Identification of Significant Genes And Pathways In ARDS Via Bioinformatical Analysis

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Research Article

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Abstract

Background and Aims: Acute respiratory distress syndrome (ARDS) is one of the most common acute thoracopathy with complicated pathogenesis in ICU. The study is to explore the differentially expressed genes (DEGs) in the lung tissue and underlying altering mechanisms in ARDS.

Methods: Gene expression profiles of GSE2411 and GSE130936 were available from GEO database, both of them included in GPL 339. Then, an integrated analysis of these genes was performed, including gene ontology (GO) and KEGG pathway enrichment analysis, protein-protein interaction (PPI) network construction, Transcription Factors (TFs) forecasting, and their expression in varied organs.

Results: A total of 39 differential expressed genes were screened from the datasets, including 39 up-regulated genes and 0 down-regulated genes. The up-regulated genes were mainly enriched in the biological process, such as immune system process, innate immune response, inflammatory response, cellular response to interferon-beta and also involved in some signal pathways, including cytokine-cytokine receptor interaction, salmonella infection, legionellosis, chemokine, and Toll-like receptor signal pathway. GBP2, IFIT2 and IFIT3 were identified as hub genes in the lung by PPI network analysis with MCODE plug-in, as well as GO and KEGG re-enrichment. All of the three hub genes were regulated by the predictive common TFs, including STAT1, E2F1, IRF1, IRF2, and IRF9.

Conclusions: This study implied that hub gene GBP2, IFIT2 and IFIT3, which might be regulated by STAT1, E2F1, IRF1, IRF2, or IRF9, played significant roles in ARDS. They could be potential diagnostic or therapeutic targets for ARDS patients.

Introduction

Acute respiratory distress syndrome (ARDS) is an acute hypoxemic respiratory failure, characterized by lung tissue oedema and injury, inflammatory responses, and compromised gas exchange following macrophage activation, surfactant dysfunction, and epithelial destruction.[1, 2]. It has been widely recognized as a clinical problem worldwide, accompanied by high morbidity and mortality[3, 4]. According to a recent international multi-centre research, the prevalence of ARDS was 10.4% of ICU admissions[5]. Over the decades, although a variety of interventions and intensive care strategies have been used in patients with ARDS, such as the protective mechanical ventilation strategy, the mortality is still high. It is urgent to identify sensitive and specific biomarkers and novel therapeutic methods for ARDS.

Multiple studies have confirmed that ARDS is related to the damage and disruption of the epithelial and endothelial cells and dysregulated inflammation [6–8]. Mutations or gene expression alteration might be involved. Some research showed that Mucin1and MCTR3 helped to activate some related signalling pathways, while Sestrin 2 variants might promote mitophagy [9–11], all of which helped alleviate the condition. Specific genes expression alteration in the diseases were suitable to serve as diagnostic or therapeutic targets. Microarrays have been used to quantify the high-throughput expression of genes for

many species quickly [12]. As a result, the data produced from microarrays were stored in some public databases. We could explore lots of valuable clues from these raw data for further experimental research. Some different bioinformatic studies have been exploited in the past few years, which provided us with abundant integrated bioinformatical methods for studies[13].

In this study, GSE2411 and GSE130936 profiles were chosen from Gene Expression Omnibus (GEO). Titles associated with ARDS were screened, and the details of these datasets, like organisms and samples, were further evaluated. In the next, the GEO2R online tool and Venn diagram software were applied to find the common differentially expressed genes (DEGs) in the two datasets. Then, we used the DAVID database to analyze these DEGs including molecular function (MF), cellular component (CC), biological process (BP), and Kyoto Encyclopedia of Gene and Genome (KEGG) pathways. Next, the protein-protein interaction (PPI) network with MCODE plugin was constructed. GO analysis and KEGG analysis were re-utilized to screen the hub genes, followed by the hub, DEGs imported into the Human Protein Atlas database to evaluate their expression level in varied organs. At last but not least, the hub DEGs were processed by iRegulon to find the common TFs. In general, some clues for the diagnosis and treatment of ARDS could be get by the bioinformatics study.

Methods

2.1 Microarray data information

The relevant gene profiles were obtained from NCBI-GEO, a public online database. Titles associated with ARDS were screened, and the details of these datasets were further evaluated. Two datasets were obtained at last, including GSE2411 and GSE130936. Microarray data of GSE2411 and GSE130936 were on account of GPL339 Platforms ([MOE430A] Affymetrix Mouse Expression 430A Array). Dataset GSE2411 included 6 wildtype mice control, and 6 wildtype mice injected intraperitoneally with LPS to induce experimental ARDS. Samples were obtained from the pulmonary tissues. They were marked from GSM45427 to GSM45432 and from GSM45439 to GSM45444, respectively. Dataset GSE130936 included wildtype mice that were induced either with saline as control (n=4) or LPS (n=3). Samples were labelled from GSM3756516 to GSM3756518 and GSM3756522 to GSM3756525 for further processing.

