Potential function and molecular mechanism of circRNAs involved in idiopathic pulmonary fibrosis

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Abstract

Background: Recent studies have found a regulatory role of circular RNAs (circRNAs) in the pathogenesis of idiopathic pulmonary fibrosis (IPF). However, the function and underlying molecular mechanism of circRNAs involved in IPF are uncertain and incomplete. This study aimed to further provide some critical information for the circRNA function in IPF using bioinformatic analysis.

Methods: We searched in the NCBI (National Center for Biotechnology Information) Gene Expression Omnibus (GEO) database to find the circRNA expression profiles of human IPF. The microarray data GSE102660 was obtained and differentially expressed circRNAs were identified through R software.

Results: 6 significantly up-regulated and 13 significantly down-regulated circRNAs were identified involved in the pathogenesis of IPF. The binding sites of miRNAs for each differentially expressed circRNA were also predicted and circRNA-miRNA-mRNA networks were constructed for the most up-regulated hsa_circ_0004099 and down-regulated hsa_circ_0029633. In addition, GO and KEGG enrichment analysis revealed the molecular function and enriched pathways of the target genes of circRNAs in IPF.

Conclusion: These findings suggest that candidate circRNAs might serve an important role in the pathogenesis of IPF. Therefore, these circRNAs might be potential biomarkers for diagnosis and promising targets for treatment of IPF, which still need further verification in vivo and in vitro.

Background

Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic, progressive, and irreversible interstitial lung disease (ILD) of unknown cause [1], regarded as a consequence of multiple interacting genetic and environmental risk factors, which leads to myofibroblast activation and consequent abnormal accumulation of extracellular matrix [2]. Patients with IPF generally suffer progressive dyspnea, a decline in lung function, decreased activity tolerance and poor quality of life [3–5]. Since current antifibrotic treatment can only delay disease progression to some extent but does not cure the disease itself, the prognosis of IPF is poor, with a 5-year survival rate between 20% and 40%, which is lower than many tumors [6–8]. Therefore, it is important to explore the molecular mechanisms responsible for IPF, aiming to find new methods to prevent and treat IPF.
Circular RNAs (circRNAs) are a group of noncoding RNAs (ncRNAs) with a closed continuous loop structure, lacking terminal 5’ caps and 3’ tails [9]. They are generated in the process of back-splicing and often dynamically expressed among various tissues and cell types [10, 11]. Generally, circRNAs regulate gene expression via different modes [12]: (a) Act as miRNA sponges by competing for miRNA binding sites; (b) interact with RNA-binding proteins (RBPs); (c) sequester mRNA translation start sites; (d) encode proteins. Recently, an increasing number of studies have found a regulatory function of circRNAs in several diseases, including cancers, cardiovascular diseases, neurological disorders, diabetes and infection [13–17]. Li et al have found some dysregulated circRNAs in IPF through microarrays [18]. However, the function and underlying molecular mechanism of circRNAs involved in IPF are uncertain and incomplete. In the present study, comprehensive bioinformatic analysis were conducted to further provide some critical information for the function of circRNAs in IPF.

**Methods**

**Identify differentially expressed circRNA profiles in IPF**

We searched in the NCBI (National Center for Biotechnology Information) Gene Expression Omnibus (GEO) database [19] to find the circRNA expression profiles of human IPF. The microarray data GSE102660 including 3 plasma samples from IPF patients and 3 plasma samples from healthy individuals was obtained and differentially expressed circRNAs were identified through R software (version 3.6.1). Limma package was used to find the different expression between control group and IPF group. P-value < 0.05 and |log₂ (fold change)| > 1 were considered to be significantly different. Subsequently, a volcano plot and a heat map were used to display the differential expression of circRNAs between the two groups.

**Verify circRNA targeting miRNAs**

The general characteristic of circRNAs was verified via circBase database [20]. circRNA targeting miRNAs were identified in CSCD database [21]. A schematic diagram of the structure was used to show the functional area of differentially expressed circRNAs, including MREs, RBPs and ORFs.

**Construct circRNA-miRNA-mRNA Interaction Networks**

Identification of target genes for circRNA targeting miRNAs is critical for characterizing the function of circRNAs. In present study, the related target genes
were predicted by integrating miRDB [22], TargetScan [23] and miRTarBase [24] databases. To make our results more convincing, only the target genes predicted by all three databases were selected for further analysis. Finally, the circRNA-miRNA-mRNA interaction network was drawn using Cytoscape software (version 3.7.1).

