In Pulmonary Arterial Hypertension, Reduced BMPR2 Promotes Endothelial-to-Mesenchymal Transition via HMGA1 and Its Target Slug

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**Background**—We previously reported high-throughput RNA sequencing analyses that identified heightened expression of the chromatin architectural factor High Mobility Group AT-hook 1 (HMGA1) in pulmonary arterial endothelial cells (PAECs) from patients who had idiopathic pulmonary arterial hypertension (PAH) in comparison with controls. Because HMGA1 promotes epithelial-to-mesenchymal transition in cancer, we hypothesized that increased HMGA1 could induce transition of PAECs to a smooth muscle (SM)–like mesenchymal phenotype (endothelial-to-mesenchymal transition), explaining both dysregulation of PAEC function and possible cellular contribution to the occlusive remodeling that characterizes advanced idiopathic PAH.

**Methods and Results**—We documented increased HMGA1 in PAECs cultured from idiopathic PAH versus donor control lungs. Confocal microscopy of lung explants localized the increase in HMGA1 consistently to pulmonary arterial endothelium, and identified many cells double-positive for HMGA1 and SM22α in occlusive and plexogenic lesions. Because decreased expression and function of bone morphogenetic protein receptor 2 (BMPR2) is observed in PAH, we reduced BMPR2 by small interfering RNA in control PAECs and documented an increase in HMGA1 protein. Consistent with transition of PAECs by HMGA1, we detected reduced platelet endothelial cell adhesion molecule 1 (CD31) and increased endothelial-to-mesenchymal transition markers, αSM actin, SM22α, calponin, phospho-vimentin, and Slug. The transition was associated with spindle SM-like morphology, and the increase in αSM actin was largely reversed by joint knockdown of BMPR2 and HMGA1 or Slug. Pulmonary endothelial cells from mice with endothelial cell–specific loss of BMPR2 showed similar gene and protein changes.

**Conclusions**—Increased HMGA1 in PAECs resulting from dysfunctional BMPR2 signaling can transition endothelium to SM-like cells associated with PAH. (Circulation. 2016;133:1783-1794. DOI: 10.1161/CIRCULATIONAHA.115.020617.)

**Key Words:** endothelial-to-mesenchymal transition ■ HMGA1 protein ■ hypertension, pulmonary

**Vascular Medicine**

Pulmonary arterial hypertension (PAH), whether idiopathic PAH (iPAH), heritable PAH, or PAH associated with other conditions, is a potentially lethal disease characterized by progressive vascular changes leading to the obliteration of distal pulmonary arteries (PAs). Abnormal muscularization and loss of precapillary PAs is followed by proliferation of vascular cells in more proximal PAs to form an occlusive neointima. The origin of the neointimal cells remains unclear; they were originally thought to be derived from the muscular media because they express α smooth muscle actin (αSMA). However dysfunctional endothelial cells (ECs) could, by endothelial-to-mesenchymal transition (EndMT), contribute to neointima formation either directly, or indirectly by transforming in a way that impedes their ability to produce factors such as apelin that control smooth muscle cell (SMC) proliferation.

Clinical Perspective on p 1794

EndMT is a process by which endothelial cells acquire a mesenchymal phenotype in association with expression of SMC genes, such as αSMA and phospho (p) vimentin, and reduction in endothelial genes such as VE-cadherin and platelet endothelial cell adhesion molecule 1 (CD144 and CD31, respectively). Endothelial fate–mapping in a mouse model of pulmonary hypertension demonstrated cells of endothelial lineage expressing SMC markers that contribute to the neointima. Pulmonary artery endothelial cells (PAECs) can acquire a smooth muscle phenotype in culture in a transforming growth factor β (TGFβ)–dependent manner. Although EndMT has been implicated in the human pathology of PAH,
the initiating factor and the pathway involved have not been described.

