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

Fangwei Li

Lanzhou University Second Hospital <https://orcid.org/0000-0002-4176-3528>

Hong Wang

No. 82, Cuiying Men, Lanzhou

Hongyan Tao

No. 82, Cuiying Men, Lanzhou

Fanqi Wu

No. 82, Cuiying Men, Lanzhou

Dan Wang

No. 82, Cuiying Men, Lanzhou

Yixin Wan (✉ wanyixinzr@163.com)

No. 82, Cuiying Men, Lanzhou

Research

Keywords: circular RNAs, Idiopathic pulmonary fibrosis, miRNA response elements, RNA-binding proteins, Open reading frames

Posted Date: February 29th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-15519/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

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. $P\text{-value} < 0.05$ and $|\log_2(\text{fold change})| > 1$ 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]. circRNAtargeting 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 circRNAtargeting 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 $P\text{-value} < 0.05$ 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 $P\text{-value} < 0.05$ and $|\log_2(\text{fold change})| > 1$ 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_0004099	1.253335	0.000152	Up	Exonic	11	DENND5A
hsa_circ_0008898	1.265496	0.000179	Up	Exonic	10	OAT
hsa_circ_0044226	1.421143	0.000686	Up	Exonic	17	CDC27
hsa_circ_0007535	1.057601	0.002525	Up	Exonic	18	ELP2
hsa_circ_0044234	1.079494	0.007508	Up	Exonic	17	CDC27
hsa_circ_0035796	1.168627	0.010805	Up	Exonic	15	HERC1
hsa_circ_0029633	1.366249	0.000090	Down	Exonic	13	ZMYM2
hsa_circ_0001861	1.307861	0.000407	Down	Exonic	9	GRHPR
hsa_circ_0043278	2.114709	0.000707	Down	Exonic	17	TADA2A
hsa_circ_0001556	1.003207	0.001254	Down	Intronic	5	ERGIC1
hsa_circ_0005465	1.167819	0.003007	Down	Exonic	12	CLIP1
hsa_circ_0049888	1.133704	0.005791	Down	Exonic	19	EPS15L1
hsa_circ_0000422	1.137055	0.018849	Down	Exonic	12	NAP1L1
hsa_circ_0000538	1.268418	0.019167	Down	Exonic	14	PSMC6
hsa_circ_0000977	1.517045	0.019465	Down	Exonic	2	NOL10
hsa_circ_0041103	1.110617	0.032446	Down	Exonic	16	TCF25
hsa_circ_0001497	1.071198	0.033607	Down	Exonic	5	GFM2
hsa_circ_0001137	1.048250	0.046675	Down	Exonic	20	DNMT3B
hsa_circ_0088220	1.414757	0.048550	Down	Exonic	9	PAPPA
circRNA circular RNA						

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_0004099	miR-4633-5p	miR-3671	miR-4755-3p	miR-665	miR-9-3p
hsa_circ_0008898	miR-1197	miR-129-5p	miR-140-5p	miR-203-5p	miR-4635
hsa_circ_0044226	miR-1233-3p	miR-759	miR-3529-3p	miR-4305	miR-5700
hsa_circ_0007535	miR-1972	miR-203-3p	miR-3158-5p	miR-544a	miR-630
hsa_circ_0044234	miR-1251-3p	miR-1302	miR-337-3p	miR-4289	miR-4425
hsa_circ_0029633	miR-124-3p	miR-223-5p	miR-3658	miR-486-5p	miR-630
hsa_circ_0001861	miR-1306-5p	miR-212-5p	miR-4437	miR-6837-3p	miR-7150
hsa_circ_0043278	miR-4659-3p	miR-526-5p	miR-6885-3p	miR-7974	miR-922
hsa_circ_0001556	miR-1288-5p	miR-383-5p	miR-4290	miR-6784-3p	miR-6823-5p
hsa_circ_0005465	miR-5091	miR-8063	miR-1290	miR-3610	miR-4428
circRNA circular RNA, MRE miRNA response element, miRNA microRNA					

Construction Of circRNA-miRNA-mRNA Interaction Networks

Next, the most up-regulated circRNA (hsa_circ_0004099) and down-regulated circRNA (hsa_circ_0029633) were chosen for further analysis. In circBase database, hsa_circ_0004099 and hsa_circ_0029633 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_0004099 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_0004099 and down-regulated hsa_circ_0029633 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_0004099, 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_0004099. 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_0004099targeting 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_0029633targeting 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.

