EDITORIAL

Circling In on Pulmonary Arterial Hypertension: Is It Time to Consider Circular RNA circ_0016070 as a Biomarker and Target for Therapy?

Laszlo Farkas, MD; Elena A. Goncharova, PhD

Pulmonary arterial hypertension (PAH) remains a deadly disease with a bleak prognosis because current therapies mainly target vasotonus, but not the extensive remodeling in the pulmonary arteries.1,2 One cause for this limitation is the incomplete understanding of the cellular mechanisms of pulmonary artery remodeling in PAH. All pulmonary artery mural cells are affected in PAH, as well as pulmonary artery smooth muscle cells (PASMCs) obtaining an aberrant phenotype that is characterized by apoptosis resistance, unchecked proliferation, and a metabolic shift toward glycolysis, similar to cancer cells.1,3 These PASMCs drive pulmonary artery remodeling by promoting the thickening of the tunica media of pulmonary arteries, and also by altered cell function, contributing to enhanced vasoconstriction.1,3 Although some of the molecular mechanisms have been defined, a comprehensive understanding of the molecular cause of the aberrant PASMC phenotype is still lacking.

With the acceleration of sequencing technology, a variety of noncoding RNAs have been identified. Among these, circular RNAs (circRNAs) were first detected about 50 years ago in plants.4,5 Since then, ongoing research has found various circRNAs in humans that have a regulatory role in human diseases, including pulmonary hypertension (PH).4,5 circRNAs derived their name from the closed circular structure that they obtain after head and tail splicing.4,5 Interestingly, circRNAs are much more abundant in eukaryote cells than linear RNA, in part because of their increased stability.4,5 circRNAs have several important functions that make them crucial for the transcriptional or posttranscriptional regulation of gene activity.4,5 First, circRNAs can act as microRNA (miRNA) sponges by absorbing miRNAs, which themselves promote degradation of specific messenger RNAs. Second, circRNAs can also act as sponges for RNA binding proteins, hence inhibiting the function of such proteins. Third, circRNAs can themselves encode peptides and proteins. Fourth, circRNAs regulate gene transcription. Several circRNAs have been implicated in the pathobiology of PH, and some circRNAs have even been discussed as potential biomarkers in PH.4 Hence, circRNAs are abundant and impact intracellular signaling and function through various pathways.

In this issue of the Journal of the American Heart Association (JAHA), Dr Huang and colleagues study a...
novel mechanism leading to a hyperproliferative and apoptosis-resistant PASMC phenotype. The authors found elevated levels of circRNA circ_0016070 in the serum of patients with PAH and hypoxia-exposed human PASMCs, and confirmed increased expression of circ_0016070 in the pulmonary arteries of the monocrotaline model of PH. In in vitro experiments, knockdown of circ_0016070 reduced proliferation and migration, and enhanced apoptosis in PASMC. In in vivo experiments, knockdown of circ_0016070 reduced pulmonary artery remodeling and PH in monocrotaline rats. The authors further identified that circ_0016070 reduces the availability of miR-340-5p, and that miR-340-5p is a negative regulator of TCF4 (transcription factor 4) expression, which forms a transcriptional complex with β-catenin activating TWIST1 (twist-related protein 1) expression. Hence, Huang et al demonstrate that circ_0016070 functions as a sponge for miR-340-5p in PASMCs, enhancing expression of TCF4, which then upregulates TWIST1 through a transcriptional complex with β-catenin.

The article by Huang et al makes important advances in our understanding of the role of circRNA circ_0016070 and provides a novel mechanism of its function in PASMC in the pathobiology of PH. These findings, however, raise several important questions, which remain to be answered about the pathobiology of circ_0016070 as well as the potential opportunity of developing circ_0016070 as a biomarker or molecular target for treatment of pulmonary vascular remodeling and overall PH.

