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OPEN PEER REVIEW

Microbial Diversity and Abundance in Pulmonary Tissue of Patients with Early-Stage Lung Cancer

Zhao Shouhua, Liu Meilan

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About this report

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1 Original submission

2 comments · 13 May 2023 – 14 May 2023

Seyed Hossein Shahcheraghi Reviewer

MAJOR REVISION 13 May 2023

The introduction of the abstract should first describe the subject, then previous studies.
p-values should be deleted from the results of the abstract.
The text has grammatical errors.
Abbreviations are incomplete.
Primers should be checked.

Associate Editor

MAJOR REVISION 14 May 2023

According to the reviewer's comments, the manuscript needs major revision.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:
Comments for authors and editorial teams:
The study entitled "Research of Microbial Diversity and Abundance in Pulmonary Tissue of Patients with Early Stage Lung Cancer" is novel and interesting. However, some minor concerns should be considered to improve the novelty as well as the flow of the manuscript:
1- Abstract: Acceptable
2- Keywords could be: Pulmonary cancer; Early stage; Microbial flora; Biodiversity; High throughput sequencing Modified
3- Introduction: Acceptable but could be better by wider literature review particularly related new & update articles from domestic researchers.Modified

4- Methodology: Acceptable but I could not see informed census and also ethical code.added

5- Results: Acceptable
6- Discussion: Could be scientifically than present level using more and wider literature review and explanation of related and domestic survey and of course then using from them as Bibliography/References.Modified
7- Bibliography/References: More domestic and update Ref(s). are necessary. Authors should do more literature review for using related domestic articles and therefore some more domestic Ref(s). (Using from domestic Ref(s). is scientific discipline for publication of academic data.)Modified
8- I could not see running title, informed census form, ethical code number and acknowledgment in manuscript. All of these compulsory.added

9- Table & Graph quality: Acceptable
10- language/English quality: I am not right reviewer for language/English quality of manuscript.Modified
Ask it from a native speaker reviewer.

Final decision: Manuscript is acceptable for publication after minor correction
Reviewer 2:
In the submitted manuscript, "Research of Microbial Diversity and Abundance in Pulmonary Tissue of Patients with Early Stage Lung Cancer" the authors provide the correlation in the microbial flora of pulmonary tissue among different histological types of lung cancer.
There are major issues that should be addressed by the authors in

order to improve this manuscript.
- There is lack of coherence in the introduction and there is no continuity between the sentences.
In general, the INTRODUCTION can be structured better.Modified

- If you compare with normal lung tissue and tumor tissue, please specify. Normal lung tissue and tumor tissue were obtained from 29 patients!!! The investigated tissues are not clear and details are missing. The authors should provide more primary data.added

- On page 7; line 116: "front and rear F/R primers" changed to "forward and reverse primers"Modified

*- What reference did you use for primer selection?*Wang K, Huang Y, Zhang Z, et al. A Preliminary Study of Microbiota Diversity in Saliva and Bronchoalveolar Lavage Fluid from Patients with Primary Bronchogenic Carcinoma. Med Sci Monit. 2019 Apr 17;25:2819-2834. doi: 10.12659/MSM.915332. PMID: 30994108; PMCID: PMC6482867.
- The name of bacteria in must be italics. Italics are used for bacteria at the level of family and below. All bacterial are italicized (for more information visit: https://wwwnc.cdc.gov/eid/page/scientific-nomenclature).Modified

- In the methods section, please added: "Total microbial genomic DNA was extracted from the fresh frozen lung tissue samples". added
- Review spaces and grammar throughout the manuscript. Many spacing or typo errors that should be corrected, as well as some English corrections. Modified

- Authors reporting experiments on humans and/or the use of human tissue samples must confirm that all experiments were performed in accordance with relevant guidelines and regulations. Additionally, the manuscript file must include a statement identifying the institutional and/or licensing committee approving the experiments, including any relevant details, in the methods section. added
- The conclusions are supported by their data; however, the paper is not focused and needs much work to improve it.Modified

Reviewer 3:
In introduction of abstract should first describe subject , then , previous studies ....Modified

p values should be deleted from result of abstract. deleted

Text has grammar errors.Modified

abbreviations are incomplete.supplement
primers should be checked.Modified

2 Revision 1

0 comments

No public comments in this round.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:
Comments for authors and editorial teams:
The study entitled "Research of Microbial Diversity and Abundance in Pulmonary Tissue of Patients with Early Stage Lung Cancer" is novel and interesting. However, some minor concerns should be considered to improve the novelty as well as the flow of the manuscript:
1- Abstract: Acceptable
2- Keywords could be: Pulmonary cancer; Early stage; Microbial flora; Biodiversity; High throughput sequencing
Revised part in the manuscript:

