**Peer Review File**

Article information: https://dx.doi.org/10.21037/jtd-23-1203

**Reviewer A**

The manuscript describes combinatorial analysis of publicly available array-based miRNA and mRNA expression datasets using several bioinformatics tools. The authors also used ESTIMATEscore and CIBERSORT tools in the present study.

**Comments**

1. The ESTIMATE tool predicts tumor purity and identifies stromal /immune cells from TUMOR TISSUES by generating three scores (stromal score; immune score; estimate score) (https://bioinformatics.mdanderson.org/public-software/estimate/). This tool requires tumor samples data to analyze that. Moreover, there are no reports where this tool was mentioned to identify immune/stromal cells in diseases other than cancer.

Reply 1: Thank you for your comments. The ESTIMATE algorithm has been applied to non-tumor diseases for the calculation of immune and stromal scores. References are listed below: Hou Y, Chen Z, Wang L, Deng Y, Liu G, Zhou Y, Shi H, Shi X, Jiang Q. Characterization of Immune-Related Genes and Immune Infiltration Features in Epilepsy by Multi-Transcriptome Data. J Inflamm Res. 2022 May 5;15:2855-2876. doi: 10.2147/JIR.S360743. PMID: 35547834; PMCID: PMC9084924. This literature calculated the immune score and stromal score of epilepsy tissue by using the ESTIMATE algorithm to assess the immune status of the disease.

Changes in the text: None.

2. The authors have generated numerous amount data in results. But the same has not been discussed properly in Discussion part. In addition to describing XPNPEP3 gene in AMI, the authors must have got some other moieties to discuss w.r.t. AMI.

Reply 2: In this study, we focused on the role of XPNPEP3 gene in AMI. However, your suggestions are valuable and we will add what you mentioned in future in-depth studies.

3. The rationale to do numerous in silico experiments were not described anywhere in manuscript.

Reply 3: Thank you for your suggestion. We have modified our text as advised (see Page 4, line 107-110, and line 122-123)

Changes in the text: Cytoscape is software that can integrate biomolecular interaction networks with high-throughput expression data and other molecular states into a unified framework that can be adapted to any system of molecular components and interactions (14).

Next, the Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) (17) algorithm evaluates gene expression profiling datasets to obtain 22 immune cell infiltration scores per patient.

4. The parameters applied in Molecular Dynamic Simulation (MDS) are not properly described.

Reply 4: We added some data in the Methods section (see Page 6, line 170-172).

Changes in the text: Where, in molecular docking, the grid coordinates in each of the XYZ directions are -10, 18.5 and 13.5, respectively, and the grid length in each direction is 20Å.

5. Similarly, the results of MDS were not discussed.

Reply 5: Thanks for your comments. In this study, MDS was used as a tool to evaluate potential drugs for the XPNPEP3 gene. The Mehods section was able to fully illustrate the significance of MDS for this study.

Changes in the text: Where, in molecular docking, the grid coordinates in each of the XYZ directions are -10, 18.5 and 13.5, respectively, and the grid length in each direction is 20Å. Specifically, the maximum allowable energy was 3 kcal/mol, the exhaustiveness was 8, and the maximum number of conformations output was 10. Results processing was performed in Pymol (23)

6. In lines 227-228, the authors described “we found that up-regulated miRNAs may contribute to the down regulated mRNA transcriptome to a large extent” but the same is not mentioned.

Reply 6: We were really sorry for our careless mistakes. The description is misrepresented. We have modified our text as advised (see Page

Changes in the text: By using bioinformatic target prediction to integrate mRNA and miRNA expression data, it helped us to realize that upregulated miRNAs may largely contribute to the transcriptome of downregulated mRNAs.

7. In line 248-25-, citations of XPNPEP3 gene with cancers are provided. How this information is related to the present context?

Reply 7: We sincerely appreciate the valuable comments. Since there is a gap in AMI regarding the XPNPEP3 gene, which is often found in cancer-related studies. The possible biological mechanisms of the XPNPEP3 gene in AMI were demonstrated by citing previous studies.

8. ROC curves of only XPNPEP3 gene provided. Similar curves of other moieites should be given to prove the significance of XPNPEP3 gene in AMI.

