REVIEW
of the official reviewer of the thesis
Akimniyazova Aigul Nurlankyzy on the title "The interaction of miRNAs with mRNAs of candidate genes of gastrointestinal tract cancer", awarded for the degree of Doctor of Philosophy (Ph.D.) in the speciality "6D074000 - Nanomaterials and Nanotechnology"

№ п/п	Criteria	Compliance with criteria	Justification of the official reviewer's position
1.	The title of the thesis (as of the date of its approval) corresponds to the directions of scientific development and/or state programs	1.1 Relevance to priority areas of scientific development or government programs:	The thesis corresponds only to the priority direction of science development: "Life and Health Science".
		1) The thesis was carried out within the framework of a project or target program financed from the state budget (specify the name and number of the project or program)	The dissertation work was carried out within the framework of the project "Development of test-systems for early diagnosis of cardiovascular, oncological and neurodegenerative diseases based on miRNA associations and their target genes" №AP05132460 MES RK. The thesis underscores the critical importance of cancer research, particularly in colorectal, gastric and esophageal cancers, which remains a significant global health concern. The research conducted aligns closely with the scientific project focused on developing test systems for early disease diagnosis. The integration of findings from the thesis contributes to the broader objectives of the project, emphasizing interdisciplinary collaboration and knowledge exchange.
2.	Importance for science	The work does not make a significant contribution to science and its importance is not well disclosed.	The thesis is dedicated to exploring the potential (virtual) interaction characteristics between miRNA and mRNA genes associated with colorectal, gastric and esophageal cancers development. The primary goal is to identify markers using miRNA and candidate genes, with the intention of developing early diagnosis methods in medicine. However, this is not addressed in the work.
3.	Principle of self-reliance	Level of independence: 2) Medium;	The author's extensive publication record (2 articles in the international journals Q1-Q2 with an impact factor) and personal contributions underscore their dedication to advancing knowledge in the field of cancer research.
4.	Principle of internal unity	4.1 Justification of the relevance of the thesis: 2) Partial justification;	The research findings hold promise for improving diagnostic accuracy and treatment outcomes in clinical practice. But the work does not perform diagnostic studies as stated in the ultimate aim of this study.
		4.2 The content of the thesis reflects the topic of the thesis: 1) Reflects;	Overall, the thesis represents a contribution to cancer research, offering valuable insights into the complex interplay between miRNA and mRNA genes in colorectal, gastric and esophageal cancers development.
		4.3 The aim and objectives are consistent with the thesis topic:	The thesis outlines clear and comprehensive research objectives aimed at understanding the interaction dynamics between miRNA and mRNA genes linked to various forms of

		1) are consistent with;	colorectal, gastric and esophageal cancers. The objectives are logically structured and align closely with the overarching research theme.
		4.4 All sections and statements of the thesis are logically interrelated: 1) fully interconnected;	The dissertation is well-structured, comprising coherent sections that provide a comprehensive overview of the research. The inclusion of supplementary materials enhances the clarity and accessibility of the thesis. The work as a whole is logically presented and complete. I do not see any significant problems in the work and I can say that I like this study as a whole.
		4.5 New solutions (principles, methods) proposed by the author are argued and evaluated in comparison with known solutions: 2) the analysis is partial;	The paper presents the results of a computer-aided search for strings in gene sequences with the miRNA base for humans. The list of genes and miRNA complementary to them was obtained. Further, it was necessary to experimentally validate the relationship between miRNAs and changes in mRNA activity of the studied genes.
5.	Principle of scientific novelty	5.1 Are the scientific results and provisions new? 2) partly new (25-75% are new);	The information presented concerning colorectal, gastric, and esophageal carcinomas is not critical. All described genes, which are allegedly related to carcinogenesis, are basic developmental genes and essential genes. But in organ development, specialized cells acquire their task and under which mRNA composition and its quantitative changes.
		5.2 Are the conclusions of the thesis new? 2) partly new (25-75% are new);	The significance of the obtained results can serve as a starting point for further experimental studies to analyze the found miRNAs in the regulation of gene expression associated with carcinogenesis. A list of miRNAs that could potentially be associated with the regulation of mRNA expression was obtained. However, it was also necessary to study other genes in the human genome with which these miRNAs could potentially interact.
		5.3 Technical, technological, economic or managerial solutions are new and justified: 3) not new (less than 25% are new)	Technologically the project is based only on the software developed in the research group. There are no published works on this software package, no description of the algorithms and no possibility to get acquainted with the code and working files. The article the candidate refers to is very old. There are no modern methods related to cell culture, molecular engineering, and biotechnology which are necessary for such research.
6.	The justification of the main conclusions	All main conclusions are not based on scientifically sound evidence or are sufficiently well-substantiated.	Virtual research without experimental confirmation and practical application cannot be considered complete and fully justified.
7.	Main statements put forward for defense	7.1 Is the statement proven? 1) proven; 7.2 Is the position trivial? 2) no 7.3 Is it novel? 1) yes;	In the work, most of the results are presented in tables and a description of which genes and miRNAs were found to be associated. This is the main result of the work, and it is fulfilled in full for all tasks. All the points put forward for defense have not been proved by experimental results but can be the basis for further experimental analysis.

