STAT3 Promotes Apoptosis of Alveolar Epithelial Cells by Inhibiting AKT Signaling

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Type II alveolar epithelial cells are a crucial component of alveolar epithelium, and transcriptional activator 3 (STAT3) have functions in regulating alveolar epithelial cell proliferation. Therefore, based on the modular approach, we analyzed the effects of silencing STAT3 on type II alveolar epithelial cells and studied its mechanism of action. Initially, in the GEO database, we downloaded data on type II alveolar epithelial cells. For transcriptome data in alveolar epithelial cell samples, we performed a differential analysis. Secondly, protein interaction network analysis (PPIs) were performed on the differential genes, and the PPIs were analyzed modularly. The module gene was subjected to enrichment analysis of GO function and KEGG pathway. Non-coding RNAs and transcription factors that regulate the module are predicted based on hypergeometric testing. Thus, we have a total of 13 dysfunction modules. These modular genes are significantly involved in biological processes such as nuclear membranes, embryonic organ development, and regulate the insulin signaling pathway and the PI3K-Akt signaling pathway substantially. We identified vital ncRNA pivots (miR-205-5p) and TF pivot (Eomes, Ets1, Nfkb1, Spi1, Stat1, Usf1) to regulate dysfunction modules significantly. Our work deciphered a co-expression network that involved essential gene regulation of type II alveolar epithelial cell apoptosis. It helps to reveal the regulation of silencing STAT3 gene on alveolar epithelial cell apoptosis and deepen our understanding of the mechanism. More importantly, we explained that the silencing gene STAT3 inhibits the apoptosis of alveolar epithelial cells by activating the AKT signaling pathway, providing a new theoretical reference for the study of alveolar epithelial cells.

Key words: alveolar epithelial cells, STAT3, AKT signal, co-expression module, regulatory factor
Tob Regul Sci.™ 2021;7(4-1): 741-748
DOI: doi.org/10.18001/TRS.7.4.1.28

INTRODUCTION

Type II cells (AEC2) of the alveolar epithelium can produce surfactants and express innate immune molecules. As an adult progenitor cell, it can maintain the homeostasis in the lung with alveolar epithelial type I cells (AEC1) [1]. Apoptosis of alveolar epithelial cells can lead to the development of diseases such as childhood pneumonia, acute lung injury associated with sepsis, and acute hypoxic respiratory failure [2,3]. From the perspective of medicine and biology, researchers have explored the pathogenesis of alveolar epithelial cells and made some progress. HDL increases phosphorylation of MAPK. Also, it triggers proliferation and migration of type II alveolar epithelial cells, as well as reduces the secretion of TNF-α/IL-1a and the expression of CFTR [5]. EMT of alveolar type II (AT2) epithelial cells affects normal alveolar development [6]. At the same time, autophagy protects against apoptosis of alveolar epithelial cells in COPD rats induced by endoplasmic reticulum stress [7]. Besides, receptors for advanced glycation end products are abundantly expressed on alveolar epithelial cells, and they are involved in innate immune responses such as apoptosis and inflammation [8].

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On the other hand, lung epithelial cells have functions in the initiation and regulation of immune responses during pulmonary infection [9]. These findings have deepened our understanding of alveolar epithelial cells and guided us on the direction of further research. Although the previous research on a series of alveolar epithelial cells has been reported, the overall effect of these results is still elusive. To further explore the role of genes in the mechanism of apoptosis in alveolar epithelial cells, we performed a systematic modular analysis to determine its dysfunction modules and core molecules. Further, we explored the regulatory factors of alveolar epithelial cells. Overall, our work details the silencing of STAT3 by activating the AKT signaling pathway to inhibit apoptosis in alveolar epithelial cells. Potential therapeutic targets and related biological processes have been identified, which may help to understand and treat apoptosis in alveolar epithelial cells.

