Peer Review File

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Reviewer A

The manuscript entitled "miR-491-5p negatively regulates deoxythymidylate kinase (DTYMK) to participate in cell cycle arrest through TP53 to promote pancreatic adenocarcinoma progression and is associated with tumor immune infiltration" presents a comprehensive study, and the results could have significant implications in the understanding of pancreatic adenocarcinoma (PAAD) and its progression.

The methodology employed, including the use of The Cancer Genome Atlas (TCGA), GeneMania, and STRING databases, is thorough and sound. The systematic exploration of the role of DTYMK in PAAD and its regulatory mechanisms is commendable. I find it impressive that the authors effectively combined different datasets and computational tools to understand the gene's prognostic impact and molecular role.

The results convincingly show that reduced DTYMK expression is associated with improved patient outcomes in PAAD. The links established between DTYMK expression, tumor tissue, immune cell infiltration, and potential miRNA regulation are intriguing, highlighting a possible role in immune escape and a negative regulation by miR-491-5p.

Answer: Acknowledgments of our manuscript are greatly appreciated.

Reviewer B

Pancreatic adenocarcinoma (PAAD) is a particularly severe form of digestive pancreatic cancer that is characterized by rapid onset, poor prognosis, and high mortality. In the manuscript “miR-491-5p negatively regulates deoxythymidylate kinase (DTYMK) to participate in cell cycle arrest through TP53 to promote pancreatic adenocarcinoma progression and is associated with tumor immune infiltration”, authors investigated the mechanism of action of deoxythymidylate kinase (DTYMK) and its effect on the prognosis of patients with pancreatic cancer.
Couple questions are required to be answered before it will be accepted.
(1) The DTYMK was the crucial topic in the study. The title of the manuscript was not suitable. My suggestion was changing the title to “deoxythymidylate kinase (DTYMK) participates in cell cycle arrest to promote pancreatic adenocarcinoma progression regulated by miR-491-5p through TP53 and is associated with tumor immune infiltration”.

Answer: Thank you for your comment. The title has been changed to "Deoxythymidylate kinase (DTYMK) participates in cell cycle arrest to promote pancreatic adenocarcinoma progression regulated by miR-491-5p through TP53 and is associated with tumor immune infiltration".
Change in the text: title.

(2) It was better to add reference (DOI: 10.21037/tcr.2020.03.03) about roles of miR-491-5p in cancer.
Answer: Thank you for your comment. We have added relevant citations and references in the discussion section.
Change in the text: see ref.39.

(3) The immune infiltration was the key topic. What were the roles of immune infiltration in the process of PAAD? Please state in the introduction.
Answer: Surgery is the main treatment for pancreatic cancer. Perioperative radiotherapy and chemotherapy may improve the survival of patients in the limited period. However, most patients are in the advanced stage at the first diagnosis, and the 5-year survival rate of surgery is not more than 20-25%. In recent years, immunotherapy has brought hope to improve the therapeutic effect of pancreatic cancer. The main purpose of our research is to explore the role of DTYMK in the biological events of pancreatic cancer, in which immune invasion is also related. Therefore, we have included the added relevant content in the discussion section, and we have also added relevant references, as highlighted in red.
Change in the text: page 7-8/line 265-274.

(4) How to construct the nomogram? Please state in the methods.
Answer: We have made additions and changes to the relevant content in method section.
Change in the text: page 4/line 134-142.

(5) The cell senescence was showed in the abstract. What were the functions of cell senescence in PAAD? Please state in the discussion.
Answer: In the discussion section, we have added the content related to Cellular senescence. Some of these mechanisms are also hot spots in tumor research, such as the direction of Cellular senescence. Cellular senescence is a stable state of terminal cell cycle arrest, which is related to various macromolecule changes, excessive secretion and proinflammatory phenotype. Cell senescence can be regarded as an obstacle to tumorigenesis, so in principle it can constitute the ideal result of any anti-cancer treatment (including pancreatic cancer). Cancer induced aging genes are aging programs driven by activated oncogenic Gene drive. Extracellular mechanisms further enhance the tumor inhibitory function of aging, including metabolic changes and aging related secretory phenotype (SASP). SASP factors, including chemokines, cytokines, growth factors and

enzymes, can induce Paracrine signaling senescence or stable proliferation stagnation of adjacent cancer cells. Some SASP factors can also enhance immune monitoring, which in turn can clear aging cells. And our research gene DTYMK also plays an important role in the tumor immune microenvironment of pancreatic cancer. Therefore, this also reflects that our research direction also has good scientific research value and evidence support for theoretical implementation.
Change in the text: page 8-9/line 297-310.