Our group applied high-throughput RNA sequencing to PAECs obtained from lungs of patients with PAH or from donor controls, and observed elevated mRNA expression of High Mobility Group AT-hook 1 (HMGA1) in the patients.10 This gene is a member of a family of architectural factors that bind AT-rich regions of DNA and alter the chromatin structure to influence transcriptional activity.11 HMGA1 is highly expressed in stem cells during embryonic development, but, as tissues mature, HMGA1 levels drop and are very low in fully differentiated tissues.11 Abnormal elevation in HMGA1 contributes to neoplastic transformation in multiple cancers12–14 by inducing epithelial-to-mesenchymal transition.12 An invasive phenotype results from repression of the epithelial junction protein E-cadherin and upregulation of mesenchymal genes. These features completely reverse with loss of HMGA1.14 Snail and Slug (Snai1 and Snai2, respectively) are closely related zinc finger

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<th>6MW (m)</th>
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Hemodynamic data from catheterization were obtained from studies performed closest to transplantation. Pulmonary arterial hypertension (PAH) medications are listed according to total drug exposure during treatment period of follow-up, not necessarily in combination. *d* indicates diastolic; HPAH, hereditary PAH; IHC, immunohistochemistry; IPAH, idiopathic PAH; m, mean; 6MW, distance (m) walked in 6 minutes; N/A, data not available; PAP, pulmonary artery pressure (mm Hg); s, systolic; PVR, pulmonary vascular resistance (dynes/s cm⁻⁵); baseline Fick PVR; and WB, Western blots.
transcription factors that have been implicated in epithelial-to-mesenchymal transition and EndMT.\textsuperscript{15–17} HMGA2, a protein closely related to HMGA1, can directly affect expression of Snail by binding the promoter and recruiting SMAD proteins to increase transcription of the SNAI1 gene.\textsuperscript{18}

Bone morphogenetic protein receptor 2 (BMPR2) mutations are found in \approx 70\% of patients with heritable PAH and 20\% of sporadic cases or IPAH.\textsuperscript{19,20} and even IPAH patients without known BMPR2 mutations have reduced expression of BMPR2, as do patients with PAH associated with other conditions.\textsuperscript{21} Silencing BMPR2 in control PAECs causes an elevation in expression of HMGA1 that is phenocopied by reducing levels of \(\beta\)-catenin, an effector of gene regulation downstream of BMPR2.\textsuperscript{22} We therefore hypothesized that dysfunctional BMPR2 signaling in PAH PAECs causes an elevation in HMGA1, promoting acquisition of a mesenchymal phenotype via a process of EndMT.

### Materials and Methods

#### Subjects and Human Primary Cell Culture

As previously described,\textsuperscript{22} we harvested and cultured ECs predominantly from small (<1 mm) PAs. We analyzed tissue sections from explanted lungs of patients with IPAH and heritable PAH, obtained at the time of transplantation, and from unused donor control lungs, via the Pulmonary Hypertension Breakthrough Initiative Network, funded by the Cardiovascular Medical and Education Fund and National Institutes of Health-National Heart, Lung, and Blood Institute. Demographic and clinical data relevant to PAH patients and controls are in Tables 1 and 2. PAECs from lung tissues were cultured in commercial EC media containing 5\% fetal bovine serum and controls are in Tables 1 and 2. PAECs from lung tissues were cultured in commercial EC media containing 5\% fetal bovine serum (Sciencell, Carlsbad, CA) and used at passages 3 to 6 \textsuperscript{22} as were commercial human PAECs (PromoCell, Heidelberg, Germany).

#### siRNA Transfection

Small interfering (si) RNAs for BMPR2, HMGA1, Slug, and nontargeting control siRNAs (GE Healthcare Dharmacon, Lafayette, CO) were transfected into subconfluent PAECs at a concentration of 20 nmol/L by using Lipofectamine RNAiMAX and OptiMEM medium (Life Technologies, Carlsbad, CA). We measured gene expression (mRNA) at 72 hours and protein levels at 7 days. In some experiments, PAECs were transfected with control nontargeting siRNA, incubated for 48 hours, and then treated with TGF\(\beta\)2 (Abcam, Cambridge, MA) for 5 days.