**Analyze Molecular Function And Enriched Pathways For circRNA-targeting Genes**

Molecular function of the circRNA-target genes was further analyzed using GO enrichment. In addition, enriched pathways of circRNA-target genes were identified using KEGG analysis. The GO and KEGG analysis were performed using R software. The P-value < 0.05 was considered significant. A bar and a bubble chart were used to present the molecular function and enriched pathways for circRNA-targeting genes.

**Results**

**Identification of differentially expressed circRNA profiles in IPF**

Based on the analysis of GSE102660, differentially expressed circRNAs were identified using R language. It was considered significantly different that P-value < 0.05 and |log₂ (fold change)| > 1 compared to control group. As shown in Fig. 1A, a volcano plot visualized the differential expression of circRNAs between the IPF group and control group. Figure 1B revealed the differential expression profiles of circRNAs in the two groups using hierarchical cluster analysis. Finally, 19 differentially expressed circRNAs were identified between the IPF group and control group, of which 6 circRNAs were significantly up-regulated and 13 circRNAs were significantly down-regulated. The details are listed in Table 1.
Table 1
The differentially expressed circRNAs in idiopathic pulmonary fibrosis

| CircRNA       | Fold change | P-value   | Regulation | CircRNA type | Chromosome | Gene symbol |
|---------------|-------------|-----------|------------|--------------|------------|-------------|
| hsa_circ_0004| 1.253335    | 0.000152  | Up         | Exonic       | 11         | DENND5A     |
| 099-898       | 1.265496    | 0.000179  | Up         | Exonic       | 10         | OAT         |
| hsa_circ_0004| 1.421143    | 0.000686  | Up         | Exonic       | 17         | CDC27       |
| 226-535       | 1.057601    | 0.002252  | Up         | Exonic       | 18         | ELP2        |
| hsa_circ_0044| 1.079494    | 0.007508  | Up         | Exonic       | 17         | CDC27       |
| 234-796       | 1.168627    | 0.010805  | Up         | Exonic       | 15         | HERC1       |
| hsa_circ_0029| 1.366249    | 0.00090   | Down       | Exonic       | 13         | ZMYM2       |
| 611-861       | 1.307861    | 0.00407   | Down       | Exonic       | 9          | GRHPR       |
| hsa_circ_0043| 2.114709    | 0.000707  | Down       | Exonic       | 17         | TADA2A      |
| 278           | 1.003207    | 0.001254  | Down       | Intronic     | 5          | ERGIC1      |
| hsa_circ_0005| 1.167819    | 0.003007  | Down       | Exonic       | 12         | CLIP1       |
| 465-888       | 1.133704    | 0.005791  | Down       | Exonic       | 19         | EPS15L1     |
| hsa_circ_0000| 1.137055    | 0.018849  | Down       | Exonic       | 12         | NAP1L1      |
| 422           | 1.268418    | 0.019167  | Down       | Exonic       | 14         | PSMC6       |
| hsa_circ_0000| 1.517045    | 0.019465  | Down       | Exonic       | 2          | NOL10       |
| 977           | 1.110617    | 0.032446  | Down       | Exonic       | 16         | TCF25       |
| hsa_circ_0001| 1.071198    | 0.033607  | Down       | Exonic       | 5          | GFM2        |
| 497           | 1.048250    | 0.046675  | Down       | Exonic       | 20         | DNMT3B      |
| hsa_circ_0001| 1.414757    | 0.048550  | Down       | Exonic       | 9          | PAPP4A      |

Verification Of circRNA-targeting miRNAs

Since circRNAs interacted with miRNAs via miRNA response elements (MREs) to reduce the miRNA expression and subsequently up-regulated the levels of miRNA target genes, the binding sites of miRNAs for each differentially expressed circRNA were predicted in CSCD database. The top 5 potential miRNA binding sites for the top 10 significantly up-regulated or down-regulated circRNAs were listed in Table 2. Of these, the top 5 potential miRNA binding sites for the most up-regulated circRNA (hsa_circ_0004099) were miR-4633-5p, miR-3671, miR-4755-3p, miR-665 as well as miR-9-3p, and the top 5 potential miRNA binding sites for the most down-regulated circRNA (hsa_circ_0029633) were miR-124-3p, miR-223-5p, miR-3658, miR-486-5p as well as miR-630.
Table 2
Top 5 miRNA binding sites for the top 10 differentially expressed circRNAs

