Peer Review File

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Reviewer A

This is a very complex analysis of transcriptomics data to shed light on possible mechanisms underlying pathological processes in the airways of asthmatic processes namely airway remodeling and Th2-related inflammation. Analyzed data include datasets from 56 bronchial epithelial samples from asthmatic patients, 27 samples from non-asthmatic patients and also samples from the upper airways of mice exposed to chlorine gas vs control mice. Series of complex analyses were conducted that revealed a total of 182 differentially expressed genes, of which 97 had higher expression and 85 showed lower expression. Analysis of samples from the mice showed 210 differentially expressed genes (mRNA) from trachea exposed to chlorine gas. The rationale of conducting this analysis is not clear in the context of Th2-mediated airway inflammation although next set of experiments suggest that it has served as reference for airway remodeling. Indeed, cross sectional analysis between human samples from asthmatic patients and stenotic tracheal samples from mouse exposed to chlorine gas was subsequently performed. The latter analysis revealed 11 DEGs; authors concluded these 11 genes are likely to be involved in tracheal remodeling: this needs to be clarified. Series additional analyses were conducted including downloaded 10 Th2-type inflammation-related factors from the Molecular Signatures Database namely IL1, IL4, IL5, IL6, IL9, IL10, IL13, IL25, IL33, and TSLP and compared to bronchial asthma set that narrowed findings to 2 DEGs, IL-6 (less expressed) and IL-9 (with higher expression) in asthmatic patients. Expression of these two genes was further correlated with “remodeling genes” showing relatively mild correlation suggesting that there may not be a regulatory relationship between them.

Interestingly, authors explored the upstream regulatory mechanisms of the IL6 and IL9 genes by analyzing 3 databases of microRNA and long noncoding RNAs of IL6 and IL-9. In these analyses they found that miR-515-5p was the core node gene, and miR-607 was the common upstream regulators of IL6 and IL9. The latter findings could be commented in the context of “reciprocal” expression of IL-6 and IL9.

Overall, this transcriptomics analysis used advanced approaches for crossectional analysis of complex genes from human and mice samples. The findings are quite intriguing; however, paper needs to be revised for clarity. It needs better rationale and justification for performed analyses. Justification needs to be provided for comparison of human samples from lower airways with Th2 inflammation with mice samples from the upper airways exposed to irritant gas.

Response: Thank you for your thoughtful and thorough comments on our manuscript. We appreciate the time and effort you've put into reviewing our work. Below, we have addressed your comments and concerns:

1. Rationale of the analysis and linkage with Th2-mediated airway inflammation**: We acknowledge that our rationale behind conducting the analysis could have been explained more clearly. Our primary goal was to uncover the potential molecular mechanisms underlying Th2-type airway inflammation and airway remodeling in asthma. The inclusion of mice exposed to chlorine gas was intended to provide an experimental model for airway remodeling, which is a significant component of the asthmatic pathology.

> The primary objective of this study is to gain a deeper understanding of the potential molecular mechanisms of Th2 type airway inflammation and airway remodeling in asthma. To achieve this goal, we will focus on studying Th2 type inflammation-related factors, leveraging bioinformatics techniques and big data mining to delve into their roles in the mechanisms of airway remodeling. By choosing mice exposed to chlorine gas as the experimental model, we aim to further uncover the intricate details of airway remodeling in the pathology of asthma. Our research is anticipated to provide new molecular targets for the prevention and treatment of asthma.

2. Involvement of 11 DEGs in tracheal remodeling**: We agree with your comment that our conclusion regarding the involvement of the 11 DEGs in tracheal remodeling needs to be clarified. These genes were identified as differentially expressed in both asthmatic patients and mice exposed to chlorine gas, making them potential candidates for further investigation. However, we acknowledge that further functional studies are required to determine their exact roles in airway remodeling. We will adjust our language to reflect this.

> 229 *ABCA13*, and *RASSF10*) (*Figure 2B*). The expression of these 11 genes in the
> 230 GSE109365 dataset was further evaluated (*Figure 2C,2D*). Based on the above, our
> 231 study reveals that these are potential candidate targets for further research. However,
> 232 we acknowledge that further functional studies are required to determine their exact
> 233 roles in airway remodeling.

3. Upstream regulatory mechanisms of IL6 and IL9: We appreciate your interest in our findings regarding the miRNAs and lncRNAs potentially regulating IL6 and IL9. We agree that the "reciprocal" expression of IL-6 and IL9 is an interesting aspect and plan to further explore this in our future studies.

4. Justification for human-mice comparison: The comparison between human and mice samples aimed to integrate the clinical features of asthmatic patients with an experimental model of airway remodeling. We acknowledge that the difference in the airway regions (lower in humans, upper in mice) and the irritant exposure in mice could introduce some variability. However, we believed this approach would allow us to explore the potential common elements of airway inflammation and remodeling across species and conditions.

We hope these responses address your concerns, and we appreciate your valuable feedback, which will greatly improve our manuscript.

Reviewer B

It is well known that the basic features of asthma include episodic airways inflammation, airways hyperresponsiveness, and mucous hypersecretion. Although we understand the basic clinical features of asthma, asthma is a heterogeneous disease process with varying phenotypes and presentations.