2.2 Data Processing and DEGs Identification

DEGs between ARDS pulmonary tissues and normal pulmonary tissues were identified by online tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/), with |logFC| > 2 and adjust *P* value < 0.05.Then, the raw data were input in Venn software online (http://bioinformatics.psb.ugent.be/webtools/Venn/) to find the common DEGs between the two datasets. At last, the genes in the common datasets with logFC>0 were considered up-regulated genes, while those with logFC<0 were considered down-regulated genes.

2.3 Gene function and pathway enrichment analysis of DEGs

Gene ontology (GO) is a systematical approach for gene annotation, RNA and protein expression[14]. KEGG is an online database of genomes, enzymatic pathways, and biochemicals. The pathway database of KEGG records molecular interaction networks in cells and changes specific to specific organisms[15]. DAVID (https://david.ncifcrf.gov/) is a biological information database that integrates biological data and analysis tools to provide systematic comprehensive biological function annotation information for the large-scale gene or protein lists, helping us to extract from them biological information[16]. In this study, the DAVID database was used to perform GO analysis and KEGG pathway enrichment analysis in helping classifying DEGs (*P*-value < 0.05).

2.4 PPI network construction of DEGs and significant module screening

Visualized protein-protein interaction (PPI) information of DEGs was evaluated by STRNG (https://string-db.org/), an online database set for retrieving interacting genes. Subsequently, the result from STRING was imported into Cytoscape software to examine the potential correlation among these DEGs (maximum number of interactors = 0 and confidence shub ≥ 0.4). Lastly, MCODE plugin of Cytoscape was utilized to screen the obvious submodules and hub genes in the PPI network (degree cutoff = 2, k-hub = 2, node shub cutoff = 0.2 and the normalized enrichment shub (NES)>12[17]).

2.5 Expression of hub genes in different human normal organs

The Human Protein Atlas (https://www.proteinatlas.org/) was a public database of the gene expression profile in human varied organs. The basic RNA and protein expression levels of specific genes could be identified from it. There were three RNA expression databases in the Human Protein Atlas, including the HPA dataset, the genotype-tissue expression (GTEx) project dataset and the Functional Annotation of the Mammalian Genome (FANTOM5) dataset. In this study, GTEx database was used to evaluate the hub genes' mRNA expression level in different organs, particularly in the pulmonary tissue.

2.6 Prediction of Transcriptional Factors(TFs) of hub genes

The Cytoscape plugin iRegulon[18] was used to analyze transcription factors regulating the hub genes. The iRegulon plugin can identify regulons using motifs and track discovery in an existing network or a set of regulated genes. The cutoff criteria were as follows: enrichment shub threshold = 3.0, ROC threshold for AUC calculation = 0.03, rank threshold = 5,000, minimum identity between orthologous genes = 0.0 and FDR = 0.001[17].

Results

3.1 Identification of DEGs in ARDS

Raw data of the microarray datasets from GEO datasets were processed by the CEO2R online tool. We extracted 224 and 56 DEGs from GSE130936 and GSE2411, respectively. Then, the common DEGs in the two datasets were identified by Venn diagram software. 39 common DEGs were obtained, including 39

up-regulated genes (log FC> 2, adjust p<0.05) and 0 down-regulated genes(log FC<-2, adjust p<0.05) in the pancreas(Figure 1).

3.2 DEGs gene ontology and KEGG pathway analysis in ARDS

All 39 DEGs were further analyzed by DAVID software. The results of gene ontology analysis showed that 1) For biological process (BP), DEGs were particularly enriched in regulation of immune system process, innate immune response, inflammatory response, cellular response to interferon-beta and so on. 2) For cell component (CC), DEGs were mainly enriched in the extracellular space, the extracellular region, the symbiont-containing vacuole membrane, and the high-density lipoprotein particle. 3) For molecular function (MF), DEGs were enriched in the response to the cytokine activity, the chemokine activity, the CXCR chemokine receptor binding, the chemoattractant activity, the Toll-like receptor 4 binding and the interleukin-1 receptor binding (Figure 2a,2c,2d). The analysis results of KEGG appeared that DEGs were enriched in multiple pathways(Figure 2b), including Cytokine-cytokine receptor interaction, Salmonella infection, Legionellosis, Chemokine signalling pathway, Toll-like receptor signalling pathway and so on (p<0.05).