Reply 8: We think this is an excellent suggestion. Since this study is a preliminary study of the biological mechanism of XPNPEP3 gene in AMI, we only provide the ROC curve of XPNPEP3 gene. In the future in-depth study, we will prove the significance of this gene in AMI by other curves and graphs.

9. It seems only one drug/compound was used in docking and it showed good results as there are no comparisons. More drugs/compounds need to be used in MDS.

Reply 9: Thank you for your comments. We have identified 7 drugs in Table 1 that may be related

to XPNPEP3 gene. Since DB06909 had the lowest docking score, we only examined the results of this drug in this study. Based on your suggestion, we will continue to delve deeper into the relationship between other drugs and this gene in future studies.

10. References are outdated.

Reply 10: Based on your suggestions, we have updated some of the references in the Discussion section (see Page 8, line 246-249).

Changes in the text: Rapid diagnosis of AMI is important for the management of patients with chest pain. There have been many studies based on a variety of technological tools and screening biomarkers to provide critical information on the accuracy of the diagnosis of AMI and its associated diseases (28-30).

**Supplementary Comments**

1. The use of ESTIMATEscoring is done in tumors. No reference was provided that it can be used in other diseases also.

Reply 1: Thanks to your suggestion. References related to the application of the ESTIMATE algorithm to non-tumor diseases are listed below: Hou Y, Chen Z, Wang L, Deng Y, Liu G, Zhou Y, Shi H, Shi X, Jiang Q. Characterization of Immune-Related Genes and Immune Infiltration Features in Epilepsy by Multi-Transcriptome Data. J Inflamm Res. 2022 May 5;15:2855-2876. doi: 10.2147/JIR.S360743. PMID: 35547834; PMCID: PMC9084924.

Changes in the text: None.

2. Rationale of doing various experiments was also not provided. Numerous thus generated data is not sufficiently discussed.

Reply 2: We have modified our Discussion as advised (see Page 8, line 246-252).

Changes in the text: Rapid diagnosis of AMI is important for the management of patients with chest pain. There have been many studies based on a variety of technological tools and screening biomarkers to provide critical information on the accuracy of the diagnosis of AMI and its associated diseases (28-30). By using bioinformatic target prediction to integrate mRNA and miRNA expression data, it helped us to realize that upregulated miRNAs may largely contribute to the transcriptome of downregulated mRNAs (31).

3. Only focus on single gene was given. No appropriate and sufficient discussion of other genes/miRNAs are given.

Reply 3: Thanks for your suggestion. Since the XPNPEP3 gene was first reported in AMI. In the present study, we only explored the potential biological mechanism of this gene for AMI to contribute new insights into AMI. Based on your suggestion, we will delve into the mechanism of action of other genes on AMI in our next study.

4. Only one drug/compound was tries in Molecular Dynamic Simulation study and it was commented to have significant results. Comparison with other drugs/compounds are needed.

Reply 4: We sincerely appreciate the valuable comments. We have identified 7 drugs in Table 1 that may be related to XPNPEP3 gene. Since DB06909 had the lowest docking score, we only examined the results of this drug in this study. Based on your suggestion, we will continue to delve deeper into the relationship between other drugs and this gene in future studies.

5. Bibliography is outdated

Reply 5: Based on your suggestions, we have updated some of the references in the Discussion section (see Page 8, line 246-249).

Changes in the text: Rapid diagnosis of AMI is important for the management of patients with chest pain. There have been many studies based on a variety of technological tools and screening biomarkers to provide critical information on the accuracy of the diagnosis of AMI and its associated diseases (28-30).

The manuscript describes use of various tools of bioinformatics to generate numerous data but fails to justify the results in discussion and focuses only on single gene. The authors need to thoroughly revise the text from a new perspective which should update sufficient knowledge and be interested for readers.

Reply: We thank the reviewers for their careful reading of this manuscript. This study is the first to investigate the role of the XPNPEP3 gene in AMI. As this gene is often seen in the study of cancer-related diseases, and in AMI is an innovative research direction. For this reason, we only explored the XPNPEP3 gene in AMI in this study. For other genes, we will discuss them in more depth in further studies.

