A KLF2-BMPER-Smad1/5 checkpoint regulates high fluid shear stress-mediated artery remodeling

Hanqiang Deng[1,2], Jiasheng Zhang[1,2], Yewei Wang[4], Divyesh Joshi[1], Xinchun Pi[5], Sarah De Val[6], and Martin A. Schwartz[1,2,3,*]

[1] Yale Cardiovascular Research Center, Yale School of Medicine, New Haven, CT, USA.

[2] Section of Cardiovascular Medicine, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, USA.

[3] Department of Cell Biology, Yale School of Medicine, and Department of Biomedical Engineering, Yale School of Engineering, New Haven, CT, USA.

[4] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA.

[5] Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX, USA.

[6] Department of Physiology, Anatomy and Genetics, Institute of Developmental and Regenerative Medicine, University of Oxford, Oxford, UK

[*] Author for correspondence: martin.schwartz@yale.edu

Abstract

Vascular remodeling to match arterial diameter to tissue metabolic requirements commonly fails in ischemic disease. Endothelial cells (EC) sense fluid shear stress (FSS) from blood flow to maintain FSS within a narrow range in healthy vessels. Higher FSS induces vessel outward remodeling to return FSS to physiological levels, but mechanisms are poorly understood. We previously reported that Smad1/5 is maximally activated at physiological FSS and suppressed at higher flow. The Smad1/5 pathway opposes activation of Akt, suggesting that inhibiting Smad1/5 may be required for outward remodeling. Here, we report that suppression of Smad1/5 at high FSS is mediated by elevated KLF2, which induces the BMP pathway inhibitor BMPER, which suppresses Smad1/5 and de-inhibits Akt. In a mouse arteriovenous fistula (AVF) model, high FSS induces arterial outward remodeling coincident with elevated BMPER expression and Smad1/5 inactivation. Endothelial BMPER deletion impaired blood flow recovery and vascular remodeling in the AVF and a hindlimb ischemia (HLI) model, with the latter reversed by BMP9/10 blocking antibodies (bAbs). In both STZ-induced type 1 and HFD-induced type 2 diabetic mice that show poor recovery from HLI, BMP9/10 bAbs improved outcomes. Thus, suppression of Smad1/5 is required for high FSS-mediated outward remodeling and is a potential therapeutic approach for ischemic disease.

Key Words: fluid shear stress, vascular remodeling, artery outward remodeling, KLF2, BMPER, Smad1/5, BMP9/10

Introduction

Cardiovascular diseases (CVD) such as coronary artery disease (CAD) and peripheral artery disease (PAD) remain the leading global cause of morbidity and mortality, usually associated with maladaptive artery remodeling that leads to tissue ischemia[1]. Artery remodeling is a complex process involving structural changes that result in inward remodeling to reduce or outward remodeling to increase lumen diameter[2]. In healthy vessels, it is an essential physiological mechanism that matches vessel diameter to blood flow requirements[3]. Pathological vessel remodeling in CAD and PAD leads to tissue ischemia and disease symptoms. Diabetes is a major risk factor for these conditions, with highly accelerated CAD and severe PAD/critical limb ischemia that can result in limb amputation[4,5].

Endothelial cells (ECs) lining the inner layer of blood vessels are continuously exposed to blood flow, which exerts a frictional drag force called fluid shear stress (FSS)[6]. Current evidence supports a model in which ECs encode an FSS set point that mediates homeostatic artery remodeling[7]. Increased or decreased flow through an artery stimulates outward or inward remodeling, respectively[8,9]. According to this view, low FSS stimulates inward remodeling, physiological FSS stabilizes vessels to suppress remodeling, and high (above physiological) FSS promotes outward remodeling[10,11]. One instance of vessel remodeling in response to sustained high FSS is autologous arteriovenous fistula (AVF), a surgical procedure connecting an artery to a vein[12]. In mice, this enables analysis of the specific segment of artery subject to high flow[13]. A similar procedure is used in patients to provide vascular access with increased blood supply for hemodialysis[14].

Bone morphogenetic proteins (BMPs) are important regulators of vascular development, integrity, inflammatory responses, and insulin sensitivity[15-17]. BMP family members bind to type I and type II transmembrane receptors that contain cytoplasmic serine/threonine kinase domains. BMP binding initiates receptor activation and C-terminal phosphorylation of Smads 1, 5 and 8, though in ECs, Smad 1 and 5 predominate[18]. C-terminal Smad1/5 phosphorylation promotes their binding to Smad4, nuclear entry and induction of target genes. BMP-binding endothelial regulator (BMPER) is a secreted protein expressed mainly in ECs that binds BMPs and BMP receptors and modulates BMP signaling in the endothelium[19] and potentially in adjacent

smooth muscle cells[20]. BMPER plays important roles in disease-associated vascular processes including angiogenesis, atherosclerosis, inflammation, and diabetes[21-24].

Artery outward remodeling is impaired in CAD and PAD, for which (in addition to diabetes), age, genetics and hyperlipidemia are major risk factors[25]. A well-established mouse PAD model is hindlimb ischemia (HLI) after femoral artery ligation[26]. After surgery, blood flow in the thigh is redirected to preexisting parallel small collateral arteries and capillaries. Higher shear stress in these vessels then induces arteriogenesis. The resultant outward remodeling and increased blood flow through these vessels bypasses the blockade to restore blood flow. The calf also undergoes vascular remodeling, mainly angiogenesis, in response to hypoxia in this region. Together, these two processes mediate blood flow recovery. In mice, both type 1 diabetes (T1D) and obesity-related type 2 diabetes (T2D) slow blood flow recovery from hindlimb ischemia[27].

We recently reported that low FSS activates Smad2/3 to induce inward artery remodeling[28]. In the current study, we set out to elucidate the mechanism that governs high flow-induced outward artery remodeling. We show that elevated Klf2 expression induces BMPER to terminate Smad1/5 activation, which de-represses Akt to permit remodeling. We also show that artificially blocking Smad1/5 potently rescues poor remodeling in both T1D and T2D mice. Elucidation of a key mechanism of artery outward remodeling thus suggests a novel therapeutic approach.

