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REVIEW

Circular RNAs in pulmonary hypertension: Emerging biological concepts and potential mechanism

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Abstract

Circular RNAs (circRNAs) are endogenous RNAs with a covalently closed single-stranded transcript. They are a novel class of genomic regulators that are linked to many important development and disease processes and are being pursued as clinical and therapeutic targets. Using the most powerful RNA sequencing and bioinformatics techniques, a large number of circRNAs have been identified and further functional studies have been performed. It is known that circRNAs act as potential biomarkers, sponges for microRNAs (miRNAs) and RNA-binding proteins (RBPs), and regulators of mRNA transcription. They also participate in the translation of peptides or proteins. Many types of circRNAs are dysregulated in plasma or lung tissues, and they may be involved in regulating the proliferation and apoptosis of pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs), leading to pulmonary vascular remodeling in pulmonary hypertension (PH). One possible mechanism is that circRNAs can regulate the function of PAECs and PASMCs by acting as miRNA sponge. However, other potential mechanisms of action of circRNAs are still being actively explored in PH. This paper presents a systematic review of the biogenesis, biological characterization, relevant underlying functions, and future perspectives for studies of circRNAs in the pathogenesis of PH.

KEYWORDS
biological characterization, circular RNAs, miRNA sponges, pulmonary hypertension

1 | INTRODUCTION

Pulmonary hypertension (PH) is a progressive pulmonary vascular disease.[1] Over-proliferation and apoptosis resistance of pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs), accumulation of peripheral connective tissues and elastic fiber, and infiltration of perivascular inflammation contribute to pulmonary arteriole contraction and progressive pulmonary vascular stenosis, followed by increased pulmonary artery pressure and right ventricular overload,

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eventually leading to right ventricular failure and even death.[2,3] PH is classified according to different etiologies into five categories such as pulmonary arterial hypertension (PAH), PH secondary to left heart disease, PH associated with hypoxia and lung disease, and chronic thromboembolic pulmonary hypertension (CTEPH).[4] Although there are many targeted drugs for the treatment of PH,[3] most of them mainly dilate pulmonary vessels and cannot completely reverse pulmonary vessel remodeling. Therefore, patients with PH have a poor prognosis, with a 3-year survival rate of only 55%–65%.[5] The etiology of vascular remodeling is extremely complex, involving genetic[6,7] and epigenetic factors,[8-10] inflammation,[11] oxidative stress,[12] and metabolic transformation.[13-15] In short, despite great progress in understanding the pathogenesis of PH, further in-depth exploration at the cellular and molecular levels is urgently needed to develop a more effective treatment regimen.

Noncoding RNAs (ncRNAs) such as circular RNAs (circRNAs), long noncoding RNAs (lncRNAs), and microRNAs (miRNAs) have significant functions in the progression of many diseases.[16] CircRNAs have been widely identified in plant viruses, yeast, mice, and humans.[17-20] With rapid development of deep sequencing technology, different types of circRNAs have been identified with multiple biological functions, such as binding miRNAs and proteins, regulating transcriptional and post-transcriptional levels, modulating parental gene expression, and coding protein in the physiological and pathological development of many organisms.[21] In recent years, circRNAs, as a special ncRNA with a covalent closed-loop structure, have attracted considerable attention because they are extensively involved in various diseases, including cardiopulmonary vascular disease.[22]

In PH, limited studies indicated that a subset of circRNAs, known as competing endogenous RNAs (ceRNAs), can regulate mRNA expression by absorbing miRNAs to further regulate the occurrence and development of PH.[23,24] The present review summarizes the research history, biogenesis, classification, biological functions, and implications of circRNAs. Our review also sumarizes current knowledge of circRNAs' underlying functions in pathogenesis of PH and highlights future perspectives for studies of circRNAs in PH.

2 | DISCOVERY AND RESEARCH HISTORY OF CIRCRNAS

CircRNAs were initially discovered approximately 50 years ago by electron microscopy (Figure 1). As early as 1971, Diener first found that the genome of viroids, which consists of a single-stranded, closed RNA molecule, can infect and cause spindle tuber disease in potato plants, leading to their death; this molecule was confirmed to be a circRNA in subsequent studies.[1,25-27] Another crucial time point came in 1976. In this year, Sanger et al. first discovered that plant viroids are single-stranded, covalently closed RNA molecules with a high degree of base pairing and self-complementarity; they named these molecules circular RNAs, thereby initiating research into circRNAs.[20]

In the next 3–4 decades, several circular forms of circRNAs had been found and observed through electron microscopy in other species, such as viruses, prokaryotes unicellular eukaryotes, and mammals (Figure 1).[18,28-30] In 1977 and 1979, base pairing between inverted complementary sequences at the 3′ and 5′ ends of linear molecules was recognized as a mechanism of forming circRNAs in the Uukuniemi virus genome, particularly in the cytoplasm and nucleus of eukaryotic cells such as HeLa cells, CV-1 cells, and Chinese hamster ovary cells.[17,29] CircRNAs were subsequently observed in yeast mitochondria and in isolated nuclei of Tetrahymena in 1980 and 1981.[19,30] In 1986, Kos et al. discovered that hepatitis delta virus in human also contained circRNAs.[28] CircRNAs were also detected in saccharomyces cerevisiae in 1990.[31] In 1993, Capel et al. found circRNAs transcripts in the Y chromosome in mouse testis.[18] In the same year, Cocquerelle et al. first reported that nuclear pre-mRNA can process circRNAs in eukaryotes (Figure 1).[32]