Table 3
GO analysis for hsa_circ_0004099targeting genes

ID	Term	P-value	Genes annotated to the term
Molecular function			
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 4
KEGG enrichment analysis for hsa_circ_0004099targeting 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...

Term	ID	Sample number	Background number	P-value	Genes
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					

Table 5
GO analysis for hsa_circ_0029633targeting genes

ID	Term	P-value	Genes annotated to the term
Molecular function			
GO:0050839	cell adhesion molecule binding	9.58E-07	CD226 DSG2 MPRIP ITGB1 HMGB1 EIF5 BZW1 PCMT1...
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 TJP2 ITSN2 PAG1 BICD2...
GO:0030674	protein binding	5.42E-05	CAV1 NCK2 SH2B3 TJP2 ITSN2 LDLRAP1 BICD2...
GO:0031490	chromatin DNA binding	0.0001	STAT3 H1FO H3F3B NKAP HMGN2 BCL6 RXRA 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 TOM1L1...
GO:0004721	phosphoprotein phosphatase activity	0.0001	PTPRE PTPN4 PTPRJ PPP1R3B PTPN9 PTPN14 PTPRB...
GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding	0.0002	SMAD5 NFIA SIX4 SP1 PRRX1 HOXA9 SOX4 RXRA...
GO:0000987	proximal promoter sequence-specific DNA binding	0.0002	SMAD5 NFIA SIX4 HOXA9 SOX4 LHX2 ZNF516 RARA...
GO:0004725	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 TOM1L1...
GO:0005070	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
Molecular function			
GO:0030295	protein kinase activator activity	0.0007	PARP16 DAZAP2 TOM1L1 CCNB1 IQGAP1 DDX3X...
GO:0005543	phospholipid binding	0.0008	SH3PXD ARAP2 JAG1 OSBP GOLPH3 PIK3C2A HMGB1...
GO:0008093	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	LDLRAP1 NCK2 ABL2 BTRC PTPN11 GRB2 SHC1
GO:0031267	small GTPase binding	0.0010	NFIA SIX4 FOXC1 SP1 EGR1 NR4A1 SOX4 CREBRF...
GO:0019209	kinase activator activity	0.0011	PARP16 DAZAP2 TOM1L1 CCNB1 IQGAP1 DDX3X...
GO:0070273	phosphatidylinositol-4-phosphate binding	0.0014	SH3PXD2A OSBPL8 GOLPH3 OSBP ARFIP1
GO:0017016	Ras GTPase binding	0.0015	SOS2 CAV1 DENND6A RNF41 ROCK1 DOCK3 SPATA13...
GO gene ontology			

Table 6
KEGG enrichment analysis for hsa_circ_0029633targeting 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 ZFP36L1 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 PDGFRA PTEN PRKCA 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 PDGFRA SOS1 LAMC1 CREB3L2 RXRA...
estrogen signaling pathway	hsa04915	15	308	0.0112	R2A PTEN PRKCA CDK4 GRB2 SGK1 RELA...
prolactin signaling pathway	hsa04917	10	308	0.0112	CCND2 SGK1 GRB2 PTEN CCNB1 BCL6 GRM1...
proteoglycans in cancer	hsa05205	19	308	0.0112	PPP1CB IQGAP1 SMAD2 PRKCA RRAS MAPK14...
relaxin signaling pathway	hsa04926	14	308	0.0130	COL4A1 RELA SMAD2 PRKCA CREB3L2 GNG10...
FOXO signaling pathway	hsa04068	14	308	0.0138	CCND2 SGK1 GRB2 PTEN CCNB1 BCL6 GRM1...
regulation of actin cytoskeleton	hsa04810	19	308	0.0143	PAK2 ABI2 PPP1CB IQGAP1 WASF2 MYH9 GSN...

