# Construction of potential idiopathic pulmonary fibrosis related microRNA and messenger RNA regulatory network

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*To the Editor*: Idiopathic pulmonary fibrosis (IPF) is a kind of lung disease characterized by chronic and progressive pulmonary fibrosis with unknown etiology, whose pathological manifestation is usual interstitial pneumonia.[1] However, the prognosis of IPF was still poor, and the median survival time after diagnosis was about 2 to 3 years.[2] Therefore, it seems important to seek and develop an effective therapeutic modality for IPF. Through base pairs of intramolecular complementary sequences of messenger RNA (mRNA), microRNA (miRNA) plays a key role in RNA silencing and post-transcriptional gene expression regulation.[3] Each miRNA can have multiple target genes and several miRNAs can co-regulate one gene. They also play an important role in organ fibrosis, especially in IPF, and the role may be related to the pathogenesis of the disease.[4,5] Construction of potential miRNA-mRNA regulatory network of IPF will help to reveal more comprehensive molecular mechanism of miRNAs in IPF.

Three microarray datasets (GSE75647, GSE27430, and GSE13316) in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) Dataset (https://www.ncbi.nlm.nih.gov/gds/) were selected to screen differentially expressed miRNAs (DE-miRNAs) between IPF samples and normal samples. Fourteen up-regulated candidate DE-miRNAs (miR-31, miR-493, miR-382, miR-410, miR-432, miR-654-3p, miR-127-3p, miR-487b, miR-409-3p, miR-495, miR-369-5p, miR-299-5p, miR-409-5p, miR-154) and six down-regulated candidate DE-miRNAs (miR-184, miR-338-3p, miR-203, miR-326, miR-375, miR-30b) were identified.

We used FunRich software, an open access standalone functional enrichment and interaction network analysis tool (http://www.funrich.org/download) to predict upstream transcription factors (TFs) of candidate DE-miRNAs. The top ten TFs for up-regulated DE-miRNAs were POU2F1, EGR1, NOBOX, MEF2A, BARHL1, PDX1, PORA, SP1, HOXA3, and SP4. The top ten TFs for down-regulated DE-miRNAs were EGR1, SP1, ZFP161, POU2F1, FOXD3, SP4, MAFB, MEF2A, NKX2-1, and ARID3A.

We used miRNet database, an integrated platform linking miRNAs, targets, and functions (https://www.mirnet.ca/) to predict the downstream target genes of candidate DE-miRNA as miRNAs take effect mainly through directly targeting 3′ untranslated region of mRNA. The downstream target genes for the up-regulated DE-miRNAs included 1285 genes [Supplementary Table 1, http://links.lww.com/CM9/A411] and the downstream target genes for down-regulated DE-miRNAs included 1411 genes [Supplementary Table 2, http://links.lww.com/CM9/A412].

We searched datasets focusing on the mRNA expression in GEO Dataset and one microarray dataset (GSE92592) was selected for subsequent analysis. Finally, 1160 up-regulated DE-mRNAs [Supplementary Table 3, http://links.lww.com/CM9/A413] and 1427 down-regulated DE-mRNAs [Supplementary Table 4, http://links.lww.com/CM9/A414] were identified.

It is widely acknowledged that an inverse relationship between miRNA and mRNA target gene. We conducted a combined analysis of 1427 down-regulated DE-mRNAs

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and 1285 target genes of up-regulated DE-miRNAs, then 49 candidate target genes were further screened for up-regulated DE-miRNAs [Supplementary Table 5, http://links.lww.com/CM9/A415]. We conducted a combined analysis of 1160 up-regulated DE-mRNAs and 1411 target genes of down-regulated DE-miRNAs, then 53 candidate target genes were further screened for down-regulated DE-miRNAs [Supplementary Table 6, http://links.lww.com/CM9/A416].

We used Enrichr database, a comprehensive gene set enrichment analysis web server (http://amp.pharm.mssm.edu/Enrichr/) to perform gene ontology (GO) function enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the screened candidate target genes of DE-miRNAs.

