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Review of Ankur Gadgil's PhD Dissertation entitled "The effect of ALS-associated FUS mutations on U7 snRNP activity and the expression of core canonical histone gene in neuronal cells"

Ankur Gadgil PhD thesis was prepared in the Laboratory of RNA Processing, Department of Gene Expression, Institute of Molecular Biology and Biotechnology at Faculty of Biology of Adam Mickiewicz University in Poznan under the supervision of dr hab. Katarzyna Dorota Raczyńska, prof. UAM.

The scientific aim of the work was to investigate the activity of U7 snRNP in the context of ALS-associated FUS mutations. This is a consequent continuation of research in the Laboratory of RNA Processing, and previous findings about FUS protein interaction with U7 snRNP. FUS is mainly nuclear protein involved in DNA repair and various RNA processing events. Its interaction with U7 snRNP is involved in regulation of expression of replication-dependent histone (*RDH*) genes, by modulating their transcription as well as 3'end maturation of their transcripts.

Mutations in *FUS* gene are causing familiar amyotrophic lateral sclerosis (ALS) that is neurodegenerative disorder involving progressive loss of motor neurons. The studies showing that mutations in *FUS* can cause ALS were published not so long ago, in 2009, therefore large scientific effort is still needed to describe specific molecular disturbances for this disease. The majority of ALS-associated mutations in *FUS* are affecting nuclear localization signal (NLS) of the protein and specific disturbances in molecular processes are resulting from mislocalisation of FUS.

Dissertation is presented as a collection of published and thematically related scientific articles. Doctoral thesis is based on two publications and also additional results from short term research stay are included. In both articles Ankur Gadgil is the first author and his contribution is described as a relevant for first authorship, as well as appropriate authorship statements are attached. It is also important to mention additional achievements received by Ankur Gadgil, including EMBO Scientific Exchange Grant and that his research was conducted in collaboration with two European teams, from King's College in London and University of Eastern Finland.

In the first part of Dissertation PhD Candidate presented 10 pages of description entitled "Main thesis including results and summary". The aims of the study are presented concisely and clearly. "Background

and introduction" section contains a summary of major information regarding ALS, FUS interactions with U7 snRNP, and FUS mutations associated with ALS. In the next two subchapters, published results are summarized. In the final subchapter, results from additional research stay of PhD Candidate are presented.

In the experimental study, published in 2021 in Scientific Reports, the hypothesis was that together with mutant FUS mislocalisation, also U7 snRNP is mislocalised. One of the expected consequences would be deregulation of expression of *RDH* genes, that could significantly affect neuronal cell functioning.

PhD Candidate performed the majority of the experiments for this study, using various molecular biology techniques and cell culture models. First, for the purpose of the study FUS knock-out cell lines were generated – to provide cellular background for overexpression of different FUS variants. These were mainly SH-SY5Y cells, that were also differentiated to neuron-like cells. In the performed experiments, the co-localisation of mutant FUS (tested in two variants) was observed with U7 snRNA, as well as with one of the components of U7 snRNP, Lsm11. Next, deregulation of *RDH* genes expression was shown, due to lowering efficiency of their transcripts processing, as well as inhibition of *RDH* genes transcription.

I would be grateful for providing additional comments concerning this study, during defense of the thesis:

- As expression of *RDH* genes is crucial at cell cycle of dividing cells, how these disruptions could affect motor neurons, mainly degenerating in ALS? An option that other cell types (glia) could be directly affected by deregulation of *RDH* genes in ALS (and this would indirectly affect neurons) was briefly mentioned and could be elaborated more.
- Is the mislocalisation of U7 snRNP potentially reversible in case of potential therapy delivering functional FUS? Is such approach feasible and whether this should at least work for the restoration of U7 snRNP interaction and activity? It would be valuable for discussion to comment autoregulation of FUS in this context – generally overexpression of FUS has adverse effect, unless the transgene has autoregulation functions restored (e.g. based on Sanjuan-Ruiz et al. Molecular Neurodegeneration 2021).
- Would the use of stress conditions in cell cultures enhance the observed effects in this study?
- In the description of experiments performed, there is a comment that using iPSCs derived from ALS-FUS patient was not successful approach. Is it possible that using neuronal cells differentiated from iPSCs could provide some significant effect for investigating U7 snRNP-FUS interaction?
- According to some reports, for ALS phenotype FUS mislocalisation is not a critical/indispensable point. There are studies indicating that mutant FUS nuclear gain-of-function mechanism can be determinant for pathogenesis, due to FUS role as important component of nuclear paraspeckles.

Are the effects of deregulation of *RDH* genes expression still expected if other FUS mutants were used, the ones that do not cause clear FUS mislocalisation?

- Finally, what is the current understanding of ALS caused by FUS mutation? Is there a dominant pathogenic effect coming from gain of aberrant interactions FUS in cytoplasm, or rather from a loss of interactions in nucleus?

Second article, is the comprehensive review of the knowledge and results concerning the use of U7 snRNA as a tool for gene therapy. In this publication extensive information was provided related to U7 snRNA and U7 snRNP and their role in the processing of transcripts of histone genes. Then, modifications in U7 snRNA that can lead to its therapeutic use are indicated – mainly concerning modification of Sm binding site in U7 snRNA. A major part of this review is the description of the examples of the use of U7 snRNP in gene therapy. Prepared table is valuable for very consistent and clear presentation of gathered information for different disorders. Publication also contains discussion of described results, as well as assessing "Limitations and benefits" for therapeutic use of U7 snRNA.

Unpublished results presented briefly in PhD Thesis are related to interesting project, focused on FUS role in prostate cancer. It is altogether an interesting point to study potential FUS roles in somehow opposite cell types: non-dividing neuronal cells (affected in ALS) and cells dividing too much (in case of cancer). Regarding some interplay between ALS- and cancer-related projects, was the proliferation rate also investigated in SHSY-5Y cells (FUS KO and overexpressing FUS mutants)?

As both publications were published in the first half of 2021, in "Summary" section it would be interesting to provide more broad comment on the results obtained, for example: how they impact on and correlate with general understanding of molecular disturbances in FUS mutation-caused ALS? During the defense of PhD thesis I would be grateful for commenting some new findings in the field and how they correspond to the results presented in this Dissertation. Also it would be interesting to point out next crucial steps in the research that can be continued based on the results described in Dissertation.

Based on the novelty of the findings and ability to independently conduct scientific work by Ankur Gadgil, I conclude that the presented dissertation fulfils the requirements for PhD thesis. Publication of presented results in Scientific Reports additionally confirms the quality and originality of the findings. The presented dissertation also fulfils the statutory requirements necessary to obtain PhD degree (Prawo o Szkolnictwie Wyższym i Nauce, Art. 187, Dz.U.2022.574). Without any doubts, I recommend to Scientific Council of the Discipline of Biological Sciences at Adam Mickiewicz University to admit Ankur Gadgil to further stages of the doctoral procedure.

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