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Invited Review

Role of circular RNAs in visceral organ fibrosis

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ABSTRACT

Circular RNAs (circRNAs) are a novel class of noncoding RNAs produced during pre-mRNA splicing and are emerging as new members of the gene regulatory network. Unlike linear RNAs, circRNAs have a unique structure with a covalently closed loop formed from the ligation of exons, introns, or both. CircRNAs are widely expressed in various organisms in a species-, tissue-, developmental stage- and disease-specific manner; circRNAs have been demonstrated to play a vital role in the pathogenesis and progression of human diseases. Fibrosis is characterized by an abnormal excessive deposition of extracellular matrix (ECM) in the extracellular space and plays important roles in many different pathologies of various organs. CircRNAs function as master regulators of gene expression to "sponge" or sequester other genes and target gene expression, transcription, splicing, etc. Increasing evidence has revealed that circRNAs are tightly associated with fibrotic diseases in various organs, including the lungs, liver, heart and kidneys. Herein, we provide the current understanding of the molecular characteristics of circRNAs and summarize the findings from circRNA studies in which the functions and mechanisms of action of circRNAs in organ fibrosis were proposed.

1. Introduction

Circular RNAs (circRNAs) were identified as transcripts with a scrambled exon order in the early 1990s; in the past three decades, reports on circRNAs have revealed their structure, function and mechanisms and have established circRNAs as a research hotspot (Chen and Huang, 2018; Beermann et al., 2016). With dramatic innovations in sequencing technologies and new computational methods of bioinformatics analysis, they have been found to be expressed in various eukaryotic organisms from flies to mammals and humans. Compared with miRNAs and lncRNAs, circRNAs have an increased degree of stability and sequence conservation among mammalian cells because they are more resistant to nucleases (Beermann et al., 2016; Zhou et al., 2018a). An in-depth study of the structure and function of circRNAs revealed that they exert a regulatory function with different mechanisms in various diseases, such as neurological diseases (Hanan et al., 2017), cardiovascular diseases (Fan et al., 2017), respiratory disease (Li et al., 2018), cancers (Su et al., 2018), and many other diseases (Han et al., 2018). Recently, circRNAs have been determined to have the potential to serve as diagnostic markers and therapeutic targets for various diseases.

Considering the importance of circRNAs in the emerging field of noncoding RNA and their potential association with organ fibrosis that has remained mostly unexplored (Yao et al., 2018a), in this review, we will summarize the published data that will be helpful for any further research in this respect.

2. Biogenesis of circRNAs

Unlike linear RNAs that are formed by classical splicing, circRNAs are produced by back-splicing (Jeck et al., 2013). Nascent circRNAs are generally detected later than linear RNAs, suggesting that most circRNAs are formed after transcription from the parental genes (Zhang et al., 2016a).

circRNAs are derived from pre-mRNAs that are transcribed by RNA polymerase II (Pol II) (Meng et al., 2017a, 2017b; Han et al., 2017). Following the process of transcription, pre-RNAs undergo either canonical splicing to produce linear mRNAs or back-splicing to generate circRNAs (Lasda and Parker, 2014) (Fig. 1). Back-splicing is a spliceosome-mediated process for forming a circRNA by ligating a

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downstream 5′ splice site of one exon to an upstream 3’ splice site of another exon (Ashwal-Fluss et al., 2014), which results in a covalently closed circRNA transcript. Most highly expressed circRNAs are usually synthesized from internal exons of pre-mRNAs and contain multiple exons (Chen and Yang, 2015). Some circRNAs are processed and excised from introns of pre-mRNAs, which are termed circular intronic RNAs (ciRNAs) (Zhang et al., 2013). CircRNAs can also contain both exons and retained introns, which are termed exon-intron circRNAs or EIciRNAs (Zhang et al., 2013).

RNA-binding proteins (RBPs) have been reported to regulate circRNA biogenesis. In humans and flies, the splicing factor muscleblind (MBL) have been shown to promote circRNA production (Ashwal-Fluss et al., 2014). Another RBP that regulates circRNA biogenesis by pre-mRNA binding is quaking (QKI), which is a dimer capable of binding two well-separated regions of a single RNA molecule and bringing the circularized exons closer together, thus promoting circRNA production (Conn et al., 2015). Many other splicing factors, such as fused in sarcoma/translocation in liposarcoma (FUS) (Errichelli et al., 2017), nuclear factor 90 and nuclear factor 110 (NF90 and splice variant NF110, both encoded by the ILF3 gene) (Li et al., 2017), and heterogeneous nuclear ribonucleoprotein (hnRNAP) (Kramer et al., 2015), have also been found to regulate the production of circRNAs.

Additionally, adenosine deaminase 1 acting on RNA (ADAR1) has been demonstrated to suppress circRNA biogenesis (Chen et al., 2015; Castello et al., 2012). Recently, ADAR1 was shown to reduce the formation of circRNA by recognizing and unpackaging the RNA double-stranded structure formed by the reverse complementary element (Ivanov et al., 2015; Aktas et al., 2017). Based on these data, it is hypothesized that the editing executed by ADAR1 before back-splicing may reduce pairing between complementary motifs present in the introns flanking the exons that can circularize, therefore selectively inhibiting the expression of a subset of circRNAs relying on such base-pairing production (Ivanov et al., 2015; Rybak-Wolf et al., 2015).

3. Functions of circRNAs

Regulatory RNAs have been proposed to function as modular scaffolds to assemble diverse combinations of regulatory proteins, thus enhancing protein-protein interactions (Wang and Wang, 2015). Regulatory RNAs can establish important biological networks through RNA-DNA, RNA-RNA, and RNA-protein interactions (Zhang et al., 2017a; Liu et al., 2017).