Results

High fluid shear stress inhibits Smad1/5 activation though KLF2.

We previously found that Smad1/5 is maximally activated at physiological FSS and suppressed at high (i.e., supraphysiological) FSS[7]. KLF2 and its close homology Klf4 are major EC transcription factors induced by FSS in a dose-dep manner[29] (add PMID 30826122). We therefore tested the role of Klf2 in Smad1/5 suppression under high FSS. Human umbilical vein ECs (HUVECs) were treated with control (siCtrl) and KLF2 (siKLF2) siRNA, then subjected to FSS at 3 (low), 12 (physiological) or 40 dynes/cm^2 (high) for 12h. We examined Smad1 nuclear translocation as a marker of its activation. In control cells, nuclear Smad1 was maximal around physiological shear stress, and then decreased at high FSS as expected; in KLF2 knockdown (KD) cells, nuclear Smad1 was similar to control under low and physiological FSS but remained nuclear under high FSS (**Fig. 1a-b**). This result was confirmed in human aortic ECs (HAECs, **Extended Data Fig. 1a-c**). Assaying Smad1/5 phosphorylation by immunostaining and Western blotting gave similar results (**Fig. 1c-f**, **Extended Data Fig. 1d-e**). Thus, KLF2 is required for suppression of Smad1 activation under high FSS.

KLF2 regulates Smad1/5 through BMPER.

KLF2 is a transcription factor, suggesting that its increased expression under high shear (confirmed in **Extended data Fig 2d**) could induce a gene that suppresses Smad1/5 phosphorylation. We therefore performed RNA-sequencing (RNAseq) on HUVECs with or without KLF2 KD, focusing on genes connected to the BMP pathway and that decreased after KLF2 KD (**Extended Data Fig. 2a-b;** affected relevant genes summarized in **2c**). Smad7 and Smad6 were both KLF2-dependent, however, when knocked down, neither affected Smad1 activation under high FSS (**Extended Data Figs. 3 and 4**). Then another candidate, BMPER, caught our attention. BMPER is an extracellular modulator of BMP signaling, which binds BMPs and BMP receptors and can block Smad activation[19,30]. BMPER showed Klf2-dependent induction at high flow (**Extended Data Fig. 2d**).

Whether BMPER is a direct Klf2 target gene could be addressed by chromatin immunoprecipitation and sequencing (ChIP-seq), however, suitable antibodies to Klf2 are not available. But ChIP-seq has been done for its co-regulated homolog Klf4 that activates many of the same target genes through the same consensus motif[31]. We

therefore re-analyzed a published Klf4 ChIP-seq dataset from ECs in which constitutively active MEK5 (caMEK5) drove induction of Klf4[32]. caMEK5 triggered increased binding of Klf4 to an intronic region within the BMPER gene, correlating with a near-consensus Klf2/Klf4 motif. This KLF4 binding peak was also enriched for the H3K27Ac chromatin modification (a marker of active enhancers) and for ERG binding, a vascular transcription factor commonly associated with endothelial enhancers[33] (**Extended Data Fig. 2e**). These data suggest that BMPER is likely a direct target gene of Klf2/4 in ECs.

To test its function, HUVECs were transfected with control (siCtrl) or BMPER (siBMPER) siRNA, then subjected to FSS at 3, 12 and 40 dynes/cm^2 for 12h. Like KLF2 KD, BMPER KD had little effect at low or physiological FSS but potently blocked the decrease in Smad1 nuclear translocation and phosphorylation under high FSS (**Fig. 2a-f**, **Extended Data Fig. 5a-e**). Together, these results show that KLF2-dependent BMPER induction is required for Smad1/5 suppression by high FSS.

High flow-induced outward remodeling in vivo.

To study high FSS in vivo in a system where the endothelium can be readily analyzed, we developed an arteriovenous fistula (AVF) model in which the right carotid artery (RCA) is connected to the external jugular vein (**Fig. 3a**), resulting in high flow through both vessels. At day 3 and 14 after surgery, serial cross sections of the proximal RCA close to the anastomosis or the comparable location in the control, unoperated left carotid artery (LCA) were examined. The circumference of the operated RCA was significantly increased compared to the control LCA at day 3, with a further increase at day 14 (**Fig. 3b**). Mice without anastomosis showed no difference in the circumference of LCA and RCA (**Extended Data Fig. 6a-b**). These results demonstrate robust outward remodeling after surgery. Suitable antibodies to Klf2 are not available, but staining for its co-regulated homolog Klf4 showed a significant increase in the RCA compared to the LCA, supporting higher shear stress in RCA after surgery (**Extended Data Fig. 6c-e**). BMPER immunostaining strongly increased in the proximal RCA while expression was barely detectable in the LCA (**Fig. 3c**). In contrast, Smad1/5 phosphorylation was low in the proximal RCA, consistent with a decrease at high shear (**Fig. 3d**). Together, those results show that induction of BMPER under high FSS correlates with Smad1/5 inactivation during artery outward remodeling.

BMPER KD impairs Akt activation under high FSS.

The Smad1/5 pathway is reported to suppress activation of VEGFR2 and Akt[34,35], suggesting that Smad1/5 suppresses VEGFR2 and Akt under physiological FSS to stabilize vessels and prevent unwanted vascular remodeling. However, suppression of Smad1/5 at high FSS would release this inhibition to enable outward remodeling. This hypothesis predicts that suppressing BMPER should elevate Smad1/5 and decrease Akt activity in arteries under high FSS. We first confirmed that Akt was activated by high FSS (**Fig. 4a-b**). To test the effect of BMPER on Akt activation under high FSS, HUVECs treated with control (siCtrl) or BMPER (siBMPER) siRNA were subjected to FSS at 40 dynes/cm^2. BMPER KD reduced Akt activation under high FSS, as assayed by immunostaining and Western blotting (**Fig. 4c-f**). Thus, BMPER induction under high FSS facilitates Akt activation under this condition.