Because PH is a serious condition with limited treatment options, early detection of PH improves both clinical outcomes and patient quality of life. Yet early diagnosis continues to be challenging because of, at least in part, a lack of reliable noninvasive biomarkers to identify patients in the early stages of the disease as well as individuals with increased risk of developing PH. Noninvasive options to monitor disease progression and responses to therapy are limited; hence, new diagnostic and prognostic biomarkers are urgently needed. Because of their high stability and resistance to endonucleases, circRNAs attract strong interest in the research community as potential biomarkers for the diagnosis and treatment of cancer and cardiovascular diseases, including PH. In patients with idiopathic PAH, increased serum levels of circ_0068481 indicate poor outcomes, and reduced levels of circular γ-secretase activating protein (circGSAP) in peripheral blood mononuclear cells are linked with a high risk of disease and poor prognosis. Elevated levels of circ_0016070 in the serum of patients with PH, as reported by Huang et al, suggest a potential use of circ_0016070 as a diagnostic biomarker. These observations, however, are limited in the current study by the single center cross-sectional study design, small sample size, and lack of evidence that circ_0016070 serum levels correlate with clinical outcomes. Confirmation and validation of circ_0016070 as a biomarker in PH would require confirmation by longitudinal multicenter studies involving large, well-characterized patient cohorts and unbiased molecular phenotyping. Such studies would also help to identify other circRNAs as diagnostic or prognostic biomarkers for different groups of PH.

Increased proliferation and resistance to apoptosis of pulmonary vascular cells in small pulmonary arteries (PAs) are key pathological components of pulmonary vascular remodeling and overall PH. The study by Huang et al reports that upregulation of circ_0016070 in PASMCs is induced by chronic hypoxia, a key pathophysiological factor in PH associated with hypoxia and lung diseases (defined as group III PH by the World Health Organization). Interestingly, recently published studies by Zhou et al demonstrate that circ_0016070 is significantly increased in lung tissues of patients with chronic obstructive pulmonary disease-associated PH compared with the chronic obstructive pulmonary disease group, supporting the potential role of circ_0016070 in group III PH.

Intriguingly, studies from the Huang and Zhou groups report different mechanisms by which circ_0016070 promotes proliferation of PASMCs in PH: miR-340-5p/TCF4/β-catenin/TWIST1 and miR-942/cyclin D1 (CCND1). Although one of the functional mechanisms of circRNAs is to act as miRNA sponges, accumulating evidence suggests that some circRNAs could directly bind with and inhibit more than one miRNA. This target promiscuity raises the possibility that PASMC circ_0016070 could act as a sponge for both miR-340-5p and miR-942, simultaneously inducing TCF4/β-catenin/TWIST1 and CCND1, and thereby synergistically promoting PASMC proliferation. Further underscoring the complexity of the circRNA-miRNA network, miRNAs can also have multiple downstream targets. For example, antiproliferative miR-340-5p inhibits PASMC proliferation and acute pulmonary embolism–associated PH via downregulating interleukin 1β and interleukin 6, and regulates the mitochondrial fission factor (MFF)-Sirtuin (SIRT) 1/3 axis to improve the mitochondrial homeostasis and proliferation-apoptosis imbalance of hypoxia-exposed PASMCs. Nevertheless, further studies are needed to determine the mechanism of the circ_0016070 action toward miR-340-5p and miR-942, and to uncover a downstream signaling network involved in PASMC proliferation in PH.

