CHARLES UNIVERSITY
Faculty of science

Re: Evaluation report – M.Sc. Thesis by Karolína Vaníčková

Thesis title: Identification of novel mechanisms controlling emergency granulopoiesis in hematopoietic stem and progenitor cells

Supervisor: Meritxell Alberich-Jorda, M.Sc., Ph.D.

Reviewer: Mgr. Zdeněk Zadražil, Ph.D.

This study deals with an important topic of emergency granulopoiesis (EG). EG is a crucial process that is involved in pathogen clearance, inflammatory response, and recovery of the hematopoietic compartment. The process of EG has been well described on the level of effector cells. However, this thesis focuses on the putative crucial changes in hematopoietic stem and progenitor cells (HSPC) which initiate EG.

Introduction

On 16 pages, the student describes the steady-state granulopoiesis (SSG) as well as EG and the Wnt/β-catenin signaling pathway in the hematopoietic system. The introduction is clear and reasonably easy to read. However, an introduction in slightly more depth would be welcome.

Some parts are not introduced here and are sufficiently described only later, in the results section, such as the transgenic dnTCF4 mouse model or TCF/LEF factors. In some cases, a term is used and subsequently introduced later in the thesis, for instance, C-EBPα (first used on page 2, introduced on page 9). The same is true for the introduction of some abbreviations. A brief introduction of markers used to differentiate hematopoietic stem cell (HSC) populations is also missing.

Aims of the study

Aims are clearly stated as follows:

1) To determine whether β -catenin gets activated in HSPCs during EG
    a) To validate a murine model to investigate the role of canonical Wnt signaling pathway in SSG and EG
    b) To determine whether active β-catenin gets accumulated in HSPCs during EG
2) To investigate early changes that happen at the level of HSPCs during EG

Materials and Methods

In this section the student presented a wide variety of methods which she used in her research. I would like to compliment the range of techniques the student learned and utilized.

Results

The results section is 22 pages long, and here the student describes her exciting original data. The individual experiments have a clear flow, and it is well reasoned why and how particular experiments were performed. However, I have some reservations regarding this section.

Throughout the thesis, there is not a single mention of the actual measured values from any experiments. These values can be deduced from the graphs of individual figures, but the actual values cannot be found in the figure legends either. Also, for the majority of experiments, there is no mention in the text or in the figure legends of the number of mice that were used in a given experiment. This is partially amended through the visualization of individual data points in most of the graphs.

In Figure 6: granulocytic response following LPS treatment, the student describes the percentages of granulocytes in bone marrow (BM) and blood. At time points 4,6, and 12 hours post LPS injection, there are only 2 data points for the measurement of granulocytes in the blood, and the same is true for the absolute cell counts in the BM at time points 6 and 12 hours post-injection.

What are the limitations of these results? And why is this not discussed in the thesis? There is the same issue in figure 8: Intracellular β-catenin staining after Pam 3CSK4 treatment; only 2 data points at 2 and 6 hours post-treatment.

Discussion

On 7 pages, the student discusses her results, possible limitations of her observations. I rate the discussion as excellent. The student also discusses putative hypotheses and compares her data to recent publications in the field.

References

The thesis cites 156 studies, including fundamental research in the field as well as numerous very recent studies.

Summary

Karolína Vaníčková wrote her thesis in English and her written skills are excellent. There are some formal issues, but I consider them relatively minor, especially compared to her research. Her work is very novel and, indeed, with further experiments, will get published soon. The student very well discusses all her results and their limitations. She also thoroughly discusses her data compared to other recent research.

The student demonstrates knowledge of many laboratory techniques, as well as complex bioinformatics analysis skills. It is also worth mentioning that she co-authored a highly impacted publication published in Blood during her studies, which is a very remarkable achievement for an undergraduate student. Karolína proved her abilities already in the first year of her studies as she was awarded the Best poster at a student poster session.

The candidate shows strong prerequisites to become a successful researcher. I highly recommend this thesis to be accepted as the fulfillment of the requirement for awarding a Master's degree to the candidate.

Questions for discussion:

1) The student shows that there is no age-dependent changes in the expression of *TCF7, TCF7L1, TCF7L2* and *LEF1* in murine whole BM. Is this an indicator that there is no age-related changes in EG in mice? How could the EG be influenced by age in humans?

2) In figure 6, the student measure EG response after a single LPS injection. The levels of granulocytes in blood showed high variability at certain time points. The student states that this is likely caused by the infiltration of the tissues. I find this statement rather generic. Could the student elaborate further?

3) The scRNA-seq data of multipotent progenitors (MPP) reveal 10 distinct subpopulations. 9 of these clusters were assigned lineage signatures. I wonder, what is cluster 1? The same goes for the scRNA-seq of hematopoietic stem cells (HSC), clusters 1 and 4? Does the student think these clusters with unknown signatures truly represent distinct subpopulations? How could these clusters be investigated further to unravel the features of these putative subpopulations?

4) The research presented here contains numerous original and novel observations. As I understand, the student will continue her Ph.D. studies in the same laboratory. I would like the student to elaborate on the near future work, which she regards as essential for her subsequent research. The student also mentions that the bioinformatic analysis of her scRNA-seq data is only preliminary. What is the further potential of the scRNA-seq data for such analysis?

5) Could the EG be influenced by circadian rhythms?

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