Deletion of BMPER in ECs in vivo.

To investigate the role of BMPER in vivo, BMPER floxed mice[24] were crossed with Cdh5-CreERT2 to generate inducible endothelial-specific BMPER knockout mice (BMPER iECKO). At 5-6 weeks, tamoxifen was injected for 5 consecutive days (**Fig. 5a**), with BMPER deletion confirmed in isolated lung ECs by Q-PCR, and in the intact carotid by immunostaining (**Fig. 5b-c**). We then tested the role of BMPER using the hindlimb ischemia (HLI) model in which ligation of the femoral artery results in high flow remodeling of small vessels parallel to the blockade, which leads to recovery of blood flow in the lower limb. BMPER iECKO and control mice were subject to HLI and blood flow recovery assessed by Laser Doppler flow-imaging (LDI) before and at various times after ligation, using the unoperated right leg as an internal control. BMPER ECKO strongly impaired blood flow recovery (**Fig. 5d-e**). Staining tissue sections for SMA to identify muscular arteries, we observed that in WT mice, thigh tissue parallel to the ligation had a higher density of muscular arteries than in the unoperated thigh, indicating arteriogenesis. In tissue from BMPER ECKO mice, muscular arteries in the ligated thigh showed no increase relative to the control group (**Fig. 5f**).

Control vs BMPER iECKO mice were also subject to AVF surgery. At day 3 post-surgery, serial cross sections of the proximal RCA close to the anastomosis or the comparable location in the control LCA showed that BMPER ECKO impaired outward remodeling (**Fig. 6a-b**). BMPER expression was induced in the proximal RCA in control group, but not in BMPER iECKO mice (**Fig. 6c-d**, **Extended Data Fig. 7a**). Smad1/5

activation was suppressed in the proximal RCA in control group but remained high in BMPER iECKO mice (**Fig. 6e-f, Extended Data Fig. 7b**). Together, these data provide in vivo evidence that BMPER upregulation suppresses Smad1/5 to permit artery outward remodeling.

BMP9/10 blocking Abs in BMPER iECKO mice in HLI.

FSS activation of Smad1/5 requires the presence of soluble BMP9 and 10[18], which are circulating factors produced by hepatocytes and cardiac myocytes, respectively[36,37]. BMP9/10 blocking antibodies (bAbs) thus prevent FSS activation of Smad1/5 ECs[18]. BMPER iECKO and control mice were subject to HLI then injected intraperitoneally (IP) once per week with control IgG or BMP9/10 bAbs (**Fig. 7a**). The poor blood flow recovery after BMPER iECKO was effectively reversed by BMP9/10 bAbs (**Fig. 7b-c**), with comparable improvement in arterialization (**Fig. 7d-e**). BMP9/10 bAbs also restored the expression of angiogenic factors Vegfa and Pdgfb in BMPER iECKO mice in HLI model (**Extended Data Fig. 8**). These results confirm that BMPER works through the BMP-Smad1/5 pathway.

BMP9/10 blocking Abs in type 1 diabetes.

BMPER plasma levels reportedly decrease in metabolic syndrome patients and obese mice, conditions that are major risk factors for peripheral artery disease (PAD). Mouse models of type 1 diabetes (T1D), also show slow recovery from hindlimb ischemia[4]. To explore whether BMP9/10 bAbs might improve blood flow recovery in T1D, we used the streptozotocin (STZ) model in C57BL/6 mice. Mice with confirmed hyperglycemia (**Extended Data Fig. 9**) were subjected to HLI surgery then injected with control IgG or BMP9/10 bAbs (**Fig. 8a**). BMP9/10 bAbs markedly improved blood flow recovery (**Fig. 8b-c**), as well as arterialization in the thigh (**Fig. 8d-e**), with slightly increased angiogenesis in the calf (**Extended Data Fig. 10**). Efficacy was similar in male and female mice. These results indicate the therapeutic potential of BMP9/10 blocking Abs to inhibit Smad1/5 pathway in ischemic disease.

BMP9/10 blocking Abs in type 2 diabetes.

Obesity-related type 2 diabetes (T2D) also impairs blood flow recovery in hindlimb ischemia[38]. To test the effect of BMP9/10 bAbs in this model, C57BL/6 mice were fed a high-fat diet (HFD, 60 kcal% fat) for 8 weeks. Body weight and blood glucose confirmed induction of obesity and hyperglycemia (**Extended Data Fig. 11**). Mice were then

subjected to HLI surgery and injected with control IgG or BMP9/10 bAbs (**Fig. 8f**). As expected, recovery from HLI was severely reduced in T2D mice, with stronger inhibition in females. But BMP9/10 bAbs substantially improved blood flow recovery in both male and female mice (**Fig. 8g-h**). Treatment also improved arterialization in the thigh (**Fig. 8i-j**) and modestly increased angiogenesis in the calf (**Extended Data Fig. 12a-b**). Akt activation and BMPER expression were also restored after BMP9/10 bAbs treatment (**Extended Data Fig. 12c-f**). Together, these results suggest that inhibiting the Smad1/5 pathway offers a novel means to improve homeostatic remodeling in ischemic disease.