| CircRNA      | MRE1          | MRE2          | MRE3          | MRE4          | MRE5          |
|--------------|---------------|---------------|---------------|---------------|---------------|
| hsa_circ_000409 | miR-4633-5p   | miR-3671      | miR-4755-3p   | miR-665       | miR-9-3p      |
| hsa_circ_000898 | miR-1197      | miR-129-5p    | miR-140-5p    | miR-203-5p    | miR-4635      |
| hsa_circ_004422 | miR-1233-3p   | miR-759       | miR-3529-3p   | miR-4305      | miR-5700      |
| hsa_circ_000785 | miR-1972      | miR-203-3p    | miR-3158-5p   | miR-544a      | miR-630       |
| hsa_circ_004423 | miR-1251-3p   | miR-1302      | miR-337-3p    | miR-4289      | miR-4425      |
| hsa_circ_002963 | miR-124-3p    | miR-223-5p    | miR-3658      | miR-486-5p    | miR-630       |
| hsa_circ_000186 | miR-1306-5p   | miR-212-5p    | miR-4437      | miR-6837-3p   | miR-7150      |
| hsa_circ_004327 | miR-4659-3p   | miR-526-3p    | miR-6885-3p   | miR-7974      | miR-922       |
| hsa_circ_000155 | miR-1288-5p   | miR-383-5p    | miR-4290      | miR-6784-3p   | miR-6823-5p   |
| hsa_circ_000546 | miR-5091      | miR-8063      | miR-1290      | miR-3610      | miR-4428      |

**Construction of circRNA-miRNA-mRNA Interaction Networks**

Next, the most up-regulated circRNA (hsa_circ_000409) and down-regulated circRNA (hsa_circ_002963) were chosen for further analysis. In circBase database, hsa_circ_000409 and hsa_circ_002963 were found located at chromosome 11: 9225206–9229179 and chromosome 13: 20625572–20641530, respectively. Their functional structures including MREs, RBPs and open reading frames (ORFs) were summarized in Fig. 2A and Fig. 3A. Then target genes of the top 5 miRNAs for hsa_circ_000409 and hsa_circ_002963 were predicted by integrating miRDB, TargetScan and miRTarBase tools. Finally, the top 10 target genes of each circRNA-targeting miRNA predicted by all the three databases were chosen to build circRNA-miRNA-mRNA interaction networks. The results are presented in Fig. 2B and Fig. 3B.

**GO And KEGG Enrichment Analysis For circRNA-targeting Genes**

The most up-regulated hsa_circ_000409 and down-regulated hsa_circ_002963 were selected for further investigation by Gene Ontology (GO; geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG; www.kegg.jp) analysis. For hsa_circ_000409, the target genes mainly participated in the molecular function of proximal promoter sequence-specific DNA binding, RNA polymerase II proximal promoter sequence-specific DNA binding and core promoter binding (P < 0.05), as shown in Fig. 4A and Table 3. KEGG pathways were also identified for the target genes of hsa_circ_000409.
Figure 4B and Table 4 presented that the enriched pathways included hepatitis B, measles, kaposi sarcoma-associated herpesvirus infection, p53 signaling pathway, thyroid hormone signaling pathway, cell cycle, and so on (P < 0.05). hsa_circ_0004099 targeting genes that participated in cell cycle and p53 signaling pathway were shown in Additional file 1: S1 and Additional file 2: S2. For hsa_circ_0029633, the molecular function of target genes was mainly enriched in cell adhesion molecule binding, cadherin binding, molecular adaptor activity, protein binding, chromatin DNA binding, and so on, as shown in Fig. 5A and Table 5. Figure 5B and Table 6 showed that the enriched pathways for target genes of hsa_circ_0029633 involved non-small cell lung cancer, cellular senescence, focal adhesion, glioma, acute myeloid leukemia, PI3K-Akt signaling pathway, estrogen signaling pathway, prolactin signaling pathway, prolactin signaling pathway, and so on.

hsa_circ_0029633 targeting genes that participated in ErbB, EGFR tyrosine kinase inhibitor resistance, FOXO, Ras, sphingolipid and PI3K-AKT signaling pathways were shown in Additional file 3: S3-Additional file 8: S8.

| ID           | Term                                      | P-value   | Genes annotated to the term |
|--------------|-------------------------------------------|-----------|-----------------------------|
| GO:0000987   | proximal promoter sequence-specific DNA binding | 8.51E-05 | NONO| CDK9| HOXA9| HEYL| SIX4| HIF1A| CREBBP| MYC... |
| GO:0000978   | RNA polymerase II proximal promoter sequence-specific DNA binding | 0.0001   | NONO| CDK9| MYC| HOXA9| ETS2| SIX4| NR3C1| SOX4... |
| GO:0001047   | core promoter binding                     | 0.0413   | MYC| POU2F1| KLF10| H3F3B| NR3C1| BAZ2A |
| GO gene ontology |                                              |           |                             |