#### Quantitative Real-Time Polymerase Chain Reaction

RNA was extracted using a spin column–based kit (Zymo Research, Irvine, CA) and quantified by using a spectrophotometer. Quantitative real-time polymerase chain reaction was performed on a CFX96 Real Time System (BioRad, Hercules, CA) using 4 ng cDNA, 1 mmol/L primers, and a SybrGreen master mix (Life Technologies). Primer sequences, designed using National Center for Biotechnology Information’s Primer-BLAST function, are shown in Table I in the online-only Data Supplement.

#### Immunoblotting

Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 4\% to 12\% Bis-Tris gradient gels (Life Technologies), transferred onto nitrocellulose membranes (Bio-Rad), and incubated with primary antibodies, diluted 1:1000 (unless noted): rabbit anti-HMGA1 (1:10000), rabbit antivimentin (1:4000), mouse anti–p-vimentin (1:250), mouse anti–

#### Immunohistochemistry

Formaldehyde-fixed, paraffin-embedded tissue sections were stained as previously described,\textsuperscript{22} using an HMGA1 antibody (1:500, Abcam) and anti-rabbit secondary antibody 3,3′-diaminobenzidine (Dako, Carpinteria, CA), and counterstained with hematoxylin.

### Table 2. Characteristics of Controls (Unused Donor Lungs)

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CVA, cerebrovascular accident; IHC, immunohistochemistry; MVA, motor vehicle accident; PEA, pulseless electrical activity; and WB, Western blots.
Immunofluorescence
Tissue sections were processed as described above and incubated with antibodies against HMGA1 (1:500, Abcam), von Willebrand Factor (1:100, Abcam), and SM22α (1:100, Abcam). Nuclei were stained with DRAQ7 (Biostatus, UK). PAECs were cultured in chamber slides, fixed in 4% paraformaldehyde, and permeabilized by using 0.1% Triton X-100 (Sigma-Aldrich). Slides were incubated with primary antibodies against αSMA (1:200, Sigma-Aldrich) and VE-cadherin (1:150, Abcam). Fluorescent-tagged secondary antibodies were used at 1:400 (Life Technologies). Slides were mounted using Vectashield with or without 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA) and imaged using a confocal microscope (Olympus, Center Valley, PA and Leica, Buffalo Grove, IL).

Transgenic Mice
The Animal Care Committee of Stanford University approved all protocols. Mice with an endothelial-specific inducible knockout of Bmpr2 were created in our laboratory (SCL-CreER120/R26R/Bmpr2-2Δ). Wild-type mice were used as controls. Murine pulmonary ECs were isolated from digested whole lung tissue using CD31 antibody-coated magnetic beads (Dynabeads; Invitrogen) as previously described.

Statistical Analysis
Values from multiple experiments are depicted in box plots showing the minimum, maximum, and median for each experiment. When values are depicted in scatterplots, the lines represent the mean±standard error of the mean. The number of experiments, IPAH, or control cells used is indicated in the figure legends. Statistical significance was determined by using 1-way analysis of variance followed by Bonferroni multiple comparisons test or by Kruskal-Wallis test followed by the Dunn post hoc test, as indicated in the figure legends. When only 2 groups were compared, statistical differences were assessed with the unpaired 2-tailed Student t test. A P value of <0.05 was considered significant.

Results
HMGA1 Is Elevated in PAECs From Patients With PAH Relative to Controls
We observed elevated HMGA1 protein in PAECs (passage 3–6) from patients who have heritable PAH with a BMPR2 mutation in comparison with those from donor control lungs and a similar trend in PAECs from IPAH patients (Figure 1A).
HMGAl immunoperoxidase staining was prominent in the PA endothelium of lung tissue sections from IPAH patients, whereas immunoreactivity was barely detectable in control PA endothelium (Figure 1B). Although HMGAl was localized to the endothelium primarily, HMGAl positive cells also appeared in the neointima, often close to the endothelial surface of the pulmonary arteries (Figure 1B). Scale bar, 25 μm (left column) and 50 μm (middle and right columns). PA indicates pulmonary artery; PAEC, pulmonary arterial endothelial cell; PAH, pulmonary arterial hypertension; and vWF, von Willebrand Factor.

