Loss of family with sequence similarity 13, member A exacerbates pulmonary hypertension through accelerating endothelial-to-mesenchymal transition

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Abstract

Pulmonary hypertension is a progressive lung disease with poor prognosis due to the consequent right heart ventricular failure. Pulmonary artery remodeling and dysfunction are culprits for pathologically increased pulmonary arterial pressure, but their underlying molecular mechanisms remain to be elucidated. Previous genome-wide association studies revealed a significant correlation between the genetic locus of family with sequence similarity 13, member A (FAM13A) and various lung diseases such as chronic obstructive pulmonary disease and pulmonary fibrosis; however whether FAM13A is also involved in the pathogenesis of pulmonary hypertension remained unknown. Here, we identified a significant role of FAM13A in the development of pulmonary hypertension. FAM13A expression was reduced in mouse lungs of hypoxia-induced pulmonary hypertension model. We identified that FAM13A was expressed in lung vasculatures, especially in endothelial cells. Genetic loss of FAM13A exacerbated pulmonary hypertension in mice exposed to chronic hypoxia in association with deteriorated pulmonary artery remodeling. Mechanistically, FAM13A decelerated
endothelial-to-mesenchymal transition potentially by inhibiting β-catenin signaling in pulmonary artery endothelial cells. Our data revealed a protective role of FAM13A in the development of pulmonary hypertension, and therefore increasing and/or preserving FAM13A expression in pulmonary artery endothelial cells is an attractive therapeutic strategy for the treatment of pulmonary hypertension.

Introduction

Pulmonary hypertension is a progressive and fatal lung disease diagnosed by a sustained elevation of pulmonary arterial pressure more than 20 mmHg [1]. Pulmonary arterial hypertension including idiopathic pulmonary arterial hypertension and pulmonary hypertension related with collagen disease is characterized by pathological pulmonary artery remodeling such as intimal and medial thickening of muscular arteries, vaso-occlusive lesions, and fully muscularized small diameter vessels that are normally non-muscular peripheral vessels. These vascular remodeling is a result from endothelial cell dysfunction, smooth muscle cell and endothelial cell proliferation, and also cellular
transdifferentiation [2]. Although detailed molecular mechanisms remain to be
elucidated, many pathogenic pathways in pulmonary arterial hypertension have been
revealed. These include TGF-β signaling, inflammation, pericyte-mediated vascular
remodeling, iron homeostasis, and endothelial-to-mesenchymal transition (EndMT) [3].
Recent genome-wide association studies identified family with sequence similarity
13, member A (FAM13A) gene as a genetic locus associated with pulmonary function
[4], and it is known to be associated with lung diseases including chronic obstructive
pulmonary disease (COPD) [5], asthma [6] and pulmonary fibrosis [7–9]. Moreover,
causative role of FAM13A in the development of COPD has been revealed. FAM13A
interacts with protein phosphatase 2A and β-catenin, leading to the promotion of
GSK-3β-mediated phosphorylation and subsequent proteasomal degradation of
β-catenin in airway epithelial cells [10]. Interestingly, FAM13A was also expressed in
adipocytes, and modulates insulin signaling through regulating the proteasomal

β-catenin is crucially involved in the epithelial-mesenchymal transition that play an
important role in the pathogenesis of cancer [12] and pulmonary fibrosis. Also, there are
many reports describing the role of β-catenin in EndMT that is implicated in the vascular remodeling for pulmonary hypertension [13–15]. These findings urged us to investigate a potential role of \textit{FAM13A} in the pathogenesis of pulmonary hypertension, and we here identified a protective role of \textit{FAM13A} in the development of pulmonary hypertension.