RESULT

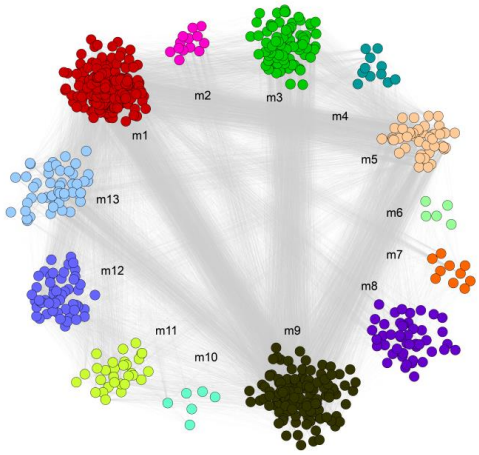
STAT3 Affects The Expression of Genes in Alveolar Epithelial Cells

We performed differential expression analysis based on microarray data to identify differential gene expression (DEG) between type II alveolar epithelial cells. Differential gene identification revealed 486 differentially expressed genes, and we believe that the presence of STAT3 in these differential genes leads to downstream regulatory genes for potential dysfunction of alveolar epithelial cells. Also, these related genes may cause possible dysfunction of alveolar epithelial cells through their interaction. Based on differential gene expression profiles, we used Cytoscape software to construct differentially expressed protein-protein interaction networks (PPIs).

Disordered Genes Have A Synergistic Expression

For each elemental gene, the module is a collection of genes with a synergistic expression relationship, and the genes of the same module have consistent expression behavior. For the genes related to apoptosis of alveolar epithelial cells, their expression behaviors are clustered into modules. It helps us to observe the complex synergy between these genes from the perspective of expression behavior. Based on the cohesive-guided clustering algorithm, we explored 13 functional modules (Figure 1). Modules in these 13 dysfunction modules may form a functional network that causes dysfunction of alveolar epithelial cells.

Figure 1 Identification of alveolar epithelial cell dysfunction module. There are 13 interaction modules, each color representing a module.



Function and Pathway Involved in Dysregulated Genes

Functions and pathways are essential media. Exploring the functions and pathways involved in the dysfunctional module gene not only helps to determine the relationship between the same pathway genes in the module but also helps to establish a molecular bridge between modules and diseases in system biology. For 13 modules of GO function and KEGG pathway, we performed enrichment analysis and obtained 20293 biological processes, 1830 cells, 3046 molecular functions, and 1036 KEGG pathways (Figure 2A, 2B). These functions were found to be mainly concentrated on biological processes such as nuclear membrane and embryonic organ development. According to the enrichment of the KEGG pathway, differential genes in alveolar epithelial cells are involved in the insulin signaling pathway and the PI3K-Akt signaling pathway. Using BinGO for path analysis, we integrated 13 modular networks(Figure 3).

Figure 2 Function and pathway enrichment analysis of module gene participation A. GO function enrichment analysis of modular genes. The color increases from blue to purple, and the enrichment increases significantly. The larger the circle, the more significant the proportion of the gene in the module that accounts for the GO function. B. KEGG pathway enrichment analysis of modular genes. The color increases from blue to purple, and the enrichment increases significantly. The larger the circle, the higher the proportion of the gene in the KEGG pathway entry.

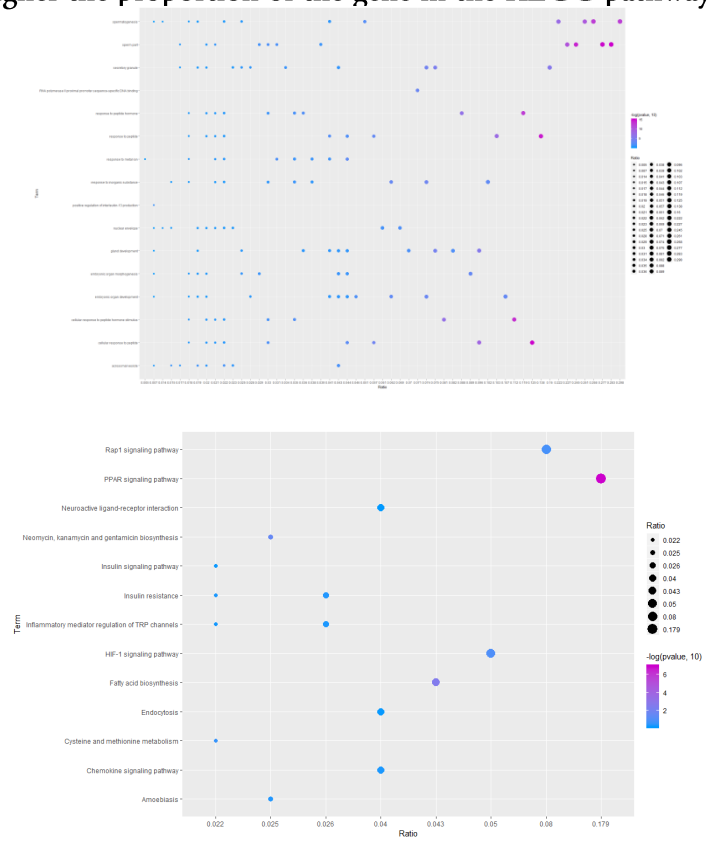
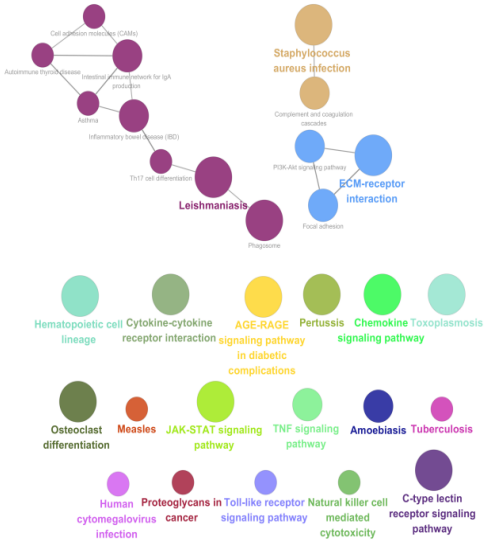


