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REVIEW of the doctoral dissertation by Michał Mazur, MSc entitled 'Exploring Cytoplasmic Polyadenylation: Regulatory Mechanisms Affecting Stability of Endogenous and Therapeutic mRNAs in Hematopoietic Cells'.

The doctoral dissertation by Michal Mazur describes cytoplasmic polyadenylation, also known as re-adenylation, mediated by the TENT5 family of non-canonical poly(A) polymerases (ncPAPs). This PhD dissertation is dedicated to the functional analysis of selected biological functions of TENT5 ncPAPs in hematological cells, namely macrophages, in the context of mRNA vaccines, and late erythrocyte progenitors in the context of hemoglobin production. These processes are very poorly understood, particularly the knowledge about the role of the recently identified TENT5 family is very limited. For this reason, the topic undertaken by the doctoral candidate is extremely interesting; the work is innovative, and its results may contribute to a better understanding of the fundamental processes regulating metabolism of both therapeutic and endogenous mRNA.

Formal assessment

The presented doctoral dissertation takes a form of a collection of two original articles – one published in the “Nature” journal, and the second in the form of a preprint available on the biorxiv platform, currently under review in the “Nature Communications” journal. In the article published in “Nature”, the doctoral candidate is the second co-author, while in the article available on biorxiv, he is the first author. Based on the information provided in the publications, it can be inferred that the doctoral candidate played a leading or role in the conducted research and simultaneously met the criteria for first and second authorship of the attached publications. In “Nature” publication PhD candidate optimized the quantification of vaccine mRNA across different tissues after intramuscular injection He performed a thorough flow cytometry analysis of muscle-infiltrating immune cells by sorting populations corresponding to dendritic cells and macrophages to quantitatively compare the vaccine mRNA stabilization between those populations using qPCR. In manuscript submitted to “Nature Communications” PhD candidate performed the majority of experiments including Oxford Nanopore Sequencing, flow cytometry sorting, TurboID proximity ligation assay.

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In addition to the attached publications, the dissertation comprises an introduction that shortly describes hematopoiesis, mRNA metabolism, the functions of non-canonical poly (A) polymerases and provides a brief overview of the manuscripts accompanying the dissertation. Additionally, unpublished data on cellular landscape of muscle infiltrating cells after intramuscular injections is also included in the dissertation. The introduction adequately introduces the reader to the research topic; however, the broad scope of the topic makes the introduction somewhat disjointed and chaotic. I have also noticed some clerical mistakes, terminology misuse, or simplifications (for example, "less adhesive macrophages"). The final part of dissertation is a short discussion section that helps to understand the broader context of the presented research and assess the scientific maturity of the PhD candidate.

Merits assessment

As part of the project, the PhD student conducted a comprehensive functional analysis of the role of polyadenylation by TENT5 polymerases in the stabilization of therapeutic and endogenous mRNA . He primarily focused on two polymerases, TENT5A and TENT5C, and their significance for the development of immune response during vaccination and hematopoiesis, respectively. Among the substrates for TENT5C, he discovered mRNA encoding globin. He also established the mechanism by which TENT5C participates in regulation of globin production and microcytic anemia phenotype upon TENT5C dysfunction.

In another part of the project, the PhD student participated in studies analyzing the re-adenylation and stabilization of target synthetic mRNA vaccines against COVID-19. These studies indicated that TENT5A is present in immune cells that uptake mRNA vaccines, and that a lack of TENT5A leads to a reduction in the production of specific immunoglobulins in response to mRNA vaccines after immunization in mice.

By conducting such extensive research, the doctoral candidate has demonstrated an impressive knowledge of various experimental methods. The analysis of the attached publications indicates that the doctoral candidate skillfully employs multiple techniques in biology and molecular genetics. The doctoral candidate has a deep understanding of methods for studying poly(A) tails, effectively chooses research techniques to meet the proposed objectives, is knowledgeable in his research field, and conducts studies that go beyond the state of the art and significantly enhance current knowledge regarding the role of cytoplasmic polyadenylation in stabilization of various mRNAs.

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As for methodological aspect, I cannot agree with the statement that “MHC-II may be used as a primary marker to discriminate between macrophages and dendritic cells”. For mouse samples, widely used markers to distinguish between these two populations include CD11b, CD11c, F4/80 and these markers were also used by PhD candidate in flow cytometry studies.

Out of pure scientific curiosity, I would also like to ask several questions:

1. Why anti-spike IgG levels in conditional knockouts of Tent5a in CD11c-positive cells 14 days after immunization of mice with mRNA-1273 are not as profoundly decreased as in Tent5A KO? Does it mean that other cells contribute to this process or Tent5A has another role in IgG production different from increasing spike protein production by CD11c-positive cells.
2. Why anti-spike IgG levels are higher in Tent5A/FLOX;CD11c-Cre mice than in Tent5a WT mice?
3. What is the rationale for using 24 mice/group in detecting spike protein production after immunization? Do these results confirm the major role of CD11c-positive cells expressing Tent5a in antigen production?
4. In flow cytometry analysis both DCs and macrophages were observed to be CD11c positive. In this context, would conditional knockout mice with Cre-induced ablation of Tent5a in CD11c+ cells properly address the role of vaccine uptake by macrophages?
5. Do Tent5a KO have the same effect as Tent5c KO in terms on the process of erythropoiesis?
6. Do Tent5a/b/c knockouts have more profound defects in globin production than Tent5c knockouts?

Summary

My comments neither decrease nor question the scientific value of the presented work. The PhD candidate has conducted innovative research, the results of which are significant for understanding and may contribute to elucidating the mechanisms regulating hematopoiesis, as well as immune responses to mRNA vaccines. The PhD student possesses extensive knowledge, as well as the ability for critical analysis and interpretation of the obtained data.

In light of this, I, the undersigned, hereby state that the doctoral dissertation of Michał Mazur meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher

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Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended). I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Michał Mazur to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

At the same time, given the exceptionally high scientific standard of the conducted research, its significant contribution to the current state of knowledge, the extensive scope of the work undertaken, and the mastery of a highly complex methodology, I recommend the doctoral dissertation for receiving a distinction.

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