71 Materials and Methods

72 Animal study

All animal experimental protocols were approved by Ethics Review Committee for Animal Experimentation of Kobe Pharmaceutical University. \textit{Fam13a} \textsuperscript{−/−} mice [\textit{Fam13a} tm1e(KOMP)Wtsi; C57BL6N background] in which LacZ cassette was knocked in at the \textit{Fam13a} gene locus were obtained from Knockout Mouse Project (KOMP) at UC Davis. Mice were maintained under standard conditions with free access to food and water. Mice at 6-7 weeks old were regularly used for experiments. For chronic hypoxia exposure, mice were put in the chamber with non-recirculating gas mixture of 10% O\textsubscript{2}
and 90% N\textsubscript{2} for 3-6 weeks.

**Hemodynamic measurements**

Mice were anesthetized with \textasciitilde2% isoflurane, and Right ventricular systolic pressure was measured by inserting 1.4 F Millar Mikro-Tip catheter transducer (Millar) into right ventricle through right jugular vein. Before the hemodynamic assessments, heart rate, fractional shortening, cardiac output, and pulmonary artery acceleration time were evaluated by echocardiography.

**Right ventricular hypertrophy assessment**

Formaldehyde-fixed dried hearts were dissected, and right ventricular wall were separated from left ventricle and septum. The Fluton’s index was presented in ratio of right ventricle to left ventricle + septum.

**Histological analysis**

Mouse lungs were inflated and fixed in 4% paraformaldehyde, followed by paraffin
Sections were cut into 3 μm and stained with hematoxylin and eosin (HE) as well as elastica van Gieson (EvG). Pulmonary artery wall thickness was assessed in HE-stained lung sections using imageJ by measuring 10 randomly selected vessels/mouse associated with alveolar duct or alveolar wall, with diameter less than 100 μm in x200 magnification. Quantitative data were presented as the wall area measurement (vessel area minus lumen area) normalized to the mean of vessel and lumen perimeters. Small pulmonary arteries number was evaluated in EvG-stained lung sections. Five fields were taken per mouse at x200 magnification and the number of distal arteries <50 μm in diameter per 100 alveoli were assessed.

To assess small pulmonary arteries muscularization, lung sections were incubated with Antigen Unmasking Solution (Citric-acid based) H-3300 (Vector Laboratories) at 90°C for 10 min, followed by incubation in PBS/0.2% Triton X-100 and subsequent blocking with 5% Skim-milk for 1 h. Sections were then incubated with antibodies for α-smooth muscle actin (1:300; Sigma) and von Willebrand factor (1:300; Abcam) at 4°C for overnight. Subsequently, section were incubated with secondary antibody labeled with Alexa Fluor 594 (1:300; Invitrogen), followed by mounting with Vectashield.
mounting medium with DAPI (Vector Laboratories). Fluorescent images were captured using fluorescence microscope (BZ-X800, Keyence). Small pulmonary artery with diameter less than 50 μm were quantified from 5e random fields at x400 magnification per mouse, and arteries with positive α-smooth muscle actin staining >75% of the circumference were classified as fully muscularized as previously described [16]. Data were presented as percentage of fully muscularized vessels normalized with total number of vessels per field.

For some immunostaining experiments, images were captured using laser confocal microscope (LSM700, Zeiss). For the assessment of colocalization, 15-20 randomly selected vessels (diameter size <50 μm) were analyzed for each group, and quantification was performed using Zen imaging software (Zeiss). Data were presented in Manders overlap coefficient as previously described [17]. To assess the nuclear accumulation of active β-catenin, >50 nuclei per group were analyzed by measuring the mean fluorescence intensity of active β-catenin using imageJ as previously described [12].
LacZ staining

Right lung was flushed with 0.2% glutaraldehyde in wash buffer (2 mM MgCl₂, 0.01% Deoxycholate, 0.02% NP-40), and incubated in wash buffer on ice for 40 min. Lung samples were then washed with wash buffer for 30 min 3 times, and then cut into pieces. Lung specimens were then incubated in X-gal staining solution (5 mM K₄Fe(CN)₆, 5 mM K₃Fe(CN)₆, 0.75 mg/ml X-gal, diluted in wash buffer) for overnight at 37°C on the rotator. After washing with 3% DMSO/PBS for 5 min twice, specimens were post-fixed with 4% PFA for overnight at 4°C. Subsequently, lung specimens were embedded with paraffin, and sections were prepared at 3 μm, followed by counterstaining with Nuclear Fast Red Solution.