**Reviewer B**

1) First, the title is misleading since the authors did not examine the prognostic role of XPNPEP3.

   Reply 1: Thank you for your suggestion. We have modified our title as advised (see Page 1, line 2).

   Changes in the text: Diagnostic and treatment value of XPNPEP3 in acute myocardial infarction.

2) Second, the abstract needs further revisions. The background did not describe the clinical needs for diagnostic biomarkers for AMI and what the clinical significance of this research focus is. The methods need to describe the clinical sample and controls in the dataset and the diagnostic criteria for AMI. The results need to report the diagnostic accuracy indicators such as sensitivity and specificity. The conclusion needs to focus on the clinical implications for the diagnosis of AMI.

Reply 2: We have modified our Abstract as advised (see Page 1, line 27-28, line 30-32, line 49-51, and line 56-57).

Changes in the text: Discovering biomarkers of AMI is important for clinical diagnosis and needs. Therefore, this study aimed to elucidate the role of XPNPEP3 as a potential biomarker for AMI.

Expression profiling data were downloaded for acute myocardial infarction patients and healthy patients in the GSE24548 and GSE24519 datasets, respectively.

Molecular docking analysis showed that DB06909 had the lowest docking score with XPNPEP3, revealing it to be a potential XPNPEP3 inhibitor.

This work discovered that XPNPEP3 is correlated with the development of AMI. These findings may provide theoretical basis for the diagnosis and treatment of AMI.

3) Third, in the introduction of the main text, the authors described that AMI is difficult to be detected in the early phase but the main analysis did not indicate that XPNPEP3 is sensitive for detecting early AMI. The authors need to review other known biomarkers for the diagnosis of AMI to suggest the needs for focusing on miRNA. A potential practical issue is the detection of XPNPEP3 is time-consuming and not feasible for the clinical emergency use.

Reply 3: We feel great thanks for your professional review work on our article. We have modified our text as advised (see Page 3, line 85-90).

Changes in the text: miRNAs are abundant and stable in the blood, and they can be aberrantly expressed under pathological conditions of cardiovascular disease (11). In addition, Chen et al. showed that miRNAs play a key role in the diagnosis of AMI and its associated symptomatic diseases, platelet activation monitoring, and prognosis prediction (11). Cardiac-rich miRNAs (such as miR-499, miR-208, and miR-1) were able to be rapidly upregulated in repertoire plasma after myocardial necrosis (12, 13).

4) Fourth, the authors need to indicate the clinical research design in the methodology of the main text and describe the patient sample and controls in the datasets in detail. If the non-AMI controls are healthy controls, the current data are not suitable to design as a diagnostic test. In statistics, please describe the calculation of diagnostic accuracy measures such as AUC, sensitivity and specificity, as well as their threshold values for a good diagnostic test.

Reply 4: We have modified our test as advised (see Page 4, line 107-110).

Changes in the text: The GEO database was used to determine the miRNA expression of the GSE24548 dataset (3 normal and 4 AMI) (14), including 3 normal samples and 4 AMI samples. The mRNA expression of the GSE24519 dataset, including 4 normal samples and 34 AMI samples.

5) Finally, please review and cite some related papers: 1. Feng L, Yang Z, Chen S, Wan J. Diagnostic value of myocardial stress detection based on feature tracking MRI in patients with

acute myocardial infarction. J Thorac Dis 2022;14(9):3454-3461. doi: 10.21037/jtd-22-973. 2. Huo H, Dai X, Li S, Zheng Y, Zhou J, Song Y, Liu S, Hou Y, Liu T. Diagnostic accuracy of cardiac magnetic resonance tissue tracking technology for differentiating between acute and chronic myocardial infarction. Quant Imaging Med Surg 2021;11(7):3070-3081. doi: 10.21037/qims-20-1109. 3. Shi LY, Han YS, Chen J, Li ZB, Li JC, Jiang TT. Screening and identification of potential protein biomarkers for the early diagnosis of acute myocardial infarction. Ann Transl Med 2021;9(9):743. doi: 10.21037/atm-20-7891.