The large majority of the annotated back-splicing circRNAs are mainly localized in the cytoplasm (Chen, 2016). CircRNAs can bind to specific miRNAs or a group of miRNAs by sequestering them and inhibiting their function in a phenomenon known as the competitive endogenous RNA (ceRNA) hypothesis (Tay et al., 2014; van Rossum et al., 2016). A number of circRNAs have miRNA response elements (MREs), which function as a new kind of ceRNA that binds and inhibits relevant miRNAs by complementary base pairing. miRNA interacting with circRNA fails to bind to its target mRNA and loses its ability to inhibit gene expression, resulting in the upregulation of its target mRNA (Altesha et al., 2019). The best characterized circRNA to support this model is CDR1as/ciRS-7, which is produced from the vertebrate cerebellar degeneration-related 1 (CDR1) antisense transcript (Hansen et al., 2011, 2013; Memczak et al., 2013). CDR1as/ciRS-7 was the first circRNA that was demonstrated to possess miRNA sponge function, which can significantly reduce the activity of miR-7 [35,36]. CDR1as harbors more than 70 conventional miR-7 binding sites acting as a designated miR-7 sponge/inhibitor and effectively inhibiting miR-7 activity

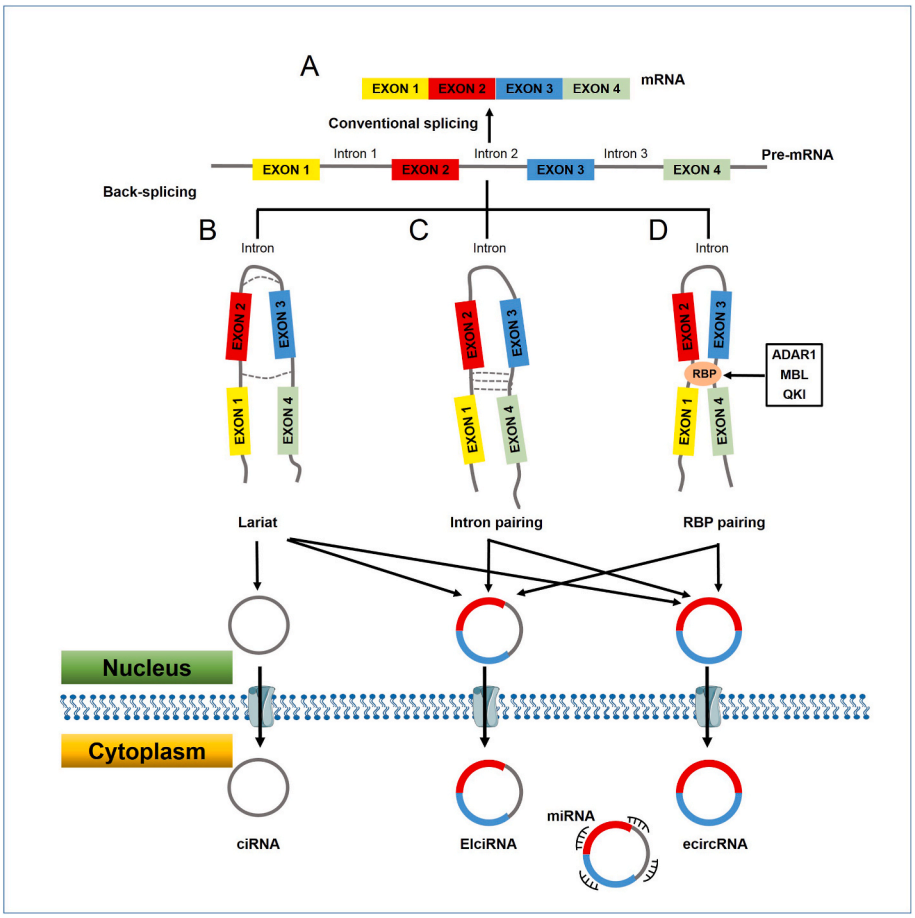

Fig. 1. Schematic representation of circRNA biogenesis. (**A**). Linear mRNA is produced conventionally through the canonical splicing machinery. (**B**). Lariat introns excised from pre-mRNA can reverse pair with complementary sequences to generate a closed loop structure termed ciRNA. (**C**). Direct base pairing of the introns flanking complementary sequences or inverted repeats can produce EIciRNA. (**D**). RBPs bind to upstream and downstream introns to form a bridge between the introns to remove some introns of the circRNA and generate exonic circRNA (ecircRNA).

(Memczak et al., 2013).

Some circRNAs might be able to be translated into proteins because most circRNAs are derived from protein-coding sequences, contain open reading frames (ORFs) and are located in the cytoplasm. EIciRNAs serve as protein-coding sequences both in vitro and in vivo when RNA molecules contain IRES elements or prokaryotic ribosome-binding sites (Granados-Riveron and Aquino-Jarquin, 2016). For instance, circ-ZNF609, an endogenous protein-coding circRNA, has been identified in murine and human myoblasts (Legnini et al., 2017). Circ-ZNF609 contains an ORF spanning from the start codon, same as the linear transcript, and an in-frame STOP codon, created upon circularization. This circRNA can be translated into a protein in a splicing-dependent and cap-independent manner, providing an example of a protein-coding circRNA in eukaryotes (Legnini et al., 2017).

Similar to lncRNAs, exonic circRNAs might sequester RBPs. Circular Foxo3 (circ-Foxo3) is an example of a circRNA-protein interaction. Both circ-Foxo3 and linear Foxo3 (Foxo3 mRNA) are encoded by the Foxo3 gene (Du et al., 2016). circ-Foxo3 binds to cell division protein kinase 2 (also known as cell division protein kinase 2 or CDK2) and cyclin-dependent kinase inhibitor 1 (p21) to form the circ-Foxo-3-p21-CDK2 ternary complex, which is involved in cell cycle progression (Du et al., 2016) and prevents cancer cell proliferation (Jeck and Sharpless, 2014).

4. Properties of circRNAs

circRNAs are abundant in many organisms ranging from flies to humans (Altesha et al., 2019), and they are predominantly located in the cytoplasm, whereas a small number of circRNAs reside in the nucleus (Zhang et al., 2017a). Multiple circRNA isoforms could be processed from a single host gene by a mechanism associated with the competition of putative RNA pairs across introns that bracket the circle-forming exons (Zhang et al., 2016b). circRNA expression profiles are significantly different between normal and pathological conditions in various diseases (Werfel et al., 2016). circRNAs are often expressed in cell type-, tissue-, and dynamic developmental stage-specific manner in organisms (Zheng et al., 2016; Xu et al., 2018). circRNAs can be potential candidates for diagnostic and prognostic biomarkers for diseases because they are stable with significantly long half-lives and resistance to RNA exonucleases due to their covalently closed circular structure (Jeck et al., 2013; Salgado-Somoza et al., 2017). They are resistant to RNA exonucleases and RNA debranching enzymes due to the absence of a 2′ to 5′ carbon linkage and free 3′ or 5’ ends, respectively (Jeck and Sharpless, 2014). Most circRNAs are produced after transcription and splicing, while only a few circRNAs are formed cotranscriptionally (Zhang et al., 2016a).