Discussion

This study aimed to elucidate essential aspects of the homeostatic high FSS outward remodeling pathway that adjusts artery inner diameter to meet tissue requirements. Previous studies showed that Smad1/5 was activated in ECs by FSS at physiological levels but then decreased at higher shear. Flow stimulation of Smad1/5 was found to occur through increased clustering of the membrane receptors Alk1 and Endoglin, which enhanced sensitivity to their circulating ligands BMP9 and 10[18]. Previous work also showed that Smad1/5 inhibits Akt activation via induction of casein kinase 2 and subsequent activation of the PI3-lipid phosphatase PTEN[39]. Smad1/5 may also limit VEGFR2 activation[35]. These data suggest a model in which Smad1/5 activation under physiological FSS confers vessel stability by limiting remodeling pathways that could otherwise be activated by transient or sub-threshold stimuli. Outward remodeling under sustained high shear thus proceeds in part by de-repressing these mediators. Loss of the Smad1/5 pathway due to mutations in the receptors Alk1 or endoglin, or Smad4 leads to vascular malformations due to loss of this stabilization pathway. A recent study showed that loss of Smad4 specifically increased sensitivity of ECs to FSS, consistent with this perspective[40]. However, in adult mice, complete loss of Alk1 or Smad4 is not sufficient for malformations, leading to the notion that additional hits are required, which helps to explain the sporadic nature of the disease[41].

Our results demonstrate that outward remodeling requires high FSS suppression of the Smad1/5 pathway via increased expression of Klf2, a well-known flow-dependent gene. Klf2 in turn induces expression of BMPER, which blocks the flow- and BMP9/10-dependent activation of Smad1/5, thereby releasing PI3-kinase and Akt from inhibition (Pathway diagram in **Extended Data Fig. 13**). Available data suggest that BMPER may be a direct Klf2 target, however, the detailed mechanism for induction only at high FSS remains to be elucidated. PI3-kinase and Akt likely act via multiple downstream effectors to promote cell cycle progression, activate eNOS to induce vasodilation, and alter integrin function and cytoskeletal organization. BMPER can also act on vascular smooth muscle cells[20], which could contribute to remodeling.

Decreased vessel lumen diameter is an essential element in ischemic disease. Atherosclerosis is widespread but generally asymptomatic due to compensatory remodeling by the unaffected part of the vessel wall that preserves lumen diameter[42]. But in severe disease, remodeling fails, lumen diameter is compromised, and tissue

ischemia ensues. The ability to remodel surrounding vessels after an occlusive event is a major factor in recovery from stroke and myocardial infarction[43,44]. However, many of the same risk factors (age, hyperlipidemia, diabetes/hyperglycemia and hypertension) that promote atherosclerosis also limit remodeling responses. Diabetes and associated hyperglycemia are especially severe, with diabetic PAD leading to acute limb ischemia and even limb amputation in some cases[4,25]. Tissue ischemia is generally accompanied by high VEGF levels and high FSS at sites of artery blockade[25,43], indicating that sensitivity to these stimuli is the limiting factor.

We therefore considered whether blocking the Smad1/5 pathway might improve vessel remodeling. In both T1D and T2D mouse models, antibodies to BMP9/10, a well characterized method for blocking this pathway[45], substantially improved recovery from hindlimb ischemia (**Extended Data Fig. 13**). We observed that T2D reduced recovery from HLI more strongly in female mice, a finding that deserves further investigation, but BMP9/10 therapy was similarly effective in both sexes. These results suggest a novel approach for therapies to improve ischemic disease. One concern here is whether inhibiting the Smad1/5 pathway will give rise to arteriovenous malformations (AVM) or telangiectasias as occurs in patients with receptor or Smad4 mutations. Although adult mice are highly resistant to complete deletion of Alk1, leading to the notion that additional genetic or environmental hits are required for lesion formation[41], ischemic tissue may be favorable for AVM formation. Careful testing in additional models and biological systems will this be critical to advancing this approach.

Limitations of this study

Pathways such as KLF2, Smad1/5 and Akt are multifunctional and perform different roles in different contexts, thus, the principles elucidated here may not apply in other settings, especially in vascular development. The in vivo experiments reported here examined a single mouse strain (C57BL/6), thus, findings should be testing additional strains and species.

Materials and Methods

The data and materials that support the findings of this study are available from the corresponding author on reasonable request.

Animals. *Bmper$^{fl/fl}$* mice and *Cdh5-CreER^{T2}* mice have been previously described[24,46]. All mice in this study are on the C57BL/6 background. To induce gene deletion, mice were intraperitoneally injected with 1.5mg tamoxifen (Sigma, T5648) for five consecutive days. All mouse protocols and experimental procedures were approved by Yale University Institutional Animal Care and Use Committee (IACUC).

Arteriovenous fistula model. Mice were anesthetized with ketamine/xylazine (100 mg/kg and 10 mg/kg), and surgical procedures were performed under 0.5-1% isoflurane anesthesia delivered via a precision vaporizer. Mice received pre-emptive analgesia of buprenorphine (Ethiqa XR) and local anesthetic bupivacaine, and surgical procedures were performed using aseptic and microsurgery techniques. A ventral midline incision (about 2 cm) was made in the neck. The right external jugular vein branch and right common carotid artery (RCA) were dissected and rinsed with saline containing 100 IU/mL heparin after the vein and artery were cut. The A-V fistula was created with end-to-side anastomoses using 11-0 monofilament sutures. The opposite left carotid artery (LCA) was approached but not cut and served as a control. The skin was closed with a 7-0 monofilament suture, 0.5 mL saline was injected subcutaneously, and the animals were kept warm until full recovery. At day 3 and 14 after surgery, proximal RCA and LCA samples were collected for further analysis.

Hindlimb ischemia model. Surgical procedures were performed in mice under anesthesia as described[47]. Briefly, a 10 mm longitudinal incision was made in the left hind limb. The left common femoral artery and its side branches were dissected and ligated with 10-0 monofilament sutures spaced 5 mm apart, and the arterial segment between the ligatures was excised. Assessment of tissue perfusion by Laser-Doppler flow-Imaging (LDI) was done by scanning rear paws with the LDI analyzer (Moor Infrared Laser Doppler Imager Instrument, Wilmington, Delaware). Low or no perfusion is displayed as dark blue, high perfusion is displayed as red. Images were quantitatively converted into histograms with Moor LDI processing software V5.3. Data are reported as the ratio of flow in the left/right (L/R) hind limb and calf regions. Measurement of

blood flow was done before and immediately after surgery and then at days 3, 7, 14 or 21.