Reply 5: We added these references in our article (see Page 8, line 246-249).

Changes in the text: Rapid diagnosis of AMI is important for the management of patients with chest pain. There have been many studies based on a variety of technological tools and screening biomarkers to provide critical information on the accuracy of the diagnosis of AMI and its associated diseases (28-30).

**Reviewer C**

The paper titled “Diagnostic and prognostic value of XPNPEP3 in acute myocardial infarction” is interesting. This work discovered that XPNPEP3 is correlated with the development of AMI, and for the XPNPEP3 protein, compound DB06909 can be considered a potential inhibitor. Those data may provide a new direction for the diagnosis and treatment of AMI. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply 1: Thanks for your suggestion. We have modified our text as advised (see Page 1-2, line 26-57).

Changes in the text: Background: At present, acute myocardial infarction (AMI) is a serious cardiovascular disease with high morbidity and mortality. Discovering biomarkers of AMI is important for clinical diagnosis and needs. Therefore, this study aimed to elucidate the role of XPNPEP3 as a potential biomarker for AMI.

2) The description of some methods in this study is too simplistic, please describe in detail.

Reply 2: We have added some descriptions in the Methods section (see Page 4, line 107-110, line 120-123; Page 5, line 135-136; Page 6, line 183-185).

Changes in the text: The GEO database was used to determine the miRNA expression of the GSE24548 dataset (3 normal and 4 AMI) (14), including 3 normal samples and 4 AMI samples. The mRNA expression of the GSE24519 dataset, including 4 normal samples and 34 AMI samples.

Cytoscape is software that can integrate biomolecular interaction networks with high-throughput expression data and other molecular states into a unified framework that can be adapted to any system of molecular components and interactions (17).

Next, the Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) (20) algorithm evaluates gene expression profiling datasets to obtain 22 immune cell infiltration scores per patient.

Identification of the strongest binding mode to ligand molecules was performed using Lamarckian algorithm (24). Where, in molecular docking, the grid coordinates in each of the XYZ directions are -10, 18.5 and 13.5, respectively, and the grid length in each direction is 20Å.

3) All figures are not clear enough. It is recommended to provide clearer figures again.

Reply 3: Based on your suggestion, we have provided a clearer figure.

4) What is the correlation between XPNPEP3 and the immune microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply 4: Thank you for your suggestion. The reports related to the XPNPEP3 gene are associated with the cancer immune microenvironment, and its report on non-tumor diseases is the first in this study. However, we think that the suggestion you provided is excellent, which reminds us to explore the role of this gene in the immune microenvironment of AMI in detail in further in-depth studies.

5) It is recommended to add in vivo experiments to study the biological function of XPNPEP3.

Reply 5: We think this is an excellent suggestion. Since the XPNPEP3 gene was reported for the first time in AMI, this study is only a preliminary investigation of the potential mechanism of action of this gene on AMI. We will add in vivo experiments in our next study to explore the biological function of XPNPEP3 in AMI in more depth.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Analysis of susceptibility genes and myocardial infarction risk correlation of ischemic cardiomyopathy based on bioinformatics, J Thorac Dis, PMID:36245596”. It is recommended to quote the article.

Reply 6: We are grateful for the valuable references provided by the reviewers. The main finding of this literature was the association of ICM susceptibility genes with myocardial infarction. We have done this by adding references about the relationship between miRNAs and AMI in the Introduction section (see Page 3, line 85-90).

Changes in the text: miRNAs are abundant and stable in the blood, and they can be aberrantly expressed under pathological conditions of cardiovascular disease (11). In addition, Chen et al. showed that miRNAs play a key role in the diagnosis of AMI and its associated symptomatic diseases, platelet activation monitoring, and prognosis prediction (11). Cardiac-rich miRNAs (such

as miR-499, miR-208, and miR-1) were able to be rapidly upregulated in repertoire plasma after myocardial necrosis (12, 13).

7) What is the future work plans? What is the guiding significance of this study?