(6) DTYMK plays a critical role in regulating nucleoside thymidine synthesis, and is correlated to DNA synthesis, replication, and nucleotide metabolism by regulating the synthesis of dTTP. Why to focus on tumor immune infiltration in the study? Please supplement in the discussion.
Answer: Pancreatic cancer is mainly treated by surgery. Perioperative chemotherapy and radiotherapy may improve the survival of patients in the limited period. However, most patients are at advanced stage when they are first diagnosed, and the 5-year survival rate even after surgery is not more than 20-25%. In recent years, immunotherapy has brought hope to improve the therapeutic effect of pancreatic cancer. Tumor immune invasion is not only a research hotspot of pancreatic cancer, but also a research hotspot of many other tumors. Metabolic changes are a major feature of cancer cells, and the increased synthesis and use of nucleotide triphosphates are key and universal metabolic dependencies in cancer cells under different types and genetic backgrounds. Many aggressive behaviors of cancer cells, including uncontrolled proliferation, chemotherapy resistance, immune evasion, and metastasis, heavily rely on enhanced nucleotide metabolism. We have added the content of tumor immune infiltration related to pancreatic cancer in the discussion part, see the red mark.
Change in the text: page 7-8/line 265-274.

(7) Missing experiments were the biggest short board in the study. It was necessary to validate the expressions of DTYMK and miR-491-5p in PAAD by experiments.
Answer: Our research mainly explores the role of DTYMK in the biological events of pancreatic cancer through bioinformatics analysis. All the research results also provide a good reference value for the later basic experimental verification. However, currently due to limitations in experimental funding resources, it is not possible to improve the relevant basic experiments. In future research and exploration, we will improve in vivo and in vitro experiments to explore and verify our research conclusions.
Change in the text: None.

Reviewer C

1) First of all, my major concern for this study is the poor predictive accuracy of the nomogram, with C-index being as low as 0.644. The predictive model also has no external validity data.

The nomogram and the predictive model is not necessary since the authors only need to examine the independent prognostic role of DTYMK.

Answer: The data in this study is mainly based on the TCGA database. Through bioinformatics analysis, we mainly explore the role of DTYMK gene in the biological events of pancreatic cancer. The construction of the column chart only aims to showcase its value from the perspective of clinical data. In later research, we will add our own clinical data for validation analysis. Through single center training set, validation set and multi center data analysis, we hope to better build a prediction model with higher accuracy and reliability, and provide better help for clinical management of pancreatic cancer patients.

Change in the text: None.

2) Second, the abstract needs further revisions. The background did not indicate the clinical significance of this research focus and what the knowledge gap is. The methods need to describe the clinical factors and prognosis outcomes in the databases used and methods for ascertaining the independent prognostic role of DTYMK. The results need to quantify the findings by reporting survival time, other statistics, and accurate P values. The conclusion needs comments for the clinical implications of the findings, not to repeat the findings again.

Answer: We have made relevant adjustments and supplements to the abstract section of the study. This study mainly utilizes bioinformatics analysis methods to explore the role of DTYMK in the OS, DSS, and PFI of PAAD patients, and therefore cannot effectively supplement relevant specific survival time. In future research, we will increase the relevant clinical data of our center to further enhance the scientific value of the research.

Change in the text: Abstract.

3) Third, in the introduction of the main text, it is necessary to review known prognostic biomarkers in PAAD, have comments on their limitations and mechanisms, explain why DTYMK is potentially important and clearly indicate the clinical significance of this study.

Answer: We have added relevant content and references in the Introduction.

Change in the text: Page 3/line 82-98.

4) Fourth, in the methodology of the main text, please first have an overview of the research procedures and the questions to be answered by these procedures. Please describe the procedures for analyzing the independent prognostic role of DTYMK. The authors need to describe the clinical factors and prognosis outcomes in the databases used. In statistics, please ensure $P<0.05$ is two-sided.

Answer: We have made relevant changes in the above questions and ensured that $P<0.05$ is bilateral in the statistics.

Reviewer D

1. Abstract

Please define GSEA in the abstract.

Reply: Thank you for your comment. GSEA has been defined in the abstract.

2. References/Citations

References 11 and 18 are the same, please delete one of them and revise both the citation in main text and reference list's order.

Reply: Thank you for your reminder. Reference 18 has been deleted and the citation in main text and reference list has been revised.

3. Figure 5

Please provide the meaning of "*, **, ***, ns" in the legend.

Reply: Thank you for your comment. "*, **, ***, ns" have been defined in the legend.

4. Figure 7

Please provide the meaning of "*" in the legend.

Reply: Thank you for your comment. "*" has been defined in the legend.

5. Table 1

Please explain UTR in the table footnote.

Reply: Thank you for your comment. "UTR" has been defined in the footnote.