Peer Review File

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Reviewer A

The most considerable workload of this manuscript lies in the clinical collection of serum data. The authors identified potential targets from the GEO analysis and additional potential indicators from the serological analysis. However, these analyzes cannot be mutually confirmed. Perhaps these superficial and scattered analyzes are the biggest flaw of this paper.

Major:

1. It is impossible to draw sound conclusions only by analyzing a single GEO dataset. At least, it needs to be validated in an independent dataset to make the results more reliable. Therefore, it is necessary to add more public data for validation. For example, the authors can find other GEO data sets or qualified populations from TCGA for proof.

Reply: Thank you for your valuable comment. Previous studies have shown that programmed cell death plays an important role in the occurrence of bone metastasis in lung cancer, and ferroptosis is a special programmed cell death like process. At the same time, there are relatively few datasets in the TCGA database containing tumor tissue and normal tissue with lung cancer bone metastasis. Therefore, we selected the intersection of GSE10799 and iron death datasets in this study to preliminarily explore the key genes that can play a role. In the future further research, we will improve the relevant clinical data validation and cytology in vitro and in vivo validation. Sorry, the author of this article was unable to find any other dataset similar to GSE10799 and consistent with the GPL platform number.

Change in the text: None.

2. Conducting wet experiments to support the conclusion is highly recommended.

Reply: Thank you for your valuable comment. The main purpose of our study is to quickly identify potential iron death key genes in the pathogenesis of lung cancer bone metastasis through simultaneous bioinformatics analysis, and to predict upstream miRNA and signaling pathways, providing better reference for future cytological experiments.

Change in the text: None.

3. Bioinformatics analysis should incorporate more algorithmic support. There are too few algorithms included in the paper.

Reply: Thank you for your valuable comment. We will further increase other datasets and add more algorithms for validation and analysis from different perspectives in the future. The data in this article has been validated according to existing algorithms available to the author, and other algorithms cannot be provided for validation at the moment.

Change in the text: None.

Minor:

1. Serum data analysis is good, which is the feature and weakness of this paper. Because this

result does not explain anything or correspond to the title, these analyses can supplement the results and be presented in a small text space.
Reply: Thank you for your valuable comment. Our research objective is to obtain the regulatory network constructed by differentially expressed iron death related genes and related miRNAs in lung cancer bone metastasis, as well as related functional enrichment analysis, providing new targets for the treatment of lung cancer bone metastasis. In the future, we will increase relevant clinical data for verification, in order to further verify the clinical and practical value of the key genes we have obtained.
Change in the text: None.

2. The introduction should be consistent with the topic's content; please rewrite it.
Reply: Thank you for your valuable comment. Our research topic is bioinformatics analysis to identify potential iron death key genes in the pathogenesis of lung cancer bone metastasis. In the first paragraph of the introduction, we elaborated on the epidemiology of lung cancer and related bone metastasis events. In the second paragraph, we elaborated on the relevant research on iron death in bone metastasis of lung cancer and its different roles in different tumors. Final, we outlined the research methods and objectives. We used data mining and analysis techniques to screen differentially expressed genes (DEGs) in lung cancer tissue with bone metastasis and normal lung tissue. Then intersect these DEGs with the iron death dataset to obtain the DEGs related to iron death. In addition, in order to identify key biomarkers and establish the pathogenesis of lung cancer bone metastasis at the molecular level, we investigated key miRNAs that may play a major role in lung cancer bone metastasis. Our research results will help to understand the iron death state after bone metastasis in lung cancer, and provide new ideas for the clinical diagnosis and treatment of bone metastasis in lung cancer.
Change in the text: Introduction section.

3. The conclusion part should discuss the results of this paper.
Reply: Thank you for your valuable comment. The discussion section of the article provides an overview of the entire research process and the research reports on the key genes related to iron death, PEBP1, IDH1, ELAVL1, and EGFR, in lung cancer. We have revised the conclusion section.
Change in the text: Page 13/line 404-408.

4. The conclusion is too exaggerated. Rewriting the conclusion section after adding more analysis or data is recommended.
Reply: Thank you for your valuable comment. We have revised the conclusion section.
Change in the text: Page 13/line 404-408.

Reviewer B

In the manuscript “Identification of the potential ferroptosis key genes in the pathogenesis of lung cancer bone metastases by bioinformatics analysis”, authors identified the potential key genes of ferroptosis in the pathogenesis of lung cancer with bone metastasis (LCBM) by

bioinformatics analysis to provide new targets for treating LCBM and an indicator for early monitoring.

Couple questions are required to be answered before it will be accepted.