Immunostaining for LacZ and endothelial cells was performed using frozen sections of the right lung by incubating with antibodies for β-galactosidase (1:1000; Abcam) and von Willebrand factor (1:250; Abcam), followed by incubation with secondary antibodies labeled with Alexa Fluor 594 (1:500; Abcam) and Alexa Fluor 488 (1:500; Invitrogen).
Quantitative Real Time-PCR

Left lung tissues were collected and homogenized in RNAiso plus (TAKARA), followed by purification with Nucleospin RNA clean-up (Macherey-Nagel). cDNA was synthesized using PrimeScript RT reagent Kit with gDNA Eraser (TAKARA). PCR reactions were prepared using FastStart SYBR Green Master (Roche Applied Science), followed by the real-time PCR analysis using LightCycler96 (Roche Applied Science). Nucleotide sequences of the primers are shown in S1 Table.

Immunoblotting

Cells were lysed in RIPA buffer, and protein concentration was measured using DC Protein Assay Kit (BioRad). Proteins of 20-30 µg were run on the SDS-PAGE gel, and the expression of target proteins were assessed by immunoblotting. Target protein expression levels were normalized to GAPDH expression levels. Primary antibodies used were as follows: FAM13A (Sigma, HPA038108), β-catenin (Cell Signaling Technology, D10A8), active β-catenin (Cell Signaling Technology, D13A1), GAPDH (Cell Signaling Technology, 14C10). All antibodies were used at 1:1000 dilution unless
otherwise mentioned.

Cell culture

Human pulmonary artery endothelial cell (PAEC) were obtained from Lonza, and cultured in Humedia-EG2 (Kurabo). To induce endothelial-to-mesenchymal transition, PAECs were treated with 10 ng/mL TGF-β1 (R&D System) and 10 ng/mL IL1β (R&D System) as previously described [18] for 6 days in the medium supplemented with 2% FBS. Medium was changed every other day during the experiments. For retrovirus infection, PAEC were grown to 70% confluency, and incubated with medium containing retrovirus carrying GFP or FAM13A gene in the presence of polybrene (8 µg/mL) for 24 h. Medium was then replaced with fresh growth medium, and cells were treated or used for experiments 48 h after initial infection.

Tube-formation assay

PAECs were plated in 96-well plate coated with 50 µl of Matrigel (Corning) with seeding density of 2x10^4 cells/well. Cells were incubated for 7 h, and images were
obtained every hour.

Cell proliferation assay

PAECs were plated in 96-well plate at the density of 0.1x10^4 cells/well. Medium was replaced after 24 h. Cell proliferation was assessed using WST-1 (Roche) 48 h after seeding.

Apoptosis assay

PAECs were plated in 96-well plate at the density of 1x10^4 cells/well, and incubated for overnight. Apoptosis was induced by serum and growth factor depletion for 24 h, followed by Hoechst nuclear staining.

Migration assay

Cell migration was analyzed using modified Boyden Chamber Assay. Cells (8x10^4 cells/insert) were seeded on the 8 µm-pore insert (Falcon) in migration buffer (serum free medium supplemented with 1% bovine serum albumin). Migration was induced by
3% FBS in the migration buffer added in the bottom chamber. After 4 hours of incubation, non-migrated cells on the top of insert membrane were removed, and the cells migrated onto the reverse face of the membrane were fixed in methanol, and stained with Hematoxylin.

Statistical analysis

All data are presented as mean ± SEM. Statistical analysis was performed using Graphpad Prism 8. The differences between groups were calculated using two-tailed Student’s t-test or one-way ANOVA, as indicated. P < 0.05 was considered statistically significant.