Although the involvement of T helper type 2 (Th2)-associated inflammatory factors in airway remodeling has been previously reported, the specific mechanisms of action is not fully determined. The currents authors have tried that roles of Th2-related inflammatory factors in tracheal remodeling by using the gene microarray database method of differentially expressed gene (DEG) screening, enrichment analysis, protein-protein interaction (PPI) network construction, machine learning, and the construction of a line graph model. Their results indicated that S100A14, KRT6A, S100A2, ABCA13, UBE2C, RASSF10, PSCA, PLAT, and TIMP1 may be involved in the airway remodeling. The epithelial-mesenchymal transition (EMT)-related genes GEM, TPM4, SLC6A8, and SNTB1 may also be associated with airway remodeling of asthma condition. The authors have concluded that the asthma gene microarray database through bioinformatics analysis and identified key genes and important pathways affecting airway remodeling in asthma patients.

As suggested by authors, gene microarray database providing new ideas to uncover the mechanism of airway remodeling due to asthma and then seek new therapeutic targets

However, the gene microarray database should be combined with the protein and its protein function analysis. The gene expression may not be associated with the protein function in vivo.

This is an attractive study. However, the limitation should be detailed described.

Response: I greatly appreciate your thoughtful and thorough commentary. I am grateful for the questions and suggestions you have raised about our research. Below, I have

addressed your comments and concerns:

Lack of protein function analysis: I agree with your perspective that an analysis based solely on gene expression data may not fully reveal the mechanisms of airway remodeling. In vivo, gene expression may not always correlate with protein function. Therefore, we plan to incorporate protein function analysis in our future research to more comprehensively unveil the mechanisms of airway remodeling. This will include validating the expression of the key genes we have identified at the protein level, as well as the functions of these proteins in the pathological process.

Limitations of the study: We acknowledge that our study has certain limitations. Firstly, our analysis mainly relies on publicly available gene expression datasets, which might be influenced by sample sources, processing methods, and experimental conditions. Secondly, our research primarily focuses on changes in gene expression, but we do not further investigate how these changes impact protein expression and function. Lastly, while our research mainly aims to reveal potential molecular mechanisms, we do not perform experimental validations. These are issues that we aim to address in our future research.

Reviewer C

I reviewed the manuscript entitled “Mechanistic analysis of Th2-type inflammatory factors in asthma”

The idea of the study is well illustrated but I have some concerns:

The title: “in” is repeated

Thanks for your comment, we have revised it.

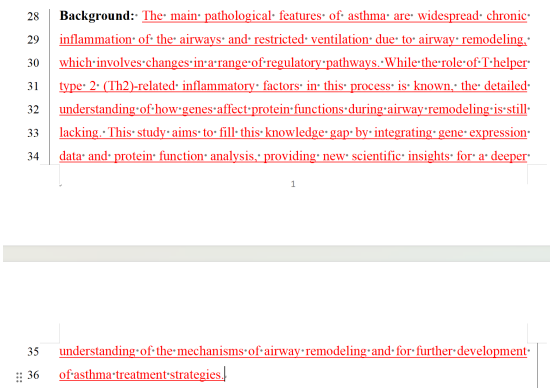
1 **Original Article**

2 **Mechanistic analysis of Th2-type inflammatory factors in asthma**

3

Abstract: the background did not show what is already known about the genes of remodeling and what your study adds

Thanks for your comment, we have revised it.



Introduction: your results should not be mentioned in the introduction, otherwise mentioning the already known interleukins and genes is important, and end your introduction with the goal of the study.

Thanks for your comment, we have revised it.

> The primary objective of this study is to gain a deeper understanding of the potential molecular mechanisms of Th2 type airway inflammation and airway remodeling in asthma. To achieve this goal, we will focus on studying Th2 type inflammation-related factors, leveraging bioinformatics techniques and big data mining to delve into their roles in the mechanisms of airway remodeling. By choosing mice exposed to chlorine gas as the experimental model, we aim to further uncover the intricate details of airway remodeling in the pathology of asthma. Our research is anticipated to provide new molecular targets for the prevention and treatment of asthma.

Discussion:

It has been stated multiple times that "airway remodeling is important in asthma pathogenesis" (essentially repeating the same point in the introduction and discussion).

You mentioned bleomycin-induced pulmonary fibrosis and its effect on EMT but did not mention its relation to asthma (this technique usually induces alveolar fibrosis).

The discussion is only mentioning your results not correlating it to other studies.

Thanks for your comment, we have revised it. And we have deleted the literature related to bleomycin.

these inflammatory factors act as secreted proteins on themselves or surrounding cells, and intracellular mRNA expression may not truly reflect their protein levels; further protein-level assays may be required. Previous research has suggested that TGF-β1, IL-13, and IL-17 are regarded as critical regulatory factors. These factors can modulate the function of airway epithelial cells, smooth muscle cells, fibroblasts, and immune cells, and may lead to structural and functional changes in the airways(24,25). In recent years, the roles of IL6 and IL9 in airway remodeling have received increasing attention. IL-6 is believed to promote airway myofibroblast and matrix synthesis through the proliferation of airway smooth muscle cells and the regulation of Th17 cell differentiation(26,27). Meanwhile, IL-9 is thought to promote airway myofibroblast and matrix synthesis, as well as the survival and activation of

As the outermost cell in the trachea, the tracheal epithelium assumes a barrier role as well as a role in the presentation and amplification of immune factors in the environment, and its EMT is generally considered the initiating factor in the onset of subsequent tracheal remodeling(35,36). EMT in the tracheal epithelium not only leads to a decrease in the tight junctions of some epithelial cells, thus reducing the epithelial barrier capacity and leading to airway hyperstress, but also leads to airway subepithelial fibrosis, increased extracellular matrix (ECM) secretion and abnormal value added by smooth muscle cells. EMT, as a transitional state between the epithelial and mesenchymal phenotypes, is a key inducer of airway fibrotic remodeling(37,38).