**Figure 3.** BMPR2 silencing in control PAECs induces increased HMGAl and EndMT markers. Commercially available PAECs were transfected with siRNA for BMPR2 (B2) or control nontargeting siRNA (Con). A, Gene expression changes were assessed by qPCR after 72 hours. B, Representative immunoblot and densitometric quantification of protein expression assessed 7 days after siRNA transfection. Snail/Slug indicates an antibody recognizing both Snail and Slug proteins. C, Representative immunoblot and densitometric quantification of protein expression assessed 7 days after siRNA transfection. Boxplots indicate minimum, maximum, and median for n=9 (A and B). Scatterplots indicate means±SEM for n=3 (C). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 in comparison with respective Con by the Student t test. EndMT indicates endothelial-to-mesenchymal transition; PAEC, pulmonary arterial endothelial cell; PECAM-1, platelet endothelial cell adhesion molecule 1; qPCR, quantitative real-time polymerase chain reaction; SEM, standard error of the mean; si, small interfering; and αSMA, α smooth muscle actin.
occurred vessels (Figure 1C). HMGA1 immunoreactivity was increased in PAs of all sizes in PAH versus control lungs and in all lesions including plexiform lesions (Figure 1C and 1D).

To determine whether HMGA1 was coexpressed with endothelial and smooth muscle markers in IPAH PAs, we performed confocal microscopy on lung tissue sections by using immunofluorescence staining. In vessels of different sizes from PAH patients, HMGA1 appeared to colocalize predominantly with the endothelial marker von Willebrand Factor, but also with smooth muscle \( \alpha \) positive cells in the neointima (Figure 2). HMGA1 expression was much less intense in control tissues.

Loss of BMPR2 Induces Elevation of HMGA1 and Changes Consistent With EndMT

To mimic BMPR2 loss in PAH patients, we transfected BMPR2 siRNA in control PAECs and evaluated whether, as a result of elevated HMGA1 previously shown, EndMT transcription factors Snail and Slug and smooth muscle markers would be increased and endothelial markers reduced. BMPR2 siRNA resulted in an increase in HMGA1, \( \alpha \)SMA, and calponin mRNA, but there was no significant change in VE-cadherin or Snail (Figure 3A). We confirmed loss of BMPR2 protein by immunoblot, and the associated elevation in HMGA1, increased Snail/Slug, \( \alpha \)SMA, and a prominent marker of EndMT, p-vimentin (Figure 3B and 3C). Platelet endothelial cell adhesion molecule 1 was reduced (Figure 3C) but not VE-cadherin (Figure 3B). There was no change in another marker of EndMT, Twist (data not shown), suggesting that Snail/Slug are the critical transcription factors regulating EndMT resulting from loss of BMPR2.

Increased \( \alpha \)SMA in PAECs transfected with BMPR2 siRNA was visualized by using immunofluorescent staining and confocal microscopy (Figure 4A). Although VE-cadherin was not reduced by loss of BMPR2, we observed fragmented VE-cadherin immunostaining on the borders of PAECs suggesting an early breakdown of the junctions (Figure 4A). Consistent with this was the change in morphology of a subset of siBMPR2 treated PAECs, from the typical cobblestone appearance to an elongated, spindle shape (Figure 4B). Many of these elongated cells stained positively for \( \alpha \)SMA (Figure 4C).

EndMT Changes Induced by Loss of BMPR2 Require HMGA1 and Slug

To determine whether HMGA1 was required for the EndMT induced by loss of BMPR2 we silenced BMPR2 and HMGA1, individually and in combination (Figure 5A). Because loss of BMPR2 increases HMGA1 mRNA, we also found that loss of HMGA1 increased BMPR2 mRNA, suggesting a feedback mechanism (Figure 5A). The increase in Slug mRNA seen with loss of BMPR2 was abolished by cotransfection with siRNA for HMGA1 and BMPR2, indicating that Slug is a target of elevated HMGA1 resulting from loss of BMPR2. Similarly, the increase in \( \alpha \)SMA mRNA caused by loss of BMPR2 was significantly abrogated by knockdown of both BMPR2 and HMGA1. Protein levels were similar to mRNA levels (Figure 5B). To determine whether Slug was required for the increase in \( \alpha \)SMA expression, we silenced BMPR2 and Slug, individually and in combination (Figure 5C). We found that the increase in \( \alpha \)SMA induced by loss of BMPR2 was completely prevented by concomitant knockdown of Slug mRNA.
indicating that Slug is required for EndMT. Similar to our observation with HMGA1, silencing Slug alone significantly increased BMPR2 mRNA suggesting that Slug plays a role in the feedback mechanism regulated by HMGA1 (Figure 5C).