One of the key regulators of hypoxic response and an important player in PH pathogenesis is a transcriptional factor, HIF-1α (hypoxia-inducible factor-1α). HIF-1α regulates cellular metabolism, proliferation, survival, and vascular tone in PH pulmonary vasculature via inducing or repressing expression of multiple genes. HIF-1α...
acts upstream and downstream of several miRNAs and cross-talks with key pro- and antiproliferative signaling pathways in PH pathogenesis, including receptor tyrosine kinases, the Akt-mechanistic target of rapamycin, p53, phosphatase and tensin homolog deleted on chromosome ten (PTEN), and HIPPO. Interestingly, TWIST1, identified by Huang et al as a downstream effector of circ_0016070, is mechanistically linked with mechanistic target of rapamycin, p53, and Akt signaling, serves as a direct target of HIF-1α in cancer cells, and plays a role in hypoxia-induced PH. In aggregate, these studies strongly suggest the existence of a mechanistic link between HIF-1α and circ_0016070 in regulating hypoxia-induced PASMC proliferation, vascular smooth muscle remodeling, and PH.

In addition to PASMCs, pulmonary vascular remodeling in PH is caused by the hyperproliferation and apoptosis resistance of other resident pulmonary vascular cells, including PA endothelial cells and PA adventitial fibroblasts, which are regulated, at least in part, by cell-cell and cell-extracellular matrix communications. The circRNAs are expressed in a cell- and tissue-specific manner, but some circRNAs are present in more than one cell type. Although molecular mechanisms driving hyperproliferation in PH could differ among different cell types, it is shown that TWIST1 promotes endothelial-to-mesenchymal transition and proliferation of PA endothelial cells in PH, suggesting that some molecular players are shared. Thus, the role of circ_0016070 in PH PA endothelial cells and PA adventitial fibroblasts remains to be determined, and the effect of PASMC-specific circ_0016070 upregulation on microenvironmental cues and behavior of other resident PA cells needs to be evaluated. A summary of potential effects of circ_0016070 in resident PA cells is provided in the Figure. In addition to the pulmonary vasculature, the role of circ_0016070 should be elucidated in the right ventricle in depth. Recent evidence shows that miR-340-5p worsens diabetes-induced cardiac dysfunction, and detailed preclinical analysis of the role of circ_0016070 and the consequences of its depletion for right ventricular structure and function in PH should also be performed.

PH affects both men and women, and a patient’s sex is an important factor influencing disease severity, response to treatment, and overall survival. The experimental models of PH also demonstrate reproducible differences between male and female animals in PH onset and PH severity, as well as in response to treatment. Given the promising effects of si-circ_0016070 in reducing experimental PH in male rats, further experiments should be performed to test a similar treatment in female animals and identify potential sex-related differences in circ_0016070 pathobiology.

In conclusion, the article from Huang et al provides novel information about the role and mechanism of the function of circ_0016070 in PH and PASMC biology and opens several exciting avenues for further investigation. Although circ_0016070 could be considered as a potential molecular target to reduce PASMC proliferation, apoptosis resistance, and overall PH, further studies will be needed to clarify the circ_0016070 molecular network in PASMCs and other pulmonary vascular cells, evaluate potential sex-specific differences.
in circ_0016070 pathobiology, and test whether circ_0016070 could be used as a diagnostic or prognostic biomarker for patients with PH. It should also be noted that currently used strategies to target circRNAs are challenging because of potential off-target gene silencing, nonspecific targeting, and immune system response, making the potential transition of circRNA-directed treatments from preclinical studies to clinical challenging. Nevertheless, in synopsis with other studies that investigate the regulatory RNA mechanism in the context of PAH, the results by Huang et al suggest circ_0016070 to be a promising candidate as a novel potential biomarker and a target for therapy in pulmonary vascular cells.

ARTICLE INFORMATION

Affiliations
Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH (L.P.F.); and Division of Pulmonary, Critical Care and Sleep Medicine, Lung Center, University of California, Davis School of Medicine, Davis, CA (E.A.G.).

Disclosures
None.

REFERENCES

1. Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger J, Nicolls M, Olschewski A, Pullamsetti S, Schermuly R, Stemmermann N, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. Eur Respir J. 2019;53:1901887. doi: 10.1183/13993003.01887-2018
2. Hassoun PM. Pulmonary arterial hypertension. N Engl J Med. 2021;385:2361–2376. doi: 10.1056/NEJMr2000348
3. Tajic T, Morrell NW. Smooth muscle cell hypertrophy, proliferation, migration and apoptosis in pulmonary hypertension. Comp Physiol. 2011;1:295–317. doi: 10.1002/cphy.c100026
4. Wang Q, Sun Y, Zhao Q, Wu W, Wang L, Miao Y, Yuan P. Circular RNAs in pulmonary hypertension: emerging biological concepts and potential mechanism. Animal Model Exp Med. 2022;5:38–47. doi: 10.1002/ame2.12208
5. Lasda E, Parker R. Circular RNAs: diversity of form and function. RNA. 2014;20:1829–1842. doi: 10.1261/rna.047126.114
6. Huang C-X, Jiang Z-X, Du D-Y, Zhang Z-M, Liu Y, Li Y-T. Hsa_circ_0016070/miR-340-5p axis accelerates pulmonary arterial hypertension progression by upregulating TWIST1 transcription via TGFβ/β-catenin complex. J Am Heart Assoc. 2022;11:e024147. doi: 10.1161/JAHA.121.024147
7. Arnaiz E, Sole C, Manterola L, Iparraguirre L, Otaegui D, Lawrie CH. CircRNAs and cancer: biomarkers and master regulators. Semin Cancer Biol. 2019;58:90–99. doi: 10.1016/j.semcancer.2018.12.002
8. Yu Z, Huang Q, Zhang Q, Wu H, Zhong Z. CircRNAs open a new era in the study of cardiovascular disease (review). Int J Mol Med. 2021;47:49–64. doi: 10.3892/ijmm.2020.4792
9. Veith C, Schermuly RT, Brandes RP, Weissmann N. Molecular mechanisms of hypoxia-inducible factor-induced pulmonary arterial smooth muscle cell alterations in pulmonary hypertension. J Physiol. 2016;594:1167–1177. doi: 10.1113/jp270689
10. 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 PASMC proliferation. Mol Ther Nucleic Acids. 2019;18:275–284. doi: 10.1016/j.omtn.2019.08.026
11. Ou M, Zhang C, Chen J, Zhao S, Cui S, Tu J. Overexpression of microRNA-340-5p inhibits pulmonary arterial hypertension induced by APE by downregulating IL-1β and IL-6. Mol Ther Nucleic Acids. 2020;21:542–554. doi: 10.1016/j.omtn.2020.05.022
12. Huang CX, Jiang Z-X, Du DY, Zhang Z-M, Liu Y, Li Y-T. The MFF-SIRT1/3 axis, regulated by miR-340-5p, restores mitochondrial homeostasis of hypoxia-induced pulmonary artery smooth muscle cells. Lab Invest. 2022;102:515–523. doi: 10.1038/s41374-022-00730-w
13. Pullamsetti SS, Mamazhakypov A, Weissmann N, Seeger W, Savai R. Hypoxia-inducible factor signaling in pulmonary hypertension. J Clin Invest. 2020;130:5638–5651. doi: 10.1172/jci137558
14. Zhao Z, Rahman MA, Chen ZG, Shin DM. Multiple biological functions of Twist1 in various cancers. Oncotarget. 2017;8:20380–20393. doi: 10.18632/oncotarget.14608
15. Mammoto T, Muylear M, Konduri GG, Mammoto A. Twist1 in hypoxia-induced pulmonary hypertension through transforming growth factor-β-Smad signaling. Am J Respir Cell Mol Biol. 2018;58:194–207. doi: 10.1165/rcmb.2016-0323OC
16. Zhu Y, Yang X, Zhou J, Chen L, Zuo P, Chen L, Jiang L, Li T, Wang D, Xu Y, et al. miR-340-5p mediates cardiomyocyte oxidative stress in diabetes-induced cardiac dysfunction by targeting Mcl-1. Oxid Med Cell Longev. 2022;2022:3182931. doi: 10.1155/2022/3182931