The above-mentioned studies only observed the presence of circRNAs using electron microscopy, with no further investigation of their formation mechanism and biological function. In 1983 and 1984, intron splicing was discovered to form circRNAs (Figure 1),[33,34] and in 1991, Nigro et al.[35] revealed that the human candidate tumor suppressor gene contained endogenous circRNAs and that the formation mechanism of circRNAs might be exon skipping between the 5′ splice donor of one exon and the 3′ splice acceptor of another exon, leading to end-to-end linking. Based on the report of Jarrell in 1993, one RNA undergoing accurate inverse splicing yields an excised exon-circRNA *in vitro*.[36] In 1994, RNA cyclase ribozymes were confirmed to play key roles in the formation of circRNAs in bacteria and yeast.[37] Two years later, Zaphiropoulos found that a new form of lariat splicing during alternative splicing of the *P450 2C24* pre-mRNA could generate circRNAs.[38] In 2014, Zhao et al. reported that reverse complementary sequences could mediate circRNA circularization, further revealing the possible mechanism of circRNA circularization (Figure 1).[39]

Functional assessment of circRNAs started in 1995,[40] when it was discovered that circRNAs could code proteins (Figure 1). Chen et al. observed that a subset of circRNAs with continuous open reading frames (ORF) could code peptides if the circRNAs contained internal ribosome entry site (IRES) elements; thus, they were first to report a mechanism of the initiation of circRNA translations.[40] In 2006, ribonuclease R (RNase R) was first used to enrich circRNAs.[41] RNase R can remove most linear RNAs leaving a rich source of circRNAs, which can be utilized for preparing intron cDNA libraries. In 2013, Hansen et al. discovered that circRNAs could absorb miRNA to mediate gene transcription, revealing the function of circRNAs as miRNA sponges (Figure 1).[42]

With the advent of RNA sequencing technology, Salzma et al. were first to detect circRNAs in the human body in 2012, a landmark in circRNA related human studies (Figure 1).[43] Research into circRNAs has rapidly expanded ever since. In 2017, some circRNAs were found to have translational functions, overturning the traditional belief