Results

FAM13A is expressed in lung vasculatures

To assess a possible involvement of FAM13A in the pathogenesis of pulmonary hypertension, we analyzed Fam13a expression in the lungs of pulmonary hypertension...
model mouse. Pulmonary hypertension was induced in mice by chronic exposure to 10% hypoxia. *Fam13a* expression was remarkably reduced in the lungs of wild-type (WT) mice exposed to chronic hypoxia comparing to that in the lungs of control mice (Figs 1A and B). It has been reported that *FAM13A* is expressed in airway cells including mucosal cells, Club cells, and alveolar cells [8,10]; however it remained unknown whether *FAM13A* is expressed in the lung vasculature as well. We have generated mice with target deletion of *FAM13A* (*Fam13a*<sup>−/−</sup>) in which LacZ cassette was inserted into the intron of the *Fam13a* gene locus. We could therefore detect cells that express *Fam13a* by using LacZ staining in the lungs. LacZ-staining was positive in airway epithelial cells as previously reported, and some of vascular cells were also positive for LacZ (Fig 1C). Immunohistochemistry using antibodies for von Willebrand factor and LacZ demonstrated that von Willebrand factor-positive endothelial cells express *Fam13a* in lung vasculatures (Fig 1D). These data suggest a potential role of *FAM13A* in the pathogenesis of pulmonary hypertension, especially in the pathological vascular remodeling.

Fig 1. *FAM13A* is expressed in lung vasculatures.
(A) Immunoblotting for *Fam13a* in the lungs isolated from WT mice exposed to either normoxia or hypoxia (10% O$_2$ for 6 weeks). (n = 5 each). (B) Quantitative real-time PCR of *Fam13a* in the lungs isolated from WT mice exposed to either normoxia or hypoxia (n=5 each). (C) Representative images of the lung sections stained with X-gal in WT and *Fam13a* −/− mice. LacZ-positive cells that are supposed to express *Fam13a* were detected in pulmonary artery (red arrow) as well as in non-vessel structure (black arrow). Bars: 50 μm. (D) Immunohistochemistry for LacZ and von Willebrand factor (vWF) in the lung. Bars: 50 μm. Data are presented as mean ± SEM. *P < 0.05 and **P < 0.01.

**Genetic loss of *FAM13A* exacerbates pulmonary hypertension**

We then explored a role of *FAM13A* in pulmonary hypertension using *Fam13a* −/− mice. Under normoxic condition, there was no significant difference in lung structures and pulmonary arterial pressure between WT and *Fam13a* −/− mice (Fig 2). When exposed to chronic hypoxia, *Fam13a* −/− mice showed deteriorated pulmonary
hypertension assessed by higher right ventricular systolic pressure and increased Fulton index (Figs 3A-C). Consistent with the exacerbated pulmonary hypertension, vascular remodeling such as increased fully muscularized small diameter vessels and loss of peripheral capillaries was worsened in the lungs of Fam13a<sup>−/−</sup> mice as compared to those in WT mice (Figs 3D-F). These data sufficiently indicate that FAM13A plays a protective role against pulmonary hypertension. 

**Fig 2. Genetic loss of FAM13A does not affect the lung structures and hemodynamics in mice.**

(A) H-E staining of the lung sections isolated from WT and Fam13a<sup>−/−</sup> mice. (B) Blood pressure in WT and Fam13a<sup>−/−</sup> mice (n = 5-6 each). (C) Hemodynamics assessed by echocardiography in WT and Fam13a<sup>−/−</sup> mice (n = 5 each).

