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Therapeutic Engagement of the Histone Deacetylase IIA–Myocyte Enhancer Factor 2 Axis Improves Experimental Pulmonary Hypertension

To the Editor:

Pulmonary arterial hypertension (PAH) is a fatal disease of the pulmonary vasculature, characterized by abnormal remodeling

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of pulmonary vessels and elevated pulmonary arterial pressure, ultimately leading to right ventricular (RV) failure and death. MEF2 (myocyte enhancer factor 2) is a transcription factor that regulates multiple genes that are important during cardiovascular development and for vascular homeostasis (1), with MEF2C known to play a crucial role in regulating endothelial integrity, survival, and angiogenesis (2). A wealth of evidence supports regulation of MEF2 activity by the class IIA subset of HDACs (histone deacetylases), which directly bind to and inhibit its transcriptional activity (3). Our previous study demonstrated the involvement of the HDAC IIA–MEF2 axis in PAH, where increased HDAC IIA activity in pulmonary artery endothelial cells (PAECs) from subjects with PAH was strongly associated with impaired MEF2 function (4). Here, we used experimental rodent pulmonary hypertension (PH) models to determine whether genetic or pharmacologic targeting of the HDAC IIA–MEF2 axis can affect PH pathogenesis. Some of the results of these studies have been previously reported in the form of an abstract (5).

Methods

To investigate the role of endothelial MEF2 function in the pathogenesis of PH, we generated adult mice with inducible, endothelial-specific deletion of the *Mef2c* allele, using a tamoxifen-responsive *Cdh5* promoter-driven Cre recombinase crossed to mice with *Mef2c*^{fl/fl} alleles (henceforth called *Mef2c*^{ECKO} mice) (6, 7). Invasive hemodynamic and morphometric parameters were assessed at baseline and after exposure to 3 weeks of chronic hypoxia (10% F_IO₂). For the SU-5416/hypoxia rat models, male Sprague-Dawley rats (200–250 g) were injected subcutaneously with SU-5416 (Sigma; 20 mg/kg body weight). For drug administration, rats were given injections of either vehicle or TMP269 (50 mg/kg of body weight intraperitoneally; DC Chemical), or Tasquinimod (10 mg/kg body weight by oral gavage; DC Chemical) daily.

Results and Discussion

Immunofluorescent staining confirmed the endothelial-specific deletion of *Mef2c* in the lungs after injection of tamoxifen (Figure 1A). These mice did not display any appreciable pulmonary vascular phenotype up to 6 weeks after *Mef2c* deletion, but on chronic hypoxia exposure, the *Mef2c*^{ECKO} mice developed significantly higher right ventricular systolic pressure (Figure 1B). We also observed increased right ventricle to left ventricle plus septum weight ratios in the *Mef2c*^{ECKO} mice, although there was variability in the extent of right ventricular hypertrophy (Figure 1B). Lung morphometric analyses showed significantly increased percentage of smooth muscle actin–positive muscularized pulmonary arterioles in *Mef2c*^{ECKO} mice compared with controls. Overall, these findings provide the first genetic evidence that endothelial MEF2C is crucial for maintaining pulmonary vascular homeostasis *in vivo*, and that its genetic deletion results in exacerbation of PH.

We sought to further investigate the efficacy of HDAC IIA inhibition in inducing MEF2 activation. Stimulation with TMP269 (8), a highly selective HDAC IIA inhibitor, led to significant increase in *KLF2* and *KLF4* expression and

Figure 1. Endothelial-specific deletion of *Mef2c* leads to worsening pulmonary hypertension. (A) Immunofluorescent staining for Mef2c (white) in the lungs of *Mef2c*^{ECKO} mice. White and red arrows designate endothelial cell nuclei. DAPI (blue) is also shown. Scale bar: 50 μ m. (B) (Left) Right ventricular systolic pressure (RVSP) measurements of control and *Mef2c*^{ECKO} mice after exposure to chronic hypoxia. *** $P < 0.001$. $n = 6$ –10 per group. (Middle) Right ventricle to left ventricle + septum [RV/(LV + S)] weight ratios for control and *Mef2c*^{ECKO} mice after exposure to chronic hypoxia. * $P < 0.05$. (Right) Quantification of muscularized pulmonary vessels in *Mef2c*^{ECKO} mice and controls. *** $P < 0.001$. Mef2c = myocyte enhancer factor 2c; Musc. Art. = muscularized pulmonary arterioles.

increased MEF2 luciferase reporter activity in two independent PAH PAEC lines (Figure 2A). Next, we found that treatment of PAECs from patients with PAH, which maintain a higher proliferation rate compared with control PAECs, displayed significantly decreased proliferation rate when treated with TMP269. TMP269 had a negligible effect on proliferation of control PAECs. To determine *in vivo* whether augmenting MEF2 activity via selective HDAC IIA inhibition can reverse PH pathogenesis, we tested TMP269, as well as a second HDAC IIA inhibitor that has been used in cancer clinical trials (Tasquinimod), in the SU-5416/hypoxia rat PH model (9, 10). Although use of male rats is a limitation, they develop more severe PH and were used for our studies. Rats were treated for 2 weeks with daily administration of either TMP269 or Tasquinimod after they were induced to develop PH (Figure 2B). We found a significant reduction in right

ventricular systolic pressure and RV hypertrophy in rats treated with TMP269 or Tasquinimod compared with vehicle controls. Morphometric analyses of the lungs demonstrated a significantly decreased number of muscularized arterioles in both the TMP269 and Tasquinimod groups. Endothelial transcriptional machinery is an emerging therapeutic target in PAH (11, 12). Overall, our findings show the importance of a transcriptional pathway mediated by HDAC IIA–MEF2 signaling in PH, via demonstration that endothelial-specific deletion of *Mef2c* exacerbates hypoxia-induced PH and two independent HDAC IIA-selective inhibitors, including one (Tasquinimod) that has been administered in a large phase 3 clinical trial, can rescue the severe SU-5416/hypoxia model of PH in rats. Although the focus of our current study has been the effect of HDAC IIA–MEF2 signaling on pulmonary vascular remodeling, future implications of this signaling axis

include the effect on the RV itself, especially given recent studies demonstrating that Mef2c expression is increased in compensated RV but lost in decompensated RV, further implicating the importance of MEF2 in preservation of RV function in PAH (13). Studies to selectively investigate the RV implications of the HDAC IIA–MEF2 axis, through use of models such as pulmonary artery banding, will be critical as we seek to better understand the RV–pulmonary artery coupling in PAH. ■

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Azithromycin for Early *Pseudomonas* Infection in Cystic Fibrosis: Do the Benefits Outweigh the Harms?

To the Editor:

We read with great interest the article by Mayer-Hamblett and colleagues regarding the efficacy of the addition of azithromycin to inhaled tobramycin when eradicating newly acquired *Pseudomonas aeruginosa* (Pa) in patients with cystic fibrosis (1). The authors are to be commended on conducting a trial with a high-quality design that addressed a clinically important question with a planned appropriately long follow-up period of 18 months. The fact that the trial was stopped early because of an increased time to first pulmonary exacerbation in the azithromycin-treated group would suggest that adding azithromycin to inhaled tobramycin has a role in eradicating newly acquired Pa. However, it is important that the benefits demonstrated in the trial outweigh the potential risks of long-term azithromycin use, such as antimicrobial resistance (especially in the era of nontuberculous *Mycobacterium* infections), and we are unsure they do.

The choice of time to pulmonary exacerbation as a primary endpoint is appropriate, given the evidence that pulmonary

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