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Review of the PhD thesis: *Regulatory mechanisms in normal and malignant granulopoiesis*

Proposed PhD thesis submitted by Miroslava Kardosova summarizes the experimental data from three published studies. Miroslava Kardosova is the first author of the paper published *in Haematologica* and co-author on the two papers published in the *Cell Death and differentiation* journal. Besides papers directly linked to the thesis, she coauthored three other manuscripts.

In her thesis, Miroslava first provides an overview steady state and emergency hematopoiesis with emphasis on granulopoiesis. I appreciate a brief methodological overview of specific blood cell surface markers at each level of the hematopoietic hierarchy and their use in flow cytometry. Miroslava also comprehensibly summarizes current knowledge of key granulopoietic transcription factors (TFs), surface protein Evi2b and micro RNA-143 in the context of leukemia.

Results part of the thesis is based on three published papers and aims to extend our knowledge on normal and malignant granulopoiesis at different regulatory levels including, TFs, surface proteins and micro-RNAs. The main projects discussed in the thesis are:

1. Function of the transcription factor *Cebpγ* in normal and stress-induced granulopoiesis

2. Characterization of *Evi2b* function in granulopoiesis

3. The role of miR-143 in normal and malignant granulopoiesis

Miroslava has clearly contributed to all three papers focused on these projects. In her first author publication she develops a new mouse model, where TF *Cebpγ* is deleted only in hematopoietic cells. Using this novel mouse strain and numerous experimental approaches Miroslava shows that the absence of *Cebpγ* has negligible consequences on production of both immature and mature granulocytes during basal and several types of demand-adapted granulopoiesis, which is in contrast to data previously using the whole body *Cebpγ* KO.

In the second manuscript, describing that *C/EBPα* controls expression *EVI2B* by direct binding to its promoter region and the role of *EVI2B* in granulopoiesis, Miroslava performed important *in vitro* experiments, showing that C/EBPα activation upregulates *EVI2B* expression in human cell lines transfected with different *C/EBPα* constructs.

The third manuscript describes the role of miR-143 in the regulation of normal granulopoiesis and shows that miR-143 accelerates neutrophilic differentiation through downregulation of ERK5. Miroslava contributed experimentally to this work by performing *in vivo* BM transplantation assays which demonstrated that LSKs with miR-143 knockdown produced less mature granulocytes.

Structure of the thesis is logical, written in a very good English and is easy to follow.

I have the following questions complementing the published reports:

1. Numerous approaches to discriminate ST-, LT-HSC and MPPs have been described in the literature. Specifically, different authors discriminate ST-HSCs using different strategies (Cabezas-Wallscheid et al. 2014. *Cell Stem Cell*, Buechler et al. 2013. *JI*, Lee-Sayer et al. 2018. *PlosOne*). In your study you have chosen the markers CD48 and CD150 according to Morrison et al. 1999. Could you comment on different, more recent strategies to discriminate ST- from LT-HSC?

2. Hirai and colleagues (Hirai et al. 2006. *Nature Immunology*) have shown, that *Cebpβ* is necessary for eliciting emergency granulopoiesis upon *Candida albicans* infection. *C. albicans* presence can be sensed by numerous Toll-like receptors including *Tlr2*, *Tlr4* and *Tlr9* (reviewed in Netea and Marodi. 2010. *Trends in Immunology*). In your system *Cebpγ* was knocked out only in hematopoietic cells and conditional *Cebpγ* KO animals did not show any difference from wt animals. Do you think this could be explained by possible role of *Cebpγ* in non-hematopoietic cells? Is the level of *Cebpγ* in wt GMPs increased similarly to *Cebpβ* upon *C. albicans* infection?

3. One of the genes downregulated in *Cebpγ* KO LT-HSCs is *Ifngr1*. Recently, embryonic HSCs have been described to show inflammatory gene signature and that sterile inflammatory signaling (including *Ifnγ* signaling) is an evolutionarily conserved pathway regulating the production of HSCs during embryonic development (Li et al. 2014. *Genes and Development*). Interestingly the decrease in HSC numbers in *Ifngr1-/-* embryos is compensated in adult animals. Have you studied the development of embryonic HSC in your *Cebpγ* conditional KOs?

4. In the *Evi2b* study you show, that *Evi2b* silencing in human CD34+ cord blood cells leads to decreased frequencies of CD11b+ and CD15+ cells when cultured in semisolid media. Specifically, sh5 causes dramatic decrease of CD11b+ cells, while CD15+ cell frequency is almost the same as in wt control. Could you comment on this?

5. Expression of miR-143 is often accompanied by miR-145 (Cordes et al. 2009. *Nature*), this is probably not the case in GSCF differentiated human CD34+ HSPCs as shown in Figure 1 of the miR-143 paper. Could you confirm, that the sequencing revealed no changes in miR-145 expression upon GCSF treatment? Besides being involved in numerous developmental processes, miR-143 is also described to be upregulated in some tumors (reviewed in (Kitade and Akao. 2010. *J Pharmacol Sci*)), could you comment on differential regulation of miR-143 expression in different cancer types?

Taken together, I highly appreciate the quality of the submitted thesis. Especially the tremendous effort to find any phenotype in conditional *Cebpγ* KO animals. During her PhD studies Miroslava Kardosova showed a proficiency in the vast array of experimental methods including demanding *in vivo* HSC repopulation assays, which is reflected by a very high quality of her research papers. In my opinion, the results of her scientific projects clearly exceed the average level of PhD candidates and she meets the requirements set by the Immunology board of PhD studies. I recommend Miroslava Kardosova to be awarded the PhD degree in the field of immunology with no reservation whatsoever.

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