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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Anna Fefilova

PhD Program: Life Sciences

Title of Thesis: Functional study of human and murine morrbid lncRNA *in vitro*

Supervisor: Associate Professor Timofei Zatsepin

Name of the Reviewer: Konstantin Lukyanov

I confirm the absence of any conflict of interest	**Signature:**  **Date: 15-11-2020**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

The summary of issues to be addressed before/during the thesis defense

Long non-coding RNAs (lncRNAs) are known to be key players in regulation of wide variety of cellular processes. The present thesis by Anna Fefilova investigates the role of human and murine CYTOR/Morrbid lncRNAs using cancer hepatocyte cell lines model.

Using CRISPR-Cas9 genome editing, Anna for the first time generated a human hepatocyte cell line with knockout of both CYTOR and hMorrbid genes. This cell line allowed to study consequences of complete elimination of these lncRNAs in detail including assessment of cell proliferation, migration and apoptosis, proteomic changes, and quantification expression of selected target mRNAs. Overall, these extensive experiments showed low differences between the wild type and knockout cells; most of the previously observed effects of RNAi-induced knockdown of hMorrbid/CYTOR were not confirmed. Anna's data call for revisiting the published roles of these lncRNAs.

To further elucidate functions of hMorrbid/CYTOR, overexpression strategy was applied. One particular variant of hMorrbid/CYTOR-encoding transcript (so called M-217) was selected, since it contains an evolutionary conserved fragment (exonCh) and its expression in different cell lines was confirmed experimentally. It was found that M-217 overexpression results in significant delay in proliferation and increase in apoptosis. Moreover, M-217-overexpressed human hepatocyte cells demonstrated elevated levels of pro-apoptotic proteins.

In addition, murine ortholog of Morrbid lncRNA (mMorrbid) was studied. mMorrbid was found to be downregulated in murine cancer hepatocytes Hepa1-6 compared to normal hepatocytes AML12. Interestingly, an antisense oligonucleotide-induced knockdown of mMorrbid resulted in strongly decreased viability and migration rate of the normal but not the cancerous hepatocytes. Downregulation of mMorrbid led to upregulation of pro-apoptotic factors; changes in expression of hundreds of genes were detected by RNA-seq and protein mass-spectrometry including those involved in signal transduction pathways, apoptosis, peroxisome and mitochondrial metabolisms, and splicing.

Regulation of NRAS oncogene (which is involved in the regulation of cell proliferation and migration) by mMorrbid was studied in detail. It was demonstrated that downregulation of mMorrbid changes alternative splicing and results in incorporation of a cassette alternative exon with a premature stop codon into NRAS mRNA. This leads to degradation of such NRAS transcripts by NMD machinery. Anna then for the first time showed that effects of mMorrbid on alternative splicing is due to direct interactions of mMorrbid with SFPQ-NONO splicing complex.

To summarize, this extensive and solid work provides significant new insights into functioning of Morrbid lncRNAs in human and murine cells. Using knockout, knockdown and overexpression approaches, Anna studied possible functions of Morrbid lncRNA in cell proliferation and apoptosis. Detailed molecular analyses allowed to proposed new mechanisms of action of this lncRNA based on direct interaction with a splicing complex that alters alternative splicing coupled with the NMD pathway. The results are published in the International Journal of Molecular Sciences (IF 4.5) with the first authorship of Anna. Second paper is published in the Molecular Therapy: Nucleic Acids (IF 7.0).

I have only a few minor remarks:

Page 110: “CYTOR is a sense lncRNA and hMorrbid is an antisense lncRNA.” Please clarify what you mean for “sense” and “antisense” terms here. It is just opposite orientation of CYTOR and hMorrbid in the chromosome relatively to each other? Or is it a difference in their positions relatively to overlapping protein-coding genes in corresponding loci (as proposed in: Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA Biol. 2013, 10, 925-933)?

Figs. S1 and S2: Please provide description of the panels A, B, C in the legend. Also, resolution of the pictures is not sufficient to see some words and numbers.

Ref. 253: A misprint in the paper title (“eleme”).

Provisional Recommendation

☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defense*

☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate’s thesis according to the recommendations of the present report*

☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*