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Examiner's Report on Mgr. Kateřina Faltusová's PhD Thesis:

The role of stem and progenitor cells in regeneration of hematopoietic tissues

This study describes an investigation regarding the cellular and molecular processes underpinning the regenerative potential of hematopietic progenitors which reside in bone-marrow (BM) and their contribution to the recovery of hematopoiesis after irradiation. This is an important and a broadly studied topic. The understanding of the principles generating essential cellular components of adult blood under stress conditions can substantially improve the efficiency of a patient's bone marrow transplantation and bears the potential of preparing customized blood supplies prepared *in vitro* conditions.

The premise of the study was to explore the original observation that after a submyeloablative dose of γ-irradiation (6 Gy), the regenerative potential of BM is largely mediated by developmentally advanced erythroid and myeloid progenitors exhibiting a dramatically altered phenotype along with intensive proliferation. It is important to emphasize that detail understanding of this mechanism is still in its infancy, and thus the thesis is a somewhat interesting "crime story" where the search for clues, such as the main triggers, cellular targets and mechanisms which direct these previously unappreciated alternative developmental pathways, are described by the defendant in an informative way and broad scientific overview combined with elegant and logically arranged and executed experiments. Overall, the work of Mgr. Faltusova represents some important steps towards the elucidation of these novel BM regenerative mechanisms. Importantly, the author of this thesis has also shown that the non-hematopoietic microenvironment of irradiated BM is actively controlling the expression of critical hematopietic markers, such as Sca-1, and thus shape the regenerative response of hematopietic precursors.

It is necessary to emphasize that the two main primary papers which are directly linked to Mgr. Faltusova's PhD thesis have been published in well respected journals, such as *Frontiers in Cell and Developmental biology* (IF = 5,186) and Biology of Blood and Marrow Transplantation (IF = 3,98) as well as in domestic journals "Transfuze a Hematologie" and "Ceskolovenska fyziologie" as review articles. Moreover, there are four other publications which are unrelated to the presented thesis, two of them as the first author and two as co-author. In this regard, the work represents an indispensable addition to scientific literature publically available on this topic.

The results from these studies are indeed interesting. From my point of view, among the most impressive results generated by Mgr. Faltusova was the discovery that not only stem cells and multipotent progenitors but also specific cell populations, such as GMPs and MEPs, which represent more differentiated and lineage-committed subsets from the hierarchical hematopoietic tree, can participate in rapid and effective hematopoietic tissue reconstitution. Using a battery of modern methods and approaches, Mgr. Faltusova provided solid and convincing evidence that this process is linked to the reconditioning of the hematopoietic environment by BM stromal cells, which results in changes in phenotype, likely reflecting reactivation and/or enhancement of regenerative potential of these subsets. While many details of this process must be further studied and elucidated, these results advocate for a fundamental importance of these previously ignored subsets, especially GMPs and MEPs, for the rapid rescue of hematopietic function after stress or injury. I am convinced that this data opens a new investigative venue for alternative ways of hematopietic reconstitution. This could be potentially far reaching for clinical practice since a step-by-step improvement in this process can potentially improve patient lives after transplantation.

The thesis was written well in English, with only few misspellings, and it is presented in a shorten version with 109 references. It contains a list of abbreviations used throughout the text, introductory chapter which highlights the main characteristics of adult as well as embryonic hematopoiesis, processes which are associated with its regeneration after irradiation, as well as an overview of the methods used. Chapters 4 and 5, “Hypothesis” and “Aims”, respectively, introduced the main assumption and goals of this study. Then, chapter #6, Materials and Methods provides a concise and complete account of the protocols, reagents, and the approaches employed. Chapter 7 provides guidance through the most important experimental results which provides the author with strong arguments for the discussion and conclusions in Chapters 8 and 9, respectively. In my view, these chapters highlight the significant achievements of Mgr. Faltusova in this relatively competitive field of research. The last Chapters 10 - 16, provide The Summary, Key words, References, List of publications and Appendices, presented as separate chapters in Czech and English (except for references).

While I feel that the conclusions of this study are very important and strong, there are several questions that could be further discussed.