5. Mechanism of organ fibrosis

Fibrosis is usually defined as an excessive accumulation of extracellular matrix (ECM) within and around chronically impaired tissue, resulting in progressive architectural remodeling in nearly all tissues and organs (Cannito et al., 2017; Kendall and Feghali-Bostwick, 2014) (Fig. 2). Although the fibrogenic mechanisms are similar, there are tremendous differences in the regenerative ability and capacity to reverse advanced fibrosis (Weiskirchen et al., 2019; Hardie et al., 2009). In different organs, including the liver, kidneys, heart and lungs, various noxious compounds trigger the initiation and progression of fibrosis. One of the profibrotic factors is transforming growth factor-β (TGF-β) (Travis and Sheppard, 2014). Other important profibrogenic mediators include members of the platelet-derived growth factor (PDGF) family and members of the connective tissue growth factor (CTGF) family (Wynn, 2008). These profibrotic factors promote the activity and proliferation of fibroblasts, thereby increasing matrix production and promoting different diseases that affect various organs, including the lungs, liver, kidneys, heart, pancreas and other organs (Kramer and Clancy, 2018).

In the following lines, we will summarize the current knowledge of the roles of circRNAs, focusing on their mechanisms of action and interaction with partner molecules in fibrosis of the lungs, liver, heart and kidneys (Table 1).

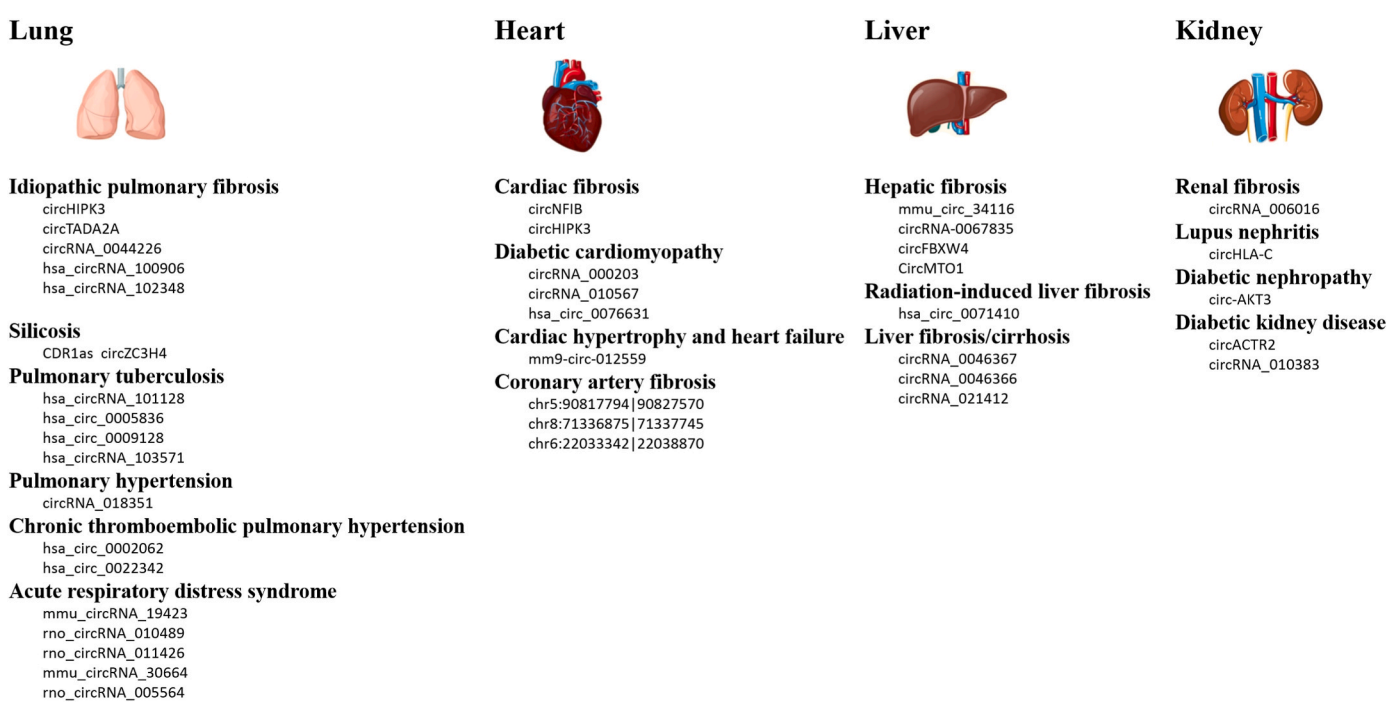

Fig. 2. CircRNA dysregulation is associated with organ fibrosis.

Table 1
List of circRNAs in organ fibrosis.