Key words: Pulmonary cancer; Early stage; Microbial flora; Biodiversity; High throughput sequencing (page2, lines 31-32)

3- Introduction: Acceptable but could be better by wider literature review particularly related new & update articles from domestic researchers.
Revised part in the manuscript:

Lung cancer is the malignant tumor with the highest mortality rate(6). At present, the research on the pathogenesis of lung cancer is not very clear, but it is believed that it may be related to air pollution, chronic infections and smoking. In recent years, continuous research (7,8) has confirmed that there has been a significant change in the microbial community in the lungs of lung cancer patients compared to healthy individuals. More and more evidence suggests that the occurrence and development of lung cancer may be closely related to this change. Polymorphic microbiomes have recently been added as one of the four new "hallmarks" of cancer (9). (page3, lines46-53)

4- Methodology: Acceptable but I could not see informed census and also ethical code.
Revised part in the manuscript:

1.4 Ethics statement

The protocol for this study was reviewed and approved by the the Ethics Committee of Tengzhou Hospital Affiliated to Xuzhou Medical University(2021-NO-11) and informed consent was obtained from all subjects. All procedures undertaken were in accordance with the ethical standards of the Helsinki Declaration (1964 and amended 2008) of the World Medical Association.All methods were carried

out in accordance with relevant guidelines and regulations.(page6, lines126-132)

5- Results: Acceptable

6- Discussion: Could be scientifically than present level using more and wider literature review and explanation of related and domestic survey and of course then using from them as Bibliography/References.

Revised part in the manuscript:

Sun Y, et al(22) found alterations in the microbial community of patients with lung cancer, whose diversity might be site and pathology dependent.(page11, lines232-234)

7- Bibliography/References: More domestic and update Ref(s). are necessary. Authors should do more literature review for using related domestic articles and therefore some more domestic Ref(s). (Using from domestic Ref(s). is scientific discipline for publication of academic data.)

Revised part in the manuscript:

9. Hanahan D. Hallmarks of cancer: new dimensions [J] . Cancer Discov, 2022, 12(1):31-46. doi:10.1158/2159-8290. PMID: 35022204(page13, lines275-276)

22. Sun Y, Liu Y, Li J, et al. Characterization of Lung and Oral Microbiomes in Lung Cancer Patients Using Culturomics and 16S rRNA Gene Sequencing[J]. Microbiol Spectr. 2023,24:e0031423. doi: 10.1128/spectrum.00314-23. PMID: 37092999. (page15, lines315-317)

8- I could not see running title, informed census form, ethical code number and acknowledgment in manuscript. All of these compulsory.

Revised part in the manuscript:

1.4 Ethics statement

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accordance with the ethical standards of the Helsinki Declaration (1964 and amended 2008) of the World Medical Association.All methods were carried out in accordance with relevant guidelines and regulations.(page6, lines126-132)

9- Table & Graph quality: Acceptable

10- language/English quality: I am not right reviewer for language/English quality of manuscript.Ask it from a native speaker reviewer.

response：We have tried our best to correct grammar errors in text.

Final decision: Manuscript is acceptable for publication after minor correction
Reviewer 2:
In the submitted manuscript, “Research of Microbial Diversity and Abundance in Pulmonary Tissue of Patients with Early Stage Lung Cancer” the authors provide the correlation in the microbial flora of pulmonary tissue among different histological types of lung cancer.
There are major issues that should be addressed by the authors in order to improve this manuscript.
- There is lack of coherence in the introduction and there is no continuity between the sentences.
In general, the INTRODUCTION can be structured better.
response：Removed the following content: The microbial community enters the lung tissue through the upper and lower respiratory tracts due to respiratory movement, resulting in a dynamic equilibrium state of the microbial community in the lungs.
Lung cancer is one of the most common malignant tumors in China and the world.Due to its non-specific clinical symptoms, early diagnosis of lung cancer is difficult, Added a new literature.
Revised part in the manuscript:

Lung cancer is the malignant tumor with the highest mortality rate(6). At present, the research on the pathogenesis of lung cancer is not very clear, but it is believed that it

may be related to air pollution, chronic infections and smoking. In recent years, continuous research (7,8) has confirmed that there has been a significant change in the microbial community in the lungs of lung cancer patients compared to healthy individuals. More and more evidence suggests that the occurrence and development of lung cancer may be closely related to this change. Polymorphic microbiomes have recently been added as one of the four new “hallmarks” of cancer (9). (page3, lines46-53)

- If you compare with normal lung tissue and tumor tissue, please specify. Normal lung tissue and tumor tissue were obtained from 29 patients!!! The investigated tissues are not clear and details are missing. The authors should provide more primary data.

response：Thank you for pointing out the ambiguous wording.