Table 3
GO analysis for hsa_circ_0004099 targeting genes
| Term                                | ID      | Sample number | Background number | P-value    | Genes                                     |
|-------------------------------------|---------|---------------|-------------------|------------|-------------------------------------------|
| Hepatitis B                         | hsa05161| 14            | 165               | 0.001494  | CYCS, MYC, MAP3K7, STAT2, DDX3X, IFNAR1...|
| Measles                             | hsa05162| 12            | 165               | 0.002412  | CYCS, MAVS, STAT5B, APAF1, CCND1, CDK6...  |
| Kaposi sarcoma-associated herpesvirus infection | hsa05167| 14            | 165               | 0.002412  | CCR5, CYCS, CCND1, MYC, ZFP36, STAT2...    |
| p53 signaling pathway               | hsa04115| 8             | 165               | 0.006778  | MDM2, CYCS, CCND1, PMAIP1, RRM2, APAF1...  |
| Thyroid hormone signaling pathway   | hsa04919| 10            | 165               | 0.010790  | ACTG1, MDM2, NOTCH2, CCND1, MYC, PLCB2...  |
| Epstein-Barr virus infection        | hsa05169| 13            | 165               | 0.010559  | CDK6, CCND2, APAF1, MAVS, STAT3, IFNAR1...|
| Hepatitis C                         | hsa05160| 11            | 165               | 0.021266  | APAF1, MAVS, IFIT1, TBK1, STAT3, IFNAR1...|
| Human cytomegalovirus infection     | hsa05163| 13            | 165               | 0.021266  | ADCY7, CDK6, VEGFA, WNT1, PLCB2, TBK1...   |
| Influenza A                         | hsa05164| 11            | 165               | 0.021266  | TBK1, IFNAR1, DNAJC3, CREBBP, KPNA6...     |
| Cell cycle                          | hsa04110| 9             | 165               | 0.025571  | MDM2, BUB3, CCND1, MYC, MAD2L1, CCND2...   |

KEGG Kyoto encyclopedia of genes and genome
| ID          | Term                                      | P-value      | Genes annotated to the term |
|-------------|-------------------------------------------|--------------|-----------------------------|
| GO:0050839  | cell adhesion molecule binding            | 9.58E-07     | CD226 | DSG2 | MPRIP | ITGB1 | HMGGB1 | EIF5 | BZW1 | PCMT1 | HSPA1A | RAB1A |
| GO:0045296  | cadherin binding                          | 4.24E-06     | MPRIP | ITGB1 | EIF5 | BZW1 | PCMT1 | HSPA1A | RAB1A |
| GO:0060090  | molecular adaptor activity                | 1.35E-05     | CAV1 | NCK2 | SH2B3 | DISC1 | TIP2 | ITSN2 | BCD2 |
| GO:0030674  | protein binding                           | 5.42E-05     | CAV1 | NCK2 | SH2B3 | ITSN2 | LDLRAP1 | BCD2 |
| GO:0031490  | chromatin DNA binding                     | 0.0001       | STAT3 | H1F0 | H3F3B | NCKAP | HMGN2 | BCL6 | RELA |
| GO:0035591  | signaling adaptor activity                | 0.0001       | NCK2 | SH2B3 | ITSN2 | LDLRAP1 | PAG1 | PTPN11 | GRB2 |
| GO:0019887  | protein kinase regulator activity         | 0.0001       | PRKAG1 | PARP16 | DAZAP2 | YWHAG | TRIB3 | TOM11 |
| GO:0004721  | phosphoprotein phosphatase activity       | 0.0001       | PTPRE | PTPN4 | PTPRJ | PPP1R3B | PTPN9 | PTPN14 | PTPRB |
| GO:0000978  | RNA polymerase II proximal promoter DNA    | 0.0002       | SMAD5 | NFIA | SIX4 | SP1 | PRRX1 | HOXA9 | SOX4 | RXRA |
| GO:0000987  | proximal promoter DNA binding             | 0.0002       | SMAD5 | NFIA | SIX4 | HOXA9 | SOX4 | LHX2 | ZNF516 | RARA |
| GO:004725   | protein tyrosine phosphatase activity     | 0.0004       | PTPRE | PTPN4 | PTPRJ | PTPN9 | PTPN14 | PTPRB | DUSP19 |
| GO:0019207  | kinase regulator activity                 | 0.0005       | PRKAG1 | PARP16 | DAZAP2 | YWHAG | TRIB3 | TOM11 |
| GO:005070   | SH3/SH2 adaptor activity                  | 0.0005       | NCK2 | SH2B3 | ITSN2 | PAG1 | PTPN11 | GRB2 | LASP1 |
| GO:0016791  | phosphatase activity                      | 0.0005       | NUDT16 | PTPRE | PTPN4 | PTPRJ | CTDSP1 | PPP1R3B | PPP2R |
### Table 5
Continued