3.3 Protein-protein interaction network (PPI) and modular analysis

A total of 33 DEGs were imported into the DEGs PPI network complex which included 17 nodes and 77 edges, including 72 up-regulated and 0 down-regulated genes (Figure 3). There were 16 genes excluded from the EDGs PPI network. Then Cytotype MCODE was applied for further analysis. It reviewed that 17 hub genes, including Cd14, Irg1, Iigp1, Gbp6, Ifit1, Ifit2, Saa3, Il1rn, Il1b, Ccl3, Cxcl10, Clec4e, Cxcl1, Cxcl2, Ifit3, Gbp2 and Rsad2, all of which were identified from the 33 nodes. Based on the PPI network analysis, GO term and KEGG pathway enrichment analysis was performed again. The result from GO enrichment analysis showed that hub genes were mostly enriched in the biological process(BP), including cellular response to interferon-beta and so on, in cell components(CC), including symbiont-containing vacuole membrane, extracellular space an extracellular region, and also enriched in the molecular function(MF), including cytokine activity and so on. KEGG pathway was mainly enriched in Salmonella infection signalling pathway and so on (Figure 4). According to the biological process analysis, GBP6, GBP2, IFIT1, IFIT3 and IIGP1 were related to the cellular response to interferon-beta.

3.4 The basic expression of hub genes in the lung and human other organs

The Human Protein Atlas database was used to evaluate the expression level of cor- genes, including GBP6, GBP2, IFIT1, IFIT3 and IIGP1 in varied human organs. IIGP1 was mus musculus specific and not expressed in human. From Figure 5a to 5d, GBP6, GBP2, IFIT1 and IFIT3 were expressed in multiple human organs with different expression levels in different tissues. But GBP6 were not detected in the human pulmonary tissue. It suggested that GBP2, IFIT1 and IFIT3 might be potential targets for ARDS diagnosis and treatment(Figure 5).

3.5 Transcription factor analysis of hub genes

The common transcription factor analysis of the 3 hub genes was conducted using iRegulon, a Cytoscape plugin, and a normalized enrichment shub (NES) >12 was considered to be significant. The transcriptional regulation network of these hub genes was shown in Figure 6. The transcription factors with NES > 12 were STAT1 (NES = 24.252), E2F1 (NES = 21.465), IRF2 (NES = 19.614), IRF1 (NES = 12.027) and IRF9 (NES=12.007).

Discussion

In the study, a total number of 39 genes, including 39 up-regulated and 0 down-regulated genes, were identified based on the two mouse microarray information. They were expressed differently in LPS-induced ARDS samples compared with control groups. In the two microarrays, there were some common hub genes and TFs were screened. GBP2, IFIT1 and IFIT3 were considered hub genes. STAT1, E2F1, IRF1, IRF2, and IRF9 were identified as the main TFs regulating gene GBP2, IFIT1 and IFIT3 at the same time. Besides, GBP2, IFIT1 and IFIT3 were confirmed expressed in varied organs including the lung.

GBP2, as a member of the human GBP GTPase superfamily, can be induced by interferon and plays a role in the fight against microbial and viral pathogens[19]. LPS could target host GBPs to the bacterial outer membrane to activate non-canonical inflammasome[20] and initiated assembly of a caspase-4 activating platform on cytosolic bacteria[21]. In addition to intracellular immunity, GBP2 might participate in the regulation of the development of multiple tumours. Shuye Yu et al have reported that GBP2 can enhance glioblastoma invasion through Stat3/fibronectin pathway[22].

IFIT1 and IFIT3 belong to the interferon-induced protein with the tetratricopeptide repeats (IFIT) protein family. They are involved in regulating immune responses and restrict viral infections through a variety of mechanisms, including the restriction of viral RNA translation[23]. Recent studies showed that IFIT3 could modulate IFIT1 RNA Binding specificity and protein stability[24, 25]. Fengchao Xu et al reported that IFIT3 transcription was dependent on NF-κB activation[26], while NF-κB played a vital role in ARDS[27, 28]. Exome-wide analysis showed that IFIT3 mutation was associated with COPD and airflow limitation[29]. All of them suggested that IFIT1 or IFIT3 mutation might be related to ARDS.

To identify the common TFs in the pulmonary tissue, STAT1, E2F1, IRF1, IRF2, and IRF9 were screened according to iRegulon, which was the plugin of Cytoscape. STAT1 is a member of the STAT family of 7 cytoplasmic proteins. It has essential effects on innate immunity via defending the host from different infections[30]. Sevoflurane could reduce LPS-induced ARDS via modulating STAT1[31]. GSK3β mediated phosphorylation of E2F1, which could activate Peg10 promoter and increase Peg10 mRNA expression. Elevation of PEG10 levels promoted lung cell proliferation and wound healing[32]. IRF1, IRF2, and IRF9 belong to the interferon regulatory factor (IRF) family. IRF-1 deficiency played a key role in the classical ROS-dependent release of NETs, which might serve as a novel target in ARDS[33]. LncRNA XIST could aggravate LPS-induced ARDS in mice by upregulating IRF2[34]. As a result, we concluded that GBP2, IFIT1 and IFIT3 could be regulated by STAT1, E2F1, IRF1, IRF2, and IRF9 in the ARDS.