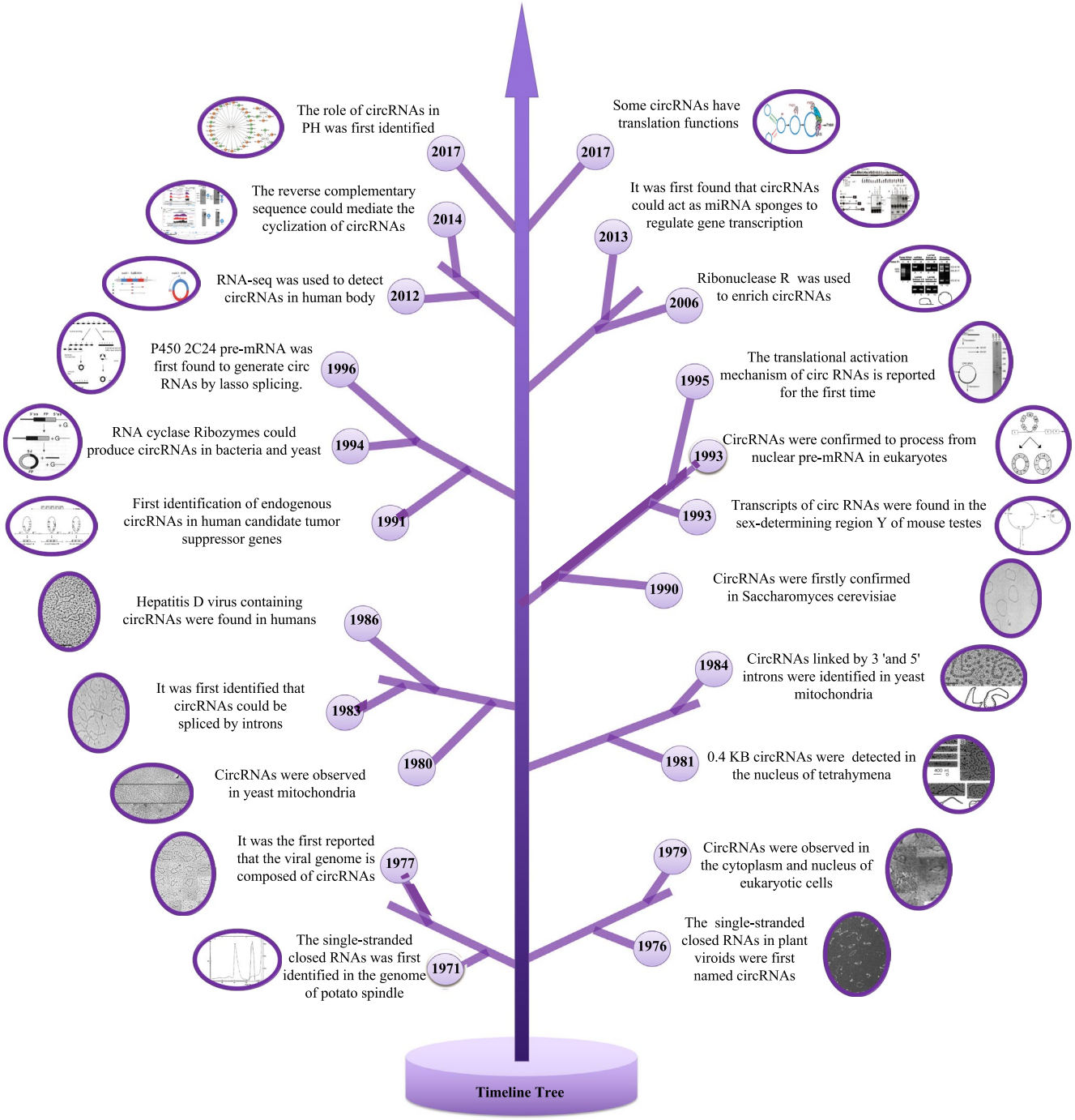

FIGURE 1 History of the discovery and development of circRNAs[1,17-20,25-33,35-47]

that circRNAs do not encode proteins.[44] By this time, circRNAs were also being studied in PH. In the same year, Miao et al. first reported that hsa_circ_0002062 and hsa_circ_0022342 might be key circRNAs in the pathogenesis of CTEPH.[45] In 2019 and 2021, two studies suggested that expression of upregulated circ_0068481 and downregulated circGSAP could be promising novel biomarkers for the diagnosis and prognosis of idiopathic PAH (IPAH, Figure 1).[46,47] However, at present, although the number of studies of circRNAs is increasing, the functions of circRNAs in PH are still poorly understood.

3 | BIOLOGICAL CHARACTERIZATION OF CIRCRNAS

CircRNAs have a closed circular structure formed by head and tail splicing.[22] Their circular structure gives these molecule a lot of biological characteristics. They are widely and highly expressed in eukaryotes.[48] Moreover, numerous circRNAs are expressed in various mammalian tissues, and about 30,000 different circRNAs have been found in human tissues.[49] By this time, circRNAs are about 10 times that of linear RNA.[50] With a closed circular structure, circRNAs do not

have a 5′ cap and a 3′ polyadenylate tail and are insensitive to nuclease, making them more stable than homologous linear RNAs.[51] The circRNAs so far examined have a median half-life ranging from 18.8 to 23.7 hours, compared with 4.0–7.4 hours for their cognate linear RNAs.[52] CircRNAs are also highly conserved. Two thousand one hundred and twenty-one circRNAs in human can be mapped to the murine genome, of which 457 generate the murine circRNAs.[53] In addition, they are tissue-specific and/or development-stage-specific.[54] CircRNAs are common expressed in both physiological and pathological conditions.

Currently, the formation mechanism of circRNAs has gained considerable attention (Figure 2A).[55] CircRNAs, which are generated by the back splicing of mRNA precursors through non-classical splicing by spliceosomes,[50] differ from common RNA canonical linear splicing patterns, as reflected in the binding of the 3′ and 5′ ends by circularization.[51] According to the sequence of their various exons and introns and different circularization mechanisms, circRNAs are classified into four categories: exonic circRNAs (EcircRNAs), intronic circRNAs