| ID               | Term                                                                 | P-value   | Genes annotated to the term                                      |
|------------------|----------------------------------------------------------------------|-----------|-----------------------------------------------------------------|
| GO:0030295       | protein kinase activator activity                                    | 0.0007    | PARP16 | DAZAP2 | TOM1L1 | CCNB1 | IQGAP1 | DDX3X... |
| GO:0005543       | phospholipid binding                                                 | 0.0008    | SH3PD2D | ARAP2 | JAG1   | OSBP   | GOLPH3 | PIK3C2A | HMGB1... |
| GO:008093        | cytoskeletal adaptor activity                                        | 0.0009    | NCK2   | BICD2  | SDCBP  | ABI2   |
| GO:0045309       | protein phosphorylated amino acid binding                            | 0.0009    | NCK2   | POU2F1 | KLF10  | H3F3B  | NR3C1  | BAZ2A... |
| GO:0001228       | DNA-binding transcription activator activity                         | 0.0009    | LDLRAPI | NCK2  | AB2L1  | BTRC   | PTPN11 | GRB2... |
| GO:0031267       | small GTPase binding                                                | 0.0010    | NFA1   | SIX4   | FOXC1  | SP1    | EGR1   | NR4A1   | SOX4   | CREB5... |
| GO:0019209       | kinase activator activity                                           | 0.0011    | PARP16 | DAZAP2 | TOM1L1 | CCNB1  | IQGAP1 | DDX3X... |
| GO:0070273       | phosphatidylinositol-4-phosphate binding                            | 0.0014    | SH3PD2D2A | OSBP1 | GOLPH3 | OSBP   | ARFIP1 |
| GO:0017016       | Ras GTPase binding                                                 | 0.0015    | SOS2   | CAV1   | DENND6A | RNF41  | ROCK1  | DOCK3   | SPATA13... |

### Table 6
KEGG enrichment analysis for hsa_circ_0029633 targeting genes

| Term                                      | ID      | Sample number | Background number | P-value | Genes                                                                 |
|-------------------------------------------|---------|---------------|-------------------|---------|-----------------------------------------------------------------------|
| non-small cell lung cancer                | hsa05223| 12            | 308               | 0.0018  | SOS2 | CCND1 | STAT3 | AKT3 | RXRA | RASSF5 | SOS1... |
| cellular senescence                      | hsa04218| 19            | 308               | 0.0018  | CCND1 | ZFP361L | AKT3 | MAPK14 | RRAS | SERPINE1... |
| focal adhesion                           | hsa04510| 21            | 308               | 0.0027  | SOS2 | CCND1 | CAV1 | ARHGAP5 | ROCK1 | AKT3... |
| glioma                                    | hsa05214| 11            | 308               | 0.0095  | SOS2 | CCND1 | PDGFR | AKT3 | CDK4... |
| acute myeloid leukemia                    | hsa05221| 10            | 308               | 0.0112  | RELA | GRB2 | CEBPA | RUNX1 | SOS1 | RARA | AKT3... |
| PI3K-Akt signaling pathway                | hsa04151| 28            | 308               | 0.0112  | PPP2 | PDGFR | SOS1 | LAMC1 | CREB3L2 | RXRA... |
| estrogen signaling pathway                | hsa04915| 15            | 308               | 0.0112  | R2A | PTK2 | PRKCA | CDK4 | GRB2 | SGK1 | RELA... |
| prolactin signaling pathway               | hsa04917| 10            | 308               | 0.0112  | CCND2 | GRB2 | PTK2 | CCNB1 | BC6G1 | GRM1... |
| proteoglycans in cancer                   | hsa05205| 19            | 308               | 0.0112  | PPP1CB | IQGAP1 | SMAD2 | PRKCA | RRAS | MAPK14... |
| relaxin signaling                         | hsa04926| 14            | 308               | 0.0130  | COL4A1 | RELA|
| pathway | pathway ID | Rank | Score | Genes |
|---------|------------|------|-------|-------|
| FOXO signaling pathway | hsa04068 | 14 | 308 | 0.0138 | SMAD2, PRKCA, CREB3L2, GNG10, |
| regulation of actin cytoskeleton | hsa04810 | 19 | 308 | 0.0143 | CCND2, SGK1, GRB2, PTEN, |
| chronic myeloid leukemia | hsa05220 | 10 | 308 | 0.0143 | CREB3L2, GNG10, |
| apelin signaling pathway | hsa04371 | 14 | 308 | 0.0156 | CCNB1, BCL6, |
| EGFR tyrosine kinase inhibitor resistance | hsa01521 | 10 | 308 | 0.0156 | SHC1, RELA, GRB2, PTEN, |
| Hippo signaling pathway | hsa04390 | 15 | 308 | 0.0156 | SASF1, FRMD6, |
| insulin resistance | hsa04931 | 12 | 308 | 0.0116 | RELA, PTPN11, |
| other types of O-glycan biosynthesis | hsa00514 | 5 | 308 | 0.0201 | PLOD3, POAFUT1, |
| ErbB signaling pathway | hsa04012 | 10 | 308 | 0.0228 | PAK2, |
|                         |            |      |      |       | SHC1, GRB2, |
|                         |            |      |      |       | PRKCA, |
|                         |            |      |      |       | AKT3, |
|                         |            |      |      |       | |