Reply: The XPNPEP3 gene is a common target in cancer. This gene is the first to be identified as a potential target in AMI. This provides a new idea for the treatment of AMI in order to be able to provide a new theoretical basis for the treatment and diagnosis of AMI patients in the future. The next step in the study will be to add in vivo experiments to delve deeper into the biological function of the XPNPEP3 gene in AMI. In addition, we will continue to explore the biological mechanisms of other genes in AMI to discover more novel targets.

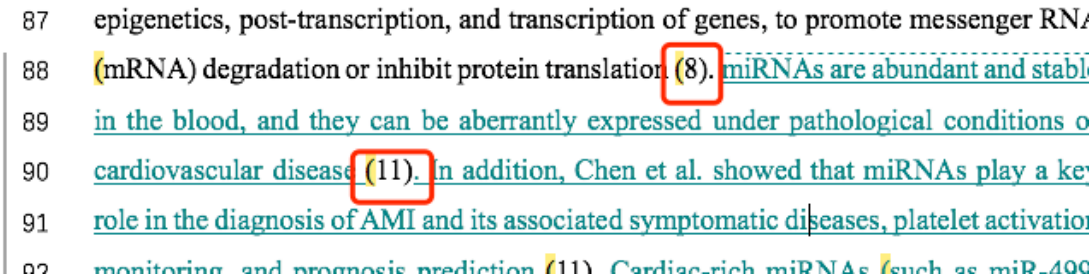
**Reviewer D**

**1. Reference/citation**

There are two versions of reference list. Please check which is correct, and remove the wrong one.

**2.** References (9, 10) should be cited between (8) and (11).

***Noted:*** *References should be cited consecutively and consistently according to the order in which they first appear in the text.*


87 epigenetics, post-transcription, and transcription of genes, to promote messenger RNA
88 (mRNA) degradation or inhibit protein translation (8). miRNAs are abundant and stabl
89 in the blood, and they can be aberrantly expressed under pathological conditions o
90 cardiovascular disease (11). In addition, Chen et al. showed that miRNAs play a ke
91 role in the diagnosis of AMI and its associated symptomatic diseases, platelet activatio
92 monitoring, and prognosis prediction (11). Cardiac-rich miRNAs (such as miR-499

Reply 5: Thanks for your comments. We have modified this error.

Changes in the text:

(mRNA) degradation or inhibit protein translation (8). ~~miR-133 has been found to be able to protect cardiomyocytes from myocardial infarction . In an AMI rat model, it has been shown that AMI could be improved via p38/SIRT1/p53 signaling pathway regulation .~~ miRNAs are abundant and stable in the blood, and they can be aberrantly expressed under pathological conditions of cardiovascular disease (9). In addition, Chen et al. showed that miRNAs play a key role in the diagnosis of AMI and its associated symptomatic diseases, platelet activation monitoring, and prognosis prediction (9). Cardiac-rich miRNAs (such as miR-499, miR-208, and miR-1) were able to be rapidly upregulated in repertoire plasma after myocardial necrosis (10, 11).

**3.** The current "**##Statistical Analysis**" is too simple. Please extend more contents.

162 ## *Statistical Analysis*

163 The Sangerbox platform supported all the analyses of this study (27).

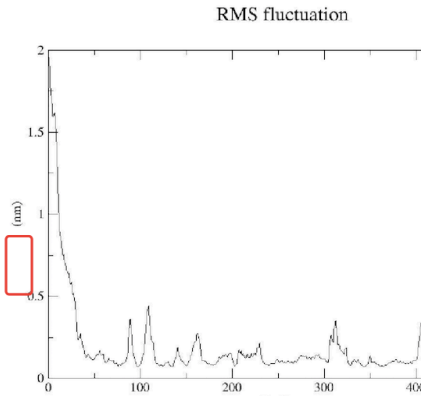
164

Reply 7: Thanks for your comments. We have added some content in our article (see Page 6, line 195-197).

Changes in the text: The statistical data of this study were all obtained using the R software (version 3.6.0). The Sangerbox platform supported all the analyses of this study (25). Notably, $p < 0.05$ was considered statistically significant.

**4. Figure 6C**

Please confirm if here should be "RMSF (nm)".




Reply 12: We have modified this figure in our article (see Figure 6-revised).

Changes in the text: