EUROPEAN RESPIRATORY JOURNAL
ORIGINAL RESEARCH ARTICLE
M. DU ET AL.

Single-cell RNA sequencing reveals that *BMPR2* mutation regulates right ventricular function *via ID* genes

Mingxia Du[1,2], Haibin Jiang[1,2], Hongxian Liu[1,2], Xin Zhao[1,2], Yu Zhou[3], Fang Zhou[2], Chunmei Piao[4], Guoqiang Xu[5], Feng Ma[6], Jianan Wang[7], Frederic Perros[8], Nicholas W. Morrell[9], Hong Gu[4] and Jun Yang[1]

[1]Dept of Physiology, and Dept of Cardiology of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. [2]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing, China. [3]Dept of General Intensive Care Unit, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China. [4]Dept of Pediatric Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China. [5]Jiangsu Key Laboratory of Neuropsychiatric Diseases and College of Pharmaceutical Sciences, Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, Soochow University, Suzhou, China. [6]Institute of Blood Transfusion, Chinese Academy of Medical Sciences, Chengdu, China. [7]Dept of Cardiology of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. [8]Université Paris–Saclay, AP-HP, INSERM UMR_S 999, Service de Pneumologie et Soins Intensifs Respiratoires, Hôpital de Bicêtre, Le Kremlin Bicêtre, France. [9]Dept of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge, UK.

Corresponding author: Jun Yang (yang_jun@zju.edu.cn)

Shareable abstract (@ERSpublications)
This study reports for the first time that inhibitor of DNA-binding protein knockout mice developed pulmonary arterial hypertension and that the *USP9X* gene was a downstream effector of ID during heart development in CHD-PAH patients with *BMPR2* mutations. https://bit.ly/3ciUNim

Cite this article as: Du M, Jiang H, Liu H, *et al*. Single-cell RNA sequencing reveals that *BMPR2* mutation regulates right ventricular function *via ID* genes. *Eur Respir J* 2022; 60: 2100327 [DOI: 10.1183/13993003.00327-2021].

Received: 2 Feb 2021
Accepted: 10 Nov 2021

Abstract

Background Mutations in bone morphogenetic protein type II receptor (*BMPR2*) have been found in patients with congenital heart disease-associated pulmonary arterial hypertension (CHD-PAH). Our study aimed to clarify whether deficient BMPR2 signalling acts through downstream effectors, inhibitors of DNA-binding proteins (IDs) during heart development to contribute to the progress of PAH in CHD patients.

Methods To confirm that IDs are downstream effectors of BMPR2 signalling in cardiac mesoderm progenitors (CMPs) and contribute to PAH, we generated cardiomyocyte-specific *Id 1/3* knockout mice (Ids cDKO), and 12 out of 25 developed mild PAH with altered haemodynamic indices and pulmonary vascular remodelling. Moreover, we generated *ID1* and *ID3* double-knockout (IDs KO) human embryonic stem cells that recapitulated the BMPR2 signalling deficiency of CHD-PAH induced pluripotent stem cells (iPSCs).

Results Cardiomyocytes differentiated from iPSCs derived from CHD-PAH patients with BMP receptor mutations exhibited dysfunctional cardiac differentiation and reduced calcium (Ca^{2+}) transients, as evidenced by confocal microscopy experiments. Smad1/5 phosphorylation and ID1 and ID3 expression were reduced in CHD-PAH iPSCs and in *Bmpr2*$^{+/-}$ rat right ventricles. Moreover, ultrasound revealed that 33% of Ids cDKO mice had detectable defects in their ventricular septum and pulmonary regurgitation. Cardiomyocytes isolated from mouse right ventricles also showed reduced Ca^{2+} transients and shortened sarcomeres. Single-cell RNA sequencing analysis revealed impaired differentiation of CMPs and downregulated USP9X expression in IDs KO cells compared with wild-type cells.

Conclusion We found that BMPR2 signals through IDs and USP9X to regulate cardiac differentiation, and the loss of ID1 and ID3 expression contributes to cardiomyocyte dysfunction in CHD-PAH patients with *BMPR2* mutations.