**Legend:**
- Rank: Position in the list of pathways.
- Score: Statistical significance of the pathway.
- Genes: List of genes associated with the pathway.
| Term                                                                 | ID       | Sample number | Background number | P-value  | Genes                                                                 |
|----------------------------------------------------------------------|----------|---------------|-------------------|----------|-----------------------------------------------------------------------|
| AGE-RAGE signaling pathway in diabetic complications                  | hsa04933 | 11            | 308               | 0.0228   | COL4A1| RELA| CDK4| SMAD2| PRKCA| SERPINE1... |
| Ras signaling pathway                                                | hsa04014 | 19            | 308               | 0.0228   | PAK2| SHC1| RASSF1| TGFA| RELA| GRB2| PTPN11...  |
| sphingolipid signalling pathway                                       | hsa04071 | 12            | 308               | 0.0280   | ROCK1| AKT3| MAPK14| GNAI2| GNAI2| SGMS1| PPP2R2A... |
| transcriptional misregulation in cancer                              | hsa05202 | 10            | 308               | 0.0288   | CCND2| RELA| ELK4| KLF3| CDK9| CEBPA| RUNX1...   |
| hepatitis C                                                           | hsa05160 | 14            | 308               | 0.0234   | CLDN1| RELA| GRB2| CDK4| PPP2R2A| SOS1| RXRA...    |
| human cytomegalovirus infection                                       | hsa05163 | 18            | 308               | 0.0313   | GNAI3| RELA| GRB2| CDK4| PRKCA| PDGFA...   |
| small cell lung cancer                                                | hsa05222 | 10            | 308               | 0.0313   | COL4A1| RELA| CDK4| PTEN| LAMC1| RXRA| AKT3...    |
| leukocyte transendothelial migration                                 | hsa04670 | 11            | 308               | 0.0386   | ARHGAP5| ROCK1| ITGB1| MAPK14| GNAI2| GNAI2| CLDN1...   |
| toxoplasmosis                                                          | hsa05145 | 11            | 308               | 0.0386   | GNAI2| LAMC1| PPIF| RELA| GNAI3| TYK2| AKT3...    |
| AK-STAT signaling pathway                                            | hsa04630 | 14            | 308               | 0.0386   | STAT3| IFNAR2| MCL1| PDGFA| GRB2| CCND2... |
| shigellosis                                                            | hsa05131 | 8             | 308               | 0.0386   | RELA| BTRC| WASF2| RHOG| MAPK14| ARPC1B... |
| prostate cancer                                                       | hsa05215 | 10            | 308               | 0.0386   | TGFA| RELA| GRB2| PTEN| PDGFA| CREB3L2... |
| endocrine resistance                                                 | hsa01522 | 10            | 308               | 0.0393   | SHC1| GRB2| CDK4| SOS1| MAPK14| JAG1...    |
| bladder cancer                                                        | hsa05219 | 6             | 308               | 0.0393   | CCND1| RPS6KA5| THBS1| DAPK1| CDK4| RASSF1... |
| dopaminergic synapse                                                 | hsa04728 | 12            | 308               | 0.0400   | GNAI3| GRIA2| PPP1CB| CLOCK| PRKCA| PPP2R2A... |

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**Discussion**

IPF is a progressive and ultimately fatal interstitial lung disease, whose available therapies are limited
Deeply to explore the pathogenesis and further to find new therapeutic options for IPF are a clear and urgent need. circRNAs are a class of the non-coding RNA family and play an important role in the development of multiple lung diseases [26–28]. Recently, studies have detect circRNAs with abnormal expression in IPF using a high-throughput microarray assay and found that several circRNAs may be potential biomarkers and promising molecular targets for the diagnosis and treatment of IPF [18, 29]. However, the function and underlying molecular mechanism of circRNAs contributing to the development of IPF remain largely uncertain and incomplete.