Term	ID	Sample number	Background number	P-value	Genes
chronic myeloid leukemia	hsa05220	10	308	0.0143	SHC1 RELA GRB2 PTPN11 CDK4 RUNX1 AKT3...
apelin signaling pathway	hsa04371	14	308	0.0156	SPHK1 RYR3 GNAI3 SMAD2 GNG10 SERPINE1...
EGFR tyrosine kinase inhibitor resistance	hsa01521	10	308	0.0156	SHC1 TGFA GRB2 PRKCA PTEN PDGFRA SOS1...
Hippo signaling pathway	hsa04390	15	308	0.0156	RASSF1 FRMD6 SNAI2 CCND2 YAP1 BMP6...
insulin resistance	hsa04931	12	308	0.0116	RELA PTPN11 PPP1CB PTEN MLXIP CREB3L2...
other types of O-glycan biosynthesis	hsa00514	5	308	0.0201	PLOD3 POFUT1 B4GALT1 GXYLT1 POGLUT1...
ErbB signaling pathway	hsa04012	10	308	0.0228	PAK2 SHC1 TGFA GRB2 ABL2 PRKCA AKT3...

Table 6
Continued

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 signaling pathway	hsa04071	12	308	0.0280	ROCK1 AKT3 MAPK14 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 PDGFRA...
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 CLDN1...

Term	ID	Sample number	Background number	P-value	Genes
toxoplasmosis	hsa05145	11	308	0.0386	GNAI2 LAMC1 PPIF RELA GNAI3 TYK2 AKT3...
JAK-STAT signaling pathway	hsa04630	14	308	0.0386	STAT3 IFNAR2 MCL1 PDGFRA 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 PDGFRA CREB3L2...
endocrine resistance	hsa01522	10	308	0.0393	SHC1 GRB2 CDK4 SOS1 SP1 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...
KEGG Kyoto encyclopedia of genes and genome					

Discussion

IPF is a progressive and ultimately fatal interstitial lung disease, whose available therapies are limited [2, 25]. 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.

Author details

Department of Respiratory Medicine, Lanzhou University Second Hospital, Lanzhou 730030, China.

Acknowledgments

The authors gratefully acknowledge contributions from R software, NCBI-GEO database, circBase database, CSCD database, miRDB database, TargetScan database, miRTarBase, Cytoscape software, GO database and KEGG database.

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.

Funding

This work was supported by Chinese National Science Foundation (No. 81960014) and Cuiying Scientific and Technological Innovation Program of Lanzhou University Second Hospital (No. CY2018-QN12).

References

1. Pleasants R, Tighe RM. Management of idiopathic pulmonary fibrosis. *Ann Pharmacother*. 2019; 53(12):1238-1248.
2. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. *Lancet*. 2017;389(10082):1941-52.
3. Doubkova M, Svancara J, Svoboda M, Sterclova M, Bartos V, Plackova M, Lacina L, Zurkova M, Binkova I, Bittenglova R, et al. EMPIRE Registry, Czech Part: Impact of demographics, pulmonary function and HRCT on survival and clinical course in idiopathic pulmonary fibrosis. *Clin Respir J*. 2018;12(4):1526-35.
4. Glaspole IN, Chapman SA, Cooper WA, Ellis SJ, Goh NS, Hopkins PM, Macansh S, Mahar A, Moodley YP, Paul E, et al. Health-related quality of life in idiopathic pulmonary fibrosis: Data from the Australian IPF Registry. *Respirology*. 2017;22(5):950-6.
5. Jo HE, Glaspole I, Moodley Y, Chapman S, Ellis S, Goh N, Hopkins P, Keir G, Mahar A, Cooper W, et al. Disease progression in idiopathic pulmonary fibrosis with mild physiological impairment: analysis from the Australian IPF registry. *BMC Pulm Med*. 2018;18(1):19.
6. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, Behr J, Cottin V, Danoff SK, Morell F, et al. Diagnosis of idiopathic pulmonary fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2018;198(5):e44-e68.
7. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based

- guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183(6):788-824.
8. Kolilekas L, Papiris S, Bouros D. Existing and emerging treatments for idiopathic pulmonary fibrosis. *Expert Rev Respir Med*. 2019;13(3):229-39.
 9. Chen LL, Yang L. Regulation of circRNA biogenesis. *RNA Biol*. 2015;12(4):381-8.
 10. Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res*. 2016;44(6):2846-58.
 11. Barrett SP, Salzman J. Circular RNAs: analysis, expression and potential functions. *Development*. 2016;143(11):1838-47.
 12. Han B, Chao J, Yao H. Circular RNA and its mechanisms in disease: From the bench to the clinic. *Pharmacol Ther*. 2018;187:31-44.
 13. Kristensen LS, Hansen TB, Veno MT, Kjems J. Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene*. 2018;37(5):555-65.
 14. Li M, Ding W, Sun T, Tariq MA, Xu T, Li P, Wang J. Biogenesis of circular RNAs and their roles in cardiovascular development and pathology. *FEBS J*. 2018;285(2):220-32.
 15. Floris G, Zhang L, Follesa P, Sun T. Regulatory role of circular RNAs and neurological disorders. *Mol Neurobiol*. 2017;54(7):5156-65.
 16. Jiang G, Ma Y, An T, Pan Y, Mo F, Zhao D, Liu Y, Miao JN, Gu YJ, Wang Y, et al. Relationships of circular RNA with diabetes and depression. *Sci Rep*. 2017;7(1):7285.
 17. Li X, Liu CX, Xue W, Zhang Y, Jiang S, Yin QF, Wei J, Yao RW, Yang L, Chen LL. Coordinated circRNA biogenesis and function with NF90/NF110 in viral infection. *Mol Cell*. 2017;67(2):214-27 e7.
 18. Li R, Wang Y, Song X, Sun W, Zhang J, Liu Y, Li H, Meng C, Zhang J, Zheng Q, et al. Potential regulatory role of circular RNA in idiopathic pulmonary fibrosis. *Int J Mol Med*. 2018;42(6):3256-68.
 19. 19. NCBI GEO database. <https://www.ncbi.nlm.nih.gov/geo/>. Accessed 20 October 2019.
 20. 20. circBase databa <http://www.circbase.org>. Accessed 21 October 2019.
 21. CSCD database. <http://gb.whu.edu.cn/CSCD>. Accessed 21 October 2019.
 22. miRDB. <http://www.mirdb.org/>. Accessed 24 October 2019.
 23. TargetScan. <http://www.targetscan.org/>. Accessed 24 October 2019.
 24. miRTarBase. <http://mirtarbase.mbc.nctu.edu.tw/php/index.php>. Accessed 24 October 2019.
 25. Collard HR, Ryerson CJ, Corte TJ, Jenkins G, Kondoh Y, Lederer DJ, Lee JS, Maher TM, Wells AU, Antoniou KM, et al. Acute exacerbation of idiopathic pulmonary fibrosis. An international working group report. *Am J Respir Crit Care Med*. 2016;194(3):265-75.
 26. Yao JT, Zhao SH, Liu QP, Lv MQ, Zhou DX, Liao ZJ, Nan KJ. Over-expression of CircRNA_100876 in non-small cell lung cancer and its prognostic value. *Pathol Res Pract*. 2017;213(5):453-6.
 27. Zhou S, Jiang H, Li M, Wu P, Sun L, Liu Y, Zhu K, Zhang B, Sun G, Cao C, et al. Circular RNA hsa_circ_0016070 is associated with pulmonary arterial hypertension by promoting PASM proliferation. *Mol Ther Nucleic Acids*. 2019;18:275-84.