**EndMT Changes Are Not Replicated by TGFβ Treatment**

To determine whether loss of BMPR2 induces an increase in TGFβ signaling that is necessary for EndMT, we treated control PAECs with TGFβ2, previously implicated in EndMT.24 TGFβ2 signaling increased phosphorylated SMAD2 relative to total SMAD2, but loss of BMPR2 did not phosphorylate SMAD2 (Figure 6). Moreover TGFβ2 increased HMGA1 but did not induce the EndMT-related increase in Snail/Slug or αSMA (Figure 6). Taken together, our results show that EndMT changes in PAECs associated with loss of BMPR2 are not acting via TGFβ2 signaling.

**Mice Lacking Endothelial BMPR2 Show Changes Consistent With EndMT**

To further characterize the relationship between reduced BMPR2 expression and EndMT, we studied EC-specific Bmpr2 knockout mice (EC-Bmpr2-KO) produced by breeding SCL-CreER<sup>TM</sup>, R26LacZ<sup>fl/fl</sup>, and Bmpr2<sup>fl/fl</sup> mice and characterized in a previous study.23 They have no significant pulmonary hypertension at baseline, but develop exaggerated right ventricular systolic pressure and right ventricular hypertrophy following exposure to chronic hypoxia23 and do not reverse pulmonary hypertension after recovery in room air.25 Pulmonary ECs isolated from these mice show increased expression of HMGA1 and Slug at the mRNA and protein level, relative to wild-type (WT) mice (Figure 7A and 7B). Although the increase in αSMA mRNA was small and not accompanied by a significant elevation in protein level, the increase in SM22α was particularly prominent both at mRNA
and protein level (Figure 7A and 7B). Immunofluorescence microscopy, however, showed a distinct increase in expression of αSMA in EC-Bmpr2-KO relative to WT pulmonary ECs (Figure 7C). Taken together, these findings suggest that loss of BMPR2 in pulmonary ECs is sufficient to promote features of EndMT in a transgenic mouse that develops more severe disease in response to a pulmonary hypertension–producing stimulus.

**Discussion**

Our study identifies a novel role for HMGA1 in promoting EndMT in PAH. We show that PAECs highly expressing HMGA1 are prevalent in human PAH PA lesions. Moreover, HMGA1 is upregulated by loss of BMPR2 in PAECs, as assessed either by using siRNA to reduce BMPR2 in cultured human PAECs, or in transgenic mice with BMPR2 deleted in ECs. Downstream effectors of the increase in HMGA1 suggest an axis in which induction of Slug increases expression of αSMA and other SM genes including SM22a and calponin (see schema in Figure 8).

Previous studies have documented elevated expression of HMGA1 in response to insults in human umbilical endothelial cells, with HMGA1 regulating genes induced by hypoxia and viral infection/inflammation. An elevation in HMGA1 is also a feature of angiogenesis in postischemia brain ECs. Following identification of elevated HMGA1 mRNA in isolated PAECs of patients with PAH by RNAseq, we sought to determine the role of elevated HMGA1 particularly with regard to EndMT in IPAH. HMGA1 colocalized primarily with endothelial and occasionally with smooth muscle markers in arteries with neointimal formation and plexogenic lesions. Although a previous study in mice used fate mapping to show αSMA-positive cells of endothelial lineage within the neointima, these cells constituted only a small subpopulation. Similarly, not all neointimal cells in the human tissue expressed p-vimentin, a marker of EndMT. Thus, it is possible that, at best, only a subset of PAECs contribute to the SM-like cells of the neointima as has been previously reported. The majority of PAECs may be transformed in a manner that impairs their ability to produce inhibitors of SMC proliferation, such as apelin. Consistent with this, we showed that PAH ECs produce less apelin in comparison with donor controls. This transition without transformation is in keeping with mesenchymal changes noted in epithelial cells infected in fibrotic lung disease. These cells promote fibrosis but do not become fibroblasts. It is conceivable that once ECs fully undergo EndMT, HMGA1 levels fall and are not detectable by immunofluorescence. This is in keeping with the drop in HMGA1 levels seen normally in tissue differentiation in development, but different from HMGA1 in cancer cells undergoing epithelial-to-mesenchymal transition, which remains highly expressed.

