INSTYTUT HEMATOLOGII I TRANSFUZJOLOGII
02-776 Warszawa, ul. Indiry Gandhi 14 • Centrala: tel. 22 34-96-100, sekretariat: tel. 22 34-96-176, fax 22 34-96-178 • www.ihit.waw.pl

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Prof. dr hab. Przemysław Juszczyński
Z-ca Dyrektora ds. Nauki
Kierownik Zakładu Hematologii Eksperymentalnej
Instytut Hematologii i Transfuzjologii
Ul. Indiry Gandhi 14
Warszawa 02-776

Evaluation
of the doctoral dissertation of Michał Antoni Mazur

“Exploring Cytoplasmic Polyadenylation: Regulatory Mechanisms Affecting Stability of Endogenous and Therapeutic mRNAs in Hematopoietic Cells”

titled “Exploring Cytoplasmic Polyadenylation: Regulatory Mechanisms Affecting Stability of Endogenous and Therapeutic mRNAs in Hematopoietic Cells”

The doctoral dissertation submitted for evaluation by Michał Antoni Mazur fits into one of the most dynamically developing areas of modern molecular biology - the regulation of mRNA stability and its consequences for the function of hematopoietic cells and modern therapies based on modified RNA. This issue has garnered particular attention in recent years, which is clearly linked to the global use of mRNA vaccines during the COVID-19 pandemic. Understanding how host cells recognize, modify, stabilize, or degrade exogenous mRNA is crucial both for the safety and effectiveness of current preparations and for designing next-generation therapies,

including vaccines, immunotherapeutics, and mRNA-based drugs for rare/orphan diseases.

In this context, Michał Mazur's dissertation addresses a biologically fundamental and clinically highly relevant problem: the role of cytoplasmic polyadenylation, mediated by enzymes of the TENT5 family, in stabilizing therapeutic and endogenous mRNAs in hematopoietic cells. The author focuses on two distinct but important biological systems: macrophages and antigen-presenting cells at the site of LNP-mRNA vaccine administration, and terminally differentiating erythroblasts whose transcriptional activity physiologically declines. Both systems share a unique dependence on post-transcriptional mechanisms — in macrophages, these mechanisms determine the stability of therapeutic mRNA and the intensity of the immune response, while in erythroblasts they determine the ability to produce enormous amounts of hemoglobin despite the gradual degradation of the transcriptional machinery.

The discovery that therapeutic mRNA undergoes re-adenylation by TENT5A, leading to poly(A) tail elongation and increased stability, is one of the most important findings of this dissertation. This result has direct implications for the future design of novel mRNA vaccines. It shows that cellular modification of exogenous RNA is not only possible but also functionally significant and can predictably influence vaccine immunogenicity. Equally important is the second major discovery of the work: the role of TENT5C in polyadenylating cytoplasmic globin transcripts in erythroblasts, which challenges the previous assumption that TENT5 enzymes act mainly on transcripts directed to the endoplasmic reticulum. This result opens new possibilities for understanding mechanisms of mRNA stabilization in terminal erythropoiesis and potential disturbances in anemias, including myelodysplastic syndromes (MDS).

The dissertation is based on the results of two major research projects, resulting in a publication in *Nature* and a manuscript under review (BioRxiv / Nature Communications). Both attest to the exceptional scientific level and originality of the

work. Structurally, the thesis is clear and follows the classical format of publication-based dissertations. The extensive introduction presents the current state of knowledge on hematopoiesis, mRNA biology, the functioning of mRNA vaccines, and the specifics of post-transcriptional regulation in erythropoiesis. The author then presents a detailed discussion of his research findings and associated publications, followed by an in-depth discussion. The dissertation concludes with materials and methods and a carefully prepared bibliography containing up-to-date, appropriately selected, high-quality scientific sources. The author accurately described his personal contribution to both projects.

The aims of the dissertation are presented clearly and coherently - the author sought to understand how TENT5A and TENT5C enzymes influence the stability of therapeutic and endogenous mRNAs in hematopoietic cells, under physiological conditions and following administration of exogenous RNA in the form of LNP-mRNA vaccines. These studies address both fundamental biological questions (the role of post-transcriptional mechanisms in mRNA stabilization) and problems of significant clinical relevance (in vivo metabolism of vaccine mRNA). The objectives were fully achieved and documented with results of high experimental value.

In the methodological section, the author applied an impressive set of advanced experimental techniques. These include modern sequencing methods (direct RNA sequencing via Nanopore and cDNA sequencing), which enabled measurement of poly(A) tail length and the dynamics of re-adenylation. The author also used FACS sorting, multiparameter cytometry, animal knockout models of TENT5 enzymes, proteomic techniques (proximity labeling), as well as bioinformatic analyses. The methods employed are not only modern and diverse but also demonstrate the doctoral student’s high level of independence and excellent technical preparation.

The most important results of the dissertation concern two distinct areas. The first is the metabolism of therapeutic mRNA following administration of mRNA vaccines. The author demonstrated that exogenous mRNA undergoes TENT5A-dependent re-adenylation in immune cells, leading to poly(A) tail elongation and increased transcript stability. This fundamental discovery shows that host cells actively modify therapeutic mRNA and that this process can predictably enhance immunogenicity.

Importantly, the author showed that this process occurs in vivo and that the greatest RNA stabilization takes place in macrophages (LAMs), which constitute the dominant cell population at the injection site. These findings have practical significance — they may be used in designing more stable and effective mRNA vaccines and optimizing their formulations.

The second set of findings concerns the role of TENT5C in erythropoiesis. The discovery that TENT5C polyadenylates cytoplasmic globin mRNAs significantly modifies the previous model of TENT5 enzyme function, whose substrates had been assumed to be primarily transcripts associated with the endoplasmic reticulum. The author shows that in terminal erythropoiesis, a process exceptionally dependent on mRNA stability, re-adenylation may play a crucial role in sustaining continuous hemoglobin production, particularly when the cell's transcriptional activity becomes limited. These findings open new research perspectives on disorders of erythropoiesis including myelodysplastic syndromes.

This work presents an original solution to a scientific problem concerning both therapeutic mRNA metabolism and the regulation of transcript stability in erythropoiesis. The discoveries substantially expand knowledge in RNA biology and have translational potential applicable to vaccine technology, erythropoiesis biology, and the diagnostics and treatment of hematological diseases.

Scientifically, technically, and conceptually, the dissertation unequivocally confirms the doctoral candidate's strong theoretical knowledge and his ability to conduct independent scientific research. The complexity and scale of the experiments, independence in analyses, correct interpretation of results, and ability to present them in a translational context indicate a highly mature scientific skillset.

Questions and comments to the Author

1. In the Introduction, the author incorrectly classifies follicular dendritic cells (FDCs) as dendritic cells. FDCs do not originate from the myeloid lineage, and their functions

differ fundamentally from those of DCs (they do not present antigen to T lymphocytes; rather, they serve as antigen reservoirs in the germinal center for B cells). At the same time, in the scRNA-seq results section, the author correctly describes DC populations, which makes this mistake surprising. This part of the Introduction should be clarified.

2. In the section on annotating cell populations identified in scRNA-seq, the author uses somewhat inconsistent macrophage nomenclature (e.g., describing M2 macrophages without mentioning M1). This inconsistency can be explained by the lack of consensus in terminology describing macrophage heterogeneity and the shift away from the M1/M2 dichotomy. Nevertheless, greater precision in this section would be advisable.

3. In the section describing variability in cell populations at the injection site based on scRNA-seq (pp. 71–73), analyses of “RNA velocity” or “pseudotime” are missing - these would show how populations change over time and which populations differentiate into which.

4. Regarding manuscript 2 (“Efficient globin production during terminal erythropoiesis depends on the synergistic action of TENT5C poly(A) polymerase and LARP4/5”), I would like to ask the Candidate following questions:

a. Were globin genes the only ones from the hemoglobin synthesis pathway and iron metabolism regulated by TENT5C in erythroblasts?

The author demonstrated strong regulation of globins, but ~55% of other transcripts change in TENT5C KO mouse erythroblasts. In the context of MDS pathophysiology, it would be crucial to determine whether genes such as ABCB7, PPOX, or TMEM14C also show polyadenylation abnormalities in TENT5C KO. This is important because increasing the half-life of these transcripts could represent a therapeutic strategy in MDS with SF3B1 mutations. Abnormal splicing of these transcripts leads to nonsense-mediated decay and reduced expression, causing anemia (MDS with ring sideroblasts). Since only a portion of transcripts undergo abnormal splicing in SF3B1mut cells, increasing the stability of the correctly spliced transcript could help counteract consequences of SF3B1 mutations.

b. How is TENT5C expression regulated in MDS? Are there publicly available datasets (e.g., TCGA, BeatAML, single-cell MDS publications) that allow assessment of TENT5C expression in erythroid precursors of MDS patients?

c. TENT5C KO mice have elevated TGFβ1 levels. Activin pathway and SMAD2/3 activation is a therapeutic target of the most effective drug for anemia in MDS — luspatercept. Could the TENT5C KO phenotype be partially reversible with pharmacological inhibition of the SMAD2/3 axis?

d. Could TENT5C overexpression alleviate anemia in MDS? iPSC-based MDS models could allow investigation of the consequences of TENT5C overexpression and assessment whether it reduces the differentiation block.

I, the undersigned, hereby state that the doctoral dissertation of Michal Antoni Mazur meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher 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 Michal Antoni 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. Last but not least – I strongly believe that the presented work is outstanding, represents a significant contribution to the development of biological sciences, and should be distinguished.

Sincerely,

Przemysław Juszczyński

Elektronicznie podpisany przez Przemysław Juszczyński
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