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Review of the doctoral dissertation of M.Sc. Ambroise Wu entitled: "Role of ABCA1 transporter in the plasma membrane reorganization in mammalian cells"

conducted under the supervision of prof. Aleksander Czogalla and dr Tomasz Trombik

at the Faculty of Biotechnology at the University of Wroclaw

Doctoral dissertation of Mr. Ambroise Wu is a classic dissertation written in the form of a monograph. Noteworthy, a large part of the research was carried out as part of the Preludium 18 project no.2019/35/N/NZ3/00633 financed by the National Science Center.
Dissertation concerns research on the cholesterol transporter ABCA1, which plays a key role in cholesterol homeostasis in mammals. ABCA1 protein participates in the modulation of cholesterol distribution in the cell membrane affecting the organization of the plasma membrane of mammalian cells. The spatial organization of lipids in the membrane affects key cellular processes related to cell resistance to therapies targeting cholesterol metabolism and plays a role in the development of certain cancers, including melanoma.
Therefore, deepening knowledge in this area has not only cognitive value, but it is also an applied research, as it may contribute to the development of new therapeutic strategies.

The reviewed dissertation has 128 pages of typescript, including: abstracts in Polish and English, list of abbreviations, introduction, aims of study, results, discussion, materials and methods, and bibliography (439 items, including the latest reports). The dissertation ends with the chapter "Publications", in which the PhD student presented a list of his own publications including two first-author publications. The text is written in the layout typical for doctoral dissertations, in the correct scientific language, theoretical issues and research results are presented in a factual manner.
In the introduction, the PhD student briefly yet comprehensively introduces to the reader the subject of cell membrane organization and how it can be impacted by transporters belonging to the ABC super family. Then, he characterizes the ABCA1 transporter in detail including its post-transcriptional regulation ,molecular structure, the mechanism of lipid transport, and the

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transcription of a gene encoding ABCA1 transporter. In the last chapter of the introduction, he describes the role of the ABCA1 transporter in the course of various diseases, including Tangier disease, atherosclerosis, and cancer.

The introduction leads to the formulation of two objectives of the work for which the PhD student clearly specifies the research hypothesis.

Chapters "Results" and "Discussion" constitute the main part of the thesis, in which the PhD student presented the results of conducted experiments, and then discusses them based on the available literature data. The substantive part of the work is summed up by the chapter "General discussion, conclusion and perspective" presenting two main conclusions, supported by the results of the experiments.

The chapter "Materials and methods" presents the experimental procedures described in a reliable and exhaustive manner.

Summing up the formal assessment of the thesis - the dissertation has been prepared thoroughly and reliably, with great editorial care and allows to familiarize the reader with the workshop and scientific achievements of the PhD student.

The main aim of the work undertaken by the PhD student was to study the function of the ABCA1 transporter in modulating the function and organization of the plasma membrane of mammalian cells.

ABCA1 protein, a member of the ABC family of transporters, is important for the redistribution and efflux of cholesterol from the cell membrane and may control the organization of the cytoplasmic membrane. In the first stage of research PhD student showed with unique biophysical techniques, that in cells expressing ABCA1, cholesterol export by the transporter in the presence of amphotericin B induced changes regarding cholesterol content and distribution. Moreover, the results showed the ability of ABCA1 to efflux its substrate to a drug and not into the apolipoprotein. Active ABCA1 at the plasma membrane enhances the efflux of plasma membrane cholesterol to amphotericin B, forming cholesterol-amphotericin structures that prevent cytotoxicity of amphotericin B.

Neoplastic processes are closely related to mutations resulting deregulation of gene expression and protein misfolding, which lead to abnormal and non-physiological behavior of cells inside the body. Recent studies have found that cancer cells have a higher cholesterol content compared to normal cells, suggesting a link between cancer development and membrane cholesterol levels. Therefore, in the next stage of research, the PhD student began experiments to clarify the role of the ABCA1 transporter in the metastasis of human melanoma. The research carried out on the Hs294T line, characterized with a high

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expression level of ABCA1, showed a correlation between ABCA1 activity and changes in the modification of the plasma membrane lateral organization, lipid order and fluidity. These changes resulted from the redistribution of cholesterol in cell membranes and caused an increased ability of cells to degrade the extracellular matrix.
Finally, he proposed a mechanism of action in which ABCA1 expressed in Hs294T controls the distribution of cholesterol content and cell membrane organization allowing digestion of the extra-cellular matrix by the cell and proper stable recruitment of focal adhesion kinase to promote active integrinβ3-dependent invasion and migration processes.

Summarizing the research results obtained by the PhD student, I believe that the objectives of the research set out at the beginning of the work have been fully realized and bring provide unique information on the crucial influence of ABCA1 on plasma membrane-associated biological within mammalian cells.

The results presented in the dissertation are convincing, comprehensively described and prove the reliable and accurate conduct of the planned research, which made it possible to achieve the goals set in the work. Nonetheless, two suggestions could have been made:

1. In the case of experiments using the Hs294T cell line, in which the PhD student knocked out the ABCA1 gene, were there any additional tests (apart from the Western Blot) performed to confirm the selectivity of gene editing in the line?
From the CRISPS/Cas9 technique description in the method chapter, indicated that the PhD student used protein Cas9 based system instead of the plasmid encoding the enzyme, which significantly reduces non-specific reactions, but one cannot completely exclude them. If no additional analysis was performed, please propose such experiments.
2. The results presented by the PhD student on the influence of the ABCA1 transporter on changes in the fluidity and organization of the cell membrane of the studied melanoma line confirm that ABCA1 expression influences the amount of lipids in the cell membrane, including cholesterol.
Considering the fact that such changes affect the organization and localization of receptors in the cell membrane in general, the question arises whether the changes of the key signaling pathways dependent on the receptors for CXCL8 or PTEN/PI3K/AKT system have been investigated.

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In my opinion, the doctoral dissertation of Ambroise Wu meets all the criteria specified in Article 13 of the Act of March 14, 2003 on academic degrees and academic title as well as academic degrees and title in the field of art (Dz. U. Nr 65, poz.595, z późn. zm.).
I apply to the Biotechnology Faculty Council of the University of Wroclaw for admit Ambroise Wu the doctoral dissertation. At the same time, considering its cognitive importance and the fact that some of the results were published in the reviewed journal of IF 9.26, I would like to ask the High Council of the Faculty of Biotechnology of the University of Wroclaw to award the assessed work with an appropriate award.

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