First, I have only two formal concerns:

1/ usually, the abstract is provided at the beginning of thesis and not at the end as the Summary. In the accompanying “dissertation panphlet” this has been corrected and the abstract is indeed at the beginning.

2/ On page 20, the reference citing the work by Voboril et al., 2020, Nat Comm. is used incorrectly.

Questions for discussion:

1/ Fig. 16 shows that while in the untreated mice MEP and GMP are largely Sca-1 negative, in regenerating BM all GMPs and a fraction of MEPs comes from Sca-1 positive LK cells. In Fig. 30 the author shows that Sca-1 re-expression is controlled by the stroma of irradiated BM. Do you think that Sca-1 re-expression is a prerequisite for the enhanced production of GMPs or it is just an accompanying phenomenon of this process? In the same figure, when comparing fraction A and B from the donor, it is obvious that A, which originally displayed no or the lowest expression of Sca-1, 48 hours after its transfer into irradiated host expressed much higher levels of Sca-1 compared to fraction B. How would you explain this? Did your RNA sequencing (Faltusova et al, 2020), which is not part of this thesis and thus is not discussed in detail, reveal any other important changes in the gene expression profile which could provide some insight into this Sca-1 re-expression/reprogramming process?

2/ From Fig. 30 it seems that not only the re-expression of Sca-1 but also the downregulation of c-kit expression in the $LS^{neg}K$ population from untreated UBC-GFP mice is caused by irradiated stroma cells. This is remarkable given that SCF mRNA after irradiation was highly upregulated throughout BM (Fig.24). Are previously described arterial endothelial cells (AECs) (Xu, C. et al, Nat Comm, 2018, 9:2449) an exclusive source of SCF also in irradiated BM? Why LK and/or LSK cells, instead of the whole BM or Lin^- subset, not used in the experiment presented in Fig. 24A and importantly in Fig. 24B? Are there any microscopically observable changes in the architecture of BM on day 15 after irradiation which could be indicative of MEPs and GMPs overproduction?

3/ Fig. 27 argues that while c-kit signalling is critical for the appearance of MEPs, GMP development and expansion in the absence of c-kit signalling can be rescued by the expression of Sca-1. How do you reconcile this discrepancy? Would blocking of GM-CSF inhibit GMP development and expansion?

4/ Table 9 and Fig.27 show that c-kit signalling is essential for regeneration of irradiated BM and act at least in two stages: first stage, which occurs within the first four days after insult, or the mice fail to regenerate BM and die; and second, at day 10 post-irradiation to maintain LS^-K population. Can you speculate whether the cellular target of c-kit signalling in this experiment is the same or represents distinct hematopoietic cell subsets?

5/ As you wrote in your thesis, your work describes a new phenomenon where the irradiation unlocks "the latent potential of developmentally advanced and committed progenitor cells to carry out the

initial phase of tissue regeneration". Can you speculate and/or explain how irradiation can trigger this alternative activation of more committed cells for robust proliferation and development? What could be the most important signal that initiates this process and which cells are responsible? What could be evolutiuonary considerations of this alternative way of hematopoiesis? Is there any indication that what you observe in mice also occurs in humans?

Conclusions and recommendation

I have identified and discussed only a few formal concerns among the many positives of this thesis. I would like to emphasize, however, that the above concerns in no way diminish the high quality of work presented in this thesis and author's publications.

The thesis of Mgr. Faltusova contains important work that has been presented in a well-written and shortened format that has brought further advancement of the understanding of how BM regeneration occurs and for the first time described alternative hematopoietic progenitors which can efficiently replace stem cells and multipotent progenitors in this process. This contribution in the long run can improve the management of patients who have undergone BM transplantation. In addition, modern experimental approaches, an open presentation, the discussion and analysis of the results demonstrate that the author is fully prepared for her professional scientific carrier. The quality of papers which have been published in well-recognized international journals lends further support to this statement. Given the quality of Mgr. Faltusova's work, I fully recommend this thesis to be accepted as a fulfilment of the requirement for awarding the degree to the candidate according to the law §47 section 4.

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