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Review report on the PhD thesis submitted to University of Gdańsk

PhD candidate: **M.Sc. Maria Camila Tovar Fernandez**
PhD thesis title: **Origin of antigenic peptides in MHC class I pathway**
Supervisor: **Prof. Robin Fahraeus, ICCVS, INSERM and University of Gdańsk**
Reviewer: **dr hab. n. med. inż. Beata PAJĄK, prof. WIHE**
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Project background

Antigen presentation is a vital immune process essential for triggering T CD8 cells' immune response. T cells recognize only fragmented antigens displayed on cell surfaces. Thus, before the antigen fragment is bound to the major histocompatibility complex (MHC), it undergoes intracellular processing. The antigens presented on MHC class I can be of endogenous origin, including cellular or viral antigens, or exogenous from bacteria. Antigens synthesis, processing, and presentation are complex processes with multidirectional, not fully understood, regulatory mechanisms. Maria Tovar Fernandez's Ph.D. thesis is devoted to investigating the origin of antigenic peptides in the MHC class I pathway and the role of: 1st part: autophagy and its role in the generation of peptide substrates, and 2nd part - the function of proteasome and alternative mRNA translation processes as a source of MHC class I antigen peptides. Autophagy is known to be engaged in the MHC class II presentation pathway. However, the link between autophagy and antigenic peptides generation for the MHC class I is still poorly understood. According to available evidence, this association could be substrate-dependent. In Ph.D. thesis, Maria Tovar Fernandez focused on two kinds of substrates: an aggregate-prone protein and a viral protein. Explaining the role of autophagy in the formation of MHC class I antigens is a novelty. It is undoubtedly an important problem for a better understanding of the complexity of this process and, at the same time, the basis of their disturbances in pathological processes. The second part of the PhD thesis focuses on alternative mRNA translation as a source of antigen peptides. The alternative mRNA translation includes the alternative translation initiation, peptide synthesis derived from intron sequences, and synthesis from the pioneer round of translation. All these molecular processes have been described relatively recently, and their detailed role in the immune response processes is still under investigation. Available papers have demonstrated that some MHC class I antigenic peptides originate from pre-spliced mRNA, and these peptides are called PTPs. In PhD thesis, Maria Tovar Fernandez tried to verify these hypotheses and elucidate the riboproteome responsible for PTPs synthesis. Taken together, I can admit that PhD thesis addresses several important issues affecting the MHC class I antigen presentation and antigen synthesis that are currently not fully explained. Thus, this thesis addresses the highly relevant and vital areas of current immunology research.

General description of the thesis

The submitted thesis presents an investigation of the molecular mechanisms of antigen synthesis via autophagy and pre-spliced mRNA translation in in vitro model of H1229 cell line. The dissertation comprises

102 pages (plus Annexes and a copy of the published paper) and does not follow the classic structure of a Ph.D. thesis in science. The two scientific goals are described as two PhD thesis parts with separate Introduction, Materials and Methods, Results and Discussion, and Perspectives sections. In my opinion, this division was not the best idea and made it rather difficult to follow. In my opinion, Introduction I and II, Materials and Methods I and II, and Discussion I and II should be presented as one text with subsequent subsections. This would allow for discussing the results more smoothly and avoid repetitions, as in the Materials and Methods section. In the current form of the doctoral dissertation, there is no summary containing the conclusions resulting from the verification of both research goals and their general importance for the phenomena of antigen presentation by MHC class I. Furthermore, the References cited in the PhD thesis have 150 items, but less than half (only 64) has been published in the last 10 years. The list of cited publications should be more recent. The dissertation contains numerous high-quality illustrations of the content discussed in the Introduction section, as well as presented results. It would be valuable if the PhD thesis included also a list of Figures at the end and their numbering was continuous throughout the content of the dissertation.