Conclusions

In summary, our bioinformatics analysis study identified three DEGs (GBP2, IFIT1 and IFIT3) in ARDS pulmonary tissues according to two different microarray datasets (GSE2411 and GSE130936). Results suggested that these three genes could be targets for the study of ARDS, and might be regulated by TFs, STAT1, E2F1, IRF1, IRF2, or IRF9. Anyway, these predictions would be verified by a series of experiments in the future. These studies have opened up new research directions for the diagnosis and treatment of ARDS

Declarations

1. Ethics approval and consent to participate

Not applicable

2. Consent for publication:

Not applicable.

3. Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

4. Competing interests:

The authors declare that they have no competing interests.

5. Funding:

Not applicable.

6. Authors' contributions:

Weina Lu analyzed and interpreted the data regarding ADRS and was a major contributor to the manuscript.

Ran Ji drafted the work and substantively revised it.

Weina Lu and Ran Ji read and approved the final manuscript.

7. Acknowledgements:

Not applicable.

8. Footnotes:

Not applicable.

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Figures

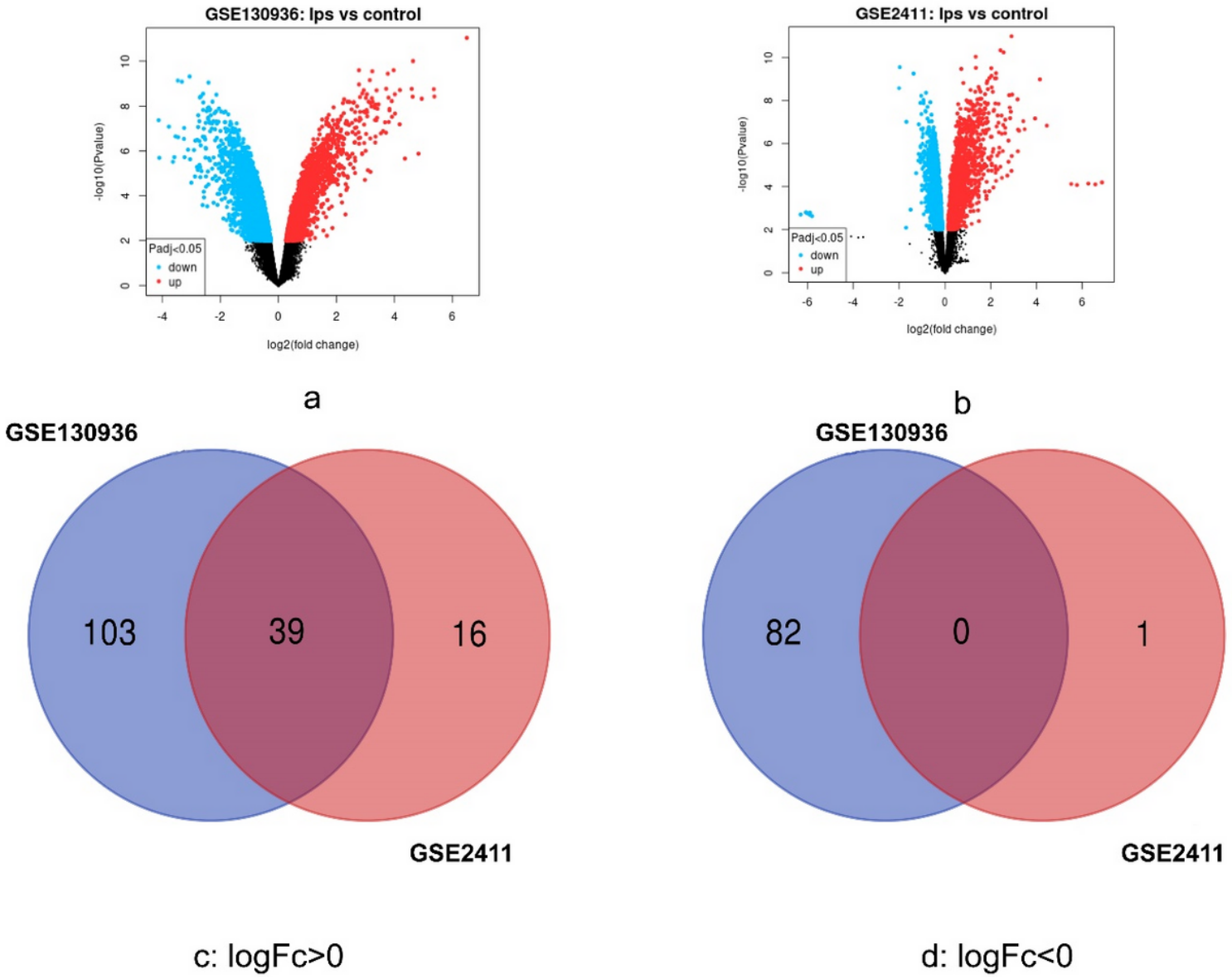

Figure 1

Authentication of 39 common DEGs in the two datasets (GSE2411 and GSE130936) through Venn diagrams software (available online: http://bioinformatics.psb.ugent.be/webtools/Venn/). Different color meant different datasets. c. 39 DEGs were up-regulated in the two datasets (logFC> 0). d. 0 DEGs were down-regulated in two datasets (logFC < 0).

