Reviewer's Dissertation Thesis Report

Disertation Thesis Title: Epigenetics in gene regulation and chromatin structure

(Epigenetika v genové regulaci a struktuře chromatinu)

Student: MSc. Dijana, Lađinović

The thesis focused on the lysine demethylases KDM2A and KDM2B especially their short isoforms lacking demethylation activity. It is now apparent that regulation of histone methylation and demethylation is essential for most of the biological processes in the context of development and cellular responses. Therefore it is very important to understand the role of alternative protein isoforms of chromatin modifying proteins in these processes. The author characterized the alternative isoform of KDM2A (KDM2A-SF) in more detail. In comparison to full-length KDM2A, KDM2A-SF created distinct nuclear bodies together with HP1b. In the last part of the thesis, Diana studied the role of these isoforms in canonical Wnt signaling pathway.

The thesis has a standard structure and consists of 99 pages that are divided into five main Chapters. The chapter Introduction provides a succinct and well-organized overview of chromatin structure, epigenetics and epigenetic modification. This chapter is written in an understandable way. Although, some parts of introduction could be described in more detail and supplemented by appropriate figures to facilitate the reading (e.g. Wnt signaling pathway). The aims of the thesis are well defined, giving the reader clear information about what questions were addressed. The materials and methods are extensively described. The results are critically interpreted and discussed. The literature cited is comprehensive and does not seem to have any flaws.

The candidate wrote one first-authored paper on the subject, with partly overlapping topics and methodology and is a co-author of one other publication. Third first-authored article is in preparation. Both papers have been published in the journal Nucleus with IF 2.2.

Since almost all data are already published in well-known international and peer-reviewed journals, there are not many concerns and comments regarding the quality of the data.

Specific comments:

- In Figure 3, histone modifications are shown separated from histones and bound to DNA. Could you explain why?
- What did you mean by the expression: double damage repair signaling (page 13)?

- In Thesis goals and Discussion, you are describing the localization of alternative promoter of the KDM2A-SF once in intron 12, second time in intron 13. Could you, please, show and clarify localization of alternative promoters for KDM2A-SF and KDM2B-SF?
- Results, Page 55-57: I would recommend bigger labeling of graphs in Figures 11, 12 and 13. Here, I miss a statistical analysis. Nevertheless, I want to ask you how many repetitions of these experiments did you provide?

General questions:

1. Do you have any explanation why different cell lines have different level and ratio of KDM2A-LF and KDM2A-SF (Figure 5C)? Is it affected by stage of tumor transformation?

2. You suggest that the KDM2A-SF isoform bound to the tested regions. What kind of experiments may you propose to verify this knowledge?

3. Only one article form the backbone of the thesis, therefore I want to ask you what was your role in the performing of experiments and in the interpretation of your data shown in your thesis.

Finally I declare that after thorough study of the submitted dissertation thesis I can conclude that its scientific level is high, new and interesting results are presented here, which were or will be published. Therefore, I am recommending the dissertation thesis for defence.

November 7, 2019

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