Clinical and experimental studies have linked reduced expression of BMPR2 to the development of PAH. Loss of BMPR2 in ECs confers susceptibility to apoptosis and to impaired EC function, as well, as evidenced by angiogenesis and migration assays. We demonstrate a novel relationship between loss of BMPR2 and increased expression of HMGA1 that is associated with morphological changes and increased expression of Slug, αSMA, and other SMC proteins. These morphological changes in cultured cells are in keeping with transmission electron microscopy studies of endothelial cells in the PAs from patients with PAH, which show increased microfilament bundles, consistent with cytoskeletal changes of EndMT. The high expression of HMGA1 in von Willebrand Factor–positive endothelial cells might suggest that, as in cancer cells, HMGA1 can contribute to the development of an apoptosis-resistant phenotype.

We previously related increased HMGA1 to elevated β-catenin resulting from loss of BMPR2. Other transcription factors or microRNAs (miRs) may be also involved. We investigated several miRs that are reduced in PAH, including miR 21 and miR 26a, but they do not appear to regulate HMGA1 (data not shown).

Cotransfection of siRNAs targeting HMGA1 and BMPR2 completely prevented the increase in Slug but only partially prevented the increase in αSMA at the mRNA and protein level. The incomplete repression of αSMA by HMGA1 siRNA versus Slug siRNA in the context of loss of BMPR2 was surprising. Perhaps another factor elicited by HMGA1 knockdown tempers the loss of Slug-related genes. HMGA1
was not previously identified as a regulator of αSMA, but it could potentiate serum response factor–dependent transcription as has been shown for HMGA1 and SM22α.42

Activation of BMPR2 signaling by BMP ligands reversed epithelial-to-mesenchymal transition in renal fibrosis43 and EndMT in cardiac fibrosis.44 In mesenchymal breast cancer cells, silencing HMGA1 promoted mesenchymal-to-epithelial transition in conjunction with decreased proliferation, migration, and invasion of the tumor cells.14 Stimulation of PAECs with BMP9 does repress HMGA1 (data not shown) suggesting that amplification of BMPR2 signaling via treatment with BMP9,45 FK506,23 or elafin46 may reverse EndMT.

TGFβ signaling induces EndMT in experimental models of kidney47 and cardiac44 fibrosis, and mature bovine PAECs in culture have been shown to undergo EndMT in response to TGFβ.1 However, loss of BMPR2 did not result in activation of SMAD2, and TGFβ was insufficient to induce EndMT, highlighting a potentially unique feature of human PAECs.

Gene expression changes consistent with EndMT were verified in mice with an inducible loss of endothelial Bmpr2 and isolated pulmonary ECs from these mice showed a propensity to undergo EndMT in culture (Figure 7). These mice showed mildly exaggerated pulmonary hypertension in chronic hypoxia,23 and could not reverse pulmonary hypertension on return to normoxia, in association with fewer distal vessels that