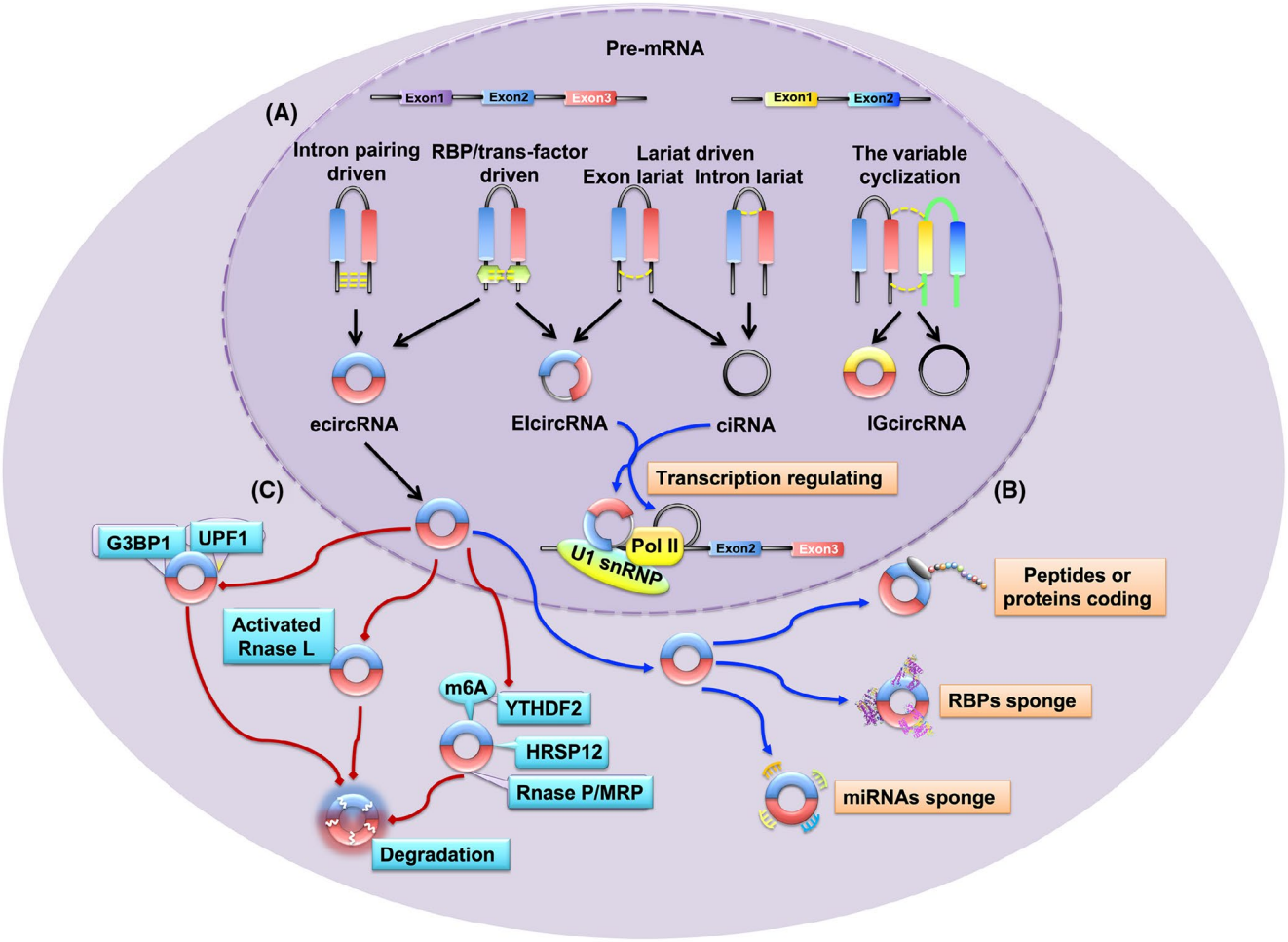

FIGURE 2 The classification, formation mechanism, degradation, and functions of circRNAs. (A) The classification and formation mechanism of circRNA biogenesis. Intron pairing–driven circularization: The intron motifs on both sides of the cyclization region show reverse complementarity, which could enable the two exons to form a loop of EcircRNA after removing or retaining certain introns. RBP/trans-factor-dependent cyclization: RBPs bridge two flanking introns close together and then remove introns to form ecircRNA or ElcircRNA. Lariat-driven circularization: The 3′ splice donor of exon 1 and the 5′ splice acceptor of exon 4 link up end-to-end by exon skipping and form an exon-containing lariat structure; after the removal of introns, ecircRNA or ciRNA is formed. Variable cyclization: Splicing selection and exon circularization may be influenced by inverted repeated ALU pairs (IRAlus) and the competition between them. (B) The functions of circRNAs. Regulation transcription: CircRNAs regulate the transcription of their parent coding genes. Protein translation: CircRNAs have a coding potential and can be translated into proteins with ribosomes. Interaction with RBPs: CircRNAs can bind to RBPs to regulate mRNA expression by altering the splicing pattern or mRNA stability. Acting as miRNA sponges: CircRNAs contain a common miRNA response element that can bind to miRNA and prevent them from interacting with mRNA. (C) The degradation of circRNAs. UPF1 and G3BP1 can bind to imperfect base-paired regions of circRNAs and induce their degradation. Upon viral infection, RNase L activated by 2′-5′-oligoadenosine(2′-5′A) causes complete degradation of circRNAs, thereby relieving PKR suppression. m^6A -containing circRNAs can be recognized by YTHDF2, which interacts with the RNase P/motility-related protein (MRP) complex bridged by HRSP12, and then the complex endoribonucleolytically cleaves circRNAs

(CiRNAs), exonic-intronic-circRNAs (EIcirRNAs), and intergenic circRNAs (IGcircRNA).[56] EcircRNAs, which include one or several exons, account for approximately 80% of circRNAs and mainly present in the cytoplasm.[57] CiRNAs are derived from introns and are predominantly found in the nucleus. EIcirRNAs are circularized by retaining introns between exons and are mostly located in the nucleus (Figure 2A).[58]