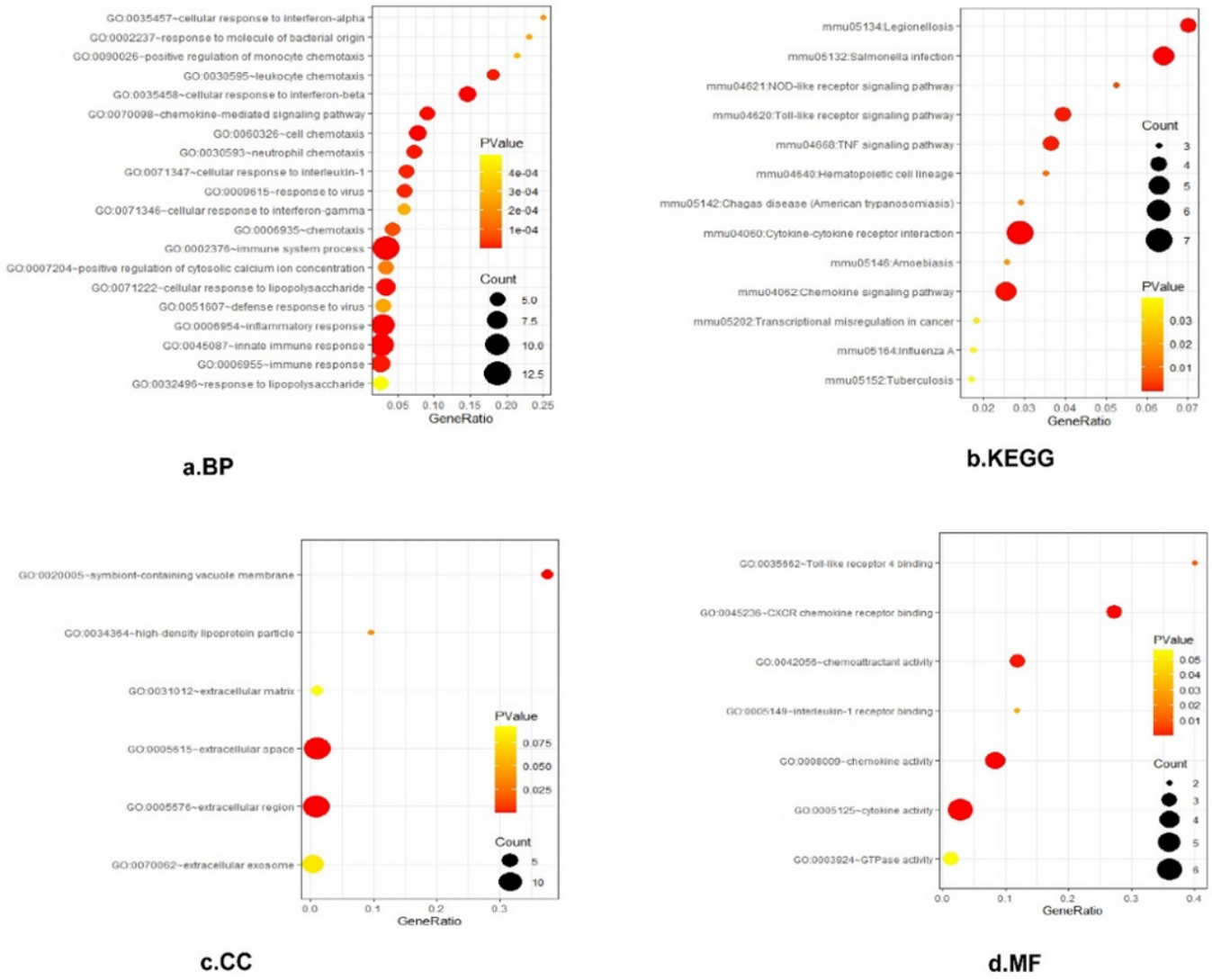

Figure 2

GO and KEGG enrichment analysis of DEGs. (a) Shows the results of biological process terms enriched by BP analysis. (b) Shows the enriched pathway by KEGG analysis. (c) Shows the results of biological process terms enriched by CC analysis. (d) Shows the results of biological process terms enriched by MF analysis. The coloured dots represent the P-value for that term, with red representing greater significance. The size of the dots represents the number of involved genes. The rich factor represents the proportion of enriched genes for each term.