**Figure 3. Genetic loss of FAM13A exacerbates pulmonary hypertension.**

(A) Pulse pressure diagram for right ventricles in WT and Fam13a<sup>−/−</sup> mice exposed to chronic hypoxia (10% O<sub>2</sub> for 3 weeks). (B) Right ventricular systolic pressure (RVSP) was measured in WT and Fam13a<sup>−/−</sup> mice exposed to either normoxia or hypoxia (n = 5 each for normoxia group, n=10 each for hypoxia group). (C) Ratio of right ventricle
compared to left ventricle + septum (Fulton’s Index) was calculated (n = 5 each for normoxia group, n=10 each for hypoxia group). (D) H-E staining of the lung sections in WT and Fam13a<sup>−/−</sup> mice exposed to chronic hypoxia. Prominent muscularization in small pulmonary artery was detected in the lungs of Fam13a<sup>−/−</sup> mice. Bars: 50 μm. Wall thickness was quantitatively analyzed (n = 8-9 each). (E) Immunohistochemistry for α-smooth muscle actin (α-SMA) and vWF in the lung sections of WT and Fam13a<sup>−/−</sup> mice exposed to chronic hypoxia. Muscularization of small pulmonary artery was deteriorated in the lungs of Fam13a<sup>−/−</sup> mice. Fully muscularized small pulmonary artery was quantified (n = 8-9 each). (F) Elastica van Gieson staining of the lung sections in WT and Fam13a<sup>−/−</sup> mice exposed to chronic hypoxia. Significant reduction in the number of small pulmonary artery (<50μm diameter) was observed in the lungs of Fam13a<sup>−/−</sup> mice. Average ratio of small pulmonary artery per 100 alveoli was calculated (n = 8-9 each). Data are presented as mean ± SEM. *P < 0.05 and **P < 0.01.

Loss of FAM13A promotes EndMT
Because *FAM13A* plays a crucial role in the regulation of β-catenin signaling that is involved in EndMT, we assessed the EndMT process in the lungs of WT and *Fam13a<sup>−/−</sup>* mice exposed to chronic hypoxia. Expression of endothelial markers such as *Pecam1* and *Cdh5* was reduced, while mesenchymal markers including *Acta1* and *Fn1* were significantly increased in the lungs of *Fam13a<sup>−/−</sup>* mice comparing to those in the lungs of WT mice (Fig 4A). Transcription factors such as *Twist1* and *Snail* showed a marked increase in the lungs of *Fam13a<sup>−/−</sup>* mice as well (Fig 4A). Furthermore, EndMT assessed by the emergence of endothelial and mesenchymal marker-double positive cells was apparently enhanced in the lungs of *Fam13a<sup>−/−</sup>* mice compared with that in WT mice (Figs 4B and C). These data suggest that loss of *FAM13A* promotes EndMT, resulting in the deteriorated pulmonary vascular remodeling and consequent pulmonary hypertension.

**Fig 4. Loss of *FAM13A* promotes EndMT.**

(A) Quantitative real-time PCR of genes involved in the EndMT in the lungs isolated from WT and *Fam13a<sup>−/−</sup>* mice exposed to chronic hypoxia. Endothelial markers (*Pecam1* and *Cdh5*) expression was reduced, while mesenchymal markers (*Acta1* and
Fn1) and their transcription factors (Twist1 and Snail) expression was enhanced in the lungs of Fam13a−/− mice (n = 9-11 each). (B) Immunohistochemistry for αSMA (mesenchymal marker) and vWF (endothelial marker) in the lungs. vWF-positive endothelial cells that are also positive for αSMA was more frequently detected in the lungs of Fam13a−/− mice. Arrows indicate the double-positive cells undergoing EndMT. Bars: 20μm. (C) Quantitative analysis for colocalization of mesenchymal and endothelial markers in pulmonary arteries assessed by the Manders overlap coefficient (n = 20 each). Data are presented as mean ± SEM. *P < 0.05 and **P < 0.01.

