Opponents' evaluation

Diploma thesis: The identification of novel MT1-MMP interaction partners

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The diploma thesis of Kateryna Turchyna is aiming on identification and confirmation of novel interaction partners of MT1-MMP. The experimental part is principally divided into identification of new binding partners using unbiased mass spektrometry (MS) approach and subsequent validation phase where the author tried to validate the interaction of MT1-MMP with desmoglein 2 (DSG2) as one of the candidate proteins.

The thesis is written in good english and is divided into standard sections. In the Introduction the author briefly discuss the invasivenees and metastasis formation, and the key focus is on MT1-MMP. Kateryna provides well rounded overview of the function of MT1-MMP in cellular biology and in the context of cancer. She is discussing the structure, isoforms and the C-terminal part and its binding partners in more details as it is crucial for the rest of the thesis. Information in the introduction is properly cited and images and illustrations complement well the written text.

Following short section is dedicated to description of the aims in a concise yet suffcient manner.

Materials and Methods section contains all the necessary information, with detailed protocols that would allow anybody to repeat the experiments. The section is structured well in logical divisions describing protocols for mammalian cells, bacterias, proteomics and molecular biology.

Results section is divided into experiments aiming on unbiased identification of novel MT1-MMP substrates and section providing overview of the approaches that were employed to validate one of the putative binding partners – desmoglein 2.

In the first part the author described two approaches, first AviTag use that did not end up being successfull and then troubleshooting strategy of using BioID2 approach that was finally used for MT1-MMP purification and subsequent MS analysis. MS analysis was performer using wild type

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and C-terminally truncated MT1-MMP as a negative control to facilitate the specificity of the pull down.

Second, more extensive part is focused on efforts to validate the interaction of MT1-MMP with a putative substate identified from MS – desmoglein 2. Validation part has proven to be much more complex and the interaction between DSG2 and MT1-MMP was not proven by the conducted experiments. Kateryna is describing multiple approaches that were undertaken to co-precipitate the two putative interacting partners however none of them yielded conclusive results.

The results and troubleshooting strategies are comprehensivelly discussed in the Discussion section, however in the first part there is a lot of information that could have been included in the introduction instead.

Comments:

1. There is one major technical weakness throughout the work and that is a complete absence of positive controls for co-immunoprecipitation experiments. Kateryna conducted a lot of immunoprecipitations but she never used any known MT1-MMP interactor to verify the technical performance of the assay. Thus it's very difficult to interpret whether MT1-MMP doesn't interact with DSG2 or the experiments did not work properly.
2. At least half of the western blot images are not labeled properly because there are only membranes on the black background. I am not sure what happened, maybe in the process of formatting.
3. Some of the GAPH loading controls are quite poor – e.g. Figure 37.

Questions:

1. What was the reasoning for using HT1080 cell line for the experiments?
2. Could you discuss what are the key advantages of using AviTag for pull downs? To me it seems that this approach was adding an unnecessary step. I completely agree with BioID2 appraoch that was used subsequently.
3. On the figure 28 (images of cells transfected with MT1-MMP variants) there are three variants (L-WT, L-LLY/AAA and L-C574A) that show very different cellular shape. Was it a general trend and majority of the cells looked like that or it is just the choice of the cell?
4. What is the phenotype of MT1-MMP knock out cells and how does it compare to the phenotype of MT1-MMP with C-terminal deletion and other mutations in the region?
5. Initial MS experiments with wt and C-terminal deleted MT1-MMP identified DSG2 as a potential interactor, which would indicate that C-terminal intracellular part is responsible for interaction. However, then complementary experiments (cell surface protein crosslinking with DMP) from your colleagues also indicated interaction of MT1-MMP and

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DSG2. Moreover, your *in silico* analysis of cleavage sites suggest that MT1-MMP cleaves DSG2. Could you comment on this discrepancy – one experiment shows that C-terminal domain is responsible for interaction, and other shows that actually the interaction happens outside of the cell and DSG2 might be cleaved by MT1-MMP in the extracellular space.

Opponents‘ conclusion:

Diploma thesis of Kateryna Turchyna represents an interesting work in the field of cellular biology. It is evident that the author put a lot of effort and dedication into the experiments to overcome challenges and obstacles. Although there is no definite answer if MT1-MMP interacts with DSG2, this work provides a good basis to continue studying this interaction. Moreover, it's clear that Kateryna learned a lot of methods that would be useful in case she decides to continue in scientific or biotechnology field.

Overall I think this diploma thesis fulfills the criteria and I recommend it for the defence

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