Figure 7. Pulmonary ECs from mice lacking endothelial Bmpr2 show changes consistent with EndMT. Pulmonary ECs isolated from mice with Bmpr2 deleted in ECs (BMPR2 KO or KO) or wild-type (WT) controls were assessed for EndMT changes at the mRNA (A) and protein (B) levels, and by immunofluorescence microscopy (C). Pulmonary ECs were isolated from 3 mice, pooled, and cultured. Assays were conducted at passages 3 to 8. A, mRNA assessed by qPCR, n=6. B, Representative immunoblot and densitometric quantification of WT or BMPR2 KO pulmonary ECs, n=3 or 4 as shown in the 2 blots (passages 2–5). C, Representative immunofluorescence images byconfocal microscopy of WT or BMPR2 KO mouse pulmonary ECs for VE-cadherin (green) and αSMA (red). Nuclei stained with DAPI (blue). Scale bar=50 μm. Quantification of αSMA fluorescence is depicted below, n=3. Boxplots indicate minimum, maximum, and median. Scatterplot indicates mean±SEM; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs control or WT by the Student t test. DAPI indicates 4′,6-diamidino-2-phenylindole; EC, endothelial cell; EndMT, endothelial-to-mesenchymal transition; KO, knockout; PAEC, pulmonary arterial endothelial cell; qPCR, quantitative real-time polymerase chain reaction; si, small interfering; SEM, standard error of the mean; si, small interfering; and αSMA, α smooth muscle actin.
EndMT Mechanism Related to Pulmonary Arterial Hypertension

Figure 8. Proposed model: Loss of BMPR2 promotes EndMT via HMGA1. Loss of BMPR2 in PAECs leads to heightened expression of HMGA1, which increases the transcription factor Slug. HMGA1 binds to DNA and may promote binding of additional pro-EndMT transcription factors. Expression of smooth muscle genes, such as αSMA, SM22α, calponin, and p-vimentin are increased, and the endothelial gene PECAM1 is decreased, reflecting a mesenchymal phenotype. EC indicates endothelial cell; EndMT, endothelial-to-mesenchymal transition; PAEC, pulmonary arterial endothelial cell; αSMA, α smooth muscle actin; and TF, transcription factor.

were highly muscularized.25 Thus, the susceptibility to EndMT may impair recovery from a pulmonary hypertensive state.

In addition to EndMT, HMGA1 overexpression in cancer cells targets inflammatory genes, increases reactive oxygen species, and reduces mitochondrial DNA repair efficiency,48,49 features that we observed in PAECs from patients with Bmpr2 mutation.25 HMGA1 is thought to act as a transcriptional regulator, linking inflammatory pathways with oncogenic potential. In PAH, HMGA1 may be the pivotal link between the proinflammatory state of PAECs and the breakdown of endothelial junctions attributable to EndMT, that permits inflammatory cell infiltration of the vessel wall and propagation of the neointima. Thus HMGA1 induces EndMT causing endothelial dysfunction and possible cellular contribution to occlusive vascular changes.

Acknowledgments
We especially acknowledge the outstanding scientific contribution of the second author, Dr Jan-Renier A. J. Moonen who, over the past year, carried out major revisions requiring additional experiments, repeat statistical analyses, and redrafting of many of the figures. IPAH and control lung tissues provided by the Pulmonary Hypertension Breakthrough Initiative were procured at the Transplant Procurement Centers at Allegheny Hospital, Baylor University, The Cleveland Clinic, Stanford University, University of California-San Diego, Vanderbilt University, and the University of Alabama at Birmingham, and deidentified patient data were obtained via the Data Coordinating Center at the University of Michigan. We are very grateful to Dr Michal Bental Roof for assistance with preparation of the manuscript and to Dr Lu Tian for statistical consultation.

Sources of Funding
Dr Hopper was supported by Pediatric Heart Center Research Program, Lucile Packard Children’s Hospital, Lucile Packard Foundation for Children’s Health, Stanford CTSAs (grant UL1 RR025744);

Dr Moonen was supported by the Dutch Heart Foundation (grant 2013T116) and the Netherlands CardioVascular Research Initiative (CVON); the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development, and the Royal Netherlands Academy of Sciences (CVON Phaedra 2012-08). Dr Diebold was funded by the Deutsche Herzstiftung e.V (S/06/11), and Dr Hennigs was funded by a fellowship of the Deutsche Forschungsgemeinschaft (He 6855/1-1). Dr Rabonovitch is funded by National Institutes of Health/National Heart, Lung, and Blood Institute grants 5UL1 HL107393 and R24 HL123767, the Cardiovascular Medical Research and Education Fund grant UL1RR024986, and the Dunlevie Chair in Pediatric Cardiology at Stanford University.