Mouse T1D model. C57BL/6J mice (12 weeks) were purchased from The Jackson Laboratory (stock no. 000664). Mice were injected intraperitoneally with streptozotocin (STZ, diluted in citrate buffer, pH 4.2-4.5; 50 mg/kg for male and 75 mg/kg for female; Sigma S1030) for five consecutive days. At 3 weeks after the first STZ injection, blood glucose was measured to confirm hyperglycemia. At 4 weeks after the first STZ injection, mice were used for hindlimb ischemia surgery.

Mouse T2D model. C57BL/6J mice (Jackson Laboratory, stock no. 000664), 12 weeks old, were fed a high-fat diet (HFD, 60 kcal% fat; Research Diet, D12492) for 8 weeks then subject to HLI surgery. Body weight and blood glucose were measured before and after HFD (before surgery).

Cell culture and siRNA transfection. HUVECs (Human Umbilical Vein Endothelial Cells) pooled from multiple donors were obtained from Yale Vascular Biology and Therapeutics tissue-culture core laboratory at Passage 1. Primary HAECs (Human Aortic Endothelial Cells) were purchased from American Type Culture Collection (ATCC, PCS-100-011). Both cell types were maintained in EGM2 Endothelial Cells Growth Media (Lonza) and used for experiments between P2 and P5. SiRNA transfection used Opti-MEM medium (Gibco, 31985070) and Lipofectamine RNAiMAX (Invitrogen). ON-TARGET plus Smartpool siRNAs from Dharmacon were used against human KLF2 (L-006928-00-0005), human Smad6 (L-015362-00-0005), human Smad7 (L-020068-00-0005), human BMPER (L-021489-02-0005).

Shear stress. HUVECs or HAECs were seeded on tissue culture plastic slides coated with 20μg/mL fibronectin and grown to confluence. Shear stress with the calculated intensities indicated in each figure was applied in parallel flow chambers as described[28,48].

Immunofluorescence. For cells, samples were fixed in 4% paraformaldehyde (Electron Microscopy Sciences) for 10 min at room temperature. For tissues, samples were fixed in 4% paraformaldehyde overnight at 4°C and incubated with 30% sucrose (Sigma) solution in PBS overnight at 4°C. Specimens were embedded in OCT medium (SAKURA) and 8-10μm sections were cut in a cryostat (Leica). For immunofluorescence, cells or sections were blocked in 5% donkey serum, 0.2% BSA,

0.3% Triton X-100 in PBS, followed by incubation with primary and secondary antibodies diluted in blocking buffer. Negative controls used non-immune species- and isotype-matched IgG. Images were taken using an SP8 confocal microscope (Leica).

Image analysis. To quantify Smad1 nucleus/cytoplasm intensity ratio, images were opened in ImageJ (National Institutes of Health) and converted to 8-bits, then thresholds adjusted to segment the nucleus and individual cell regions. Nuclear translocation was determined by masking the cell nuclei (from DAPI images), and then the integrated fluorescence intensity in the nucleus and cytoplasm were measured, respectively. The nucleus/cytoplasm intensity ratio was then calculated.

In the hindlimb ischemia model, arteriogenesis and angiogenesis were evaluated in the muscle territories located in the thigh and calf, respectively. To assess arteriogenesis, thigh muscle around the proximal femoral artery excision site was sectioned and stained with smooth muscle actin (SMA) antibody. The number of SMA-positive vessels was counted in five randomly selected fields from each mouse, and the mean value was used as a single data point for each mouse. To assess angiogenesis, gastrocnemius muscle in calf area was sectioned and stained with CD31 antibody. Angiogenesis was evaluated by calculating the CD31 positive area using ImageJ. This measurement was determined in five randomly selected fields from each animal, and the average value was used as a single data point for each mouse.

RNA isolation and quantitative real-time PCR. RNA was extracted from cells with RNeasy Plus Mini Kit (QIAGEN) according to the Manufacturer's instructions, and reverse transcription performed with the iScript Reverse Transcription Supermix for RT-qPCR (BIO-RAD). cDNA was amplified by real-time PCR with iQ SYBR Green Supermix (BIO-RAD). Expression of target genes was normalized to the housekeeping gene *GAPDH*. Primers for qPCR were listed in Extended Data Table 1.

RNA-Sequencing Analysis. Total RNA was extracted from HUVECs treated with Ctrl and KLF2 siRNA (four samples for each group) and quantitated by NanoDrop. RNA integrity number was measured with an Agilent Bioanalyzer. Samples were subjected to RNA-sequencing using Illumina NextSeq 500 sequencer (75bp paired end reads). The base calling data from sequencing were transferred into FASTQ files using bcl2fastq2 conversion software (version 2.20, Illumina). The raw reads were aligned to the human reference genome GRCh38 using HISAT2 (version 2.1.0) alignment software and

processed using HTSeq (version 0.11.1) to generate read counts for every gene. DESeq2 (version 1.24, using default parameter) was used to pre-process raw data to remove the noise, normalize each sample to correct the batch effect, perform principal component analysis (PCA) for observing the similarity of replicates, and identify differential expression genes. P values obtained from multiple tests were adjusted using Benjamini-Hochberg correction.

Western Blotting. Cells were harvested and incubated in RIPA buffer containing complete mini protease inhibitors and phosphatase inhibitors (Roche) for 30 min on ice. Lysates were centrifuged at 14000 rpm for 10 min at 4°C, supernatants transferred to new 1.5 ml EP tubes, 4X loading buffer (250mM Tris-HCl pH 6.8, 8% SDS, 40% glycerol, 20% β-mercaptoethanol, 0.008% bromophenol blue) added and heated to 95°C for 5 min. Cell lysates were resolved by a 4-15% CriterionTM TGXTM Precast Gels (BIO-RAD) SDS–PAGE electrophoresis, and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore). The membranes were blocked with 5% non-fat milk and incubated with indicated antibodies diluted in 5% BSA, followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. Protein bands were imaged using Immobilon Western Chemiluminescent HRP substrate (Millipore). ImageJ was used for densitometry quantification.