Organ fibrosis	Disease	CircRNA name	CircRNA expression level	Target miR	Target protein	Function	Sample	Reference
Pulmonary fibrosis	IPF	circHIPK3	↑	miR-338-3p	SOX4/COL1A1	FMT and fibroblast proliferation	Male C57BL/6 J mice, WI-38 cells, HEK-293T cells	Zhang et al. (2019)
		hsa_circRNA_100906 hsa_circRNA_102348	↓	miR-324-5p miR-30	Not identified	TGF-β1, Wnt, VEGF, MAPK	Plasma of IPF patients	Li et al. (2018)
		circTADA2A	↓	miR-526b, miR-203	Cav1 and Cav2	Inhibition of ECM accumulation	IPF primary human lung fibroblasts and human IPF fibroblastic cell lines	Li et al. (2020)
		circ0044226	↑	miR-7	Sp1	FMT	C57BL/6 mice, human lung fibroblasts	Zhang et al. (2020)
	Silicosis	CDR1as	↑	miR-7	TGFβR2, Smad2/3, E-cadherin, α-SMA, CTGF	EMT	Male C57BL/6 mice, HBE, A549 cells	Yao et al. (2018b)
		circZC3H4	↑	miR-212	ZC3H4, NOS2, SOCS3	Fibroblast proliferation and migration, EMT, endoplasmic reticulum (ER) stress	Human macrophage cell line, AMOs, RAW264.7, alveolar epithelial cells of mice and patients with silicosis	(Yang et al., 2018; Jiang et al., 2019)
	Pulmonary tuberculosis	hsa_circRNA_101128	↑	let-7a	Not identified	Not identified	Peripheral blood mononuclear cells (PBMCs)	Fu et al. (2019)
		hsa_circ_0005836 hsa_circ_0009128	↓	Not identified	Not identified	Not identified	Peripheral blood mononuclear cells (PBMCs)	Zhuang et al. (2017)
		hsa_circRNA_103571	↓	miR-29a miR-16	Not identified	Not identified	Blood plasma	Yi et al. (2018)
	Pulmonary hypertension	circRNA_018351	↓	miR-207 miR-665	Not identified	Not identified	Murine model of pulmonary hypertension	Wang et al. (2018)
	Chronic thromboembolic pulmonary hypertension	hsa_circ_0002062 hsa_circ_0022342	↓	hsa-miR-942-5p hsa-miR-940	Not identified	Not identified	Peripheral blood samples from patients	Miao et al. (2017a)
	Acute respiratory distress syndrome (ARDS)	mmu_circRNA_19423, rno_circRNA_010489, rno_circRNA_011426, mmu_circRNA_30664	↑	Not identified	Not identified	Not identified	Rat lung of LPS-induced ARDS model	Wan et al. (2017)
		rno_circRNA_005564	↓	Not identified	Not identified	Not identified	Rat lung of LPS-induced ARDS model	Wan et al. (2017)
Hepatic fibrosis	Liver fibrosis	mmu_circ_34116	↓	miR-22-3p	α-SMA, collagen-1, bone morphogenetic protein 7 (BMP7)	Cell autophagy, synthesis and metabolism of retinoic acid, retinol dehydrogenase activity, ubiquitin-like protein ligase activity, histone methylation	Mouse liver fibrosis model induced by CCL_4, TGF-β1-stimulated JS1 model, LPS-induced RAW264.7 cell model	(Zhou et al., 2018b, 2019)
		circRNA-0067835	↑	miR-155	Akt/FOXO3a	Not identified	Mouse liver fibrosis model induced by CCL_4, human hepatic stellate cell line LX2	Zhu et al. (2018)
		circFBXW4	↓	miR-18b-3p	TGF-β1, α-SMA, Col1A1	Inhibition of liver fibrogenesis	Mouse liver fibrosis model induced by the CCL_4, human hepatic stellate cell line LX2	Chen et al. (2020)
		CircMTO1	↓	miR-17-5p	Smad7, α-SMA, Col1A1	HCC cell proliferation	Serum from patients with chronic hepatitis B (CHB), LX-2	Wang et al. (2019)
			↓	miR-181b-5p	PTEN, α-SMA, Col1	Inhibition of HSC activation	hepatic stellate cells (HSCs), male C57BL/6J mice	Jin et al. (2020)

(*continued on next page*)

Table 1 (*continued*)

Organ fibrosis	Disease	CircRNA name	CircRNA expression level	Target miR	Target protein	Function	Sample	Reference
	Radiation-induced liver fibrosis (RILF)	hsa_circ_0071410	↑	miR-9-5p	α-SMA	Glycosaminoglycan degradation, pentose phosphate pathway and phosphatidylinositol signaling	Irradiation of human hepatic stellate cell line LX2	Chen et al. (2017)
	Liver fibrosis/ cirrhosis	circRNA_0046367 circRNA_0046366	↓	miR-34a	PPARα	Lipid peroxidation, mitochondrial injury, cell proliferation, apoptosis	High-fat-induced steatosis in HepG2 cells	(Guo et al., 2017b, 2018)
		circRNA_021412	↓	miR-1972	LPIN1	Transcriptional regulation	High-fat-induced steatosis in HepG2 cells	Guo et al. (2017a)
Cardiac fibrosis	Cardiac fibrosis	circRNA–circNFIB	↓	miR-433	AZIN1, JNK1	Cardiac fibroblast proliferation and myofibroblast Differentiation	Cardiac fibroblasts from male adult C57BL/6N mice	Zhu et al. (2019)
		circHIPK3	↑	miR-29b-3p	COL1A1, COL3A1, α-SMA	Cardiac fibroblast proliferation and migration	Cardiac fibroblasts and heart tissues after treatment with angiotensin II and TGF-β	Ni et al. (2019)
	Diabetic cardiomyopathy	circRNA_000203	↑	miR-26b-5p	Col1a2, Col3a1, α-SMA	Cardiac fibroblast proliferation	Mouse cardiac fibroblasts from male diabetic mice	Tang et al. (2017)
		circRNA_010567	↑	miR-141	Collagen 1, collagen 3 and α-SMA	Cardiac fibrosis	Cardiac fibroblasts isolated from male diabetic db/db mice	Zhou and Yu (2017)
		hsa_circ_0076631	↑	miR-214-3p	Caspase-1	Pyroptosis in cardiomyocytes	High-glucose-treated cardiomyocytes, serum of diabetic patients	Yang et al. (2019b)
	Cardiac hypertrophy and heart failure	mm9-circ-012559	↓	miR-223	ARC	Attenuates the development of cardiac hypertrophy and heart failure	Isoproterenol-induced cardiac hypertrophy and heart failure in mice	Wang et al. (2016)
	Coronary artery fibrosis	chr5:90817794|90827570, chr8:71336875|71337745, chr6:22033342|22038870	↑	Not identified	Not identified	EndMT	TGF-β1-treated rat coronary artery endothelial cells	Huang et al. (2018)
Renal fibrosis	Renal fibrosis	circRNA_006016	↑	miR-423-5p, miR-3573-5p, miR-297	Slc20a2	Not identified	Kidney tissue of hypertensive rats	Cheng and Joe (2017)
	Lupus nephritis	circHLA-C	↑	miR-150	Not identified	Not identified	Kidney tissues of patients with lupus nephritis	Luan et al. (2018)
	Diabetic nephropathy	circ-AKT3	↓	miR-296-3p	E-cadherin	Inhibition the expression of fibronectin, collagen I and collagen IV	Spontaneous diabetic db/db mice (C57BL/KsJ-db/db), mouse mesangial cells (SV40-MES13)	Tang et al. (2020)
	Diabetic kidney disease (DKD)	circACTR2	↑	Not identified	Not identified	Increase in pyroptosis, interleukin (IL)-1β release, collagen IV and fibronectin production	Human renal proximal tubular epithelial cell line HK-2	Wen et al. (2020)
		circRNA_010383	↓	miR-135a	TRPC1	Inhibition of ECM	diabetic kidneys of db/db mice	Peng et al. (2020)

6. CircRNAs and respiratory fibrosis

6.1. CircRNAs and idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and fatal disease due to the aberrant accumulation of fibrosis in the lung parenchymal tissue (Zhang et al., 2019). Although IPF has been considered rare, the incidence of this disease is similar to that of brain, stomach and testicular cancers. The prognosis of IPF is poor and is worse than that of various types of cancers, with a median survival time from the time of diagnosis of 2–4 years. The pathogenesis of IPF is not completely understood, and current therapies are limited to reducing the rate of pulmonary functional decline in some patients (Shaw et al., 2017). Mounting evidence has shown that circRNA expression is associated with several human diseases, especially proliferative diseases, such as tumorigenesis. Recently, several reports have investigated the relationship between circRNAs and IPF.

