



## Early View

Research letter

# Iron mobilization pattern in airway epithelium: tipping the immune balance in asthma

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Please cite this article as: Zhang J, Liang Z, Zhang D, *et al.* Iron mobilization pattern in airway epithelium: tipping the immune balance in asthma. *ERJ Open Res* 2023; in press (<https://doi.org/10.1183/23120541.00475-2022>).

This manuscript has recently been accepted for publication in the *ERJ Open Research*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

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**Iron Mobilization Pattern in Airway Epithelium: Tipping the Immune Balance  
in Asthma**

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Running title: Iron in Asthma Airway Epithelium

## **Iron Mobilization Pattern in Airway Epithelium: Tipping the Immune Balance in Asthma**

To the Editor,

The barrier functions of the airway epithelium are centrally involved in regulating tissue homeostasis in the lung, and abnormal responses of the airway epithelium to the environment, including metal exposure, underlies the rise in allergic conditions and chronic airway diseases<sup>1,2</sup>. Notably, injury-induced epithelial cell apoptosis and injury with resultant lack of normal restitution of epithelial barriers are increasingly recognized as critical events in the pathogenesis of airway diseases such as asthma<sup>1,3</sup>. Evidence accumulated in recent years points to a connection between iron regulation and asthma pathogenesis<sup>4,5</sup>. However, the role of iron in asthmatic airway epithelium remains incompletely understood.

As a starting point for investigating the potential role of iron level and regulation in the asthmatic airway epithelium, we identified 14 essential iron transport-related genes in the airway epithelium through combined UniProt database screening and ‘iron transport’ genesets annotated by GeneOntology. Next, we assessed their expression to reflect iron metabolism by integrating different omics data sources. The data expression profiles of asthma were searched in the Gene Expression Omnibus (GEO) database, and two independent mRNA datasets of research on the airway epithelium in human asthma, GSE67472 and GSE43696, respectively, were selected. Differential mRNAs were screened in airway epithelial samples obtained from biopsy in patients with and without asthma. By probing the GSE67472 dataset which

included 43 healthy subjects and 62 patients with asthma, we were surprised to find that all upregulated genes (*TFR2*, *SLC39A8*, and *SLC39A14*) were associated with iron uptake. Intriguingly, an external validation in the GSE43696 (comprising 108 subjects) was performed, and the same conclusions were obtained (Figure 1A). The aggregate of the above results together suggests that an iron ‘input mode’ exists in the asthmatic bronchial epithelium. Our attempts (data not shown) to detect iron levels in the airway epithelium using Prussian blue staining were unsuccessful (no coloration in the epithelium). Variability in tests of iron content has been an issue in the field, and direct evidence on this issue is still lacking.

To evaluate the effects of the disturbances in iron metabolism on airway epithelium, epithelial cell iron-overload and -reversal models were respectively constructed (Figure 1B) using ferric ammonium citrate (FAC) combined with/without deferoxamine (DFO) in human bronchial epithelial (HBE) cells (obtained from Shanghai Fuheng Biology Co., Ltd). The model was optimized by parameters including different concentrations (10  $\mu$ M, 50  $\mu$ M, or 100  $\mu$ M) of iron supplement (FAC) and DFO, and incubation time, to reach overload and followed complete reversal. Intracellular total iron and ferrous ion levels were quantified using a colorimetric assay (Elabscience, Wuhan, China). As can be seen in Figure 1B, FAC significantly influenced the total intracellular content of iron and ferrous iron. It can be seen in Figure 1B that DFO administration (100  $\mu$ M) significantly reversed the concentration changes (including total iron and ferrous ions) after FAC treatment. RNA sequencing among the three groups was performed to gain insights into the

underlying molecular mechanisms. The trending analysis indicated that most data points after screening ( $p < 0.05$ ;  $|FC| > 1.2$ ) varied dynamically over iron concentration. These 'iron-responsive' genes (IRGs) exhibited dynamic expression as cellular iron concentrations changed. The combined analysis yielded 473 results, including 225 iron-activated and 248 iron-repressed genes. Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis and followed network plot for the pathways' interaction revealed that IRGs were significantly enriched in apoptosis, IL-17 signalling, and TNF signalling pathway (Figure 1C).

To identify causative genes during iron mobilization that could be involved in the pathogenesis of asthma, we performed a cross-comparison of IRGs with asthma-associated immune genes from the GeneCards, DisGeNT, and Immport databases. A total of 12 intersected genes in four groups were extracted, as shown in Figure 1D. The change trends in only three genes (*HMOX1*, *ACTG1*, *VDR*) were consistent with the cellular iron change trend, while the opposite was observed for the remaining. Moreover, protein–protein interaction (PPI) analysis using the STRING database revealed three effective hub genes (*CXCL8*, *CCL2*, *CSF2*) located at the core of the interaction network (Figure 1D). To further validate the results obtained from the screening analysis, we next performed qRT-PCR in two types of human bronchial epithelial cell lines (HBE and BEAS-2B). Six genes (*CCL2*, *HMOX1*, *IRF7*, *TFRC*, *VDR*, and *MAPK8*), which vary dynamically over iron concentration, were confirmed with qPCR in both cell types (data not shown). These provide a degree of validation for both sets of sequencing results. Notably, most of these alterations show a trend

towards improved inflammation status and retard asthma progression. Contrary to our expectations, these observations appear to support a beneficial effect of iron mobilization in asthmatics' airway epithelium rather than a detrimental action.

To our knowledge, this is the first study to provide evidence about the existence of iron mobilization mode in asthmatics' airway epithelium. Disordered iron metabolism appears critical in maintaining the airway epithelial barrier function, regardless of whether the self-protection mechanism or normal iron metabolism is disrupted by allergen stimulation. In previous studies, the asthmatic bronchial epithelium has been recognized to be more susceptible to oxidant-induced apoptosis and is considered a crucial factor in asthma development<sup>6,7</sup>. Based on our *in vitro* studies, iron mobilization patterns in the airway epithelium against the background of asthma pathogenesis may play a pivotal role in the progression of cellular apoptosis. Many human studies have observed increased TNF- $\alpha$  and IL17A secretion in asthmatic airways<sup>8,9</sup>. Iron overload induced by upregulated proteins involved in iron trafficking in asthmatics' epithelium may further aggravate these processes. Available evidence indicates that iron may assume differential or even opposite roles in different lung cells (e.g., macrophages, fibroblasts, and epithelial cells) during asthma progression. This may explain some of the previous diverging results regarding iron supplementation in animal and cell models<sup>4,10,11</sup>. Given the study's exploratory nature, we believe that our results will lay the foundation for further research.

## **ACKNOWLEDGEMENTS**

This study was supported by grants from the Key Research and Development Program of Shandong Province grants number 2021SFGC0504 (to L.D.) and Shandong Provincial Natural Science Foundation grant number ZR2021LSW015 (to L.D.).

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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## Figure legend

Figure 1. Effects and mechanisms of the perturbed iron homeostasis on airway epithelium. (A) Differential expression analysis of iron transport-related genes in discovery and validation datasets (GSE67472 and GSE43696). (B) The iron-overload and –reversal cellular model was constructed using FAC combined with/without DFO for 24h incubation. (C) The combined analysis yielded pathway-gene network graphs. (D) Protein-protein interaction network showing asthma-associated immune and hub genes regulated by iron.