In this study, 6 significantly up-regulated and 13 significantly down-regulated circRNAs were identified based on the analysis of GSE102660 through R software. Of these, the most up-regulated circRNA (hsa_circ_0004099) and down-regulated circRNA (hsa_circ_0029633) were chosen for further analysis. The top 5 potential miRNA binding sites for hsa_circ_0004099 were miR-4633-5p, miR-3671, miR-4755-3p, miR-665 as well as miR-9-3p, and the top 5 potential miRNA binding sites for hsa_circ_0029633 were miR-124-3p, miR-223-5p, miR-3658, miR-486-5p as well as miR-630. Next, target genes of the top 5 miRNAs for hsa_circ_0004099 and hsa_circ_0029633 were predicted and the circRNA-miRNA-mRNA interaction networks were constructed. Finally, GO analysis showed that target genes of hsa_circ_0004099 were enriched in proximal promoter sequence-specific DNA binding, RNA polymerase II proximal promoter sequence-specific DNA binding as well as core promoter binding, and target genes of hsa_circ_0029633 were enriched in many biological processes, such as cell adhesion molecule binding, molecular adaptor activity, chromatin DNA binding, signaling adaptor activity as well as phosphoprotein phosphatase activity, and so on. In KEGG pathway analysis, the target genes of hsa_circ_0004099 were mainly located in p53 signaling pathway, thyroid hormone signaling pathway, Epstein-Barr virus infection and cell cycle signaling pathways; while the target genes of hsa_circ_0029633 were mainly located in focal adhesion signaling pathway, PI3K-Akt signaling pathway, FOXO signaling pathway, EGFR tyrosine kinase inhibitor resistance signaling pathway, Hippo signaling pathway, ErbB signaling pathway as well as Ras signaling pathway, and so on. These results indicated that hsa_circ_0004099 and hsa_circ_0029633 might regulate crucial biological processes during the development of IPF.
Several studies have shown that dysregulation of miR-4633 and miR-9 is involved in the pathogenesis of IPF [30, 31], and increased expression of IGFBP5 and ITGB1 plays a vital role in the development of IPF [32, 33]. Our present results suggested that up-regulated hsa_circ_0004099 might promote the development of IPF by decreasing the expression of miR-4633 or miR-9, and further increasing the expression of IGFBP5 or ITGB1, respectively. MAP3K7, TBK1 and ETS2 have been proved crucial factors in IPF progression [34–36], and these genes were predicted as downstream targets of miR-3671, which was negatively modulated by hsa_circ_0004099 in our analysis, suggesting that over-expression of hsa_circ_0004099 induced pulmonary fibrosis via reduction of miR-3671 and consequent increase of MAP3K7, TBK1 or ETS2.

In our analysis, many genes such as SMAD5, CAV1, JAG1, ROCK1 and STAT3 were positively predicted as downstream targets of miR-124, which was regarded as a potential miRNA binding site of hsa_circ_0029633. Previous studies have demonstrated that miR-124 plays a key role in multiple diseases including IPF by targeting SMAD5, CAV1, JAG1, ROCK1 or STAT3 [37–42], which is consistent with our findings. Previous studies have also shown that FOXC1 and HSPA1A are direct target genes of miR-223 [43, 44], and these genes contribute to the pathogenesis of IPF via activating various signaling pathways. Our present results suggested that down-regulated hsa_circ_0029633 might promote the development of IPF by increasing the expression of miR-223, and further decreasing the expression of FOXC1 and HSPA1A. In addition, we found that hsa_circ_0029633/ miR-486-5p and hsa_circ_0029633/ miR-630 signaling axes were also involved in the development of IPF via targeting CADM1 and OLFM4, as well as PDGFRA and YAP1, respectively.

Several published studies have demonstrated that up-regulation of miR-486-5p and miR-630 contributes to IPF progression [45, 46] and CADM1, OLFM4, PDGFRA as well as YAP1 genes play a crucial role in the pathogenesis of IPF [47–50].

Some limitations should be addressed when interpreting the results: (a) The differentially expressed circRNAs were identified only based on microarray data GSE102660 and they still needed further verification in animal and human lungs; (b) the function of the differentially expressed circRNAs in IPF was predicted only using bioinformatic
analysis, and there was no further study in vivo or in vitro to demonstrate the roles of candidate circRNAs in the pathogenesis of IPF; (c) generally, circRNAs regulate gene expression via several modes [12], such as acting as miRNA sponges, interacting with RBPs, sequestering mRNA translation start sites and encoding proteins, while the current study was performed only according to the miRNA sponge function of circRNAs.