A high-throughput microarray assay identified a total of 67 significantly dysregulated circRNAs in the plasma of patients with IPF; among them, 38 were upregulated, whereas 29 were downregulated (Li et al.,

2018). Validation of those circRNAs by PCR analysis showed that hsa_circRNA_100906, hsa_circRNA_102100 and hsa_circRNA_102348 were robustly upregulated, whereas hsa_circRNA_101225, hsa_circRNA_104780 and hsa_circRNA_101242 were downregulated in the plasma samples from IPF patients (Li et al., 2018). Furthermore, hsa_circRNA_100906 and hsa_circRNA_102348 directly bound to miR-324-5p and miR-630, respectively, which were downregulated in IPF patients (Li et al., 2018). In both IPF primary human lung fibroblasts and human IPF fibroblastic cell lines, circTADA2A suppressed lung fibroblast activation via miR-526b/Caveolin (Cav)-1 and inhibited lung fibroblast proliferation via miR-203/Cav2, thus reducing the excessive deposition of ECM and relieving IPF (Li et al., 2020). Experiments on bleomycin (BLM)-induced pulmonary fibrosis in mice showed that circHIPK3 and circ0044226 are upregulated in lung tissue samples, and silencing these circRNAs improved fibroblast proliferation by sequestering miR-338-3p and miR-7, respectively (Zhang et al., 2019, 2020). Another study identified 16 circRNAs connected to 27 miRNAs and one single circRNA connected to several miRNAs in BLM-induced IPF in rats and demonstrated that circRNAs are involved in pulmonary fibrogenesis mediated by the TGF-β and Notch signaling pathways, which are involved in EMT and fibroblast activation (Yang et al., 2019a).

6.2. CircRNAs and silicosis

Silicosis is an irreversible and incurable lung disease caused by the inhalation of dust containing crystalline silica particles, leading to the development of chronic inflammation followed by fibrosis (Sato et al., 2018). It is a fatal lung condition, and there are no effective diagnostic tools for its early detection and no effective therapeutic methods to prevent its progression. Evidence from our laboratory and others indicated that ZC3H4, a member of the CCCH-type zinc finger protein family, is involved in pulmonary fibrosis induced by silica (Yang et al., 2018). Administration of silica concomitantly increased circZC3H4 RNA expression and increased ZC3H4 protein levels in an alveolar macrophage cell line and murine lungs. circZC3H4 RNA and the ZC3H4 protein participate in SiO_2-induced macrophage activation, fibroblast proliferation and migration via the circZC3H4 RNA/ZC3H4 pathway (Yang et al., 2018). Our recent study demonstrated that EMT was involved in pulmonary fibrosis, which was accompanied by increased migratory characteristics. The involvement of circZC3H4 RNA in ZC3H4 expression and EMT regulation revealed the role of this novel functional circRNA in SiO_2-induced lung fibrosis and suggested that the circZC3H4/ZC3H4 pathway may provide a promising treatment strategy for silicosis patients (Jiang et al., 2019). Evidence from other laboratories demonstrated that circRNA CDR1as was time-dependently upregulated in HBE and A549 cell lines after silica administration. This circRNA could sponge miR-7 to release TGFBR2, suggesting that the interaction of CDR1as and miR-7 exerts important functions and provides potential therapeutic targets in pulmonary fibrotic diseases (Yao et al., 2018b). It has been demonstrated that miR-7 was decreased in breast cancer stem cells by inhibiting cell invasion and reversing EMT partially by targeting the oncogene (Zhang et al., 2014). The inhibition of miR-7 was also found to be related to localized scleroderma due to excess accumulation of collagen in normal fibroblasts (Zhang et al., 2014). Furthermore, miR-7 reversed EMT through AKT/ERK1/2 inactivation in ovarian cancer (Zhou et al., 2014).

6.3. CircRNAs and pulmonary tuberculosis

Pulmonary tuberculosis (PTB) is a communicable disease caused by infection with *Mycobacterium tuberculosis* (Mtb) (Pai et al., 2016). Traditional diagnostic methods are limited to sputum smear microscopy, bacteriological detection and polymerase chain reaction, X-ray diagnosis and purified protein derivative testing. Because of their low sensitivity and poor specificity, these methods cannot diagnose PTB effectively. Therefore, it is necessary to identify novel diagnostic biomarkers and therapeutic targets to improve patient survival (Fu et al., 2019; Zhang et al., 2017b). Evidence from recent studies revealed that circRNAs play critical roles in various pathological processes and that their dysregulation is associated with the occurrence and progression of tuberculosis (TB). Several recent studies have developed circRNA detection as a blood transcriptomic signature to differentiate active PTB patients from healthy patients and other pulmonary disease cohorts and have predicted the prognosis of PTB.

The detection of circRNA expression by whole-transcriptome sequencing in the blood of 3 PTB patients and 3 healthy individuals revealed that 170 circRNAs were dysregulated (Zhang et al., 2017b). Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis proved that several immune system pathways, including ubiquitin-mediated proteolysis, endocytosis pathways in cancer, mitogen-activated protein kinase signaling pathway, and human T-lymphotropic virus type 1 (HTLV-1) infection, were involved in PTB pathogenesis. Construction of a ceRNA network revealed that dysregulation of ceRNA expression induces changes in target gene expression by miRNA-mediated circRNA-associated ceRNA crosstalk interactions. Taken together, these results suggest that circRNA-miRNA-mRNA interactions play potential roles in PTB (Zhang et al., 2017b). Other studies have shown that circRNAs are widely expressed in human peripheral blood mononuclear cells (PBMCs). Analysis of circRNA expression profiles in PBMCs identified 171 circRNAs dysregulated in the PBMC samples of TB patients (Fu et al., 2019). Specifically, circRNA_103017, circRNA_059914 and circRNA_101128 were increased, while circRNA_062400 was decreased in the PBMC samples of patients with TB (Fu et al., 2019). Moreover, circRNA_101128 potentially targets let-7a, which can regulate the immune response to Mtb infection and control the bacterial burden via modulation of the NF-κB pathway (Kumar et al., 2015). CircRNAs in plasma were also found to be dysregulated in active TB patients (Yi et al., 2018). Hsa-circRNA_103571 was significantly decreased in active TB plasma, and it potentially interacted with TB-related miRNAs such as miR-16 and miR-29a (Yi et al., 2018). Another study found that the expression of the 7 circRNAs in the PBMCs of active PTB patients was significantly higher than that in healthy controls. Compared with host linear RNA transcripts, the circRNAs in several pathways, including "Cytokine-cytokine receptor interaction", "Chemokine signaling pathway", "Neurotrophin signaling pathway", and "Bacterial invasion of epithelial cells", were strongly upregulated in patients with PTB (Qian et al., 2018). Additionally, hsa_circ_0005836 and hsa_circ_0009128 expression levels were significantly downregulated in the PBMCs of APTB compared to the expression in the healthy controls (Zhuang et al., 2017), indicating that these two circRNAs might serve as potential novel biomarkers for Mtb infection.

6.4. CircRNAs and pulmonary hypertension

Pulmonary hypertension (PH) is a frequent and severe lung disease with an increase in pulmonary arterial pressure caused by vasoconstriction and extensive vascular remodeling, including enhanced proliferation of pulmonary artery smooth muscle cells. PH has been reported to complicate the course of numerous fibrotic pulmonary diseases, including idiopathic pulmonary fibrosis, chronic hypersensitivity pneumonitis and nonspecific interstitial pneumonitis (Collum et al., 2017). The pathogenesis of PH remains poorly understood, and there are no potential biomarkers for the diagnosis and therapeutic strategy of PH patients. Recently, microarray analysis demonstrated that 64 circRNAs were differentially expressed in a murine model of hypoxia-induced PH compared with the circRNAs in the control groups, of which 23 circRNAs were upregulated, and 41 circRNAs were downregulated (Wang et al., 2018). CircRNA-miRNA-mRNA network analysis and GO and KEGG analyses showed that dysregulation of circRNAs may play important roles in the pathogenesis of hypoxia-induced PH (Wang et al., 2018). CircRNA target prediction analysis found that all of the differentially

expressed circRNAs contained MREs to sponge different miRNAs, including miR-152-3p, miR-742-3p, miR-6373, miR-880-5p, and miR-298-3p (Wang et al., 2018). Although there is no report to show the role of miRNAs in hypoxia-induced PH, hypoxia suppressed miR-152 expression in human umbilical vein endothelial cells, and miR-152 was demonstrated to target ADAM17 to inhibit cell proliferation and migration (Wu et al., 2014). miR-152 was found to target SIRT7 and promote senescence in stem cells from human dental pulp (Gu et al., 2016). Since hypoxia upregulated mmu_circRNA_004592 expression but downregulated miR-152 in a murine model of PH, it can be hypothesized that inhibition of miR-152 expression promotes proliferation and inhibits apoptosis and senescence of endothelial cells, smooth muscle cells and fibroblasts in the pulmonary artery, therefore further raising the pressure in the pulmonary vessels (Wang et al., 2018).

Additionally, circRNA microarrays identified 351 (122 upregulated and 229 downregulated) differentially expressed circRNAs in peripheral blood samples from patients with chronic thromboembolic pulmonary hypertension (CTEPH) (Miao et al., 2017a). GO and KEGG pathway enrichment analyses of differentially expressed circRNAs showed that upregulated circRNAs might mainly affect the ribonucleotide biosynthetic process in CTEPH, whereas downregulated circRNAs may regulate the cellular response to stress, DNA damage stimulus, and gene expression. Among the dysregulated circRNAs, hsa_circ_0002062 sponges hsa-miR-942-5p targeting CDK6, which is mainly enriched in cancer-related pathways, while hsa_circ_0022342 sponges hsa-miR-940 targeting CRKL, which is mainly enriched in the ErbB signaling pathway (Miao et al., 2017a). The literature has shown that CDK6 is related to cell cycle and cell growth in the development of PH (Sang et al., 2016), whereas the ErbB signaling pathway can lead to increased apoptosis and loss of cell proliferation, which are also involved in the pathogenesis of PH (Miao et al., 2017b). Therefore, hsa_circ_0002062 and hsa_circ_0022342 may play critical roles in the development of CTEPH, and the modulation of their signaling pathway may provide a novel therapeutic strategy for CTEPH.

6.5. CircRNAs and acute respiratory distress syndrome

Acute respiratory distress syndrome (ARDS) in infants is a common and fatal disease caused by dysregulated inflammation and an impaired pulmonary gas exchange ability leading to refractory arterial hypoxemia and respiratory failure (Matthay et al., 2012). Diffuse alveolar injury and progression to pulmonary fibrosis are pathological features of ARDS. Studies on ARDS mainly focus on its pathogenesis and potential targets for therapy, and the function of circRNAs and their overall contribution to the pathogenesis of ARDS remain largely unknown. Recently, a circRNA microarray was performed to explore the expression profiles of circRNAs during lipopolysaccharide (LPS)-induced ARDS in rats and showed that among 13438 circRNAs in total, 395 and 562 circRNAs were significantly up- and downregulated in the LPS group vs. the control group, respectively (Wan et al., 2017). The mechanisms and function of circRNAs in ARDS need to be clarified in the future to identify novel biomarkers and to develop novel therapeutic strategies for the clinical management of ARDS.