(1) The running title of “Pathogenesis of bone metastases from lung cancer” was not suitable. It can be changed to “ferroptosis in lung cancer with bone metastasis”.

Reply: Thank you for your valuable suggestion. We have changed the running title to "Identification of the potential ferroptosis key genes in lung cancer with bone metastasis."

Chang in the text: Title.

(2) What were the roles of ferroptosis in the lung cancer and bone metastasis? Please state in the introduction.

Reply: Thank you for your valuable comment. Currently, there is little research on the relevant mechanisms of ferroptosis in bone metastasis of tumors (including lung cancer). Based on relevant research, it can be clarified that ferroptosis plays an important role in biological events of lung cancer. Therefore, further exploration of the mechanisms involved is also what we need to do in the future. We will further increase relevant in vivo and in vitro cytology experiments in the future, so as to further improve our research results.

Chang in the text: None.

(3) It was better to add related reference (DOI: 10.21037/tlcr-22-408) about the ferroptosis related prognostic risk in lung cancer.

Reply: Thank you for your valuable suggestion. We have added this reference in the preface, "TXNIP can serve as a potential biomarker for predicting the prognosis and efficacy of chemotherapy combined with immunotherapy in SCLC patients”.

Chang in the text: page 4/line 121-122.

(4) The research was a bioinformatics analysis. It was more convincing to validate representative ferroptosis genes and key microRNAs in lung cancer bone metastasis by experiments.

Reply: Thank you for your valuable suggestion. Our research objective is to obtain the regulatory network constructed by differentially expressed iron death related genes and related miRNAs in lung cancer bone metastasis, as well as related functional enrichment analysis, providing new targets for the treatment of lung cancer bone metastasis. In the future, we will increase relevant in vitro and in vivo experiments, and conduct prospective clinical trials in order to further validate the clinical and practical value of the key genes we have obtained.

Chang in the text: None.

(5) In the paper, the IL-17 signaling pathway was the key result. What were the functions of IL-17 signaling pathway in lung cancer bone metastasis? Whether it was correlated with ferroptosis? Please state in the discussion.

Reply: Thank you for your valuable suggestion. In the miEAA database, we found that targeting miRNAs may lead to the occurrence and development of lung cancer bone metastasis by influencing Hippo signaling pathway, AMPK signaling pathway, arginine biosynthesis, IL-17 signaling pathway, pyruvate metabolism, p53 signaling pathway, MAPK signaling pathway,

and other aspects. Based on the previous bioinformatics analysis, we have identified many potential signaling pathways that may play a role. In the future, we will conduct relevant in vitro and in vivo cytology experiments to further clarify the underlying mechanisms and pathways.
Chang in the text: None.

(6) Why to test ALP and NSE in the study? And what were the correlations between ALP or NSE and ferroptosis in lung cancer? Please state in the discussion.

Reply: Thank you for your valuable comment. Currently, due to limitations in experimental conditions, we have not conducted relevant wet experiments to explore and verify. In future research, we will improve and make up for these shortcomings.
Chang in the text: None.

(7) In the study, 15 ferroptosis-related genes were identified and differentially expressed in lung cancer bone metastasis. Why to analyze ALP and NSE overexpression were associated with bone metastasis, not the 15 ferroptosis-related genes?

Reply: Thank you for your valuable comment. At the beginning, we intersected GSE10799 with iron death related genes to obtain 15 differentially expressed genes related to iron death. In subsequent MCODE module analysis, we identified four key genes, namely PEBP1, IDH1, ELAVL1, and EGFR. These four key genes are also included in the first 15 genes, which is equivalent to further screening of 15 genes. In future research, we will add relevant clinical data, cytological experiments, and nude mouse tumorigenesis experiments to further support our research results.
Chang in the text: None.

Reviewer C

1. You've mentioned "studies", while only one reference was cited in this sentence.

335 skeletal system is a predilection site for distant lung cancer metastasis (18). Studies
336 have shown that the incidence of LCBM is about 10–15%, while the incidence of bone
337 metastasis in advanced lung cancer is as high as 30–40% (19). After lung cancer cells

Reply: Thank you for your comment. sorry for the clerical error, we have revised "Studies" to "A study".

