UAM

UNIWERSYTET IM. ADAMA MICKIEWICZA W POZNANIU
Wydział Biologii

dr hab. Michał Szcześniak, prof. UAM
Adam Mickiewicz University in Poznan
Faculty of Biology
miszcz@amu.edu.pl

Poznań, Jan 10th, 2024

Review of Elyas Mohammadi's PhD thesis entitled

„CELL-TYPE RESOLUTION DATA ANALYSIS USING DIFFERENT TRANSCRIPTOMICS APPROACHES FOR IMPROVEMENT OF CURRENT METHODS"

The thesis submitted for review was supervised by dr hab. Jakub Mieczkowski and is associated with the Laboratory of 3P Medicine at the Medical University of Gdańsk. It has a classic structure, divided into eleven chapters, such as Aims, a short but clear Introduction, Results, Conclusion and References. However, its core consists of three peer-reviewed publications that deal with different aspects of transcriptomic data analysis. The papers are evaluated below.

1. Evaluation of the thesis

1.1 *A brief overview of published results*

Paper I: ***Size matters: the impact of nucleus size on results from spatial transcriptomics***

IF: 8.45, **MNiSW**: 100.

Spatial transcriptomics methods rely on the integration of transcriptomic profiles from tissue sections. However, this approach is severely hampered when the size of the nucleus exceeds the thickness of a tissue section. To overcome this problem, the authors proposed the application of a previously developed statistical approach called CSDI (Consecutive Slices Data Integration), which turned out to significantly improve outcomes of data analysis. For example, better consistency of expression patterns across slices was observed compared to data analysis without CSDI. Importantly, this has direct application to transcriptional profiling of cells with relatively large cell bodies or nuclei, such as human brain neurons, underscoring the importance of the research presented.

Comments and remarks:

- Elyas Mohammadi is the first author of the publication, together with Katarzyna Chojnowska. Mr Mohammadi's substantial contribution is further confirmed by the "Author contributions" statement in the publication itself, as well as by individual statements of co-authors presented in the thesis.
- The authors have shown that CSDI helps to increase the accuracy of cell detection and to better reveal tissue layers (human brain) when the size of cell nuclei exceeds the thickness of successive slices. How have the other researchers tackled this problem so far? Are there alternative methods with similar or better results, or has it simply been neglected? Perhaps technologies beyond 10x Genomics Visium would be useful?

Minor remarks:

- The methodology lacks details. For example, the reads were mapped using STAR, but no tool version nor settings are provided.
- When it comes to this part: "…spots with a number of spatial features of more than 7000 and less than 200 were removed; spots that encompassed more than 15% of mitochondrial genes also were omitted…": please justify the applied criteria.
- Fig. 2A: I cannot really see the difference between WM (white matter) and GM (grey matter) in the histological image provided.

Paper II: ***4-1BBL-containing leukemic extracellular vesicles promote immunosuppressive effector regulatory T cells***

IF: 7.64, **MNiSW**: 40

In this paper, the authors investigate the role of extracellular vesicles in the stimulation of immunosuppressive regulatory T cells (so-called Tregs) and the progression of leukaemia. Using a variety of experimental approaches and an ex vivo mouse model, they show that inhibiting the secretion of leukaemic extracellular vesicles attenuates Tregs, which could be used to develop improved therapies for myeloid leukaemia.

Comments and remarks:

- As stated in the authorship section of the paper, Mr Mohammadi's contribution was the analysis of the RNA sequencing data together with Jakub Mieczkowski, but no further details were given in the manuscript, including individual author statements, which only vaguely describe it as "developing the concept, analysis and presentation of the transcriptomics data (Fig. 4)". It is a pity that Mr Mohammadi did not explicitly describe his own contribution in detail. In any case, his contribution must be very limited, as he is a tenth author out of nineteen, while the cumulative contribution of the four authors who provided the statements is 85%.
- The methods related to the transcriptomic data analysis are not present in the Methods section of the publication, but are provided in the thesis (page 28).
- As a fairly standard data analysis workflow was used, there is an inconsistency with the title of the thesis ('...improvement of current methods').

Paper III: ***Improvement of the performance of anticancer peptides using a drug repositioning pipeline***

IF: 5.08, **MNiSW**: 100

Anticancer proteins (ACPs) are seen as a complementary strategy in cancer treatment, but the efficiency of their action is compromised by high levels of cell surface components such as heparan sulphate (HP) and chondroitin sulphate (CS). One possible strategy would be to find drugs that reduce the expression of these two components, thereby improving the action of ACPs. Keeping this in mind, the authors developed a new computational workflow that exploits knowledge regarding gene perturbations with drugs from the LINCS L1000 project. As a result, six chemicals were identified and highlighted as potential repositioning drugs with the potential to improve the performance of ACPs.

Comments and remarks:

- Mr Mohammadi is the first author and has contributed to virtually every step of the data analysis and publication of the manuscript.
- This is a valuable piece of research, with an interesting methodology. However, not everything is clear. In particular, please explain the choice of gene-gene correlation method.
- Although it is formally correct, it is quite unusual for the doctoral candidate to have a publication in their thesis without the formal involvement of their supervisor.

1.2 A summary for the three pieces of research

In my opinion, Paper I and Paper III represent a valuable contribution to the field of transcriptomics, especially that new methodologies are developed and demonstrated to be useful and effective. Of note, they represent completely different fields of research, which highlights Mr Mohammadi's ability to undertake and successfully deal with versatile research objectives and methodologies. I appreciate the use of a range of statistical and computational approaches, which were absolutely not trivial. Last but not least, the PhD candidate is the first author in both of them, which demonstrates he is able to take responsibility for a research project and finish it with a scientific publication as well as that he possesses the skills necessary to effectively work in a team.

When it comes to Paper II, for abovementioned reasons I am not taking it into consideration when evaluating the thesis.

1.3 Conclusions

According to the current regulations, including *art. 187 ust. 3 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce*, the review of PhD thesis is expected to answer the following three questions:

1. **Does the dissertation present and document general knowledge of its author in the research area and discipline?**
   The presented dissertation in the discipline of "Biological Sciences" definitely shows Mr Elyas Mohammadi's broad knowledge and deep understanding of the phenomena studied. First of all, there is an introductory part at the beginning of the dissertation, including an explanation of the biology, methodology and technologies related to the studied phenomena. Also, the peer-reviewed works themselves well document the general knowledge of the author.
2. **Evaluation whether the dissertations shows its author's ability to conduct scientific research on his own**
   On the basis of the authors' declaration of contribution, I have no doubt that the PhD student's role was critical in two papers in which he is the first author. In particular, the author designed, applied and tested new or modified approaches to transcriptomic data analysis.
3. **Evaluation whether the presented dissertation constitutes an original solution to a scientific problem**
   The PhD candidate's findings and methodological advancements presented in the thesis and briefly characterized above, clearly demonstrate solutions to scientific problems.

2. Final remarks

I find the presented thesis valuable and the PhD candidate's results of interest to the broad scientific community and my minor doubts and remarks raised above do not nullify my overall good impression. I therefore conclude that the dissertation meets all the requirements for the doctoral thesis and strongly recommend proceeding to the following steps of the doctoral defense.

Michał Szcześniak
....