7. CircRNAs and hepatic fibrosis

Liver fibrosis is a common pathological stage in the development of liver cirrhosis, liver cancer and liver failure. Hepatic stellate cells (HSCs) play critical roles in the progression and development of hepatic fibrosis. As there are no effective chemical or biological drugs against liver fibrosis, it is of great importance to continue the study of liver fibrosis pathogenesis and to develop novel treatment methods. One study of a circRNA microarray screened a total of 10389 circRNAs in the hepatic tissue of a classic mouse liver fibrosis model induced by CCl4 and identified 14 upregulated and 55 downregulated circRNAs (Zhou et al., 2018b). Circ_34116 was significantly decreased and the expression of α-SMA and collagen 1 significantly increased after TGF-β1 stimulation of the mouse stellate cell line JS1 [81]. This circRNA contains the MRE of miR-22-3p and can competitively bind to miR-22 to regulate the transcription of its target gene BMP7 (Zhou et al., 2018b, 2019), suggesting that circ_34116 would inhibit HSC activation via the "circ_34116/-miR-22-3p/BMP7" signaling axis. Inhibition of HSCs by treatment with thymosin β4 (Tβ4), an anti-inflammatory and antifibrotic peptide with 43 highly conserved amino acids, induced a total of 644 differentially expressed circRNAs in LX-2 cells (Zhu et al., 2018). Among these circRNAs, circ_0067835 was significantly increased in HSCs from Tβ4-depleted LX-2 cells and CCL4-induced liver fibrosis in mice, indicating that there is an association between circ_0067835 and liver fibrosis (Zhu et al., 2018). Circ_0067835 sponges miR-155 to modify the pathogenesis of liver fibrosis via the Akt/FOXO3a signaling pathway (Zhu et al., 2018), and circFBXW4 inhibited HSC activation and proliferation, induced HSC apoptosis, and attenuated mouse liver fibrogenesis through the circFBXW4/miR-18b-3p/FBXW7 axis (Chen et al., 2020). In another study, the circMTO1 levels in the serum were negatively correlated with the progression of liver fibrosis in patients with chronic hepatitis B (Wang et al., 2019). circMTO1 suppresses TGF-β1-induced HSC activation by binding to miR-17-5p, which targets Smad7 and inhibits liver fibrosis (Wang et al., 2019). More recently, circMTO1 has been shown to inhibit HSC activation through miR-181b-5p-mediated phosphatase and tensin homolog (PTEN) (Jin et al., 2020)

Radiation-induced liver fibrosis (RILF) is a serious complication of radiation therapy for liver cancer, and the molecular mechanism of RILF is still poorly understood. CircRNA expression profiles in fibrotic HSCs induced by irradiation revealed that 179 circRNAs were upregulated and 630 circRNAs were downregulated compared with the normal control. qRT-PCR analysis validated that irradiated HSCs overexpressed hsa_circ_0071410, which harbors three binding sites for miR-9-5p. Knockdown of hsa_circ_0071410 enhanced the expression of miR-9-5p and reduced the mRNA and protein expression levels of α-SMA, resulting in the attenuation of irradiation-induced HSC activation (Chen et al., 2017). Thus, the inhibition of hsa_circ_0071410 sheds light on the molecular functions of circRNAs in RILF and provides novel diagnostic and therapeutic targets for RILF.

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease, and its spectrum comprises simple steatosis and nonalcoholic steatohepatitis (NASH), which can lead to hepatic fibrosis and cirrhosis (Sulaiman et al., 2019). A circRNA profiling experiment performed in HepG2 cells with hepatic steatosis induced by high-fat stimulation revealed that a total of 357 circRNAs were dysregulated and that the expression of circRNA_021412 was reduced; furthermore, circRNA_021412 is correlated with miR-1972-based inhibition of a transcriptional regulatory factor, LPIN1 (Guo et al., 2017a). LPIN1 can selectively activate fatty acid oxidation and mitochondrial oxidative phosphorylation and act as a key component of multiple signaling transductions involved in lipid homeostasis (Everitt et al., 2013). Another study by the same group analyzed circRNA expression and function in NAFLD and found that circRNA_0046367 and circRNA_0046366 were reduced and miR-34 was increased in hepatic steatosis. miR-34a can reduce the expression of PPARα, and this effect can be reversed by the presence of circRNA_0046367 or circRNA_0046366, which is consistent with the evidence of improvement of mitochondrial function and prevention of hepatotoxicity (Guo et al., 2017b, 2018). These identifications and proof-of-concept studies suggest that the circRNA-miRNA coregulatory network is involved in hepatic lipid regulation and shed light on understanding the molecular regulation underlying the early stage of NAFLD pathogenesis.

8. CircRNAs and cardiac fibrosis

Cardiac fibrosis is a pathological feature of most adverse ventricular remodeling in myocardial infarction. A circRNA investigation of TGF-β-treated cardiac fibroblasts and myocardial infarction samples of mice

showed that circNFIB (mmu_circ_0011794) was downregulated, indicating a strong connection between the expression of circNFIB and cardiac fibrosis both in vivo and in vitro (Zhu et al., 2019). Overexpression of circNFIB attenuated cardiac fibroblast proliferation by sponging miR-433 and reversed the expression levels of target genes in downstream signaling pathways of miR-433, including antizyme inhibitor 1 (AZIN1) and c-Jun N-terminal kinase 1 (JNK1) (Zhu et al., 2019). Knockdown of AZIN1 promoted the proliferation and differentiation of cardiac fibroblasts into myofibroblasts by activating the TGF-β-Smad3 signaling pathway. Inhibition of JNK1 was responsible for the profibrotic effects via activation of ERK and p38 kinase, which is in parallel with the activation of Smad3 (Tao et al., 2016). Another circRNA study showed that circHIPK3 expression was markedly elevated in cardiac fibroblasts and heart tissues after treatment with angiotensin II and TGF-β. Suppression of circHIPK3 expression attenuated cardiac fibroblast proliferation and migration induced by Ang II and TGF-β in vitro. Mechanistically, circHIPK3 functions as a miR-29b-3p sponge and reduces the expression of its target genes (COL1A1, COL3A1, and α-SMA), indicating that circHIPK3 can be used as a potential new target for the prevention of cardiac fibrosis (Ni et al., 2019).

