Department of Biology
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Ph.D. Thesis Review

Author: M.Sc. Oľga Babošová

Title: Molecular basis of clonal heterogeneity of hematological diseases

Reviewer: Doc. Mgr. Monika Horváthová, Ph.D.

Oľga Babošová thesis is focused on the molecular mechanisms involved in the clonal heterogeneity of hematological diseases. The research topic is very relevant in the context of up-to-date research. Study of molecular heterogeneity of myeloproliferative neoplasms (MPN) and particularly understanding the genetic predisposition to hematologic malignancies has become a hot topic in the field of clinical and molecular hematology.

The thesis consists of eight chapters, is well structured, the length is appropriate with respect to objectives. The style and language are accurate with some spelling/typing errors. The most obvious one is in the Abstract, where the candidate uses abbreviation DRR instead of DDR (for DNA damage response). As a native Slovak speaker I can find some language imperfections in the "Slovak" abstract.

The Introduction represents an overview of the topic, is well written, and covers all aspects studied or discussed in the thesis. The methods are appropriate, modern, and advanced and indicate that the candidate is a skilled researcher. The list of references is extensive and demonstrates that the candidate is familiar with the literature relevant to her research, including the most recent articles.

The aims of the thesis are clearly defined and encompass: 1) Identify the role of inherited mutations in the JAK2 gene in the initiation and progression of myeloproliferative neoplasms; (2) Identify the protective role of KAP1 protein in the progression of myeloproliferative neoplasms; (3) Identify the role of EGLN hydroxylases/PHDs and FOXO3A transcription factor in mantle cell lymphoma.

The strongest part of the thesis is primarily related to Aim 1 and represents a high-quality research with two excellent publications in high impact factor journal (*Blood*). These two publications resulted from collaborations with leading experts in the field of MPN; JT Prchal (University of Utah) and S Constantinescu (Université catholique de Louvain- de Duve Institute) and with the contribution of a group of V Divoký (Palacký University). Of importance is the description of an association of gain-of-function germline JAK2 mutations coexisting with JAK2 V617F in subjects with MPN and the characterization of functional consequences of coexisting JAK2 mutations on JAK2 signaling. Both articles gain insight into the pathophysiology of MPN.

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The presented findings also raise important questions to be answered: 1) whether the germline JAK2 mutations may predispose to acquisition of the JAK2 V617F and 2) what is the role of germline JAK2 mutations in MPN progression. The results and discussion related to this aim give a critical analysis and interpretation of obtained data. It is obvious that the presented articles passed the peer review process. I have only one minor comment which, however, does not reduce the significance of the presented research. Although the candidate clearly declares which experiments were performed in the collaborative laboratories, according to my opinion, the figures presenting these experiments should be omitted from this part of the thesis.

Aim 2 is a weak part of the thesis. The candidate proposed to "Identify the protective role of KAP1 protein in the progression of myeloproliferative neoplasms." Although the presented experiments showing the creation of a model cell line are extensive and the candidate experienced some technical difficulties in the generation of suitable model system, she does not present any results which would analyze the role of KAP1 in DDR in JAK2 V617F-positive cells. Therefore, this aim is rather "generation of a model system" than "identification of the protective role of KAP1 protein in the progression of myeloproliferative neoplasms."

The last aim of the thesis "Identify the role of EGLN hydroxylases/PHDs and FOXO3A transcription factor in mantle cell lymphoma" does not fit to the topic of clonal heterogeneity of hematological diseases. Nevertheless, this aim deals with an important question related to possible therapeutic implication of iron chelation and prolyl hydroxylase (PHD) inhibition for mantle cell lymphoma (MCL). The research has produced results showing that iron chelation decreases cyclin D1 level and proliferation rate, and induces cell cycle arrest and apoptosis. Similar results of anti-tumoral effect of iron chelation have already been published (Vazana-Barad et al., Leuk Lymphoma. 2013). Nevertheless, the novel finding presented in the thesis is that the observed effect is not mediated via ENGL2/PHD1-FOXO3A pathway.

The Conclusion part is not very well written and is disproportional. While the summary of Aim 1 is clear and straightforward, the summary of Aim 3 is too long-winded repeating some details presented in the results. Finally, the conclusion of Aim 2 represents rather future research plan.

With respect to the presented results I have following questions to the candidate:

1. What are the frequencies of T108A JAK2 and L393V JAK2 alleles in normal population? Have the authors screened also patients with hereditary erythrocytosis or hereditary thrombocytosis for the presence of these two JAK2 mutations?

2. In the second paper, the authors showed that the sensitivity of V617F/R1063H double mutant cells to Ruxolitinib is higher than the sensitivity of V617F-positive cells (which was comparable with wild-type cells). I am wondering whether the effect of Ruxolitinib is always stronger for JAK2 double-mutant cells compared

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to V617F-positive cells or it is specific for this particular V617F/R1063H combination. Have the authors performed sensitivity assays with JAK2 inhibitors also for V617F/T108A and V617F/L393V double mutants?

3. Did the authors have a chance to test whether Ruxolitinib mitigates any of the phenotypic effects observed in V617F/R1063H double mutants that are presented in Figures 10 or 11A,B?

In summary, the thesis fulfills all the requirements for the dissertation thesis. The above-mentioned comments and critiques do not reduce the overall quality of the work. I recommend the thesis for defense and after successful defense I recommend to confer the candidate M.Sc. Oľga Babošová title "doctor" (Ph.D.) in accordance with paragraph 47 of the Higher Education Act. No. 111/1998 Coll.

Olomouc, January 16th, 2019

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