Revised part in the manuscript:

Normal lung tissue and tumor tissue were obtained from 29 patients. (page5, lines91)

- On page 7; line 116: “front and rear F/R primers” changed to “forward and reverse primers”

Revised part in the manuscript:

Take 25μL high fidelity DNA polymerase, 3μL(10uM) forward and reverse primers, 10μL DNA template, 3μL DMSO、6μL ddH_2O. A total of 50uL was used as the PCR reaction system. (page5, lines101)

- What reference did you use for primer selection?

response：The primer selection is from the reference: Wang K, Huang Y, Zhang Z, et al. A Preliminary Study of Microbiota Diversity in Saliva and Bronchoalveolar Lavage Fluid from Patients with Primary Bronchogenic Carcinoma. Med Sci Monit. 2019 Apr 17;25:2819-2834. doi: 10.12659/MSM.915332. PMID: 30994108; PMCID: PMC6482867.

- The name of bacteria in must be italics. Italics are used for bacteria

at the level of family and below. All bacterial are italicized (for more information visit: https://wwwnc.cdc.gov/eid/page/scientific-nomenclature).

Revised part in the manuscript:

Pseudomonas, Meiothermus, Enterobacteriaceae, Halomonas, Staphylococcus, Enterococcus, Clostridium, Sphingomonas, Propionibacterium Faecalibacterium, Shewanella, Prevotella, Acinetobacter, Escherichia_Shigella, Brevibacterium, Enhydrobacterium, Stenotrophomonas, Blastomonas. (page8, lines159-162)

we found that the abundance of *Micrococcaceae* and *blastomonas* in tumor tissue was significantly higher than that in normal lung tissue (P values were 0.02 and 0.003, respectively), as shown in Figure 4.(page9, lines186)

- In the methods section, please added: "Total microbial genomic DNA was extracted from the fresh frozen lung tissue samples".

Revised part in the manuscript:

Total microbial genomic DNA was extracted from the fresh frozen lung tissue samples.

- Review spaces and grammar throughout the manuscript. Many spacing or typo errors that should be corrected, as well as some English corrections.

response：We have tried our best to correct grammar errors in text.

- Authors reporting experiments on humans and/or the use of human tissue samples must confirm that all experiments were performed in accordance with relevant guidelines and regulations. Additionally, the manuscript file must include a statement identifying the institutional

and/or licensing committee approving the experiments, including any relevant details, in the methods section.

Revised part in the manuscript:

1.4 Ethics statement

The protocol for this study was reviewed and approved by the the Ethics Committee of Tengzhou Hospital Affiliated to Xuzhou Medical University(2021-NO-11) and informed consent was obtained from all subjects. All procedures undertaken were in accordance with the ethical standards of the Helsinki Declaration (1964 and amended 2008) of the World Medical Association.All methods were carried out in accordance with relevant guidelines and regulations.(page6, lines126-132)

- The conclusions are supported by their data; however, the paper is not focused and needs much work to improve it.

Revised part in the manuscript:

In conclusion, through high-throughput sequencing of 29 surgical specimens from patients with early lung cancer, we found the major microbial components in tumor tissue and normal lung tissue respectively. The differences in lung microbiota among different histological types of lung cancer has been found in this study,which providing a basis for studying the mechanism of lung cancer occurrence and development.(page12, lines242-247)

Reviewer 3:

In introduction of abstract should first describe subject , then , previous studies ....

Revised part in the manuscript:

Background The composition of lung tissue microorganisms in patients with different tissue types of lung cancer has not yet been determined. Previous studies have shown changes in the composition of pulmonary microbial flora in patients with lung cancer. **Objective** The purpose of this study was to investigate the differences and correlation in the microbial flora of pulmonary tissue among different histological types of lung cancer. (page1, lines11-16)

p values should be deleted from result of abstract.

response：Thank you for your reminder. We have deleted p values from result of abstract.

Text has grammar errors.

response：We have tried our best to correct grammar errors in text.

abbreviations are incomplete.

response：We have added some abbreviations in paper.

Complicated with bronchial asthma, pulmonary tuberculosis, obstructive pneumonia, chronic obstructive pulmonary disease(COPD), (page4, lines71-72)

A total of 50uL was used as the PCR(polymerase chain reaction) reaction system (page5, lines102)

primers should be checked.

Revised part in the manuscript:

Select primers for PCR amplification of the V4 hypervariable region of 16S rDNA was performed with the forward primer 515F

(5’-GTGCCAGCMGCCGCGGTAA-3’) and reverse primer 806R

(5’-GGACTACHVGGGTWTCTAAT-3’). (page5, lines99-100)

3 Revision 2

0 comments

No public comments in this round.