In addition, differential expression of circRNAs has been found in various diseases.[59] Therefore, circRNAs may contribute to the pathogenesis of many diseases. In terms of molecular mechanisms, circRNAs are reported to serve as miRNA sponges, RBP sponges, key mediators at the transcriptional and post-transcriptional levels, parental gene expression regulators, and can translate amino acids into peptides and proteins (Figure 2B).[35-39,41-44]

Recently, the mechanisms of circRNA degradation have been studied (Figure 2C).[60,61] One of the possibilities is that m^{6}A-modified circRNAs could be recognized by heat responsive protein 12 (HRSP12), which could interact with RNase P/MRP endonuclease complex to trigger circRNA degradation.[60] In addition, miR-1270 is believed to participate into the degradation of small interfering RNA-mediated circRNAs, as miR-1270 is highly complementary to the conserved binding site on *CDRlas* and consequently induces argonaute2 (Ago2)-mediated degradation. In the same study, RNase L was also proved to completely degrade circRNAs.[62] In support of this observation, another study identified that genome-wide circRNAs were degraded due to the activation of endonuclease RNase L upon poly (I:C) treatment or viral infection.[61] Apart from intracellular degradation, circRNAs can also be excreted from cells.[63] The degradation of circRNAs remains insufficiently understood at present and future research on this aspect is warranted.

4 | THE FORMATION MECHANISMS OF CIRCRNAS

The mechanisms of circularization are extremely complex. Some factors, such as RNA-binding proteins (RBPs), are required to participate in its regulation. A competitive balance exists between the linear splicing mode and the circular splicing mode.[55] The generally best known circularization mechanisms of circRNAs are as follows:

4.1 | Intron pairing-driven circularization

In 2014, Lasda et al. reported that cyclization is driven by complementary intron sequences.[64] CircRNAs are generated through the pairing of complementarity intron motifs, such as ALU, in the transcript.[50] The intron motifs on both sides of the circularization region are reverse complementarity, causing the two exons to form the loop of EcircRNAs after removing or retaining certain introns (Figure 2A).[50]

4.2 | Lariat-driven circularization

In 2013, Jeck et al. reported a lariat-driven circularization model.[50] According to this model, some RNAs that are partially folded close to the exons could bind the upstream splicing acceptor to the downstream splicing donor via a 3′, 5′-phosphodiester bond to form a lariat structure, secondary splicing or intron splicing to form a loop, and then cut introns to form an exon-containing lariat (Figure 2A), leading to generation of EcircRNAs. This circularization mechanism is also called the exon-skipping splicing model.[65] Furthermore, intron lariats with a 7-nucleotide guanine uracil-rich element at the 5′ splice site and cytosine rich element at the branching point can generate CiRNAs without being affected by debranching enzymes.[66]

4.3 | RBP/trans-factor-driven circularization

In 2014, Ashwal-Fluss et al. discovered that circularization was driven by RBPs.[58] Circularization is driven when RBPs and dimerized trans-factors bind to specific motifs present in flanking intron (Figure 2A). For example, muscle-blind proteins, fused in sarcoma (FUS), and quaking proteins are typical RBPs that bind to specific binding sites of pre-mRNA to regulate the circularization that could generate EcircRNAs and EIcircRNAs.[58,67] Meanwhile, the RNA-editing modifier AdAR1 inhibits circRNA formation by interfering with RNA matching, suggesting that circRNAs formation is negatively correlated with AdAR1 expression.[68]

4.4 | Variable circularization

In 2014, Zhang et al. proposed the variable circularization model.[39] CircRNAs formation also depends on cis-regulatory elements. Competitive pairing of different CIS-intron complementary pairing sequences can generate multiple circRNAs at a gene locus.[43] Other unknown mechanisms of circularization still need to be explored in the future studies.

5 | THE FUNCTIONS OF CIRCRNAS IN PH

Using RNA seq, it has been demonstrated that circRNA levels generally increase or decrease in different types of PH.[69,70] Although the limited number of PH-related circRNAs studies to date have only focused on their functions as biomarkers and miRNA sponges, the data clearly indicate that circRNAs had a critical effect on the pathogenesis of PH, which are summarized in Figure 3.[46,71]

5.1 | CircRNAs as potential biomarkers

The levels of multiple circRNAs were altered in serum, peripheral blood mononuclear cells (PBMCs) and lung tissues of patients with

