

Reviewer's report on PhD thesis by Dijana Lađinović

Subject of doctoral thesis by Dijana Lađinović are epigenetic modifications and their regulation. More specifically, it is histone demethylase KDM2A, its isoforms, their localizations and function. Goals of the thesis are related to shorter, nonstandard, isoform of KDM2A and its possible role in regulation of canonical Wnt signalling. As may be judged from the cited literature, the subject of the thesis is actual, and may be also important as the studied gene may contribute to regulation of gene expression in stem cells. The thesis is written in a good English on nearly hundred pages out of which nearly 25 contains references. The text of the thesis contains minimum spelling mistakes but there are still some parts requiring clarification (like the first sentence of the last paragraph on page 58) and figures 1-3 have confusing citations – in Fig.1, it is not clear from which publication was the picture taken, Fig. 2 lacks its source completely. I also miss list of abbreviation, which, in my opinion, would facilitate reading for those who are not completely familiar with the subject. Also, the figure legends should be somehow separated from the main text, at least by usage of smaller font.

Introduction is very well structured and leads a reader toward dimethylation on lysine 36 of histone H3 and its demethylase KDM2A. Broad spectra of used methods prove that author has got practice in basically all standard techniques used in molecular biology lab, like cloning, gene expression, PCR/qRT PCR, western blot, microscopy, RNAi, luciferase reporter assays as well as with advanced techniques like chromatin immunoprecipitation.

Results are on 14 pages and basically show what Diana published about short isoform of the enzyme and unpublished results summarizing influence of KDM2A on expression of Wnt pathway target genes. There is a noticeable difference between these two parts although I miss statistical analysis of presented charts in both parts. Whereas published results are clean and straightforward the unpublished results in section 6.5 raise some concerns.

I would be very careful in making solid conclusion out of the presented results. At first, more than one housekeeping gene should be used for qRT PCR analysis. Second, more than one Wnt target gene should be used. Only *Axin2* can be regarded as genuine Wnt target gene and its expression was not even induced in NIH3T3 cells (Fig. 11b). *c-Myc* and *CyclinD1* are regulated by other pathways as well, and their expression do not depend solely on Wnt signalling although they are broadly accepted as Wnt target genes. Thus, Figs. 11 – 13 show basically only one target gene and activity of the reporter. The author cites that Yu et al. showed in 2016 that KDM2A inhibits expression of *SFRP2*, so, third, it might be worth to verify how KDM2A regulates expression of this gene or some other previously described genes regulated by KDM2A in the model of MCF-7 and NIH3T3 cells. Forth, in the experiment summarized in Fig.11, the expression of the Wnt target genes was not induced. Why not? Why was then the Wnt signalling activated in complementary experiment shown in Fig. 12? Why MCF-7 data show only effect of KDM2A knock down not the KDM2B knock down? Graphs in figures 11 and 12 are lacking Y axis labels so it is not clear what these graphs show (arbitrary units?). More experiments like coIP of KDM2A/2B with β -catenin or colocalization with β -catenin, or CHIP on Wnt target gene promoters, should be performed to prove that KDM2A/2B are operating on promoters of Wnt target genes.

Fifth, literature shows that histone demethylases KDM2A/2B also demethylate lysine on β -catenin which inactivates it and so inhibits Wnt target gene activation. This is described as a main mechanism of action of KDM2A on Wnt target genes. Since this option is not possible for KDM2A/2B-SF the author should explain more why it is worth to look if short isoforms repress the Wnt signalling. Also, mechanism of KDM2A/2B repression of Wnt target genes is not properly discussed. Only one possible explanation is mentioned and that is the inhibition through interaction with Tcf3 (TCF7L1),

know Wnt signalling repressor. This section should be more elaborative, and the author should bring up more than one hypothesis, including the description of experiments that would test it, for the defence.

Discussion of the thesis is, like the introduction, very thorough and touches basically all the subjects that should be covered. Nevertheless, as mentioned above, some parts should have been described in more detail.

I have several more questions for the defence:

Are there any KDM2A/2B knock out mice? What is their phenotype?

How may be the expression of KDM2A/2B-SFs regulated? What may drive it? Are the publicly available data for KDM2A expression distinguishing the isoforms?

Since KDM2A/2B are paralogues complementing each other, how would cells react if both proteins would be downregulated simultaneously?

Do KDM2A/2B create some protein complex with other chromatin remodelling enzymes?

Since hypoxia regulates expression of KDM2A, and somatic stem cells are known for living in hypoxia, are there any data that would show that KDM2A is important for stem cells?

Dijana Lađinović presented a thesis in which she well describes an alternative isoform of KDM2A histone deacetylase. She recognizes that the protein is produced by alternative start site and not by alternative splicing, she identifies translations start codon and nuclear pattern of the protein, which is different from full length protein and dependent on heterochromatin protein 1. This part is novel and it definitely deserved to be published. On the contrary, the part related to regulation of Wnt signalling target genes would require more data to support the statements. In summary, work of Dijana Lađinović highlights importance of distinguishing protein isoforms as functions and/or localizations of these variants may be very different. Despite all the above mentioned concerns, the presented work holds all the formal and informative qualities of a dissertation thesis therefore I recommend, if successfully defended, to grant MSc. Dijana Lađinović a PhD degree.

In Brno 11.11.2019



Mgr. Bohumil Fafílek PhD.