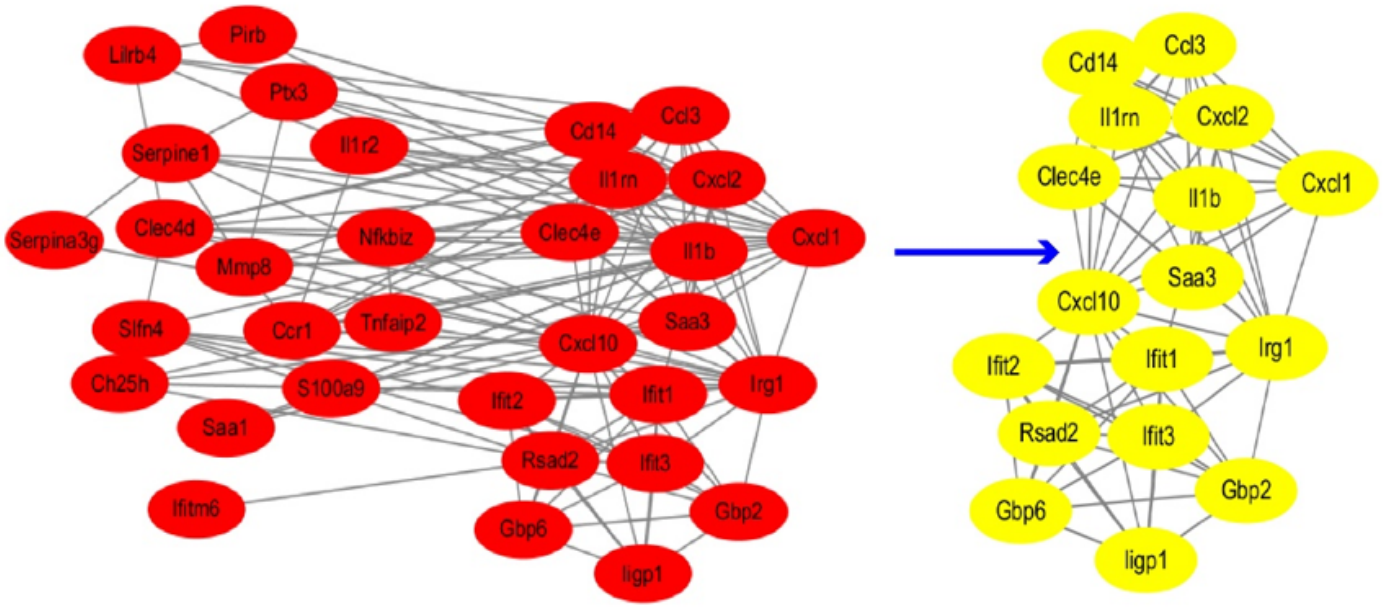

Figure 3

Common DEGs PPI network constructed by STRING online database and Module analysis. There was a total of 33 DEGs in the DEGs PPI network complex. The nodes meant proteins; the edges meant the interaction of proteins; blue circles meant down-regulated DEGs and red circles meant up-regulated DEGs. Module analysis via Cytoscape software (degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max.).