**FAM13A reduces active β-catenin in endothelial cells, and decelerates the EndMT**

We explored a role of FAM13A in EndMT using human pulmonary artery endothelial cells (PAEC) *in vitro*. When EndMT was induced by IL-1β and TGF-β1 treatment, FAM13A expression was significantly reduced in PAEC (Fig 5A). We then overexpressed FAM13A in PAEC using retrovirus-mediated gene transfection, and
subsequently treated cells with IL-1β and TGF-β1 to induced EndMT. Overexpression of *FAM13A* inhibited the induction of mesenchymal markers, whereas reduction of endothelial markers was not affected (Fig 5B). These data strongly suggest an inhibitory role of *FAM13A* in the EndMT process. In contrast, endothelial angiogenic capacities such as tube-formation, migration, proliferation, and apoptosis did not affected by *FAM13A*-overexpression in PAEC (Figs 5C-F).

We then examined whether *FAM13A* modifies β-catenin signaling in PAEC. Overexpression of *FAM13A* significantly reduced the non-phosphorylated active β-catenin after EndMT induction, while total β-catenin protein levels did not change in PAEC (Fig 4G). Furthermore, nuclear accumulation of active β-catenin was significantly reduced in PAEC that overexpress *FAM13A* compared to the control cells after EndMT induction (Fig 5H). Considering a crucial role of β-catenin in promoting EndMT, *FAM13A* decelerates the EndMT process at least partially through inhibiting the β-catenin signaling.

**Fig 5. FAM13A decelerates EndMT through inhibiting β-catenin signaling.**

(A) Quantitative real-time PCR of *FAM13A* in PAEC. EndMT was induced by the
treatment with TGF-β1 (10 ng/mL) and IL-1β (10 ng/mL) for 6 days. *FAM13A* expression was reduced after EndMT induction in PAEC (n = 4 each). (B) Quantitative real-time PCR of genes involved in the EndMT in PAEC transfected with either GFP or *FAM13A* in the presence or absence of EndMT induction. EndMT was induced by the treatment with TGF-β1 (10 ng/mL) and IL-1β (10 ng/mL) for 6 days. Overexpression of *FAM13A* reduced mesenchymal markers and their transcription factors expression in PAEC after EndMT induction (n=4-6 each). (C) Tube-formation analysis in PAEC transfected with either GFP or *FAM13A*. (D) Proliferation assessed by WST-1 assay in PAEC transfected with either GFP or *FAM13A* (n = 7 each). (E) Modified Boyden chamber assay in PAEC transfected with either GFP or *FAM13A* (n = 3 each). (F) Apoptosis was induced by serum and growth factor depletion for 24 h in PAEC transfected with either GFP or *FAM13A* (n = 7 each). (G) Immunoblotting for *FAM13A*, active β-catenin, β-catenin, and GAPDH in PAEC transfected with either GFP or *FAM13A* in the presence or absence of EndMT induction. Overexpression of *FAM13A* reduced active β-catenin in PAEC after EndMT induction (n=3 each). (H) Immunohistochemistry for active β-catenin in PAEC transfected with either GFP or
FAM13A in the presence or absence of EndMT induction. Bars: 50 μm. Nuclear accumulation of active β-catenin was quantified (n ≥ 50 nucleus / group). Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001.

Discussion

Pulmonary arterial hypertension is a chronic and progressive disease that eventually leads to the right ventricular heart failure and premature death. Despite the recent progress in clinical treatment including the endothelin receptor antagonism and new class of vasodilator, there are still significant unmet clinical needs in the treatment of pulmonary hypertension. Identification of new and/or unknown pathways in the pathogenesis of pulmonary hypertension is therefore important to improve the therapeutic strategies. In this manuscript, we revealed a previously undescribed role of FAM13A in the development of pulmonary hypertension. Given that Fam13a was reduced in the lungs of pulmonary hypertension mice model, and genetic loss of FAM13A exacerbated pulmonary hypertension, enhancing and/or preserving FAM13A

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in the lungs might have a therapeutic potential.