		7.4 Level for application: 1) narrow; 7.5 Is it proven in the article? 1) yes;	**Statement 1** – Create a database for miRNA nanomolecules and genes involved in the development of gastrointestinal tract cancer. The results are presented as Table C1 - The database of candidate GI tract cancer genes, from Supplementary materials C. **Statement 2** – To study the characteristics of the interaction of miRNA nanomolecules with mRNA genes involved in the development of esophageal cancer. The results of the research are presented in the following paper: piRNAs may regulate expression of candidate genes of esophageal adenocarcinoma Akimniyazova, A.N., Niyazova, T.K., Yurikova, O.Y., ...Zhanuzakov, M.A., Ivashchenko, A.T. ***Frontiers in Genetics***, 2022, 13, 1069637 **Statement 3** – To study the characteristics of the interaction of miRNA nanomolecules with mRNA genes involved in the development of gastric cancer. The results of the research are presented in the following paper: Predicting associations of miRNAs and candidate gastric cancer genes for nanomedicine Akimniyazova, A., Pyrkova, A., Uversky, V., Ivashchenko, A. ***Nanomaterials***, 2021, 11(3), pp. 1–16, 691 **Statement 4** – To study the characteristics of the interaction of miRNA nanomolecules with mRNA genes involved in the development of small intestinal cancer. In the work, most of the results are presented in tables and a description of which genes and miRNAs were found to be associated. **Statement 5** – To study the characteristics of the interaction of miRNA nanomolecules with mRNA genes involved in the development of colorectal cancer. The results of the research are presented in the following paper: miRNA Binding Site Clusters in mRNAs of Colorectal Cancer Candidate Genes Akimniyazova, A.N., Pyrkova, A.Y., Aisina, D.E., Ivashchenko, A.T. ***Nanotechnologies in Russia***, 2020, 15(11-12), pp. 807–818

			Statement 6 – To establish the associations of miRNA nanomolecules and mRNA genes involved in the development of gastrointestinal tract cancer. In the work, most of the results are presented in tables and a description of which genes and miRNAs were found to be associated. Found three articles in the Scopus database with the student as first author: https://www.scopus.com/authid/detail.uri?authorId=57194415971 The results obtained in the thesis were published in international journals such as ***Nanomaterials*** (Scopus 81% percentile), ***Frontiers in Genetics*** (Scopus 53% percentile). The article for 2020 was published in the journal ***Nanotechnologies in Russia*** (Scopus 41 percentile in 2020). However, this journal (***Nanotechnologies in Russia***) since 2021 is not included in the Scopus database. Therefore, only two articles in the Scopus database can be presented for the thesis defense.
8.	Principle of reliability Validity of sources and information provided	8.1 Choice of methodology - justified or methodology is described in sufficient detail 2) no	Using only computer analysis and modeling methods represents a sound approach for studying miRNA-mRNA interactions. The use of the MirTarSeq program for hydrogen bond modeling demonstrates a methodical and systematic approach to data analysis. The MirTarSeq program has not been published in any Scopus/WoS articles, and no evidence of algorithm performance is provided. There is no indication of what role the student plays in the design or optimization or another role in the development of this program. There are no laboratory methods confirming the potential sites in the mRNA genome associated with carcinogenesis in one way or another.
		8.2 The results of the thesis work are obtained using modern methods of scientific research and techniques of data processing and interpretation with the use of computer technologies: 2) no	The work is based on computer analysis of human gene sequences using software developed by the research team. Comments on this part are indicated below.
		8.3 Theoretical conclusions, models, identified interrelationships, and regularities are proved and confirmed by experimental research (for areas of training in pedagogical sciences, the results are proved on the basis of pedagogical experiment): 2) no	The theoretical significance lies in the establishment of quantitative traits in miRNA-mRNA interactions, paving the way for advancements in medicine and early cancer diagnosis. Since the work is exclusively virtual, there is no practical result of this research. Therefore, the practical significance is underscored by the potential application of findings in the development of test systems and therapeutic interventions for colorectal, gastric, and esophageal cancers.