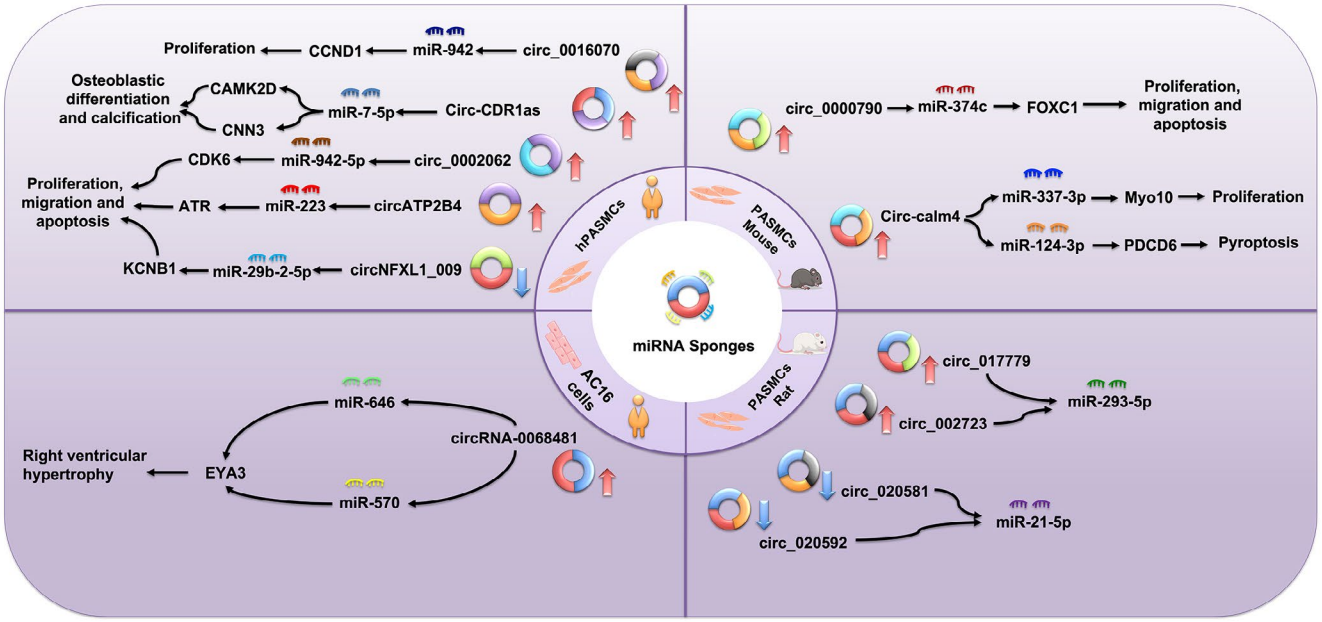

FIGURE 3 Summary of the studies of circRNAs in PH

PH, indicating that circRNAs may be potential prognostic biomarkers.[9,46] Zhang et al. found that serum circ_0068481 levels were significantly increased in patients with IPAH.[46] The patients with high levels of circ_0068481 had poorer outcomes, indicating that serum circ_0068481 may be a new noninvasive biomarker for prognosis in IPAH. Our previous study demonstrated that patients with IPAH displayed a lower level of circGSAP in PBMCs,[9] which was associated with high IPAH risk and poor prognosis.[9] Thus, lower circGSAP levels in PBMCs may be an emerging biomarker for the diagnosis and prognosis of IPAH. However, it is not clear whether the changes in circ_0068481 and circGSAP are the pathogenic factors of PH.

Of course, these single center clinical studies with small sample sizes need to be further verified by multicenter studies of PH with a larger sample size. In the future, multiple circRNAs may be combined with clinical indicators to accurately predict the occurrence and prognosis of PH, which may lead to more detailed molecular phenotyping of PH. Other functions of circRNAs in PH will be discussed in the following sections.

5.2 | CircRNAs as miRNA sponges

CircRNAs, as endogenous RNAs, can absorb miRNAs to regulate the function of mRNA.[9,72] In different types of PH, studies of multiple circRNAs as miRNA sponges in PASMCs have been described (Figure 3).

Pulmonary vascular intima injury is regarded as the primary factor causing pulmonary artery contraction and characteristic pathological changes of PH.[73] Xu et al. reported that circ_002723, circ_008021, circ_016925, and circ_020581 may be the potential ceRNAs regulating the *PDCD4*/caspase-3 axis of PAECs through sponge-mediated miR-23a or miR-21 in hypoxic PH (HPH) rat models.[23] In line with the possible role of circRNAs in PAEC functions, our previous study also showed that the circGSAP levels were downregulated in cultured human PAECs in response to hypoxia.[9] Therefore, some circRNAs may induce dysfunction of PAECs in the development of PH, which causes imbalance in the synthesis and release of vasoactive substances, loss of intimal stability, imbalance in PAEC apoptosis and proliferation, abnormal coagulation, and abnormal secretion of cytokines and growth factors. These alterations ultimately lead to vasoconstriction, lumen stenosis, obstruction, and hypertrophy of media layer and adventitia of pulmonary vessels, and are responsible for the occurrence and development of PH.