All forms of pulmonary arterial hypertension are characterized by vascular remodeling and dysfunction, of which mechanism includes multiple factors [2,3]. EndMT is one of the factors that cause abnormal vascular remodeling. EndMT is a cell transdifferentiation process in which endothelial cells lose endothelial specific markers and acquire mesenchymal properties. EndMT causes the loss of cell-cell adhesion, highly migratory and proliferative capacities in endothelial cells, leading to physiological and pathological cellular processes during embryonic development and disease onset. It has been reported that EndMT is involved in a variety of cardio-pulmonary diseases such as atherosclerosis [19], cardiac fibrosis [20], pulmonary fibrosis [21], and pulmonary hypertension [15,22,23]. In the remodeled vasculatures in pulmonary arterial hypertension, α-smooth muscle actin-expressing mesenchymal-like cells accumulate, especially in obstructive pulmonary vascular lesions. A fraction of these mesenchymal-like cells are derived from endothelial cells through the EndMT. Furthermore, alteration in BMPR-II signaling, which is critically involved in the pathogenesis of pulmonary hypertension, is linked to EndMT [22]. These findings
indicate a crucial role of EndMT in pulmonary hypertension, and strongly suggest that EndMT is a promising therapeutic target.

Previous genome-wide association studies suggest a strong association between \textit{FAM13A} and chronic lung diseases. \textit{FAM13A} is expressed in various types of tissues and cells, including airway and alveolar epithelial cells in the lung, pulmonary vascular cells, and mature adipocytes in adipose tissue [10,11,24]. Accordingly, \textit{FAM13A} has been involved in multiple biological processes such as epithelial cell regeneration, tumor cell proliferation and survival, and insulin signaling. \textit{FAM13A} is known to harbor a Ras-homologous GTPase-activating protein (RhoGAP) domain that is important for proliferation and survival in lung adenocarcinoma cell A549 [24], and this domain could activates RhoA which can affect actin cytoskeleton and promotes epithelial-to-mesenchymal transition in cystic fibrosis lung [8]. \textit{FAM13A} also has two coiled-coil domains that often play a role in the protein-protein interaction. Indeed, \textit{FAM13A} binds to insulin receptor substrate-1 in a coiled-coil domain-dependent manner, while it binds to protein phosphatase 2A (PP2A) independently of their coiled-coil domain [11]. Other study in COPD has revealed the importance of
interaction between *FAM13A* and PP2A in bronchial epithelial cells that leads to β-catenin degradation through GSK3β-mediated phosphorylation, although the coiled-coil domain dependency is not clear [10].

In the current study, we have identified a protective role of *FAM13A* in the progression of pulmonary hypertension by utilizing mice in which *Fam13a* was genetically deleted. To our knowledge, this is the first report that identifies *Fam13a* expression in the lung vasculature. *FAM13A* has been reported to regulate the β-catenin signaling in airway epithelial cells [10,25], and we found that *FAM13A* negatively regulates β-catenin activity in endothelial cell as well. β-catenin signaling has been involved in epithelial-to-mesenchymal transition in pulmonary disease and cancer [8,12,26]. Also, β-catenin has been reported to promote EndMT through nuclear accumulation and subsequent activation of TCF/Lef transcription factors [13,14]. In the current study, we revealed that overexpression of *FAM13A* decelerates the EndMT process in association with reduced active β-catenin levels and its nuclear accumulation in endothelial cells. These data strongly suggest that *FAM13A* decelerates EndMT process at least partially through inhibiting β-catenin signaling. It has been reported that
β-catenin accumulation promotes survival and proliferation in PAEC through enhancing RhoA-Rac1 signaling [27]; however, we did not detect significant difference of angiogenic capacity in PAEC overexpressing FAM13A, despite the reduction in active β-catenin.

Because FAM13A is expressed in a variety types of cells in the lungs, other FAM13A-mediated cellular processes might be involved in the pathogenesis of pulmonary hypertension. Nonetheless, our in vivo data using Fam13a−/− mice clearly showed that loss of FAM13A exacerbated pulmonary hypertension, and thus FAM13A is an attractive pharmacotherapeutic target for the treatment of pulmonary hypertension.

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Figure 1

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Figure 2

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B

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Figure 5

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