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EVALUATION OF DOCTORAL DISSERTATION

M.Sc. Kishor Gawade

“FUS-dependent processing of snoRNAs into sdRNAs and regulation of ribosomal RNA modifications: implications in Amyotrophic Lateral Sclerosis (ALS)”

made

at the Faculty of Biology Adam Mickiewicz University in Poznan, Institute of Molecular Biology and Biotechnology (IBMiB) in the Department of Gene Expression and Laboratory of RNA Processing under the supervision of prof. AMU, dr. habil. Katarzyna Dorota Raczyńska.

The review was prepared at the request of the Senat of Adam Mickiewicz University from June 28 2021 and the Scientific Council of the Biological Sciences discipline from July 11 2023.

1. Formal characteristics of the dissertation

Doctoral dissertation submitted for a review by M.Sc. Kishor Gawade entitled "FUS-dependent processing of snoRNAs into sdRNAs and regulation of ribosomal RNA modifications: implications in Amyotrophic Lateral Sclerosis (ALS)” is a compilation of two articles published in 2023 and unpublished results. One article included in the doctoral dissertation is an experimental work published in *Scientific Reports*, the second one - a review article published in *Wires RNA*. The PhD student is the first author of both publications. M. Sc. Gawade also included in the thesis the information about the funding of the research

The doctoral dissertation of Kishor Gawade is structured in a slightly different form than the standard dissertation. The thesis includes the typical and necessary chapters, such as: Summary in Polish and English, Introduction and List of cited literature. The description of

obtained results remains the core of the dissertation. The Introduction part is well-considered and is written carefully based on very good literature sources. This chapter is written in a coherent style which perfectly introduces the reader to the presented work. The only part which could be improved in the introduction are the drawings of a better quality.

The dissertation also includes the required publications and the corresponding declaration of co-authorship with a specified percentage contribution of each author in the presented work.

Precise statements regarding all other co-authors' contributions in given publications show that the doctoral student's contribution to the research presented in the thesis and its design - as a comprehensive approach to the issue of the understanding of the FUS-dependent processing of snoRNA into the small RNAs (sdRNAs) and regulation of rRNA modifications in ALS is fundamental and far exceeds the contribution of other co-authors. Therefore, the presented doctoral thesis can be considered as the author's individual work as a PhD student.

2. Subject and objectives of the dissertation and the research methodology

In the work, using high-throughput sequencing, it was identified that FUS regulates snoRNAs in SH-SY5Y (neuroblastoma) cell line. Since snoRNAs are involved in guiding rRNA and snRNA modifications, quantitative, next-generation sequencing (NGS)-based techniques, RiboMeth-seq and HydraPsiSeq were used to map changes in 2'-O-Me and pseudouridine levels in wild-type and FUS-depleted cells (FUS KO). Many fractionally modified 2'-O-Me sites on ribosomal RNAs showed a higher proportion of modification in FUS-depleted cells, and a subset of guide C/D box snoRNAs were also upregulated. Furthermore, pseudouridine changes in the FUS-depleted cells were subtle, but an overall increase in the modification of rRNAs was noticeable, along with changes in guide H/ACA box snoRNAs. Next, SH-SY5Y cells carrying ALS-associated FUS R495X mutation that lack an NLS also displayed significant changes in snoRNAs and 2'-O-Me and pseudouridine levels compared to wild-type control. In addition, ALS-patient-derived fibroblasts with FUS mutations and age-sex-matched controls were used to explore if 2'-O-Me changes are also observed in ALS patients with FUS mutations. As expected, fibroblasts carrying 'strong' FUS P525L mutation displayed the highest number of significantly changed 2'-O-Me sites, whereas 'mild' FUS mutations R521C and R521L displayed fewer sites. These results were complemented by 2'-O-Me data from an isogenic pair of induced pluripotent stem cells, neural progenitor cells and motor neurons carrying FUS P525L mutation. Interestingly, most of the 2'-O-Me and pseudouridine sites mapped to the outer periphery of the 80S ribosome, suggesting that

depending on their modification levels, these fractionally modified sites may regulate the binding of ribosomal proteins or other factors.

I am convinced, the goal of the dissertation has been achieved. However, I hesitate whether the main goal of the research has been precisely defined in the dissertation. Instead, the few detailed questions were formulated. Based on that and the further data one can assume that the main idea of the project has been achieved.

The PhD student conducted his research in the following steps:

1. identification of the expression profiles in the wild-type and FUS knockout cells;

2. establishing the modification pattern of the rRNA in the ALS cell lines;

3.confirmation of the snoRNA processing into short RNA- sdRNAs in FUS-dependent manner in neuroblastoma cell lines.

To achieve the assumed research goals, the PhD student used a number of techniques that, in my opinion, were appropriate for their implementation. Partially, they have been described in great detail in the proper chapter of "Materials and methods" which constitutes the basis of the doctoral dissertation.

However, in this point, I have to mention, that in terms of the unpublished results, the corresponding “Materials and methods” sections would have to be describe more in details.

