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Reviewer A

Rakhmetullina and colleagues describe the potential target regulation of maize and wheat miRNAs within the human transcriptome. Using miRTarget software they evaluate where the binding site occurs and generate several energy bidding parameters of each interaction and discuss their potential implication in human health. The concept of cross-kingdom regulation is a hot topic in miRNA and diet research with many very well-conducted for and against studies. The manuscript, as here presented, is based only on predictive models and lack of novelty without any single experimental validation. Moreover, it is very skewed toward positive potential effects. There are several experimental studies against that belief that has not been considered in this version of the manuscript.

Reply

We would like to thank the reviewer for careful and thorough reading of this manuscript and for the thoughtful comments and suggestions.

The miRTarget program use physicochemical characteristics to evaluate the interactions of nucleotides. For a better understanding, we offer an article (Yurikova, O.Yu., et al. The Interaction of miRNA-5p and miRNA-3p with the mRNAs of Orthologous Genes. Mol Biol 53, 612–623 (2019). <https://doi.org/10.1134/S0026893319040174>), which clearly shows the adequacy of calculation results with experimental data. None of our many publications has been questioned in determination of quantitative characteristics of miRNA-mRNA interactions (Bari A, Orazova S, Ivashchenko A. MiR156- and miR171-binding sites in the protein-coding sequences of several plant genes. Biomed Res Int 2013;2013:1-7; Bari AA, Sagaidak AI, Pinskiy IV, et al. Binding of miR396 to mRNA of genes encoding growth-regulating transcription factors of plants. Russ J Plant Physiol 2014;61:807-10; Atambayeva S, Niyazova R, Ivashchenko A, et al. The binding sites of miR-619-5p in the mRNAs of human and orthologous genes. BMC Genomics 2017;18; Myrzabekova M, Labeit S, Niyazova R, et al. Identification of bovine miRNAs with the potential to affect human gene expression. Front Genet 2021;12:705350).

Our predictions of the interaction of miRNA and mRNA nucleotides have been confirmed experimentally (miRNA156a binding described in: Tian Y, Cai L, Tian Y, Tu Y, Qiu H, Xie G, Huang D, Zheng R, Zhang W. miR156a Mimic Represses the Epithelial-Mesenchymal Transition of Human Nasopharyngeal Cancer Cells by Targeting Junctional Adhesion Molecule A. PLoS One. 2016 Jun 24;11(6):e0157686. doi: 10.1371/journal.pone.0157686. PMID: 27341697; PMCID: PMC4920421), cited in our manuscript as ref 49.

Note that there are no biochemical methods for determining the interaction of miRNA and mRNA nucleotides with the determination of specific physicochemical characteristics of their interaction. We believe that in order to examine the possible effect of plant miRNAs on humans, we first need to know whether these miRNAs can bind to mRNAs of human genes. This is an independent and primary problem that needs to be solved. Then, it will be crucial to determine which biological processes in humans can be affected by plant miRNAs. Therefore, we believe our contribution is a necessary step for the future research on the subject.

The reviewer notes the lack of novelty in the manuscript. We are sorry to hear that. We do believe that understanding , of even putative, interactions of edible plants with human body, and in particular with human genome is very interesting. From this point of view the research described in our manuscript is relevant and the novelty element is the full list of such possible interactions for two plants and the relation of the results to human diseases.

Specific comments

Comment 1: The title is misleading and should indicate that this is a bioinformatic prediction approach or something similar (i.e., in silico analysis, etc). That is to say, readers must know from reading the title that there is nothing experimentally validated here.

Reply 1: We have modified the title as advised: “*In Silico* analysis of Maize and Wheat miRNAs as Potential

Regulators of Human Genes”

Changes in the text: see Page 1, lines 2,3

Comment 2:

Abstract should be modified accordingly and indicate that the study is computational. Also, a phrase like “experimental validation of these results are guaranteed or are necessarily” could be included in the final part of the abstract.

Reply 2: We have changed the abstract and added a phrase as advised: “As a result, in order to use identified wheat and maize miRNAs in medicine, experimental validation in combination with our in silico studies will be beneficial to understand the impact of these miRNAs on changes in human gene expression levels”

Changes in the text: see Page 1, lines 35-38

Comment 3:

“The miRNAs from maize and wheat have effective BSs in the mRNA of human genes” must be changed to “might have” or “have potential to” etc. Please reduce the tone throughout the manuscript.

Reply 3: High complementarity of miRNA interaction with mRNA has been shown for many human genes. (Yurikova OY, Aisina DE, Niyazova RE, et al. The Interaction of tae-miRNA-5p and miRNA-3p with the mRNAs of Orthologous Genes; Myrzabekova M, Labeit S, Niyazova R, et al. Identification of bovine miRNAs with the potential to affect human gene expression. *Front Genet*; Atambayeva S, Niyazova R, Ivashchenko A, et al. The binding sites of miR-619-5p in the mRNAs of human and orthologous genes. *BMC Genomics*). We have reduced the tone throughout the manuscript and changed the sentences as advised.

Changes in the text: see Page 1, lines 32,33,42; Page 12, lines 313; 346

Comment 4:

In the results and discussion section authors indicate that there is a high complementarity of plant miRNAs and their target genes. It is important to know that the main function of miRNAs in mammals is through complementary binding of the seed sequence. Of course, in some cases there are complementary binding sites between nucleotide 11-17. However, high complementarity (or complete complementarity) for miRNA-related function is only observed in plant cells. In mammalian cells when a high complementarity is achieved, miRNAs are degraded through different mechanisms including the ubiquitin ligase mediates target-directed microRNA degradation PMID: 33184237 PMID: 33184234. Of course there are examples in the literature that there is repression with high complementarity, but this is not the general case, those are specific examples. Please reduce the tone that a high complementarity is desired.