		8.4 Important statements are partially supported by references to relevant and reliable scientific literature.	The thesis presents potential robust supporting the development of test systems for early cancer diagnosis based on miRNA-gene associations. The identification of specific miRNA-gene associations for different types of gastrointestinal tract cancer provides a solid foundation for future research and clinical applications.
		8.5 The references used are sufficient for a literature review	The literature review provides compelling evidence of the cancer worldwide, emphasizing the urgent need for advancements in diagnostics and treatment strategies.
9	Implementation of the principle of practical utility	9.1 The thesis is of theoretical significance: 2) no	The thesis offers valuable insights into the quantitative aspects of miRNA-mRNA interactions, establishing associations between miRNA and candidate genes for colorectal, gastric and esophageal cancers diagnosis. The identification of binding sites and the characterization of interactions contribute significantly to the understanding of cancer pathology. The novelty of the study is primarily due to the analysis of exon sequences. However, experimental validation of miRNA-mRNA interactions was not performed in this study.
		9.2 The thesis is of practical significance and there is a high likelihood of the findings being applied in practice: 2) no	The research findings have been presented and discussed at various international conferences, indicating a strong commitment to academic dissemination and engagement with the scientific community.
		9.3 Are the proposals for practice new? 3) not new (less than 25% are new)	Such studies have been conducted for quite a long time, and much material has already been accumulated. The software packages used by the authors were developed quite a long time ago, and I did not find new approaches implemented in them.
10.	Quality of writing and presentation	Quality of academic writing: 2) average;	There are problems with the text. All comments are indicated below.

Comments on the text of the thesis:

The work is much focused on the term nanomolecules, for example:
“to nanosized mRNA molecules and block the synthesis of nanosized proteins on ribosomes";
"These processes are the foundation of nanomedicine.";
Table 1 - The size of different biological nanoobjects.
- I suppose the term "nanomolecules" could be removed entirely from the text.
However, macromolecules as nucleic acids are studied in different fields of knowledge (biochemistry, biophysics, and molecular biology), and the emphasis on nanomolecules and miRNAs is not grounded. The size of molecules in nanometres, like mRNA or miRNA, is not fundamental in this study, since the interaction between RNA molecules is based on the laws of complementarity of nucleotide bases, not on the laws of nanomolecules.
By stating "miRNA nanomolecules" a hundred times, it will not make this study in any way related to nanomolecules.
It is essential to note that miRNA is a double-stranded RNA, hairpin structure. That is, it has an intervertible repeat in forming the stem sequence. The two sequences from both complementary strands, after cutting, are mirrored, and therefore, identical. The presence of miRNA formation in exon sequences is allowed, but since the total length of exons is incomparably tiny compared to the length of exons, the probability of this is extremely low.
Hairpin formation for miRNA is potentially possible for any cellular RNA, and after degradation of this RNA, part of the double-stranded hairpin remains intact and can maturation to miRNA.
It is not clear what "nanomedicine" is. Human disease is a disease of the entire human body, not of some parts of it, much less nano-parts.

“Extensive evidence has been gathered demonstrating significant changes in miRNA expression profiles within tumor cells [228-231]. “
-The target for the miRNA could be any other gene, including the gene from which this RNA was synthesized. Therefore, one must consider that miRNA synthesis occurs on one gene, and the target will be another. This relationship must be considered in the study of developing cancer biomarkers. If there is evidence that the synthesis of specific miRNAs in tumor tissues is increased, this may suggest that the synthesis of introns from which the miRNA reads has also increased. In addition, it is necessary to compare the synthesis of these miRNAs in other normal tissues (cells). This could indicate a specific process that also occurs in normal cells in organs at different levels of development.

“The miRNA nanomolecules of the future are located within this hairpin [188].”
- I believe it should be stem instead of hairpin.

