-0C20-GS17-23) financed by University of Gdansk in UGrants START 2023 program

3. “Korelacja pomiędzy aktywacją autofagii a odpowiedzią immunologiczną w Mukopolisacharydozie typu IIIB” (533-0C20-GS54-24) financed by University of Gdansk in UGrants START 2024 program

Evaluation of the Doctoral Dissertation:

The evaluation of the doctoral dissertation included an assessment of the substantive and methodological aspects of the work, as well as a summary and final conclusions.

Msc. Estera Rintz's doctoral dissertation was constructed based on five publications with a total impact factor (IF) of 30,132. The published works that form the basis of Msc. Estera Rintz's dissertation stemmed from research projects titled “Mechanizm degradacji glikozoaminoglikanów pod wpływem resweratrolu w mysim modelu neuronopatycznej choroby z grupy mukopolisacharydoz” (2019/35/N/NZ2/00505) financed by National Science Center in Poland in PRELUDIUM 18 and conducted by doctoral applicant Msc. Estera Rintz, and also project founded by Sanfilippo Foundation (but author forgot put the title of project? – my only minor remarks) conducted by prof Grzegorz Węgrzyn from the Molecular Biology Department, Faculty of Biology, University of Gdansk, as well as projects founded by many individual institutions such as Austrian MPS Society, A Cure for Robert, Inc., The Carol Ann Foundation, Angelo R. Cali & Mary V. Cali Family Foundation, Inc., The Vain and Harry Fish Foundation, Inc., The Bennett Foundation, Jacob Randall Foundation, and Nemours Funds. S.T. (supported by an Institutional Development Award from the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health).

The submitted PhD thesis for review comprises 160 pages with a traditional structure, including a summary in English, Polish and references list, manuscripts list that constitute the doctoral dissertation included in PhD thesis with statements from co-authors of the publications as well as funding of scientific PhD work and academic achievements of the candidate for the doctoral degree.

The dissertation includes five publications that are the basis for the doctoral thesis such as:

1. Rintz, E., Pierzynowska, K., Podlacha, M., Węgrzyn, G. Has resveratrol a potential for mucopolysaccharidosis treatment? (2020) European Journal of Pharmacology. 888:173534. Impact Factor = 4.432 (2020), MNISW Punctation = 70

2. Rintz, E., Podlacha, M., Cyske, Z., Pierzynowska, K., Wegrzyn, G., Gaffke, L. (2023) Activities of (Poly)phenolic Antioxidants and Other Natural Autophagy Modulators in the Treatment of Sanfilippo Disease: Remarkable Efficacy of Resveratrol in Cellular and Animal Models. Neurotherapeutics. 20(1):254-271. Impact Factor = 5.7 (2023), MNISW Punctation = 140

3. Rintz, E., Węgrzyn, G., Fujii, T., Tomatsu, S. (2022) Molecular Mechanism of Induction of Bone Growth by the C-Type Natriuretic Peptide. International Journal of Molecular Sciences. 23(11):5916. Impact Factor = 5.6 (2022) MNISW Punctation = 140

4. Rintz, E., Herreño-Pachón, A.M., Celik, B., Nidhi, F., Khan, S., Benincore-Flórez, E., Tomatsu, S. (2023). Bone Growth Induction in Mucopolysaccharidosis IVA Mouse. International Journal of Molecular Sciences. 24(12):9890. Impact Factor = 5.6 (2023) MNISW Punctation = 140

5. Rintz, E., Celik, B., Nidhi, F., Herreño-Pachón, A.M., Khan, S., Benincore-Flórez, E., Tomatsu, S. (2024) Adeno-associated virus-based gene therapy delivering combinations of two growth associated genes to MPS IVA mice. Molecular Therapy and Nucleic Acids. 35(2):102211 doi.org/10.1016/j.omtn.2024.102211, Impact Factor = 8.8 (2024) MNISW Punctation = 140

This PhD thesis focuses on addressing the challenge of delivering therapeutic agents to hard-to-reach tissues in mouse models of MPS. The PhD Candidate proposed to use small molecules capable of crossing biological barriers to effectively target these tissues. She investigated the potential of resveratrol to cross the blood-brain barrier (BBB) in the treatment of MPS IIIB, and CNP for targeting avascular chondrocytes in MPS IVA. Both therapies demonstrated a significant potential in improving treatment outcomes for mice suffering from these forms of MPS by enhancing drug delivery to otherwise inaccessible sites within the body. This approach not only aims to improve the efficacy of current treatments but also to pave the way for the development of new therapeutic strategies for MPS and potentially other lysosomal storage disorders.

After a thorough analysis of the aforementioned scientific articles, I would like to express my highest appreciation for the PhD student's significant achievements. Her substantial contribution to laboratory work with international cooperation, using cutting-edge molecular methods of the

highest scientific caliber, represents significant utilizing advanced and innovative molecular methods as well as exemplifies the highest scientific standards. This research is of particular importance in the field of treating ultra-rare genetic diseases in children.

Conclusion:

I would like to emphasize that the doctoral candidate has demonstrated a high ability for critical analysis of her own research results in relation to findings from the global literature, as well as a professional understanding of the significance of molecular tools in pathomechanism of genetic disease, in genetic therapy and a scientific responsibility for formulating practical implications of her research.

In summary, I would like also to highlight that the doctoral dissertation of Msc. Estera Rintz constitutes a genuine contribution to the advancement of knowledge in the discussed areas. The dissertation has also been very carefully prepared in terms of editing. I found only a few editorial errors (such as lack of title of funding project), which, of course, do not diminish the scientific value of this work.

In my opinion, Msc. Estera Rintz's doctoral dissertation meets the requirements for such dissertations as specified in the artcle. 187 sec. 1-4 of the Act on Higher Education and Science of July 20, 2018 (Journal of Laws of 2018, item 1668, as amended). Therefore, I appeal to the Scientific Council of Biological Sciences Discipline at the University of Gdańsk to allow Msc. Estera Rintz to proceed to the next stages of her doctoral process.

Additionally, I am proposing to the Scientific Council of the Biological Sciences Discipline at the University of Gdańsk to award the doctoral dissertation prize to Msc. Estera Rintz, due to its really very high scientific value and innovation approaches.

Wrocław, 04.10.2024 r

Prof. dr hab. Robert Śmigiel