28. Huang Z, Cao Y, Zhou M, Qi X, Fu B, Mou Y, Wu G, Xie J, Zhao J, Xiong W. Hsa_circ_0005519 increases IL-13/IL-6 by regulating hsa-let-7a-5p in CD4(+) T cells to affect asthma. *Clin Exp Allergy*. 2019;49(8):1116-27.
29. Bachmayr-Heyda A, Reiner AT, Auer K, Sukhbaatar N, Aust S, Bachleitner-Hofmann T, Mesteri I, Grunt TW, Zeillinger R, Pils D. Correlation of circular RNA abundance with proliferation—exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues. *Sci Rep*. 2015;5:8057.
30. Fisher AJ, Cipolla E, Varre A, Gu H, Mickler EA, Vittal R. Potential mechanisms underlying TGF-beta-mediated complement activation in lung fibrosis. *Cell Mol Med Open Access*. 2017;3(3). pii: 14.
31. Dai WJ, Qiu J, Sun J, Ma CL, Huang N, Jiang Y, Zeng J, Ren BC, Li WC, Li YH. Downregulation of microRNA-9 reduces inflammatory response and fibroblast proliferation in mice with idiopathic pulmonary fibrosis through the ANO1-mediated TGF-beta-Smad3 pathway. *J Cell Physiol*. 2019;234(3):2552-65.
32. Sellares J, Veraldi KL, Thiel KJ, Cardenes N, Alvarez D, Schneider F, Pilewski JM, Rojas M, Feghali-Bostwick CA. Intracellular heat shock protein 70 deficiency in pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2019;60(6):629-36.
33. Sen N, Weingarten M, Peter Y. Very late antigen-5 facilitates stromal progenitor cell differentiation into myofibroblast. *Stem Cells Transl Med*. 2014;3(11):1342-53.
34. Liu B, Jiang T, Hu X, Liu Z, Zhao L, Liu H, Liu Z, Ma L. Downregulation of microRNA30a in bronchoalveolar lavage fluid from idiopathic pulmonary fibrosis patients. *Mol Med Rep*. 2018;18(6):5799-806.
35. Qu H, Liu L, Liu Z, Qin H, Liao Z, Xia P, Yang Y, Li B, Gao F, Cai J. Blocking TBK1 alleviated radiation-induced pulmonary fibrosis and epithelial-mesenchymal transition through Akt-Erk inactivation. *Exp Mol Med*. 2019;51(4):42.
36. Baran CP, Fischer SN, Nuovo GJ, Kabbout MN, Hitchcock CL, Bringardner BD, McMaken S, Newland CA, Cantemir-Stone CZ, Phillips GS, et al. Transcription factor ets-2 plays an important role in the pathogenesis of pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2011;45(5):999-1006.
37. Lu Y, Zhang T, Shan S, Wang S, Bian W, Ren T, Yang D. MiR-124 regulates transforming growth factor-beta1 induced differentiation of lung resident mesenchymal stem cells to myofibroblast by repressing Wnt/beta-catenin signaling. *Dev Biol*. 2019;449(2):115-21.
38. Jin J, Zhai HF, Jia ZH, Luo XH. Long non-coding RNA HOXA11-AS induces Type I Collagen synthesis to keloid formation via sponging miR-124-3p and activating Smad5 Signaling. *Am J Physiol Cell Physiol*. 2019;317(5):C1001-C1010.
39. Yang S, Liu X, Li X, Sun S, Sun F, Fan B, Zhao S. MicroRNA-124 reduces caveolar density by targeting caveolin-1 in porcine kidney epithelial PK15 cells. *Mol Cell Biochem*. 2013;384(1-2):213-9.
40. Zhou H, Gao L, Yu ZH, Hong SJ, Zhang ZW, Qiu ZZ. LncRNA HOTAIR promotes renal interstitial fibrosis by regulating Notch1 pathway via the modulation of miR-124. *Nephrology (Carlton)*. 2019;24(4):472-80.