Figure 3. Path analysis of the integrated module network

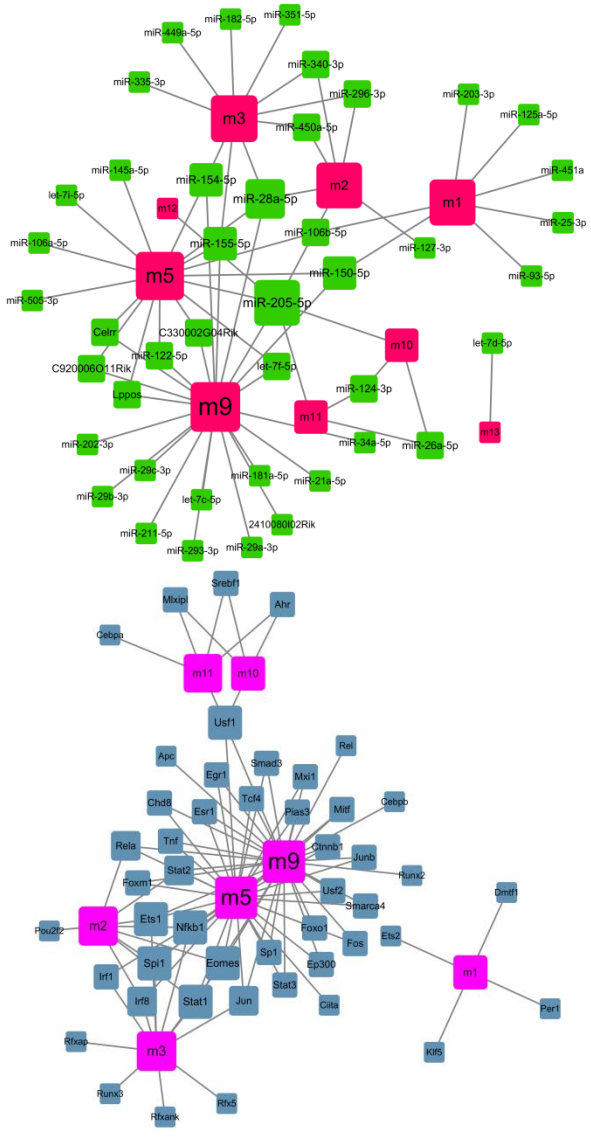


Drive Module Gene TF and ncRNA

Transcription and post-transcriptional regulation of genes have been considered critical. Transcription factors and ncRNA are regulators of common expression and function. In this study, based on the targeted regulation relationship of TF and ncRNA to the module gene, we performed a pivotal analysis of the conventional module. At the same time, we explored the key transcriptional

regulators that regulate the progression of apoptosis in alveolar epithelial cells. The predicted results showed that a total of 43 ncRNAs involved 69 ncRNA-module regulatory pairs and 48 transcription factors affected 97 TF-module target pairs. The above results were introduced into Cytoscape to observe the adjustment of the adjustment factor in the dysfunctional module (Figure. 4A, 4B). Also, we performed a statistical analysis of the pivot control module to obtain ncRNA (miR-205-5p) and TF (Eomes, Ets1, Nfkb1). These transcription factors and ncRNAs regulate the process of alveolar epithelial cell dysregulation through STAT3-mediated dysfunctional module genes. Thus, we identified these potential regulatory factors as likely regulators of apoptotic progression in alveolar epithelial cells. Furthermore, the expression level of key genes key genes with maximum differential multiplier was verified by qPCR. We found that the expression trend of key genes was consistent with the previous results.