“The known mechanisms you've collected mainly focus on miRNA action on mRNA sites situated in the 3'UTR. ” – a strange form of “you've collected ”.

“The miRNAs can be encoded in any region of the genome. Most of the miRNA nanomolecules genes (61%) are located in the intron regions of the genes; however, miRNA genes can be located in exons or intergenic regions.”
- If miRNAs are present mainly in introns, there must be a mechanism for their synthesis without the synthesis of the main gene. In mRNA synthesis, introns are not synthesized, but only the exon part of genes forms mRNA. Introns can be 1 million nucleotides, but synthesis is impossible and does not occur in a real cell. Although theoretically, it is believed that pre-mRNA should be synthesized, for some genes, it takes place, but rather as an exception.

2.2 Methods

Using the MirTarget program, we have found binding sites in 5'UTR, CDS, and 3'UTR of many human, animal and plant genes.

- I have not found anything about the MirTarget program, and I have not been able to find any Scopus/WoS articles that have anything about it, except for the mention of an article here:

https://www.bioinformation.net/010/97320630010423.htm

But there are many problems with this paper, and most importantly, this journal is not a trusted journal.

Figure 3 - Comparison of miR-1273d,e,f binding sites with mRNA of IL11 gene in miRTarget and TargetScan programs.

-Here is a comparison of the two software packages. In the miRTarget software, the complementary pair A-C is labeled on par with the canonical pairs, which is not correct. This pair introduces a significant destabilization in this section, so it should not be labeled as a complementary pair. Whereas the pair G-U is quite stable, as actually all pairs with G.

In Figures 4, 5, and 6, comparisons are given, but you have to specify in the figures the complementary pairs that TargetScan and DIANA-MicroT-CDS do not have.

I am not sure if this is correct: “The process of searching for target genes for miRNAs remains consistent when using the MirTarget program, which was developed in our laboratory. ”

“These sequences were analyzed through the created in our laboratory Lextractor, MirTarget, and MirTarSeq programs.” - It is necessary to indicate the authors and a link to the article or the repository. Otherwise, it is virtual software, which may not exist.

On what the 90% value is based:“The miRNAs-mRNAs binding sites with a ΔG/ΔGm value of 90% or higher were selectively chosen. ”

“The language used is formal, with a clear and concise structure.” – what is meant here?

It's a mistake: “G-U, and A and C through hydrogen bonding. The distance between A and C is equal to that of G-C, A-U, and G-U nucleotides.”

- A and C do not form hydrogen bonds that lead to a stable pair.

In miRNA/mRNA structures, mismatches in non-complementary pairs are allowed since the temperature of these complexes will be stable at 37 degrees.

Instead of ΔG/ΔGm value, focusing only on dG and Tm values for the duplex was necessary. The number of nucleotide mismatches may vary but is unlikely to be 3 as indicated by the author (for 22 nt), instead 1-2 at most ("within three nucleotides at a length of 22 nt.").

This isn't easy to parse and understand:

“This program operates when files containing binding site schemes acquired from MiRTarget's site search (scheme.mres) and gene files (Gene.gene) are present.”

In Figure 7, the complementary pairs A-C are indicated, as I wrote above, this is incorrect. But what could be indicated as a potential pair is G-A, in some cases, and the A-A pair is not a problem either since the dG of such a pair is about zero, which is a neutral event, not destabilizing as for A-C pairs.

Non-canonical pairs are stable pairs that do not introduce destabilization into the duplex. These pairs include all possible pairs with nucleotide G (G-C, G-U, G-A, G-G).

Figures 10-15, nucleotide sequences of mRNA regions of orthologous genes containing clusters of binding sites of miRNAs are shown. But I see that these sequences are about 100% CG. It's hard to imagine how such miRNAs could exist since their denaturation/annealing is unlikely, and their secondary structure would be stable to bind to the target mRNA. It is unlikely that miRNAs with 100% composed of CG nucleotides could exist.

Figure 19 indicates 2 cases of a single nucleotide deletion in the complementary site; this is also a problem.

The Decision: based on published scientific papers in which student Akimniyazova Aigul Nurlankyzy is the first author, I recommend awarding the Doctor of Philosophy (PhD) in the major only after all of the above comments have been addressed.

Official Reviewer:
Professor, Leading Researcher,
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(signature)

R.N. Kalendar