Regardless of the comments regarding the form of the PhD thesis, I would like to return to its merits. The submitted thesis is prepared neatly, and the content is formulated clearly and precisely. The dissertation introduces the reader to the general background of MHC class I and the mechanisms of antigen presentation. Then autophagy process, its characteristics, and its importance in the immune response mechanisms are described. Finally, the known mechanisms of alternative mRNA translation are presented. The diagrams included in the Introduction section illustrate the content described and help understand them well. The Introduction ends by indicating the missing elements, which constitute the explanation for implementing the PhD candidate's research goals.

To verify above mentioned hypothesis, Maria Tovar Fernandez used several molecular biology techniques, such as cell culture, cell transfection with plasmids and siRNA, flow cytometry, Western blot, immunofluorescence and RT-PCR. Moreover, biochemical assays were applied, such as measure antigen presentation assay and IL-2 release ELISA. The methods are generally well described. The minor point concerns the concentrations of antibodies used in Western blot and immunofluorescence techniques. I would also like the Candidate to explain the statistical thresholds used for statistical differences indications. Usually, significant differences are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. In submitted thesis, the candidate used * $p < 0.0332$, ** $p < 0.0021$, *** $p < 0.0002$ and **** $p< 0.0001$. Please explain such values.

In the autophagy assay, the well-known autophagy inhibitor Chloroquine was used. Cells were treated with relatively high CQ concentration [30 uM] for 36 hours. Does the Author verify CQ toxicity by, for example, measuring viability after CQ treatment?

In general the presented results seems to be reliable and well documented, although, as always in cutting edge research, the discussion can be extended and more-in-depth. As mentioned previously, the summary addressing all analyzed processes would be advantageous.

Based on the performed experiments, the Author concluded that autophagy engagement in the MHC class I pathway antigenic synthesis is not a universal process and depends on substrate. In the case of viral proteins, autophagy appeared to be connected with the MHC class II pathway rather than MHC class I. On the other hand, in the case of protein aggregation induced by ovalbumin overexpression, autophagy inhibition by Atg5/12 silencing downregulated the antigen presentation mediated via MHC class I. Importantly, this part of PhD results has already been published in the Cellular Immunology journal (IF 4,178). The above conclusions suggest the next question, is autophagy not involved in MHC class I response in all viral infections or is it also virus-specific? Is MHC class I viral-escape the mechanism of viral infection? Could the PhD candidate present her opinion about it?

The second part of PhD research revealed exciting observations that MHC class I antigenic peptides can be derived from intron sequences, and pre-spliced mRNA antigenic peptides sequences were identified. I have to admit that my scientific expertise is focused on the autophagy process and its importance in various physiological states. Thus, this section of PhD thesis was an exciting lecture, providing clear evidence for more complex regulation of MHC class I pathway action in immune response, including intron translation theory. Professor Fahraues's Team is a world leader in research in this field and has already published several highly cited papers describing the various aspects of pre-mRNA processing in gene expression and antigen presentation. I am sure that the presented in Ph.D. thesis results will also meet the great interest in the scientific community.

Final evaluation statement

This thesis represents a great deal of work. The results are well presented, and their interpretation is at a high scientific level. I really appreciate the Candidate's expertise in the field of MHC antigen presentation processes characterization and their complex regulatory machinery. The research it describes is of the international standard. This thesis is ready to be defended and certainly meets the requirements laid down for the degree of PhD in biology by the status in the Journal of Laws of the Republic of Poland (Dz. U. z 2018 r. poz. 1668 z późń. zm).

Ja, niżej podpisany stwierdzam, że recenzowana rozprawa doktorska mgr Marii Camili Tovar Fernandez spełnia warunki określone w art. 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 r. poz. 1668 z późń. zm.) i wnioskuję do Rady Dyscypliny Nauki biologiczne Uniwersytetu Gdańskiego o dopuszczenie mgr Marii Camili Tovar Fernandez do dalszych etapów przewodu doktorskiego.

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18/08/2022
date

signature