41. Cui M, Wang J, Li Q, Zhang J, Jia J, Zhan X. Long non-coding RNA HOXA11-AS functions as a competing endogenous RNA to regulate ROCK1 expression by sponging miR-124-3p in osteosarcoma. *Biomed Pharmacother.* 2017;92:437-44.
42. Chen G, Shi Y, Zhang Y, Sun J. CircRNA_100782 regulates pancreatic carcinoma proliferation through the IL6-STAT3 pathway. *Onco Targets Ther.* 2017;10:5783-94.
43. Henn D, Abu-Halima M, Wermke D, Falkner F, Thomas B, Kopple C, Ludwig N, Schulte M, Brockmann MA, Kim YJ, et al. MicroRNA-regulated pathways of flow-stimulated angiogenesis and vascular remodeling in vivo. *J Transl Med.* 2019;17(1):22.
44. Tang Q, Yuan Q, Li H, Wang W, Xie G, Zhu K, Li D. miR-223/Hsp70/JNK/JUN/miR-223 feedback loop modulates the chemoresistance of osteosarcoma to cisplatin. *Biochem Biophys Res Commun.* 2018;497(3):827-34.
45. Ji X, Wu B, Fan J, Han R, Luo C, Wang T, Yang J, Han L, Zhu B, Wei D, et al. The anti-fibrotic effects and mechanisms of microRNA-486-5p in pulmonary fibrosis. *Sci Rep.* 2015;5:14131.
46. Shentu TP, Huang TS, Cernelc-Kohan M, Chan J, Wong SS, Espinoza CR, Tan C, Gramaglia I, van der Heyde H, Chien S, et al. Thy-1 dependent uptake of mesenchymal stem cell-derived extracellular vesicles blocks myofibroblastic differentiation. *Sci Rep.* 2017;7(1):18052.
47. Yang IV, Luna LG, Cotter J, Talbert J, Leach SM, Kidd R, Turner J, Kummer N, Kervitsky D, Brown KK, et al. The peripheral blood transcriptome identifies the presence and extent of disease in idiopathic pulmonary fibrosis. *PLoS One.* 2012;7(6):e37708.
48. Arthur G, Bradding P. New developments in mast cell biology: Clinical implications. *Chest.* 2016;150(3):680-93.
49. Li R, Bernau K, Sandbo N, Gu J, Preissl S, Sun X. Pdgfra marks a cellular lineage with distinct contributions to myofibroblasts in lung maturation and injury response. *Elife.* 2018;7. pii: e36865.
50. Gokey JJ, Sridharan A, Xu Y, Green J, Carraro G, Stripp BR, Perl AT, Whitsett JA. Active epithelial Hippo signaling in idiopathic pulmonary fibrosis. *JCI Insight.* 2018;3(6). pii: 98738.

Figures

Figure 1

Differentially expressed circRNA profiles in IPF: A 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 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.

Figure 3

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.

A

B

Figure 5

GO and KEGG enrichment analysis for target genes of hsa_circ_0029633. A Molecular function for target genes of hsa_circ_0029633 was identified by GO enrichment analysis. B Pathways for target genes of hsa_circ_0029633 were verified by KEGG enrichment analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [S1.tif](#)

- [S7.tif](#)
- [S6.tif](#)
- [S8.tif](#)
- [S5.tif](#)
- [S2.tif](#)
- [S4.tif](#)
- [S3.tif](#)