At present, the research on circRNAs for PASMCs mainly focuses on hypoxic induced animals and cell models.[74] Hsa_circ_0016070 was found to promote pulmonary vascular remodeling through the miR-942-*CCND1* axis to regulate the viability and G1/G0 phase of PASMCs in PH associated with chronic obstructive pulmonary disease (COPD) (Figure 3).[75] Decreased levels of hsa_circNFXL1_009 affect the expression and activity of K^+ channels by targeting the hsa-miR-29b-2-5p-*KCNB1* axis in human PASMCs (Figure 3).[76] In the study by Zhang et al., circ_Calm4 expression was upregulated in HPH mice and hypoxia-cultured PASMCs, and the circ_calm4/miR-337-3p/*Myo10* pathway regulated the proliferation of hypoxia-cultured PASMCs (Figure 3).[71] Their subsequent study indicated that circ_Calm4 also acted as a ceRNA, regulating the expression of miR-124-3p/*PDCD6* to modulate the pyroptosis of hypoxia-cultured PASMCs (Figure 3).[69] Moreover, Yang et al. reported that mmu_circ_0000790 competitively bound to miR-374c, thus upregulating *FOXC1* expression and activating notch pathway in the pulmonary vascular tissues of HPH mice and hypoxia-cultured PASMCs (Figure 3).[77] Guo et al. reported that circATP2B4/miR-223/*ATR* axis regulated the proliferation, migration, and apoptosis of PASMCs induced by

hypoxia.[24] Upregulation of mmu_circRNA_004592 and downregulation of mmu_circRNA_018351 could inhibit target miRNA levels and promote the proliferation and suppress aging and apoptosis of PASMCs.[78] Ma et al. observed that increased levels of circ_*CDR1*as promoted PASMC calcification by sponging miR-7-5p to increase the expression of *CAMK2D* and *CNN3* under hypoxia (Figure 3).[72] All told, these studies indicated that some specific circRNAs affect pulmonary vessel remodeling by regulating the phenotypic changes and functions of PASMC under hypoxia, which is another potential mechanism by which circRNAs are involved in PH vascular injury.

Pulmonary thromboembolic obstruction is also one of the vascular injury manifestations in some PH patients, such as patients with CTEPH.[79] A previous study identified the abnormal expressions of hsa_circ_0022342 and hsa_circ_0002062 in the pathogenesis of CTEPH,[45] and indicated that the hsa_circ_0002062-miR-942-5p-*CDK6* pathway and hsa_circ_0022342-miR-940-*CRKL-ErbB* pathway may participate in the occurrence and development of CTEPH.[45] Miao et al. revealed a critical role for cir_0022342-miR-503-5p-*SLC2A3* axis in eosinophil regulation and for circ_0002062-miR-92b-3p/miR-92a-3p-*MAN2A1* axis in regulatory T cell regulation in CTEPH,[80] linking epigenetic reprogramming with dysregulated immunity in CTEPH. These results suggest that circRNAs can also regulate the function of inflammatory cells and participate in the vascular injury process of CTEPH, which might open a new avenue for the treatment of CTEPH.

Beside pulmonary arterial remodeling or obstruction, right ventricular hypertrophy (RVH) is a common pathological feature of PH, which ultimately cause right-side heart failure.[81] In fact, right ventricular function is the most critical factor in determining the prognosis of PH patients. Guo et al. found that circRNA_0068481 can be used as a sponge for miRNAs such as miR-646, miR-570, and miR-885 in cardiomyocytes (Figure 3).[47] These miRNAs target eye absent transcription coactivator and phosphatase 3 (EYA3) mRNA and participate in RVH development in patients with PH.[47] The data indicated that circRNAs may also affect the function of cardiomyocytes involved in RVH in PH.

5.3 | CircRNAs as RBP sponges

CircRNAs acting as protein bait participate in pathophysiological processes such as stress responses.[82] For example, circFoxo3 is increased in the heart of aged patients and mice. CircFoxo3 is mainly distributed in cytoplasm and can bind to the anti-aging protein-inhibitor of differentiation/DNA binding 1 (ID-1), transcription factor-E2F transcription factor 1, anti-stress protein-focal adhesion kinase, and hypoxia inducible factor-1α (HIF-1α). In addition, circFoxo3 is highly expressed in non-cancer cells and is related to the cell cycle.[83] By combining with cell division protein kinase 2 (CDK2) and P21 protein, circFoxo3 forms an RNA-protein complex that inhibits CDK2 function. CDK2 interacts with cyclin A and cyclin E to facilitate cell cycle entry in normal conditions. Disruption of the CDK2–cyclin A–cyclin E interaction thus arrests cell cycle progression.[84] A study by Zeng et al. showed that circ-Amotl1 was upregulated in a neonatal heart, possibly enhancing myocardial function.[82] By binding AKT1 and PDK1, circ-Amotl1 induced AKT1 phosphorylation and pAKT nuclear transport, promoted cell survival and proliferation, and played a protective role in doxorubicin-induced cardiomyopathy, implying that circ-Amotl1 could be a potential therapeutic agent for myocardial repair.[82] Thus, the function of circRNAs as RBP sponges should also be a key aspect of future studies in PH.