Mouse lung EC isolation. Mouse lungs were collected and digested in a solution of 2 mg/ml collagenase (Sigma). The cell suspension was filtered through a 70μm sterile cell strainer (Falcon). ECs were isolated using magnetic beads coated with anti-rat IgG (Invitrogen) then incubated with rat anti-mouse CD31 antibody (BD Biosciences). Isolated cells were then lysed, and RNA isolated using PicoPure RNA isolation kit (Applied Biosystems) according to the manufacturer's instructions.

Antibodies. We used the following antibodies for immunofluorescence (IF) and immunoblotting (IB): Rat anti-Mouse CD31 (BD 550274; IF 1:200), GAPDH (Cell Signaling 5174S; IB 1:2000), Smad1 (Cell Signaling 9743S; IB 1:1000, IF 1:400), phospho-Smad1/5 (Cell Signaling 13820S; IB 1:1000, IF 1:400), Akt (Cell Signaling 2920S; IB 1:1000), phospho-Akt S473 (Cell Signaling 4060S; IB 1:1000, IF 1:400). BMPER (Abcam ab73900; IB 1:1000, IF 1:400), KLF4 (Abcam ab215036; IF 1:400). Antibodies for BMP9 (MAB3209), BMP10 (MAB2926), IgG2A (MAB003) and IgG2B (MAB004) were purchased from R&D Systems.

Statistical analysis. Statistical analysis was performed using GraphPad Prism software (GraphPad software Inc.). Data were analyzed for normality and equal variance using the Shapiro-Wilk test and Brown-Forsythe test, respectively. If both tests were passed, statistical significance was analyzed by unpaired t test for two group comparison or one-way ANOVA with Tukey's post hoc test for multiple group comparison. Statistical significance between two groups plus treatment was calculated by two-way ANOVA with Tukey's or Sidak's multiple comparison tests. A P value less than 0.05 was considered significant (*P < 0.05, **P < 0.01, ***P < 0.001).

Author contributions

H.D. performed most of the experiments, analyzed data, and prepared figures and manuscript. J.Z. performed mouse surgery. Y.W. performed RNAseq analysis. D.J. assisted with mice samples collection. X.P. shared the *Bmper* floxed mice. S.D.V performed ChIP-seq analysis. M.A.S. supervised and supported the project, analyzed data, and wrote the manuscript.

Acknowledgments

This work was supported by National Institutes of Health (NIH) Grant R01 HL135582 (M.A.S.) and American Heart Association (AHA) career development award 24CDA1268658 (H.D.).

Competing Interests

A patent application (M.A.S. and H.D.) related to this work was filed. All other authors have declared no competing interests.

Figure Legends

Fig. 1: KLF2 mediates high FSS-induced Smad1/5 suppression. (a-b) HUVECs transfected with control or KLF2 siRNA were subjected to FSS at the indicated magnitudes. At 12 h, Cells were fixed and stained for total Smad1. Scale bar: 25μm. Nucleus/Cytoplasm intensity ratio of Smad1 was quantified, n=100 cells per group from 3 independent experiments, data showing all points from min to max. (c-d). Immunostaining of phospho-Smad1/5. Scale bar: 25μm. Nuclear intensity of Smad1/5 was quantified (normalized to siCtrl 3 dyn/cm^2 group), n=100 cells for each group from 3 experiments, data showing all points from min to max. (e-f) Western blotting for p-Smad1/5 and total Smad1, n=5 experiments, data are presented as mean ± s.e.m. Statistics calculated by two-way ANOVA with Sidak's multiple comparison tests.

Fig. 2: BMPER suppresses Smad1/5 activation under high FSS. (a-b) HUVECs transfected with control or BMPER siRNA were subjected to FSS at indicated magnitudes for 12 h. Cells were fixed and stained for Smad1. Scale bar: 25μm. Nucleus/Cytoplasm intensity ratio of Smad1 was quantified, n=100 cells for each group from 3 experiments, data showing all points from min to max. (c). Immunostaining of phospho-Smad1/5. Scale bar: 25μm. Nuclear intensity of Smad1/5 was quantified (normalized to siCtrl 3 dyn/cm^2 group), n=100 cells for each group from 3 independent experiments, data showing all points from min to max. (d-e) HUVECs transfected with control or BMPER siRNA were subjected to FSS at indicated magnitudes for 12 h. Western blotting analysis and quantification of p-Smad1/5, total Smad1 and BMPER, n=5 experiments, data are presented as mean ± s.e.m. Statistics calculated by two-way ANOVA with Sidak's multiple comparison tests.

Fig. 3: AVF model of high FSS-mediated outward remodeling. (a) Schematic of AVF model: the right carotid artery (RCA) was connected to the jugular vein through a branch. The blue double headed arrows indicate the regions that were excised and sectioned. (b) H&E staining and quantification of cross-sectional circumference from the RCA and LCA. Scale bar: 100μm. (c) Immunostaining of BMPER and quantification in the RCA and LCA, n=6 mice. Scale bar: 25μm. (d) Phospho-Smad1/5 staining and quantification in the RCA and LCA, n=6 mice. Arrowheads are the nuclei and p-Smad1/5 positive area in artery ECs. Scale bar: 25μm. Data are presented as mean ± s.d. Statistics calculated by two-tailed paired t tests.

Fig. 4: BMPER regulates Akt activation under high FSS. (a-b) Immunostaining for Akt S473 phosphorylation and quantification in the RCA and LCA sections from the AVF model, n=6 mice, data are presented as mean ± s.e.m. Scale bar: 25μm. (c-f) HUVECs transfected with control or BMPER siRNA were subjected to high FSS for 24h. Akt S473 phosphorylation was assayed by immunostaining (c-d) and Western blotting (e-f). Scale bar: 25μm. (d) n=60 cells for each group from 3 experiments, data showing all points from min to max; (f) n=4 experiments, data are presented as mean ± s.e.m. Statistics calculated by two-tailed unpaired t tests (b) or two-way ANOVA with Tukey's multiple comparison tests (d, f).

