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May 27th, 2024
Prague, Czech Republic

Diploma Thesis by Viktoriia Kulinich
Opponent: Meritxell Alberich-Jorda, Ph.D

Confidential report:

The Diploma thesis entitled "The role of T-cell Receptor in Lymphoma" by Viktoriia Kulinich explores the relationship between TCR and T-cell lymphoma pathogenesis.

TCR is a protein complex expressed on the surface of T cells. It is responsible for recognizing antigens bound to MHC molecules, thus translating extracellular signals from the microenvironment to the inside of T-cells. Activation of TCR signaling helps to fight infections and cancer, and is also a major determinant of T-cell fate. TCR alterations and/or hyperactivation are common in T-cell lymphoma, however how aberrant TCR signaling might contribute to T-cell lymphoma is poorly understood. In the present thesis, Viiktoria hypothesizes that TCR signaling in T-cell derived malignancies could support growth and survival of tumor cells. Thus, the specific aims are to determine:

1. Whether TCR supports growth of TCR positive T-cell derived lymphomas
2. What are the phenotypic consequences of TCR knockout in tumor cells

The thesis starts with an introduction and a literature overview which contains several sections: general overview on T-cell lymphoma and a classification of the different subtypes, overview of the lymphoma models, and a detailed description of the TCR complex in lymphoma (composition, signaling). The introduction is well written and follows a logical structure, providing sufficient information to justify the rationale of the study. The only thing that I missed is a short section of the distinct genetic aberrations that occur in T-cell lymphoma. It follows a concise section of the thesis which clearly states the knowledge gap and the thesis aims. Next, a detail description of the materials and methods employed in the thesis is provided. Here, it got my attention the numerous and variable number of methods that Viktoriia performed, including cloning, FACS sorting and analysis, cell cycle and apoptosis assays, FRET-based AKT activity assessment, and RNAseq profiling. I appreciate that it is clearly stated that some constructs used in the thesis were cloned by other lab

members, providing a clear information about the contribution of Viktoriia to the work. The results are presented in a logical and clear manner, going over each step of the worked performed. All the results are supported by figures, and the graphical presentation is quite clear. As a small remark, in some cases the difference between WT and KO are very small, and even if statistically significant, it is questionable whether this could have some biological consequences. The discussion section analyzes the results obtained in deep, and it also evaluates the results in a larger context. Several aspects are discussed, from different perspectives and point of views, provided a rich and meaningful discussion on the topic. The thesis concludes with a summary of the results and provides the list of references.

I would like to congratulate Viktoriia Kulinich on the present work, and ask her few additional questions:

1. Could you briefly summarize the distinct genetic aberrations which occur in T-cell lymphoma?
2. The many sub-types of T-cell lymphoma are in part determined by the differentiation stage of the cell where the genetic aberration takes place. What is the earliest cell type where mutations in T-cell lymphomas occur? And what type of disease they generate?
3. Where do mutations in the TCR complex occur and which domains are affected by these mutations?
4. The hypothesis is based on the fact that TCR expression is retained in T-cell lymphomas, however the loss of TCR is a frequent feature of many subtypes of T-cell lymphoma. Could you please indicate how frequent TCR expression is retained in this pathology? And is it a characteristic of a specific subtype(s) of T-cell lymphoma?
5. In the results section it is mentioned that the absence of TCR or CD3 expression is associated with the loss of surface expression of the other. Does it mean that they stabilize each other at the surface? If the read out would include intracellular staining would you get the same result?
6. Are the results shown in Fig 22-27 statistically significant?
7. Overall the cell lines with KO seem to grow slower. Could this phenotype be driven by non-specific CRISPR targeting? How could you assess it?
8. In several parts of the results, you mention a potential effect of electroporation itself on your results. Can you think about an additional control to check for that?
9. Cell cycle analysis was assessed by FxCycle. Does this method allow you to assess the percentage of cells in G_0? Is that important for you analysis?
10. The measurement of AKT kinase activity was done in the acute or chronic KO cell lines? What other method could be used to assess AKT signaling activity?
11. In your experimental design you assess the effects of the KO in steady state conditions, giving you a mild phenotype. Do you think you could enhance those differences upon TCR activation? Did you try to perform similar experiments upon antigen presentation or PMA stimulation?
12. In the gene expression profile you picked up WNT9A. Are Wnts, and in particular 9A, being secreted by your cell lines?
13. If you were about to continue with this project, what are the next steps you would like to take? How would you continue with this project?
14. On the long run, what could be the clinical implications of your work?

To conclude, the present thesis by Viktoriia Kulinich addresses an interesting topic in the field of T-cell transformation, and opens an attractive view for developing novel strategies in certain T-cell lymphomas. **Viktoriia Kulinich demonstrated scientific creativity and competence. The thesis meets the standard requirements for the diploma dissertation in the given field and I recommend it for the defense (according to §47 of the Act No. 111/98 Sb).**

Sincerely,

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