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Assessment of doctoral dissertation

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pt. „Functional dissection of the immunoglobulin heavy chain enhancers RNAs in B-cell non-Hodgkin lymphomas”

The doctoral thesis titled "Functional dissection of the immunoglobulin heavy chain enhancers RNAs in B-cell non-Hodgkin lymphomas" describes the role of non-coding sequences, enhancing the expression of the *IGH* gene in non-Hodgkin lymphomas.

1. Work aim

The aim of the work is to establish the IGH enhancers involvement in B cells lymphomagenesis and controlling expression of the translocated oncogenes. The purpose of the work was clearly defined and presented. Including the review work in the doctoral dissertation additionally helped to understand the context of the research conducted.

2. Methods

The work used a significant number of molecular biology methods, including the relatively new CRISPRi method. This is a technique for transcriptional repression in various biological systems. A significant number of cell lines were used in the described studies, which makes the obtained results credible. The amount of collected data and their diversity is a definite advantage of the work, but also a challenge for the author of the analysis.

3. Results

The main substantive value is the use of eRNA (enhncer RNA) as markers of regulatory elements that can be a therapeutic target. The results of the published work (manuscript no. 3) validated the method described in manuscript no. 2. This confirms the correctness of the presented analysis methodology and the significance of the obtained results. The aim of the work was achieved, and the methodology used can be used in research of a different nature.

4. Questions and critical comments

- The second of the presented manuscripts ("CRISPRi screen identifies core regions in IGH enhancers essential for non-Hodgkin lymphoma cells survival") seems to present results that are significant from the point of view of the third presented manuscript. However, in the Ph.D. Abstract it is identified as a paper in preparation. Does this mean that the conclusions drawn from it may still change?
- The dissertation touches on very important molecular issues, in particular regarding the regulation of gene expression through the activity of enhancers. Although one of the manuscripts contains information about histone modifications within enhancers, their analysis is quite superficial. Why was it not decided to epigenetically characterize enhancers and their surroundings, e.g. by using the 3C method and whole-genome methods such as ATAC-seq and nucleosome positioning? Also, analysis of the activity of transcription factor binding sites would allow us to say more about the regulation of gene expression.
- eRNA appears to be a useful marker. Has the uniqueness of the sequences of the obtained markers and their possible similarity to other eRNAs been verified?
- I understand that the main goal of the work concerned intracellular processes and expression regulation. However, in a living organism, cells do not function independently of others. For this reason, it is worth checking whether, for example, intercellular communication does not activate signaling processes/pathways that change intracellular processes. Why was there no research on primary cell lines or co-culturing in this work?

5. Conclusions

The doctoral dissertation was summarized with correctly formulated conclusions.

6. Summary

In the final assessment, I state that the work was performed to a high standard. The assumed goals of the work have been achieved, and some of the results have already been published. A thoughtful presentation of the topic of the thesis and the collection of the results into three manuscripts has a positive impact on the readability of the dissertation and the reviewer's understanding of the topic. Applies for the PhD student to be admitted to further stages of the doctoral process.

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