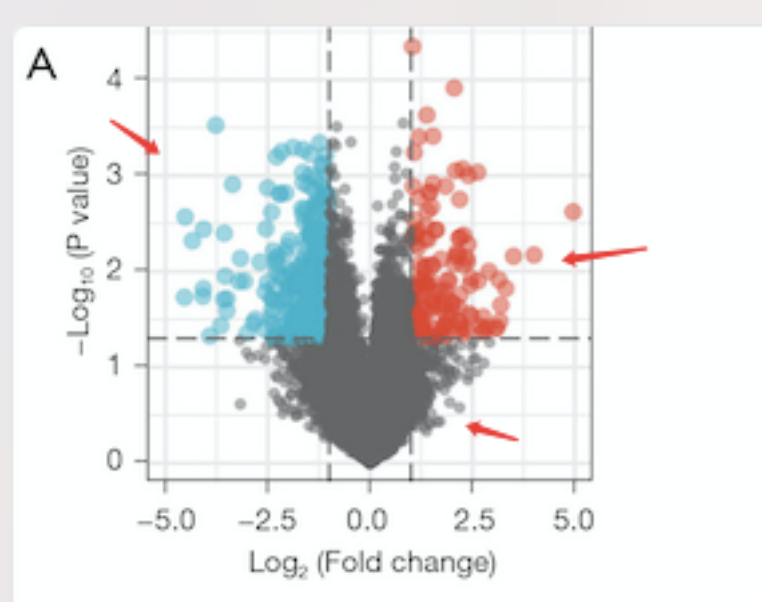
2. Please check if any references are missing in below sentences since you've mentioned "Studies". Please supplement.

396 Studies have shown that under normal physiological conditions, bone formation and
397 bone resorption are balanced processes, which are mediated by osteoblasts and
398 osteoclasts, respectively. When a malignant tumor metastasizes to bone, it can

427 neuroendocrine cells and can produce NSE. Studies have reported that some non-small
428 cell lung cancers also have neuroendocrine functions, often accompanied by NSE
429 secretion. In addition, in the process of LCBM, tumor cell proliferation is accelerated

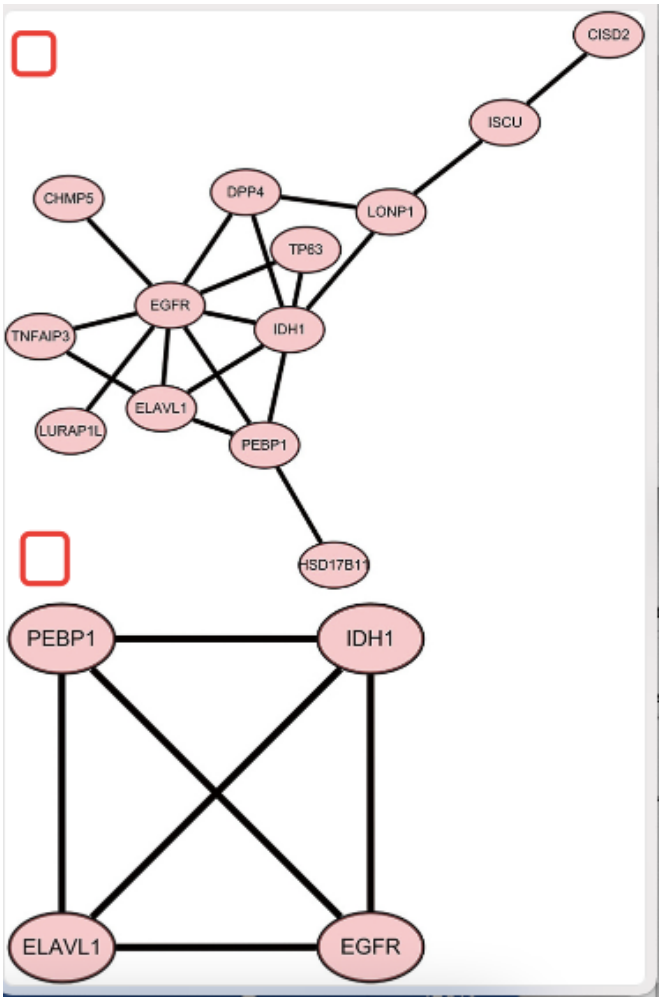
Reply: Thank you for your comment. We have added references 32.

3. Figure 1: Please define those blue, red, and black dots either inside the figure or in figure legends.



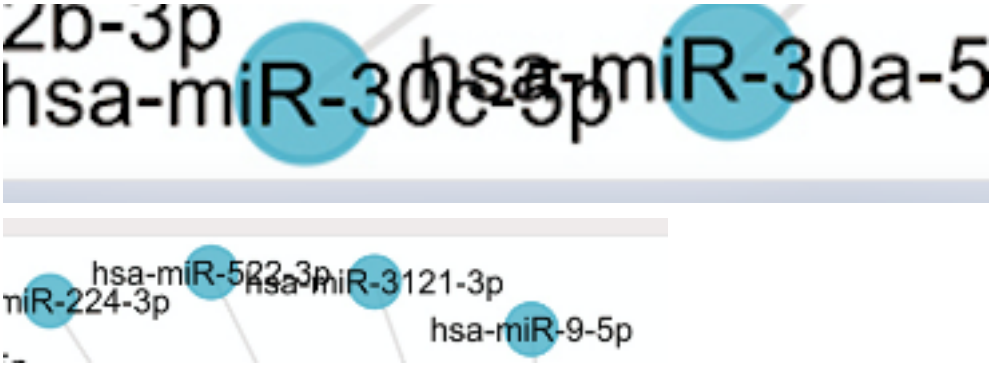
Reply: Thank you for your comment. We have defined blue, red, and black dotsin the figure legend.