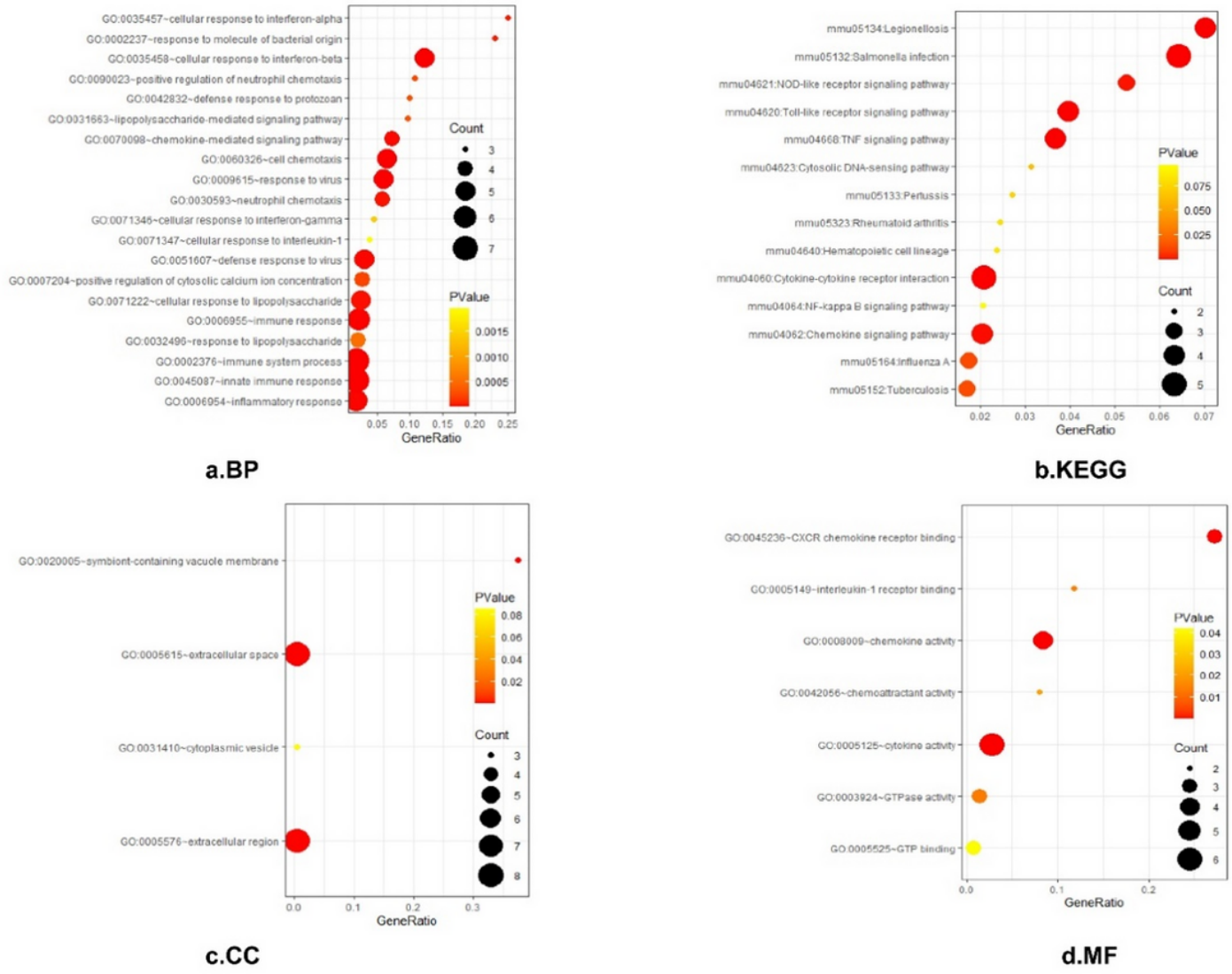

Figure 4

GO and KEGG enrichment re-analysis of DEGs. (a) Shows the results of biological process terms enriched by BP analysis. (b) Shows the enriched pathway by KEGG analysis. (c) Shows the results of biological process terms enriched by CC analysis. (d) Shows the results of biological process terms enriched by MF analysis. The coloured dots represent the P-value for that term, with red representing greater significance. The size of the dots represents the number of involved genes. The rich factor represents the proportion of enriched genes for each term.