Fig. 5: BMPER iECKO impairs blood flow recovery in the HLI model. (a) $BMPER^{flox/flox}$;Cdh5-CreERT2 and Ctrl mice at 6 weeks were injected with tamoxifen. (b) BMPER deletion was confirmed by Q-PCR analysis of isolated lung ECs, n=3 mice per group, data are presented as mean ± s.d. (c) BMPER immunostaining in carotid arteries. Scale bar: 25μm. (d) Representative images and (e) quantification of blood flow recovery from Ctrl and BMPER iECKO mice at indicated days, n=8 mice per group, data are presented as mean ± s.e.m. (f) Representative images and quantification of SMA staining of sections from ligated and control thighs, n=8 mice per group, data are presented as mean ± s.e.m. Scale bar: 100μm. Statistics calculated by two-tailed unpaired t tests (b) or two-way ANOVA with Sidak's multiple comparison tests (e, f).

Fig. 6: BMPER iECKO blocks outward remodeling in the AVF model. (a-b) H&E staining and quantification of vessel circumference in the RCA and LCA. Scale bar: 100μm. (c-d) Representative images and quantification of BMPER staining in Ctrl and BMPER iECKO mice. (e-f) Representative images and quantification of phospho-Smad1/5 in Ctrl and BMPER iECKO mice. Scale bar: 25μm (c, e). n=6 mice per group (b, d, f). Arrowheads are the nuclei and BMPER or p-Smad1/5 positive area in artery ECs. Data are presented as mean ± s.e.m. Statistics calculated by two-way ANOVA with Tukey's multiple comparison tests.

Fig. 7: BMP9/10 blocking Abs restore blood flow recovery in the BMPER iECKO HLI model. (a) Experiment timeline: 8-week-old mice were injected with tamoxifen on 5 consecutive days. At 10 weeks of age, the left femoral artery was ligated. BMP9/10 bAbs or IgG (2.5 mg/kg) were injected intraperitoneally every week. (b-c) Representative images and quantification of blood flow recovery from Ctrl and BMPER

iECKO mice treated with IgG or BMP9/10 bAbs at indicated days. Statistical comparisons: * for BMPER iECKO+BMP9/10 bAbs vs. BMPER iECKO+IgG, $P < 0.05$; # for BMPER iECKO+IgG vs. Ctrl+BMP9/10 bAbs, $P < 0.05$. (d-e) Representative images and quantification of SMA staining of sections from ligated and control thighs. Scale bar: 100μm. Data are presented as mean ± s.e.m. Statistics calculated by two-way ANOVA with Tukey's (c) or Sidak's (e) multiple comparison tests.

Fig. 8: BMP9/10 blocking Abs improves blood flow recovery and vascular remodeling in diabetic mice. (a) Timeline for T1D experiment: 12-week-old mice were injected with STZ for 5 consecutive days. At 16 weeks, the left femoral artery was ligated. BMP9/10 bAbs or IgG (2.5 mg/kg) were injected intraperitoneally every week. (b-c) Representative images and quantification of blood flow recovery from IgG or BMP9/10 bAbs treated male (n=4 per group) and female (n=5 per group) mice at indicated days, data are presented as mean ± s.d. (d-e) Representative images and quantification of SMA staining of sections from ligated and control thighs, data are presented as mean ± s.e.m. (f) Timeline for T2D experiment: 12-week-old mice were fed a 60 kcal% high fat diet for 8 weeks. The left femoral artery was ligated and BMP9/10 bAbs or IgG (2.5 mg/kg) injected intraperitoneally every week. (g-h) Representative images and quantification of blood flow recovery from IgG or BMP9/10 bAbs treated T2D male (n=5 per group) and female (n=5 per group) mice at indicated days, data are presented as mean ± s.d. The grey dotted line with open circle symbol (h) shows blood flow recovery from control mice treated with isotype IgG (n=5 mice per gender). Statistical comparisons: * for T2D+BMP9/10 bAbs vs. T2D+IgG; # for T2D+BMP9/10 bAbs vs. Ctrl+IgG. (i-j) Representative images and quantification of SMA staining of sections from ligated and control thighs, data are presented as mean ± s.e.m. Scale bar: 100μm. Statistics calculated by two-way ANOVA with Sidak's multiple comparison tests.