Conclusion

6 significantly up-regulated and 13 significantly down-regulated circRNAs were identified involved in the pathogenesis of IPF in present study. The binding sites of miRNAs for each differentially expressed circRNA were also predicted and circRNA-miRNA-mRNA networks were constructed for the most up-regulated hsa_circ_0004099 and down-regulated hsa_circ_0029633. In addition, GO and KEGG enrichment analysis revealed the molecular function and enriched pathways of the target genes of circRNAs in IPF. These findings suggest that candidate circRNAs might serve an important role in the pathogenesis of IPF. Therefore, these circRNAs might be potential biomarkers for diagnosis and promising targets for treatment of IPF, which still need further verification in vivo and in vitro.

Abbreviations

IPF: idiopathic pulmonary fibrosis; ILD: interstitial lung disease; circRNAs: circular RNAs; ncRNAs: noncoding RNAs; RBPs: RNA-binding proteins; MREs: miRNA response elements; ORFs: open reading frames; NCBI: National Center for Biotechnology Information; GEO: Gene Expression Omnibus.

Additional Files

Additional file 1: S1. hsa_circ_0004099-targeting genes participated in cell cycle signaling pathway. Red squares represent the hsa_circ_0004099-targeting genes

Additional file 2: S2. hsa_circ_0004099-targeting genes participated in p53 signaling pathway. Red squares represent the hsa_circ_0004099-targeting genes

Additional file 3: S3. hsa_circ_0029633-targeting genes participated in ErbB signaling pathway. Red squares represent the hsa_circ_0029633-targeting genes

Additional file 4: S4. hsa_circ_0029633-targeting genes participated in EGFR tyrosine kinase inhibitor resistance signaling pathway. Red squares represent the hsa_circ_0029633-targeting genes

Additional file 5: S5. hsa_circ_0029633-targeting genes participated in FOXO signaling pathway.
Red squares represent the hsa_circ_0029633-targeting genes

**Additional file 6: S6.** hsa_circ_0029633-targeting genes participated in Ras signaling pathway. Red squares represent the hsa_circ_0029633-targeting genes

**Additional file 7: S7.** hsa_circ_0029633-targeting genes participated in sphingolipid signaling pathway. Red squares represent the hsa_circ_0029633-targeting genes

**Additional file 8: S8.** hsa_circ_0029633-targeting genes participated in PI3K-AKT signaling pathway. Red squares represent the hsa_circ_0029633-targeting genes

Declarations

**Authors’ contributions**

Fangwei Li and Yixin Wan: designed the study. Hong Wang and Hongyan Tao: analyzed and interpreted the data. Fanqi Wu and Dan Wang: organized the results. Fangwei Li: wrote the manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

We searched in the NCBI (National Center for Biotechnology Information) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) to find the circRNA expression profiles of human IPF. The general characteristic of circRNAs was verified via circBase database (http://www.circbase.org). circRNA-targeting miRNAs were identified in CSCD database (http://gb.whu.edu.cn/CSCD). The target genes of related miRNAs were predicted by integrating miRDB (http://www.mirdb.org/), TargetScan (http://www.targetscan.org/) and miRTarBase
(http://mirtarbase.mbc.nctu.edu.tw/php/index.php) databases.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Figures
Figure 1

Differentially expressed circRNA profiles in IPF: A volcano plot shows the differentially expressed circRNAs between IPF group and control group. Red and green points represent the up-regulated and down-regulated circRNAs, respectively. B Hierarchical cluster analysis reveals the expression profiles of the dysregulated circRNAs between IPF group and control group.
Figure 2

A schematic diagram of the structure and circRNA-miRNA-mRNA interaction network for hsa_circ_0004099. A schematic diagram of the structure shows the functional area of hsa_circ_0004099. B Cytoscape software presents putative circRNA-miRNA-mRNA interaction network of hsa_circ_0004099.
A schematic diagram of the structure and circRNA-miRNA-mRNA interaction network for hsa_circ_0029633. A A schematic diagram of the structure shows the functional area of hsa_circ_0029633. B Cytoscape software presents putative circRNA-miRNA-mRNA interaction network of hsa_circ_0029633.
Figure 4

GO and KEGG enrichment analysis for target genes of hsa_circ_0004099. A Molecular function for target genes of hsa_circ_0004099 was identified by GO enrichment analysis. B Pathways for target genes of hsa_circ_0004099 were verified by KEGG enrichment analysis.
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