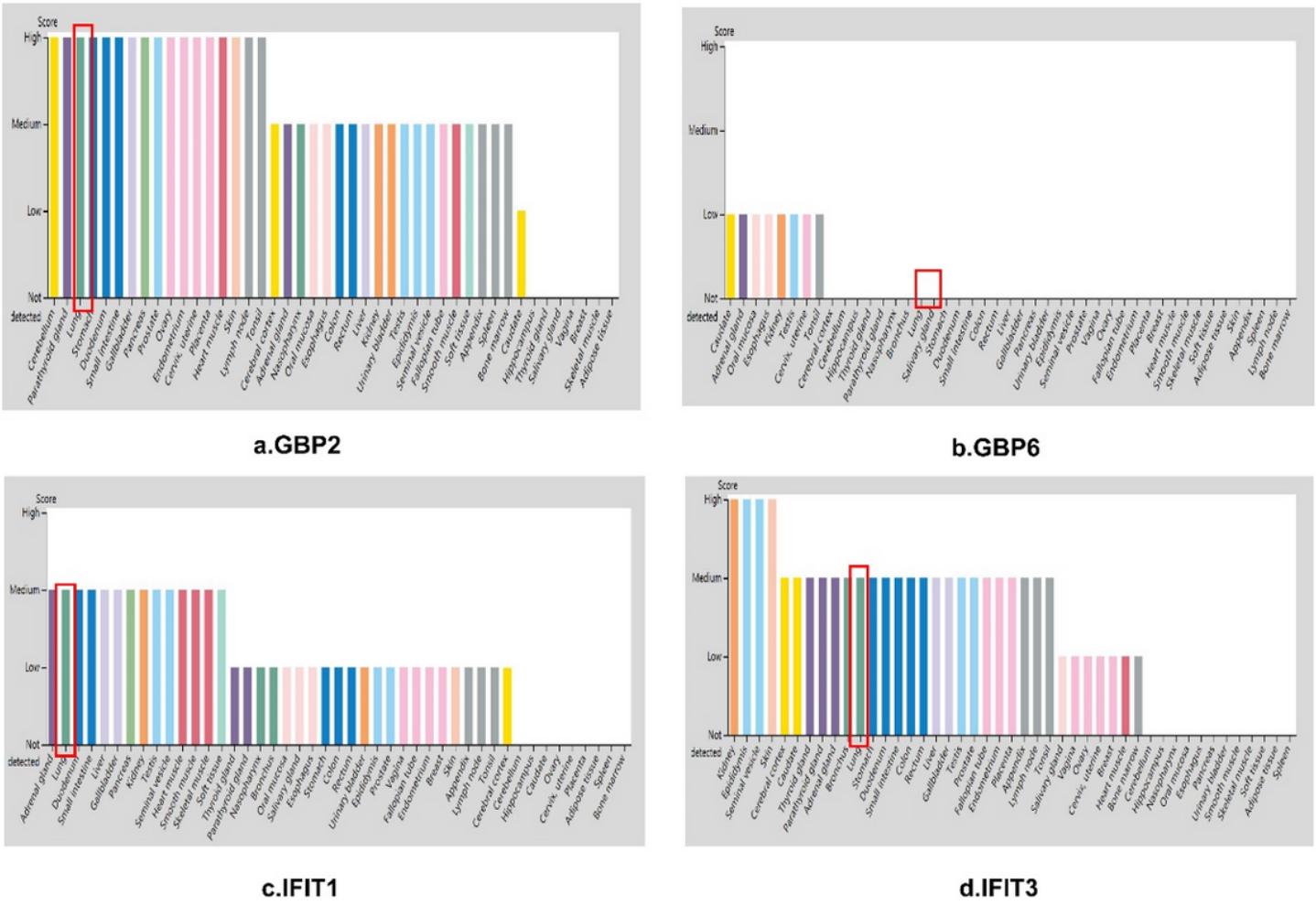

Figure 5

Basic expression of hub genes in the lung and other human organs via The Human Protein Atlas database. RNA expression level in different human organs, especially in the lung was evaluated with genotype-tissue expression (GTEx) project dataset from The Human Protein Atlas database.

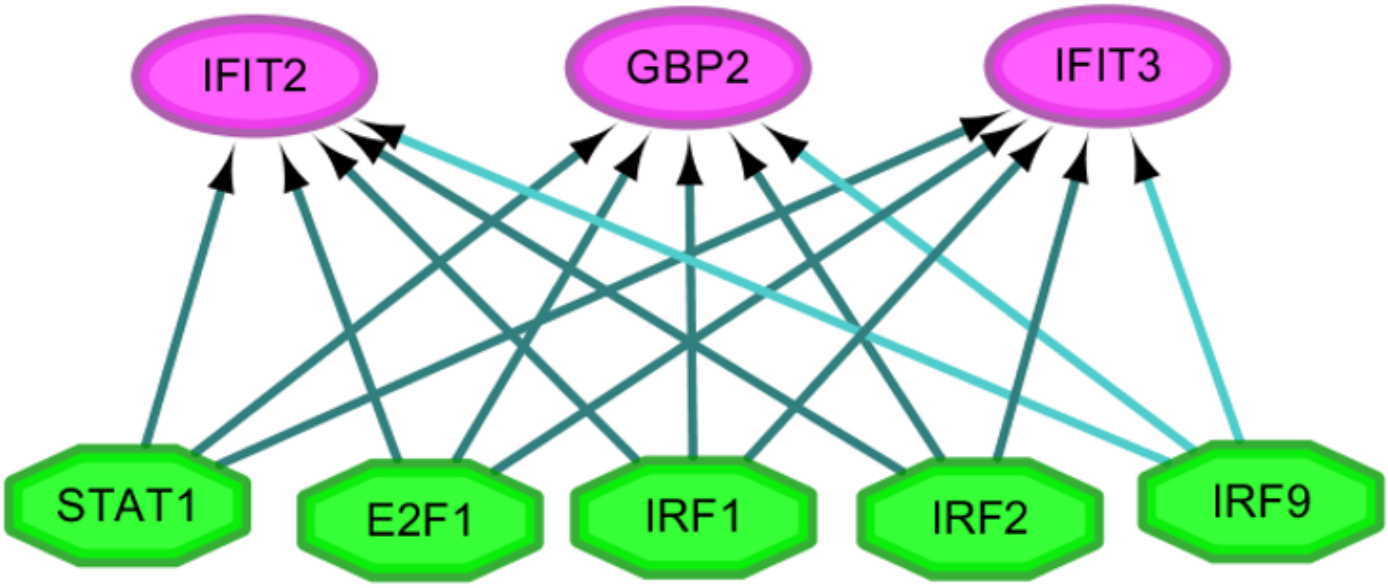

Figure 6

Common TFs among GBP2, IFIT2 and IFIT3 were screened by the iRegulon plugin of Cytoscape software. STAT1, E2F1, IRF1, IRF2, or IRF9 could modulate the three genes at the same time.