5.4 | CircRNAs as peptide or protein encoders

The role of circRNAs as protein encoders was initially discovered in hepatitis D virus.[85] Recently, circRNAs have been reported to encode proteins in eukaryotic cells. CircZNF609 contains start codons and stop codons and can produce proteins through ribosomal translation in human muscle cells.[86] Using a ribosome-imprinting technology, Pamudurti et al. discovered that a group of circRNAs is associated with ribosomes, and could encode proteins.[87] According to Gao et al., circFBXW7 can be translated into protein FBXW7-185aa, which coordinately regulates *c-Myc* expression, thereby inhibiting the proliferation of mammary epithelial cell lines.[88] In addition, m^6A-modified circRNAs can promote the initiation of circRNA translation.[44] The introduction of an IRES into the circRNA sequence can initiate protein translation independently of the 5′ cap structure. CircRNAs have translation functions even without IRES and the specific sequences required for translation ('cap' structure and poly A 'tail').[89] Yang et al. found a circNlgn produced by a neuroglobulin gene was upregulated in myocardial tissues of patients with congenital heart diseases. CircNlgn was found to translate into Nlgn173 and other peptides in the heart. Nlgn173 was functionally independent of full-length Nlgn distributed in the brain. Peptides derived from circNlgn included a unique 9-amino acid motif fostering the nuclear localization of Nlgn173. Furthermore, Nlgn173 bound the promotors of ING4 and SGK3, and eventually led to cardiac fibroblast over proliferation, reduced cardiomyocyte viability, abnormal collagen deposition, and induced cardiac remodeling.[90] However, the remodeling associated proteins or peptides encoded by circRNAs require further functional studies in PH.

5.5 | CircRNAs as transcription regulators

CircRNAs can regulate maternal gene transcription during the processes of the transcription or post-transcription.[91] A subtype of circRNAs mainly located in the nucleus of HeLa cells and H9 cells binds to the small nucleoprotein particle U1 to regulate the activity of RNA polymerase II and increase the transcription level of its parent gene.[65] CircRNAs such as circ-ankrd52 and circEIF3J can also regulate the transcription of maternal genes. CircEIF3J regulates its transcription efficiency by specifically binding to RNAp01 II transcribed from its parent gene *ANKRD52*. It exists in the nucleus and interacts with the promoters of small nuclear ribonucleoprotein u1

and EIF3J in the promoter region to improve the transcription efficiency of EIF3J.[92]

CircRNAs can also bind to the host gene at synthesis sites by forming Rnadna hybrids (R-loop structure) to cause transcription pause or termination, or upregulate exon-skipped or -truncated transcripts.[93] CircSEP3, which is derived from exon 6 of *SEPALLATA3*, binds to the host DNA site to form Rnadna hybridization to increase homologous exon 6 skip mutations, leading to transcription pause and splicing factor recruitment.[93] In addition, circSMARCA5 causes termination of *SMARCA5* exon 15 transcription by forming R-loops; it also upregulates truncated nonfunctional subtypes.[94] Moreover, circRNAs regulate the translation of their linear counterpart by binding to RBP.[87] Among these circRNAs, circ_PABPN1 is one of the most significant HuR enrichment targets and competitively inhibits *PABPN1* translation by inhibiting the binding of HuR and *PABPN1* mRNA in HeLa cells.[95] Hu et al. found that HuR knockout in cardiomyocytes enhanced isoproterenol-induced cardiac remodeling.[96] Werfel et al. observed that the dozens of cardiac circRNAs arising from *TTN* gene regulation were different between neonatal and adult rats, meaning that cardiac circRNA generation could be involved in the *TTN* splicing.[97] Therefore, an in-depth study of the transcriptional function of circRNAs in cardiomyocytes might provide important in understanding RVH in PH.

5.6 | Other functions

A recent study on the transcriptome-wide map of m^6A circRNAs reported that m^6A-mediated circ_Xpo6 and circ_Tmtc3 were downregulated in the lung tissues of HPH rats.[70] Under hypoxia, the reduction of m^6A in circ_Xpo6 and circ_Tmtc3 may be caused by HIF-dependent and ALKBH5-mediated m^6A demethylation of NANOG mRNA.[98] Whether circRNAs are involved in regulating the pathogenesis of patients with PH through modification of their methylation needs to be further confirmed and studied.

6 | CONCLUSION

The human genome has abundant noncoding sequences. Some noncoding sequences are transcribed by circRNAs to regulate various biological functions. The key roles of circRNAs have attracted the attention of researchers worldwide, and progress in understanding these roles has been achieved. However, the biological characterization of circRNAs in cardiovascular diseases is still insufficiently understood, especially in the pathogenesis of PH. Most circRNA-related PH studies focus on their functions as a biomarkers in blood samples or as miRNA sponges in PAECs and PASMCs of PH, but specific functions of these circRNAs are still unclear. Importantly, among the thousands of circRNAs identified to date, which circRNA might be better biomarkers for the occurrence and outcomes PH, and which are key drivers to promote pulmonary vascular intimal injury, media thickening, inflammatory infiltration of adventitia in PH? How does the crosstalk between circRNAs and signaling pathways influence the development of PH? Do circRNAs regulate the function of right ventricular cardiomyocytes? And of course, what are the mechanisms of circRNAs as RBP sponges, key mediators at the transcriptional and post-transcriptional levels, parental gene expression regulators, and in translation into peptides and proteins? Answers to these questions will further help us understand the roles of circRNAs in the pathogenesis of PH and enable us to find better therapeutic strategies for the treatment of PH via targeting circRNAs.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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