4. Please indicate “A” and “B” inside the figure and resend us updated Figure 3.



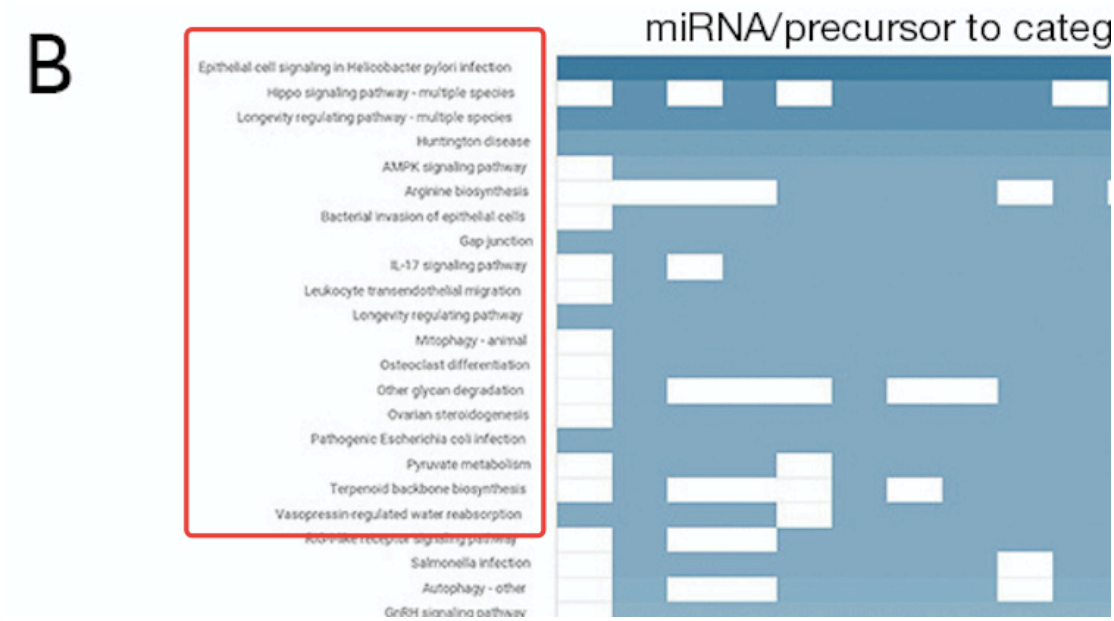
Reply: Thank you for your comment. We have indicated “A” and “B” inside the figure and resent updated Figure 3 to you.

5. Figure 4: Those words got covered, please check and revise.



Reply: Thank you for your comment. We have resent the updated figure to you.

6. As shown below, Figure 5B is rather vague.



Reply: We have resent you an update figure 5 with higher resolution.

7.Table 1: Please define ALL abbreviations in table footnote. Like “GO”, “KEGG”, “BP”, “CC”.

Reply: We have defined all abbreviations in table footnote.

8. Table 2: How were these data presented in your table 2? Please also define them.

Clinical feature	Bone metastasis (n=39)	Non-bone metastasis (n=66)	χ^2	P
Gender			1.018	0.313
Male	28 (71.79)	41 (62.12)		
Female	11 (28.21)	25 (37.88)		
Age (years)	57.85±3.48	58.03±3.29	0.265	0.791
Pathological feature			0.486	0.784
Adenocarcinoma	22 (56.41)	37 (56.06)		
Squamous cell carcinoma	10 (25.64)	20 (30.30)		
Small cell lung cancer	7 (17.95)	9 (13.64)		
ECOG score			4.171	<0.001
0	5 (12.82)	26 (39.39)		
1	16 (41.03)	33 (50.00)		
2	18 (20.51)	7 (10.61)		
Serum ALP (U/L)	254.73±15.24	235.50±12.45	7.029	<0.001
Serum NSE (μg/L)	16.62±3.18	13.26±2.83	5.599	<0.001

Reply: We have added ($\bar{x}\pm s$) in table title.