Figure 4. Regulatory effects of regulators on dysfunction modules. A. nc-RNA pivot regulates the module. Red squares represent modules, and green squares represent ncRNAs. B. TF pivot's regulation of the module. Purple squares represent modules, and blue squares represent TF.



DISCUSSION

Alveolar epithelial cell apoptosis and inflammatory response are important pathological processes in many lung diseases [10]. In this study, we collected genes related to type II alveolar epithelial cells on the NCBI Gene Expression Omnibus database (GEO Dataset), which involved knockout of the STAT3 gene and the non-knockout STAT3 gene. Based on the differential gene expression profile data of STAT3-related alveolar epithelial cells, we dissected the relevant modules of alveolar epithelial cell genes, which are driven by transcription factors and ncRNA regulators. Module genes are significantly involved in biological processes, including embryonic organ development, while significantly participating in signaling pathways, including the insulin signaling

pathway (PI3K-Akt). Among them, BCG is a human tuberculosis vaccine that stimulates the release of PGE (2) by in vitro activated macrophages [11]. Insulin attenuates pulmonary edema and enhances AFC by inhibiting the expression of ENdC, which is dependent on the PI3K/Akt pathway, by inhibiting Nedd4-2 [12]. On the other hand, by modulating the PI3K / Akt / mTOR signaling pathway, epithelial cells attenuate Toll-like receptor-mediated monocyte derivation [15]. Also, signaling pathways including Wnt signaling, interleukin and apoptosis are critical for differentiation of lung epithelial stem cells and lung epithelial cells [16]. By pretreatment, we not only significantly attenuated the early stage of pathological mechanical stretch induction, but also the release of pro-inflammatory cytokines in alveolar epithelial cells. Finally, the expression of NF-κB can be significantly reduced [17]. Besides, hydrogen activates the PI3K / Akt signaling pathway. Hyperoxia-induced apoptosis in type II alveolar epithelial cells [18]. On the other hand, at the molecular level, we predicted that 43 ncRNAs are involved in the apoptosis of alveolar epithelial cells through a mediator module. Based on statistical analysis, we determined that miR-205-5p has a significant effect on the six dysfunctional modules. Among them, by targeting ZEB1, miR-205-5p inhibits cell migration and invasion in prostate cancer. Moreover, the miR-205-5p / ZEB1 axis has the potential for development in prostate cancer treatment strategies [19]. Long non-coding RNA-derived miR-205-5p regulates human endometrial cancer by targeting PTEN [20]. The MALAT1 / miR-205-5p axis regulates MPP + -induced apoptosis in MNPD cells by targeting LRRK2 [21]. The effect on apoptosis of alveolar epithelial cells was not found in the study on miR-205-5p. However, our analysis showed that it significantly regulates the apoptosis of alveolar epithelial cells, which is the ncRNA that regulates the most dysfunctional modules and is the direction of further research in the future. Finally, we identified 48 transcription factors that differentially expressed and significantly regulated alveolar epithelial dysfunction. According to the regulatory analysis, Eomes, Ets1, Nfkb1, Spi1, Stat1, and Usf1 significantly regulate four modules, all of which may have functions in the apoptosis of alveolar epithelial cells. The transcription factor Eomesodermin have functions in regulating the cytotoxic, developmental and survival functions of immune cells [22]. Through the Ets1 dependent pathway, overexpression of thrombin in interstitial lung disease (ILD) increases CHOP expression in primary AEC and A549 cells [23]. By activating genes critical for the NF-κB and JNK pathways, FOXM1 is required for K-Ras-mediated lung tumorigenesis [24]. Studies have found that SPI-1 is important for macrophage (PAM) and induces the formation of spacious phagosomes in the invasion of porcine alveoli [25]. Also, EGCG inhibits epithelial cell apoptosis in alveolar cells by inhibiting the STAT1 -caspase-3 / p21-related pathway [26].