Disclosures

None.

References


### CLINICAL PERSPECTIVE

Transition of endothelial cells to take on features of smooth muscle cells, a process called endothelial-to-mesenchymal transition, is increasingly appreciated as a mechanism integral to vascular pathobiologies, including pulmonary arterial hypertension. The transformed endothelial cells may contribute to the expanding neointima either directly or by failing to produce inhibitors of smooth muscle cell proliferation. In this report, we relate endothelial-to-mesenchymal transition to reduced expression of the receptor for bone morphogenetic protein (BMPR2) that occurs either related to a mutation or independent of a mutation in patients with pulmonary arterial hypertension. We show that loss of BMPR2 causes an elevation in a chromatin remodeling and scaffolding protein, High Mobility Group AT-hook 1 (HMGA1), that has been implicated in the transition of cancer cells. Elevated HMGA1 leads to an increase in a transcription factor called Slug that upregulates expression of smooth muscle genes such as smooth muscle actin and SM22α. At the same time, impaired structure and reduced expression of endothelial cell junctional proteins, CD-31 (platelet endothelial cell adhesion molecule 1) and CD-144 (VE-cadherin), respectively, lead to morphological changes that promote the smooth muscle cell phenotype.
In Pulmonary Arterial Hypertension, Reduced BMPR2 Promotes Endothelial-to-Mesenchymal Transition via HMGA1 and Its Target Slug

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_Circulation._ 2016;133:1783-1794; originally published online April 4, 2016; doi: 10.1161/CIRCULATIONAHA.115.020617

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/133/18/1783

Data Supplement (unedited) at:
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SUPPLEMENTAL MATERIAL

In Pulmonary Arterial Hypertension, Reduced BMPR2 Promotes Endothelial-to-Mesenchymal Transition Via HMGA1 and its Target Slug

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## Supplemental Table 1: Primers Used

<table>
<thead>
<tr>
<th>Gene (HUMAN)</th>
<th>Primer</th>
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| HMGA1       | F – GCTGGTAGGGAGTCAGAAGGA  
              | R - TGTTGGTTTTCCGGGTCTTG   |
| SLUG        | F – CGAAGTTGGACACACATACAGTG  
              | R - CTGAGATCTCTGGGTGTGGT   |
| SNAIL       | F – TCGGAAGCCTAACTACACAGCGA  
              | R - AGATGAGCATTGGCAGCGAG   |
| αSMA        | F – CTATGAGGGCTATGCCTTGCC  
              | R - GCTCAGCCAGTAGAAGGAAGGA |
| V-E-CADHERIN| F – TTGGAACCAGATGCACATTGAT  
              | R - TCTTGCAGACTCAGCCTTGAC  |
| CALPONIN    | F – CTGTCAGGCCGGATGTAAGGAAC  
              | R - GAGGCCGTCCTACATGGTTT   |
| BMPR2       | F – CTGCAGGCTCAGCTGAAGGTAATGT  
              | R - TTGGTGTGTCAGGAGGTGG    |
| β-ACTIN     | F – CATGCCATCTGCTGCTGGA   
              | R - CCGTGGCCATCTCTTGCTG    |

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<tr>
<th>Gene (MOUSE)</th>
<th>Primer</th>
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| HMGA1       | F – GGTCGGGAGTCAGAAAGAGC  
              | R - ATTCGGTCTCCCTTTGGTCG   |
| SLUG        | F – CAGCGAACTGGACACACACA  
              | R - ATAGGGGTGATGCTCCCGAG   |
| αSMA        | F – CCCAGACATCAGGGAGTTATGG  
              | R - TCTATCGGATACGTCAGGTCA  |
| SM22α       | F – CCAACAAGGGTCATCTCCTAG  
              | R - ATCTGAGGCCTACATCA      |
| BMPR2       | F – TCGCATCTGCTTGGTCTCCT  
              | R - TAGGAGCTGCTTTTCGTCT    |
| β-ACTIN     | F – ATGTTGGATCAGCAAGCAGGA  
              | R - AAGGGTGAACACGAGCTCA    |