Reply 4:

The use of seed region as the basis for the search for miRNA target genes has been practiced for more than 30 years. This method is a profound misconception, as a result of which many false positive target genes are found (Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005 Jan 14;120(1):15-20. doi: 10.1016/j.cell.2004.12.035. PMID: 15652477; Broughton JP, Lovci MT, Huang JL, Yeo GW, Pasquinelli AE. Pairing beyond the Seed Supports MicroRNA Targeting Specificity. *Mol Cell*. 2016 Oct 20;64(2):320-333. doi: 10.1016/j.molcel.2016.09.004. Epub 2016 Oct 6. PMID: 27720646; PMCID: PMC5074850; Sheu-Gruttadauria J, Xiao Y, Gebert LF, MacRae IJ. Beyond the seed: structural basis for supplementary microRNA targeting by human Argonaute2. *EMBO J*. 2019 Jul 1;38(13):e101153. doi: 10.15252/embj.2018101153. Epub 2019 Apr 26. PMID: 31268608; PMCID: PMC6600645.), and as a result, not a single reliable method for diagnosing human diseases using this technology has been created over the years. Only correlations between changes in the amount of some miRNAs and the expression of target genes were revealed, which cannot serve as a reliable method of a direct interaction between miRNA and target gene. Incomplete complementarity of miRNA interaction with mRNA inevitably leads to the identification of many false positive target genes. Even the creators of programs using seed region (Betel D, Koppal A, Agius P, Sander C, Leslie C. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol*. 2010;11(8):R90. doi: 10.1186/gb-2010-11-8-r90. Epub 2010 Aug 27. PMID: 20799968; PMCID: PMC2945792.) write about it. High complementarity is necessary for high selectivity of miRNA–mRNA

interaction, and due to this interaction, the uniqueness of the interaction of miRNA with mRNA is achieved. mRNA degradation occurs, not miRNA.

Comment 5:

Include in the discussion section that this in silico analysis is a first step for gene repression regulation and that there are other obstacles and challenges that dietary miRNAs need to surpass to exert a biological effects, including digestion. The literature describes that digestion is an important part of possible function of miRNAs both in mammalian dietary miRNAs PMID: 34716465 and plant-derived miRNAs PMID: 34358423. Transport of dietary miRNAs within exosomes could help reduce the degradation of dietary miRNAs PMID: 36182038.

Reply 5: We have modified the discussion as advised.

Changes in the text: see Page 12, lines 327-332

Comment 6:

Indicate that a validation is required as there are some examples in the literature indicating that dietary miRNAs have no biological effects.

Reply 6:

We do not claim that all plant miRNAs have a biological effect. Of all known maize and wheat miRNAs, miRNAs have been identified that have potential to interact with human mRNA genes. We do understand that experimental verification is necessary in cases where one concentrates on particular miRNA and its interactions, which we show also in our previous contributions e.g.:

Kasarello K, Köhling I, Kosowska A, Pucia K, Lukasik A, Cudnoch-Jedrzejewska A, Paczek L, Zielenkiewicz U, Zielenkiewicz P. The Anti-Inflammatory Effect of Cabbage Leaves Explained by the Influence of bol-miRNA172a on FAN Expression. *Front Pharmacol.* 2022 Mar 24;13:846830. doi: 10.3389/fphar.2022.846830. Our experience (see the above mentioned reference)

shows that computational methods correctly predict interactions of plant miRNAs with human mRNA genes.

Changes in the text: see reference under number 45

Comment 7:

Include a section of limitations of the study. In this section authors should indicate that an experimental validation is needed and that there are several aspects that need to be considered before these finding reach the real biological effect including technological processing (i.e., cooking), GI digestion, absorption, biodistribution and more importantly a minimum amount of miRNA that need to be reached within the cell to exert a biological effect.

Reply 7:

We have included recommended suggestions in the discussion section. The effect of interactions of plant miRNAs and human mRNA genes depend on many factors: for example, on the concentration of miRNAs in cells of various human organs, on the concentration of target genes in the cells, since a low concentration of plant miRNAs cannot significantly change the expression of the target gene.

Changes in the text: see Page 12, lines 333-336

Comment 8:

I would suggest to include references of ExRNA Journal. There are very good manuscript on the topic on 2021 and 2022.

Reply 8: We have included references of ExRNA Journal

Changes in the text: See references under number 27,28,31,33,34,35,59,99.

Reviewer B

1. The link: <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis> in the Main Text shows "Page Not Found". Please check.

Reply: We have changed the link: (<https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa/>) (Page 4, line 159)

2. “A previous **study** showed that the slight reduction in miRNA content that occurs during storage, processing, and preparation of plant-based foods is one factor impacting the entry of plant miRNA into the human body (16,42).”

Singular of study was used, but two references were cited. Please confirm.

Reply: We have modified the sentence (Page 2, line 85)

3. HIV/IPA should be defined upon first use in the Main Text.

Reply: We have defined "HIV/IPA (Page 2, line 63; Page 4, line 158)

4. All genes should be italicized.

Reply: All genes have been italicized

5. Supplementary tables should also be cited consecutively in the text and numbered in the order in which they are discussed.

Reply: we have modified and cited all supplementary tables as Table S1-14 in the order in which they are discussed.

6. In the last page of the supplementary material file, there are a couple of paragraphs. Please confirm whether they are for Table S17.

Reply: we have modified and cited all supplementary tables as Table S1-14 in the Main Text. The couple of paragraphs are now for Table S1.