By the accumulation of TGF-β1 production in melanoma cells, overexpression of USF1 promotes the EMT process [27]. The apoptotic mechanism of alveolar epithelial cells is essentials, which is the next research direction. At the same time, other transcription factors that significantly regulate the dysfunction of alveolar epithelial cells may also be involved in the apoptotic process.

MATERIALS AND METHODS

Data Resource

The NCBI Gene Expression Omnibus database (GEO Dataset) [28] includes a broad classification of high-throughput experimental data. There are single-channel and dual-channel microarray-based assays for mRNA abundance, genomic DNA and protein molecules. From GEO, we collected gene expression profiles for alveolar epithelial cells, numbered GSE6846 [29]. The data included an autopsy sample of 7 AD patients and six normal subjects. We downloaded all human protein-protein interaction data from the STRING v10 database [30] to construct differential gene-related PPIs. The STRING database is a search tool for retrieving interacting genes, and it helps us to discover and annotate functional interactions in living systems fully. We screened the ncRNA-mRNA interaction pair with score >= 0.5 from the RAID v2.0 database [31], including 431937 interaction pairs involving 5431 ncRNAs. The RAID v2.0 database recruited more than 5.27 million RNA-related interactions. Among them, more than 4 million RNA-RNA interactions and more than 1.2 million RNA-protein interactions are included. It refers to 130 000 RNA/protein symbols in 60 species, which can help us to observe various RNA-related interactions comprehensively. At the same time, all human transcription factor target data were downloaded and used in the general database TRRUST v2 database of transcriptional studies [32], involving 2492 transcription factors and 9396 interaction pairs.

Differentially Expressed Genes

The differential expression analysis of the gene expression profile and miRNA expression profile data of this study was performed using the R language limma package [33-35]. We used the Correct background function to perform background correction and normalization of the data. The control probe and the low expression probe were filtered using the normalize Between

Arrays function quantile normalization. Then, the differentially expressed genes and differentially expressed miRNAs were identified based on the lmFit and eBayes functions, respectively, using default parameters.

A Protein-Based Network Identification Module

We mapped all differentially expressed genes into a human protein-protein interaction network. Then we extracted only the interaction pairs containing these differential genes and then constructed a PPI that knocked out the STAT3 gene and the differential gene of alveolar epithelial cells that did not knock out the STAT3 gene. We shared the differential expression of PPIs and their genes into Cytoscape [36] for demonstration. According to the cohesion algorithm and the neighbor selection strategy, we use the default parameter plug-in ClusterONE [37] for class identification of protein complexes. In the ClusterONE algorithm, the higher the cohesion score, the more likely the protein is a protein complex. Among them, Cytoscape is used in network visualization software, which can help us observe various material interaction networks.

Enrichment Analysis

Exploring the functions and signaling pathways involved in genes often helps to study the molecular mechanisms of disease. Based on the genes of the dysfunctional module, we performed enrichment analysis of functions and pathways, which can help explore the underlying mechanisms of alveolar epithelial cell apoptosis. Therefore, we used the R language Clusterprofiler package [38] for Go function and KEGG pathway enrichment analysis for the 13 modules. The cluster profile is a Bioconductor software package for visual analysis of gene sets/gene clusters. In addition, we use Cytoscape's BinGO [39] application to perform path analysis on the integrated module network.

Regulator Analysis

Non-coding genes and transcription factors often drive the transcription and post-transcriptional regulation of genes. Therefore, we have scientifically predicted and tested the role of the alveolar epithelial dysfunction module. Pivot regulators are defined as modulators that have significant regulatory functions in modules during alveolar epithelial cell apoptosis, including ncRNA and TF. More than two control connections between the regulator and the module are required. Based on hypergeometric testing and the significance of the enriched target in the module, the p-value is supposed to be less than 0.01.

Verification of Key Genes by qPCR

Specifically, total RNA in whole blood was extracted, transcribed into cDNA using a reverse transcription kit, and qPCR reaction was carried out using the SYBR qPCR Detection Kit. The qPCR program begins the initial 3 minute denaturation step at 95 ℃to activate the hot-start iTaqTM DNA polymerase. This was followed by 45 cycles of denaturation at 95 ℃ for 10 seconds and annealing and extension at 60 ℃ for 45 seconds. The internal reference gene is beta-actin.

FUNDING

This work was supported by Outstanding Youth Project of Education Department of Hebei Province(YQ2014008)

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