GO biological process (BP) analysis showed that down-regulated candidate target genes (candidate target genes of up-regulated DE-miRNAs) were significantly enriched in negative regulation of DNA binding, regulation of adiponectin secretion, positive regulation of cell migration by vascular endothelial growth factor signaling pathway, and so on. GO molecular function (MF) analysis showed that candidate target genes of up-regulated DE-miRNAs were significantly enriched in transcription corepressor binding, G-protein coupled receptor activity, postsynaptic density protein-95, Drosophila disc large tumor suppressor, and zonula occludens-1 (PDZ) domain binding, and so on. GO cellular component (CC) analysis showed that candidate target genes of up-regulated DE-miRNAs were significantly enriched in integral component of plasma membrane, clathrin-coated endocytic vesicle membrane, clathrin-coated endocytic vesicle, and so on.

GO BP analysis showed that up-regulated candidate target genes (candidate target genes of down-regulated DE-miRNAs) were significantly enriched in positive regulation of gene expression, positive regulation of nucleic acid-templated transcription, negative regulation of neuron apoptotic process, and so on. GO MF analysis showed that candidate target genes of down-regulated DE-miRNAs were significantly enriched in metalloendopeptidase activity, TF activity/RNA polymerase II core promoter proximal region sequence-specific binding, transcriptional activator activity/RNA polymerase II core promoter proximal region sequence-specific binding, and so on. GO CC analysis showed that candidate target genes of down-regulated DE-miRNAs were significantly enriched in platelet alpha granule membrane, platelet alpha granule, dendrite, and so on.

KEGG pathway enrichment analysis was further conducted for candidate target genes of DE-miRNAs. Candidate target genes of up-regulated DE-miRNAs (down-regulated candidate target genes) were significantly enriched in pathways in cancer, signaling pathways regulating pluripotency of stem cells, breast cancer, and so on. Candidate target genes of down-regulated DE-miRNAs (up-regulated candidate target genes) were significantly enriched in bladder cancer, central carbon metabolism in cancer, transcriptional misregulation in cancer, and so on.

We mapped these candidate target genes into the STRING database (https://string-db.org/). STRING is one of ELIXIR's Core Data and a web server to retrieve and display the repeatedly occurring neighborhood of a gene. We found out that 44 candidate target genes of up-regulated DE-miRNAs in the network could have protein interaction and 116 edges represented the interaction between proteins, while 51 candidate target genes of down-regulated DE-miRNAs in the network could have protein interaction and 209 edges represented the interaction between proteins.

Then we input these nodes and edges data into Cytoscape 3.6.0 software and used the "Network Analyzer" tools to do topology analysis. Then the hub genes were identified. The top 12 hub genes in the target genes of up-regulated DE-miRNAs were *VEGFA*, *FOS*, *IFNG*, *MAPK4*, *MAP2*, *FABP4*, *PTGDR*, *GRM3*, *KLF6*, *SPRY4*, *PER2*, and *HEY1*; the top 12 hub genes in the target genes of down-regulated DE-miRNAs were *MMP9*, *SOX2*, *MMP2*, *CDH2*, *TOP2A*, *HIF1A*, *MMP1*, *IGFBP5*, *CALU*, *HMGA2*, *ITGB3*, and *CLU*. According to the miRNA and candidate target gene pairs analyzed above, we found a link between miRNA and hub genes [Figure 1].

Until now, we have constructed potential IPF related miRNA-mRNA regulatory network, which include miR-31-5p-SPRY4, miR-127-3p-MAPK4, miR-382-5p-PTGDR, miR-369-5p-FABP4, miR-409-3p-IFNG, miR-410-3p-KLF6 VEGFA/HEY1, miR-495-3p-VEGFA/PER2, miR-493-5p-FOS, miR-493-3p-MAP2, and miR-487b-3p-GRM3 of up-regulated miRNA and down-regulated mRNA regulatory network; miR-203a-3p-IGFBP5/MMP1/TOP2A, miR-375-CALU/SOX2, miR-326-CLU/HMGA2, and miR-338-3p-CDH2/HIF1A/ITGB3/MMP2/MMP9 of down-regulated miRNA and up-regulated mRNA regulatory network. There are still relatively few reports on these regulatory networks and IPF related research has barely been reported in particular.