While the section devoted to the description of the published data contains the appropriate information, the sections containing the unpublished results, in my opinion, is too superficial. It is not written neither like the manuscript nor like the full description of the standard dissertation. This, unfortunately, I found as a weak point of the thesis.

What is also missing in the dissertation, is the conclusion part. Since the work, in a great majority is a bioinformatical study, I think, it would be good to conclude obtained data, to show also the further research directions. Also, in the biomedical context, it would be also good to outline the significance and the further perspectives of the obtained results.

3. The most important results obtained during the work

The dissertation presents the results of many extensive well-planned works.

The most important results obtained as a result of the work, in my opinion, are:

1. FUS has an impact on the snoRNA profile both in the proliferating and differentiated cell states;

2. differentially expressed snoRNAs have a significant downstream effect on ribosomal RNA modifications. As captured by the Ribo-Meth-seq and HydraPsiSeq the snoRNAs have a special impact on the 2’-O-ribose methylation (2’- O-Me) and pseudouridylation;

3. snoRNA can be processed into sdRNAs in a FUS-dependent manner in neuroblastoma cells under proliferating and differentiated conditions;

4. the obtained FUS-knock down cell lines can be used for a further research of any RNAs, where FUS protein can have a biological meaning, such as e.g. circRNAs.

4. Significance of the results obtained

The works presented as part of the doctoral dissertation and the research performed by the PhD student and the research team are a valuable contribution showing the functional and potential clinical importance of snoRNAs and sdRNAs in ALS and neuroblastoma.

FUS is a DNA/RNA binding protein involved in many aspects of RNA metabolism. Moreover, mutations within the nuclear localization signal (NLS) of FUS result in the mislocalization of this protein into the cytoplasm, resulting in the formation of cytoplasmic aggregates, and it is associated with amyotrophic lateral sclerosis, a neurodegenerative disease. snoRNAs guide site-specific 2'-O-methylation (2'-O-Me) and pseudouridylation of ribosomal rRNAs and snRNAs. These epitranscriptomic modifications provide stability and maintain the structural fidelity of the ribosomes. Additionally, in the contrary to the previous belief, about two-thirds of these sites on the rRNA are fractionally modified; this provides another layer of generating ribosomal heterogeneity. Not limited to only guiding rRNA and snRNA modifications, both C/D and H/ACA box types of snoRNAs can be moreover processed into smaller, stable fragments called sdRNAs. These sdRNAs may function as miRNAs and regulate gene expression at transcriptional and translational levels. Moreover, the role of FUS in the biogenesis of microRNAs is known and well documented, but its role in regulating snoRNA expression and processing into sdRNAs is not explored.

Taken this into account, the data obtained within the doctoral project, provide the new layer of understanding of the potential functional impact of the snoRNAs and sdRNAs in neuroblastoma and ALS, especially in the context of FUS function. The identification of the FUS-dependent changes within the expression profile of sno-RNA, sdRNAs and the rRNA modifications indeed opens a great avenue for a further research.

5. Questions and comments

1. The most important question is: how would you specify the main goal of you research, taking into account the data presented in the thesis?

2. Please, explain why did you use the given cell lines? What justify the comparison od the HEK cells, neuroblastoma and ALS cell lines? What will be the link between all of these analyses?

3. One of the questions asked within the research was: "Are there quantifiable changes in 2'-O-Me in ALS patient-derived fibroblasts compared to age-sex-matched control samples?". I would like to ask- how the samples were collected and matched?

4. The authors showed the changes in the 2'-O-Me position in WT and FUS P525L iPSCs, smNPCs and motor neurons. They noticed the changes within the cells, but in the contrary to the previous expectations. I'm wondering, if there is possible that the changes observed in motor neurons can be related to the progressive lost of the cells? And the question also is- how the changes of the 2'-O-Me level in one type of the cells, can be impacted by the others cells' type?

5. The presented results show that within the cell differentiation protocol there is snoRNA can be processed into sdRNA. M.Sc Gawade concludes, that the retinoic acid impacts the processing within the protocol as the external cue. I am wondering, whether the observed changes are the response of the external stimuli or these changes are caused by the downstream effects of the differentiation events? The question arises, whether some other external cues could impact also the snoRNA processing? I would like to ask for some comment on that issue.

6. Mr. Gawade discussed the potential function of sdRNAs based on the similarity to miRNAs. Are there some data showing the functional sdRNAs working within the different mechanism than miRNAs? If not, could you speculate what, in your opinion, could be the other possible mechanism/mechanisms of sdRNAs' action?

6. The final conclusion

W mojej ocenie przedłożona do recenzji rozprawa doktorska M.Sc. Kishor Gawade spełnia wszystkie warunki stawiane kandydatom w Ustawie – Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2020 r. poz.85 z późniejszymi zmianami). Zwracam zatem się do Rady Naukowej dyscypliny nauki biologiczne UAM w Poznaniu o dopuszczenie mgr Kishora Gawade do dalszych etapów przewodu doktorskiego.

dr habil. Katarzyna Rolle, prof. ICHB PAN

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