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**REVIEW**

**Ph.D. thesis by Ankur Gadgil**

**"The effect of ALS-associated FUS mutations on U7 snRNP activity and expression of core canonical histone genes in neuronal cells".**

The Ph.D. thesis was performed in the Laboratory of RNA Processing, Department of Gene Expression, Faculty of Biology at Adam Mickiewicz University (AMU) in Poznan, and was supervised by dr. hab. Katarzyna Dorota Raczynska, prof. AMU.

The main part of the thesis is two articles (listed below), both published in well-recognized international journals indexed in Journal Citation Reports (Clarivate).

1) Gadgil A, Walczak A, Stępień A, Mechtersheimer J, Nishimura AL, Shaw CE, Ruepp MD, Raczyńska KD.
*ALS-linked FUS mutants affect the localization of U7 snRNP and replication-dependent histone gene expression in human cells.*
**Scientific Reports (SPRINGER NATURE)** 2021 11: 11868.

2. Gadgil A, Raczyńska KD.
*U7 snRNA: A tool for gene therapy.*
**Journal of Gene Medicine (WILEY)** 2021 23: e3321.

The first article is a research experimental article about the effect of amyotrophic lateral sclerosis (ALS)-associated *FUS* mutations on the mislocalization of U7 small nuclear ribonucleoprotein (U7 snRNP) in cell and its further disease-related consequences. The second article is a comprehensive review article about the biological function of U7 snRNA and its use as a tool in molecular therapies for different diseases. The main element that spans both articles is U7 snRNA, a small nuclear non-coding RNA that is a key component of the U7 snRNP complex, playing an important role in the processing and maturation of

histone mRNAs. Generally, the thesis combines interesting elements of basic biology (maturation of histone mRNAs) with aspects that may have practical implications, such as the explanation of the pathological mechanism of ALS-associated mutations, and technical considerations on the U7 snRNA-based molecular therapeutic approaches.

The study is well rationalized by the previous study of the supervisor dr. Raczynska, who demonstrated the role of the FUS protein in the regulation of histone genes replication/expression via interaction with U7 snRNP (Raczynska KD, Nucl Acids Res 2015).

In both articles, Ankur Gadgil was the first author and according to the letters of co-authors and the author contributions notes, included in the articles, Ankur Gadgil contributed substantially to both papers and was the leading author regarding the experimental analyses in the first article and preparation of the manuscript in the second article. In both cases under the supervision of dr. Dorota Raczynska, who was the senior/corresponding author.

The main achievement of the study described in the first article is the observation that the FUS mutants form cytoplasmic aggregates sequestering U7 snRNA together with proteins constituting the U7 snRNP complex. Consequently, U7 snRNA/RNP is mislocalized in the cytoplasm (instead in the nucleus). The decreased level of functional FUS and U7 snRNP in the nucleus results in decreased transcription efficiency and expression (mRNA level) of histone genes and affects U7 snRNP-mediated histone pre-mRNAs processing (usually elongating the mature transcripts). The experiments were performed *in vitro* in 3 cellular models, i.e., HeLa cells, neuron-like SH-SY5Y cells, and rat primary brain neurons, transfected with appropriate vectors with either the wild-type or mutants *FUS* gene. The experiments were also performed in iPSCs derived from ALS patients with the R514G FUS mutation, however, the results were not conclusive and were not included in the article.

As mentioned above the second article is a comprehensive review of current knowledge and up-to-date studies describing the biological function of U7 snRNA/RNP and the use of its engineered version, termed U7 smOPT, in splicing-correction therapies for different human diseases, including myotonic dystrophy type 1 (DM1), Duchenne muscular dystrophy (DMD), ALS, HIV, and many others. The most important examples of U7 snRNA-based therapies are described in detail, others are listed and shortly characterized in a large comprehensive table (Table 1). An important element of this article is a discussion of the limitations of U7 snRNA-based therapies. In my opinion, this part of the article is very comprehensive, honest, and mature, confirming the maturity and knowledge of the authors.

Both articles are very interesting, I enjoyed reading them, I had no comprehension problems and I learned a lot from them.

I do not have any serious comments on the studies/articles, maybe except for the border-line visible small fonts in some of the figures, and the lack of p-values indicated in figure S3 in the first article (no need for addressing these minor comments during the presentation).

Nevertheless, I have one question that I would like to be answered during the thesis defense. One of the mutations tested in the study is the nonsense mutation R495X. As a nonsense mutation, it may potentially induce nonsense-mediated decay (NMD) process and thus may lead to complete loss of the FUS protein. If so, is it still possible that FUS will form cellular aggregates? Are there some other ALS-associated deleterious (nonsense or frameshift) mutations in *FUS* that may induce NMD? If yes, is the phenotype of ALS patients with deleterious *FUS* mutation different from those with missense mutations?

Except for the above-mentioned articles, the thesis is supplemented by the abstracts in Polish and English, a summary of the study (including unpublished experiments), and several formal elements of the thesis, including Ph.D.-candidate's and other coauthors' letters confirming the leading role of Ankur Gadgil in the articles consisting the dissertation. I do not have any comments on these parts.

To summarize, I am convinced that Ankur Gadgil deserves and meets all the requirements to receive a Ph.D. degree. Therefore, I urge the Council of the Faculty of Biology of Adam Mickiewicz University (AMU) in Poznań to grant the Ph.D.-candidate access to further stages of the procedure. Also, based on the arguments mentioned above and the fact that the work of Ankur Gadgil was published in two articles in well-recognized journals of which the Ph.D.-candidate is the first author, I strongly recommend the Faculty of Biology AMU to honor the Ph.D. thesis with appropriate distinction and/or award.

Piotr Kozłowski