Cardiac fibrosis is one of the main pathologies of diabetic cardiomyopathies because diabetes mellitus (DM) induces biochemical, functional and morphological abnormalities in cardiomyocytes. Myocardial fibrosis decreases myocardial shortening, enhances myocardial stiffness and induces atrial and ventricular arrhythmias. However, the mechanism involved in myocardial fibrosis due to DM remains unclear. Several studies have revealed a consistent association between the presence of cardiac hypertrophy and myocardial fibrosis in patients with DM. A circRNA profiling array revealed that circRNA_000203, which is transcribed from the myo9A gene, was upregulated in the diabetic mouse myocardium and cardiac fibroblasts treated with angiotensin II. CircRNA_000203 can enhance the expression of Col1a2, Col3a1 and α-SMA in cardiac fibroblasts by specifically sequestering miR-26b-5p, which can interact with the 3′UTRs of Col1a2 and CTGF, suggesting that suppressing the function of circRNA_000203 might provide a novel therapeutic strategy to treat cardiac fibrosis (Tang et al., 2017). Another study detected 24 upregulated and 19 downregulated circRNAs in diabetic mouse myocardia and cardiac fibroblasts. Bioinformatics analysis showed that circRNA_010567, one of the most upregulated circRNAs, contains binding sites for miR-141, which is predicted to modulate TGF-β1 function. Inhibition of circ_010567 expression reduced the expression of fibrosis-related proteins, such as collagen 1, collagen 3 and α-SMA, in mouse cardiac fibroblasts. These results imply that circRNA_010567/miR-141/TGF-β1 may play a pivotal role in myocardial fibrosis and thus provide novel insight into and a novel therapeutic strategy for cardiopathy pathogenesis (Zhou and Yu, 2017). Hsa_circ_0076631, also named caspase-1-associated circRNA (CACR), was increased in both the serum of diabetic patients and in high-glucose-treated cardiomyocytes. Silencing hsa_circ_0076631 significantly alleviated pyroptosis in high-glucose-treated cardiomyocytes by sponging endogenous miR-214-3p to counteract high-glucose-induced caspase-1 activation, suggesting that hsa_circ_0076631 might be a novel therapeutic target via the CACR/miR-214-3p/caspase-1 pathway in diabetic cardiomyopathy patients (Yang et al., 2019b).

mm9-circ-012559, also termed heart-related circRNA (HRCR), was demonstrated to target miR-223 and function as an antihypertrophic molecule to inhibit cardiac hypertrophy and heart failure induced by isoproterenol in mice. Overexpression of HRCR attenuated hypertrophic responses upon isoproterenol treatment, such as decreased cardiac stress-related gene expression, interstitial fibrosis, cardiomyocyte size and heart weight-to-body weight ratio (Wang et al., 2016). Another study investigated the roles of differentially expressed circRNAs during TGF-β1 treatment in rat coronary artery endothelial cells and found 66 upregulated and 36 downregulated circRNAs. Furthermore, three circRNAs (chr5:90817794|90827570, chr8:71336875|71337745, and chr6:22033342|22038870) were confirmed to be significantly upregulated in TGF-β1-treated rat coronary artery endothelial cells (Huang et al., 2018).

9. CircRNAs and renal fibrosis

To explore the role of circRNAs in renal fibrosis, a number of circRNAs were detected to be differentially expressed in the kidneys of the rat model of hypertensive nephropathy by genome-wide circRNA profiling, suggesting that circRNAs may contribute to the pathogenesis of kidney injury (Cheng and Joe, 2017; Cui et al., 2020). Further confirmation experiments by qRT-PCR showed that circRNA_006016 was downregulated more than 30-fold in hypertensive rats compared to normal control rats. This circRNA was predicted to bind miR-423-5p, miR-3573-5p and miR-297, and all three microRNAs were predicted to target the Slc20a2 gene. Renal Slc20a2, also known as Pit-2, plays an important role in blood pressure control by mediating the process of dietary potassium deficiency via a circRNA-miRNA interaction (Breusegem et al., 2009). Similarly, another study established 142 upregulated and 29 downregulated circRNAs in renal biopsies of patients with lupus nephritis compared with healthy control patients (Luan et al., 2018). Among these circRNAs, renal circHLA-C was particularly enhanced in lupus nephritis patients and displayed a tendency of negative correlation with miR-150, which could suppress lupus nephritis pathogenesis, suggesting that circHLA-C may play an important role in the pathogenesis of lupus nephritis by sponging miR-150 [104].

Diabetic nephropathy is a leading cause of lethal diabetic complications and primary end-stage renal disease around the world. The pathological features of diabetic nephropathy are thickened glomerular membranes, progressive mesangial hypertrophy and accumulation of extracellular matrix and proteins, including fibronectin and collagen (Ni et al., 2015). Recent studies have investigated the critical roles of circRNAs for understanding the pathogenesis of diabetic nephropathy and provided new therapeutic targets for this disease. Circ-AKT3 reduces ECM accumulation in mesangial cells in diabetic nephropathy via modulating miR-296-3p/E-cadherin signals in spontaneous diabetic db/db mice (C57BL/KsJ-db/db) and mouse mesangial cells (SV40-MES13) (Tang et al., 2020). circACTR2 is upregulated and might be involved in inflammation pyroptosis in glucose-stressed human renal tubular epithelial cells (HK-2), while knockdown of circACTR2 significantly reduces pyroptosis, interleukin (IL)-1β release, and fibronectin and collagen IV production, thus providing novel insights into and therapeutic strategies for diabetic kidney disease (Wen et al., 2020). In addition, circRNA_010383 functions as a sponge for miR-135a to promote the accumulation of ECM proteins and downregulate the expression of transient receptor potential cation channel, subfamily C member (TRPC1), and inhibit proteinuria and renal fibrosis in db/db mice (Peng et al., 2020).

10. Summary

CircRNAs are a hot topic in RNA research, although they were previously considered to be the result of transcriptional errors. With the development of new technologies, the unique properties and powerful functions of these molecules are being acknowledged by researchers. Increasing evidence suggests that circRNAs, as a novel class of endogenous noncoding RNAs, play a critical role in the pathogenesis and disease progression of various forms of visceral organ fibrosis, including pulmonary fibrosis, cardiac fibrosis, hepatic fibrosis and renal fibrosis. Under these conditions, circRNAs present different expression patterns and different roles function as important regulators of gene expression to "sponge" or sequester other genes and alter gene expression, transcription, splicing, etc. CircRNA dysregulation has been associated with a wide range of biological processes, such as cell proliferation, apoptosis, and EMT. Those biological processes are related to fibrotic diseases. However, the techniques for circRNA identification and validation are

largely experimental, and these approaches remain to be standardized. Future in-depth research, such as studies on the adenosine methylation of circRNAs, investigations of the predictive actions and functions of circRNAs and tests of the therapeutic potential of circRNAs in visceral organ diseases and animal models, are needed. In summary, circRNAs may serve as prognostic evaluators and potential biomarkers for the diagnosis of various types of visceral organ fibrosis and could represent a target for treatment strategies.

Author contributions

X.D. interpreted the data, prepared the figures and wrote the manuscript. Y.C, C.W. and J.H. interpreted the data. J.C. provided the funding and laboratory space and designed and monitored all the experiments. All the authors carefully read, discussed and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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