For our next study to be more targeted, GEO Dataset was still used to detect the 24 hub genes expression levels. We searched datasets focusing on the gene expression in GEO Dataset. Finally, GSE72073 was selected for subsequent analysis. The results showed that in GSE72073 dataset the expression level of *VEGFA* was significantly lower in IPF tissues than that in normal tissues and the expression levels of *SOX2*, *MMP2*, *CDH2*, *TOP2A*, *HIF1A*, *MMP1*, *CALU*, and *ITGB3* were significantly higher in IPF tissues than those in normal tissues. Based on the preliminary validation, a more accurate potential miRNA-mRNA regulatory network contributing to IPF was established, including miR-410-3p/miR-495-3p-VEGFA, miR-375-SOX2/CALU, miR-338-3p-MMP2/CDH2/HIF1A/ITGB3, and miR-203a-3p-TOP2A/MMP1 regulatory pathways, which could be further studied in clinical and basic experiments, combining the predicted TFs, GO enrichment analysis and KEGG pathways analysis above.

In conclusion, we reveals a comprehensive potential mechanisms of miRNA-mRNA regulatory axes in pathogenesis of IPF and established a potential IPF related miRNA-mRNA regulatory network, which may help to know the underlying mechanism and new research pathway of IPF.

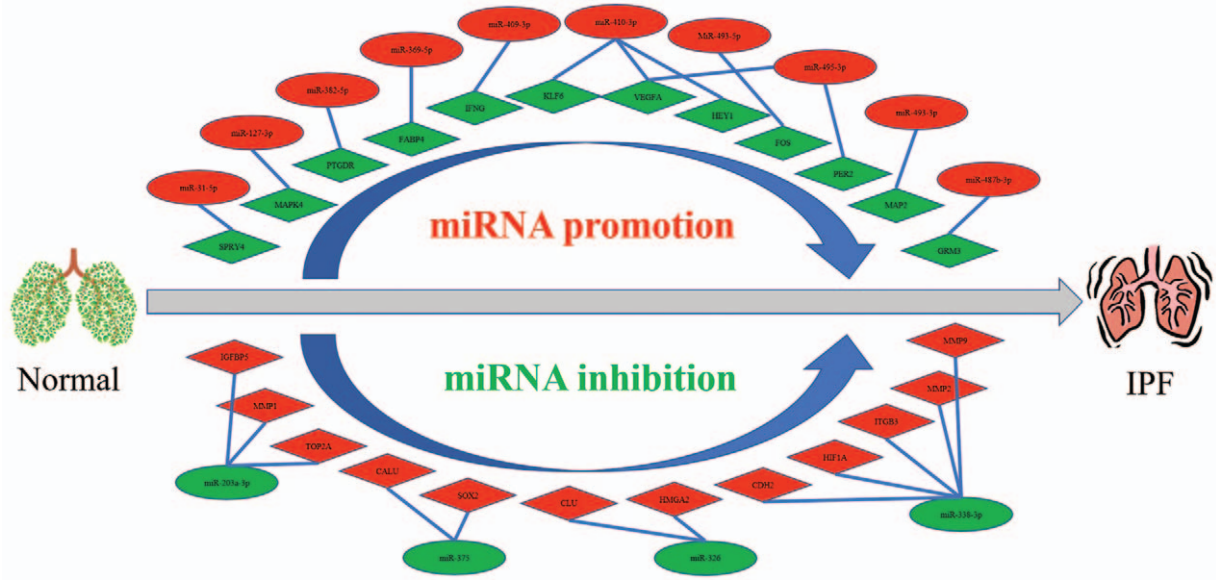


**Figure 1:** Candidate potential miRNA-mRNA regulatory network in IPF. IPF: Idiopathic pulmonary fibrosis; miRNA: MicroRNA; mRNA: Messenger RNA.


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## *Conflicts of interest*

None.

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