50 LAT
INSTYTUT
GENETYKI CZŁOWIEKA
POLSKIEJ AKADEMII NAUK

ul. Strzeszyńska 32
60-479 Poznań

tel. +48/61/657 91 00
fax +48/61/823 32 35
e-mail: igcz@man.poznan.pl

www.igcz.poznan.pl

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Prof. dr hab. Maciej Giefing
Director of the Institute of Human Genetics PAS
Head of the Department of Cancer Genetics

Review

of the doctoral dissertation authored by mgr Estera Rintz

entitled „Molecular therapies for mucopolysaccharidosis in mouse models"

The aim of the reviewed dissertation is to improve current treatment approaches for mucopolysaccharidoses (MPS). Ms. Rintz investigated the small molecule resveratrol, capable of crossing the blood-brain barrier to alleviate the symptoms of MPS but also proposes a novel combined gene therapy to improve bone growth tested in a newly developed MPS IVA mouse model. The aims of the dissertation are clearly presented and the topic is beyond doubt very ambitious, interesting and up-to-date as there is currently no approved therapy that could fully reverse the symptoms of MPS manifested in skeletal and central nervous systems. There is a clear clinical need to either improve the currently available therapies by enhancing the used drug delivery methods or develop new approached based on gene therapies. Thus, the reasons to undertake the research are well justified.

The reviewed dissertation is 160 pages long and is a compilation of five previously published manuscripts of good to very good impact factor ranging from 4.4 - 8.8 and a cumulative IF > 30. Ms. Rintz is the first author of all five publications. It must be

emphasized that this is an impressive achievement which could easily be a basis for a habilitation thesis. Three publications from the compilation are original research papers and two are review articles. Taken together, the compilation builds a coherent dissertation with good interconnection behind the respective parts. Each publication is accompanied by the statements of contribution given by the co-authors which show the leading role of Ms. Rintz among the authors. The dissertation is editorially very well prepared, written in good English and includes informative figures. There are two summaries included, in English and Polish, which should be regarded more as short introductions into the topic of the dissertation rather than usual summaries. Further parts consist of the table of content, list of funding towards the performed research and the publication and attended conference records. The proposed structure is adequate for a doctoral dissertation but I would appreciate to see short introductions preceding each publication to stress the connections between these papers and allow smooth reading of the entire work. However, I have to admit that the review articles included in the thesis do actually function as introductory publications to the original research.

Despite the mentioned minor shortcomings **the summaries, the included review publications as well as the introductions of the original publications allow to state that the dissertation fully demonstrates a very good overall theoretical knowledge of the PhD student in the discipline of biological sciences.**

Going into a more detailed analysis of the individual publications I must admit that I am impressed by the numerous experimental techniques used in these studies. They range from standard laboratory techniques, elaborate functional assays performed on cell cultures, behavioral and biochemical experiments on mouse models to advanced genetic engineering. This clearly shows how many laboratory techniques are mastered by Mr. Rintz. Importantly, the experiments and individual laboratory techniques described in the dissertation are adequately used to answer specific scientific questions and are correctly designed.

I therefore state that the doctoral dissertation fully demonstrates the doctoral student's ability to conduct independent research work.

I would like to stress that the presented publications deliver numerous valuable results. Naming all these important findings is beyond the scope of this review, nevertheless I would like to highlight some of these. There is a clear benefit shown both on the cell line model as well as on the mouse model of the resveratrol treatment. The treatment was shown to significantly reduce levels of glycosaminoglycan (GAG) in cell lines and to significantly improve GAG clearance in mice. Moreover it showed a positive effect on the behavior of

mice suggesting a reduction of adverse effects of the disease on the CNS. This is a novel and important finding with possible clinical applications for the treatment of the Sanfilippo disease. I wonder if a respective technology transfer office at the University of Gdańsk was informed about these findings? Also the results on a combined treatment using adeno-associated virus to deliver and express GALNS enzyme and a natriuretic peptide C gene - *NPPC* are of high importance. This is reflected by the fact that these findings were published in the prestigious *Molecular Therapy and Nucleic Acids* journal. The author demonstrated that this approach results in a synergistic effect on the mouse model and a clear benefit compared to delivering each of the transgenes separately. These effects were verified experimentally on multiple levels. I also appreciate the important caution of the author who correctly observes the necessity to control the levels of the CNP expressing vector in patients in order to avoid similar problems like the excessive growth observed in mice. All this together indicates the scientific maturity of the doctoral student.

I have only minor comments to some of the presented experiments and their results. In publication number two, Fig. 3, the relative GAG levels in the control and MPS fibroblasts treated with the tested compounds shown on the y axis should be shown using the same scale for all the graphs. This would allow better comparison between the effectiveness of the individual compounds tested.

In the publication number four I lacked experiments or discussion of possible elimination or epigenetic silencing of the used AAV8 vector for gene therapy. Such elimination / silencing can results in attenuation of expression of the transgene in clinical settings and diminish the therapeutic effect. I also asked myself the question if the AAV8 transduced mice did show any immune responses against the vector that similarly could diminish the therapeutic effect. There is also the open, unanswered question if the therapy has any cancerogenic effect for the patients that could not be observed in mice analyzed only for a short period of time. These are important question that must be answered prior to introducing the novel therapies into routine clinical applications. I lacked a more elaborated discussion on these questions in the dissertation.

There is also one characteristic of the used MPS IVA mouse model that pinpointed my attention which is however deeply discussed by Ms. Rintz. This is why the model shows no differences compared with the WT mice in respect to body weight and length. This is surprising and stresses the general observation that in genetical studies results obtained on animal models cannot be directly translated to the human organism.

There are also some surprising results shown in Fig.6 of the fourth publication; for example the bone volume are higher in the MPS IVA model than the WT mouse. This is contra intuitive and reduces the significance of the findings presented in this publication.

To say few words about the conclusions of the dissertation presented in the individual publications I admit that they correspond well with the aims of the dissertation. I would however appreciate a short, one page long chapter, dedicated to conclusions that the author draws from the entire presented research. This would allow to follow what findings are regarded as most important for Ms. Rintz.

Nevertheless, as stated before, these are only minor issues that do not change my very good opinion on the entire dissertation. To sum up, I state that the results of the reviewed doctoral dissertation constitute an original solution of the considered scientific problem.

Concluding the review, I state that the doctoral dissertation prepared by mgr Estera Rintz is an extremely interesting work, written in beautiful English, well and carefully edited and importantly, performed with up-to-date and well-designed experiments. It needs to be stressed that the results of these dissertation contribute not only to basic biological sciences but also may have impact on putative novel therapies to alleviate patients suffering from mucopolysaccharidoses.

Given the above my assessment of the doctoral dissertation authored by mgr Estera Rintz entitled „Molecular therapies for mucopolysaccharidosis in mouse models" is with full conviction positive. The dissertation fulfills the criteria specified in art. 187 sections 1 and 2 of the Act of 20 July 2018 - Law on Higher Education and Science.

Furthermore, due to the importance of the presented findings, the in-depth and complex analyses reflected by the strong impact factor of the entire dissertation and its significant contribution on putative novel therapies of mucopolysaccharidoses, I recommend to the Council of the Discipline of biological sciences of the University of Gdańsk to award it as *summa cum laude*.

Maciej Giefing