References

1. Heusch G, Libby P, Gersh B, Yellon D, Bohm M, Lopaschuk G, Opie L. Cardiovascular remodelling in coronary artery disease and heart failure. *Lancet*. 2014;383:1933-1943. doi: 10.1016/S0140-6736(14)60107-0
2. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *The New England journal of medicine*. 1994;330:1431-1438. doi: 10.1056/NEJM199405193302008
3. Lucitti JL, Jones EA, Huang C, Chen J, Fraser SE, Dickinson ME. Vascular remodeling of the mouse yolk sac requires hemodynamic force. *Development*. 2007;134:3317-3326. doi: 10.1242/dev.02883
4. Howangyin KY, Silvestre JS. Diabetes mellitus and ischemic diseases: molecular mechanisms of vascular repair dysfunction. *Arteriosclerosis, thrombosis, and vascular biology*. 2014;34:1126-1135. doi: 10.1161/ATVBAHA.114.303090
5. Spinetti G, Kraenkel N, Emanueli C, Madeddu P. Diabetes and vessel wall remodelling: from mechanistic insights to regenerative therapies. *Cardiovasc Res*. 2008;78:265-273. doi: 10.1093/cvr/cvn039
6. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nature reviews Molecular cell biology*. 2009;10:53-62. doi: 10.1038/nrm2596
7. Baeyens N, Nicoli S, Coon BG, Ross TD, Van den Dries K, Han J, Lauridsen HM, Mejean CO, Eichmann A, Thomas JL, et al. Vascular remodeling is governed by a VEGFR3-dependent fluid shear stress set point. *eLife*. 2015;4. doi: 10.7554/eLife.04645
8. Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science*. 1986;231:405-407. doi: 10.1126/science.3941904
9. Wong CY, de Vries MR, Wang Y, van der Vorst JR, Vahrmeijer AL, van Zonneveld AJ, Roy-Chaudhury P, Rabelink TJ, Quax PH, Rotmans JI. Vascular remodeling and intimal hyperplasia in a novel murine model of arteriovenous fistula failure. *Journal of vascular surgery*. 2014;59:192-201 e191. doi: 10.1016/j.jvs.2013.02.242
10. Tiezzi M, Deng H, Baeyens N. Endothelial mechanosensing: a forgotten target to treat vascular remodeling in hypertension? *Biochemical pharmacology*. 2022:115290. doi: 10.1016/j.bcp.2022.115290
11. Deng H, Rukhlendo OS, Joshi D, Hu X, Junk P, Tuliakova A, Kholodenko BN, Schwartz MA. cSTAR analysis identifies endothelial cell cycle as a key regulator of flow-dependent artery remodeling. *bioRxiv*. 2023. doi: 10.1101/2023.10.24.563764
12. Yang B, Shergill U, Fu AA, Knudsen B, Misra S. The mouse arteriovenous fistula model. *Journal of vascular and interventional radiology : JVIR*. 2009;20:946-950. doi: 10.1016/j.jvir.2009.03.044
13. Browne LD, Bashar K, Griffin P, Kavanagh EG, Walsh SR, Walsh MT. The Role of Shear Stress in Arteriovenous Fistula Maturation and Failure: A Systematic Review. *PloS one*. 2015;10:e0145795. doi: 10.1371/journal.pone.0145795
14. Manov JJ, Mohan PP, Vazquez-Padron R. Arteriovenous fistulas for hemodialysis: Brief review and current problems. *The journal of vascular access*. 2022;23:839-846. doi: 10.1177/11297298211007720
15. Lowery JW, de Caestecker MP. BMP signaling in vascular development and disease. *Cytokine & growth factor reviews*. 2010;21:287-298. doi: 10.1016/j.cytogfr.2010.06.001
16. Morrell NW, Bloch DB, ten Dijke P, Goumans MJ, Hata A, Smith J, Yu PB, Bloch KD. Targeting BMP signalling in cardiovascular disease and anaemia. *Nature reviews Cardiology*. 2016;13:106-120. doi: 10.1038/nrcardio.2015.156
17. Perera N, Ritchie RH, Tate M. The Role of Bone Morphogenetic Proteins in Diabetic Complications. *ACS pharmacology & translational science*. 2020;3:11-20. doi: 10.1021/acsptsci.9b00064
18. Baeyens N, Larrivee B, Ola R, Hayward-Piatkowskyi B, Dubrac A, Huang B, Ross TD, Coon BG, Min E, Tsarfati M, et al. Defective fluid shear stress mechanotransduction mediates hereditary

hemorrhagic telangiectasia. *The Journal of cell biology*. 2016;214:807-816. doi: 10.1083/jcb.201603106

19. Moser M, Binder O, Wu Y, Aitsebaomo J, Ren R, Bode C, Bautch VL, Conlon FL, Patterson C. BMPER, a novel endothelial cell precursor-derived protein, antagonizes bone morphogenetic protein signaling and endothelial cell differentiation. *Mol Cell Biol*. 2003;23:5664-5679. doi: 10.1128/MCB.23.16.5664-5679.2003
20. Satomi-Kobayashi S, Kinugasa M, Kobayashi R, Hatakeyama K, Kurogane Y, Ishida T, Emoto N, Asada Y, Takai Y, Hirata K, et al. Osteoblast-like differentiation of cultured human coronary artery smooth muscle cells by bone morphogenetic protein endothelial cell precursor-derived regulator (BMPER). *The Journal of biological chemistry*. 2012;287:30336-30345. doi: 10.1074/jbc.M111.329110
21. Moreno-Miralles I, Ren R, Moser M, Hartnett ME, Patterson C. Bone morphogenetic protein endothelial cell precursor-derived regulator regulates retinal angiogenesis in vivo in a mouse model of oxygen-induced retinopathy. *Arteriosclerosis, thrombosis, and vascular biology*. 2011;31:2216-2222. doi: 10.1161/ATVBAHA.111.230235
22. Pi X, Lockyer P, Dyer LA, Schisler JC, Russell B, Carey S, Sweet DT, Chen Z, Tzima E, Willis MS, et al. Bmper inhibits endothelial expression of inflammatory adhesion molecules and protects against atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2012;32:2214-2222. doi: 10.1161/ATVBAHA.112.252015
23. Helbing T, Rothweiler R, Ketterer E, Goetz L, Heinke J, Grundmann S, Duerschmied D, Patterson C, Bode C, Moser M. BMP activity controlled by BMPER regulates the proinflammatory phenotype of endothelium. *Blood*. 2011;118:5040-5049. doi: 10.1182/blood-2011-03-339762
24. Mao H, Li L, Fan Q, Angelini A, Saha PK, Wu H, Ballantyne CM, Hartig SM, Xie L, Pi X. Loss of bone morphogenetic protein-binding endothelial regulator causes insulin resistance. *Nature communications*. 2021;12:1927. doi: 10.1038/s41467-021-22130-2
25. van Golde JM, Ruiter MS, Schaper NC, Voo S, Waltenberger J, Backes WH, Post MJ, Huijberts MS. Impaired collateral recruitment and outward remodeling in experimental diabetes. *Diabetes*. 2008;57:2818-2823. doi: 10.2337/db08-0229
26. Niiyama H, Huang NF, Rollins MD, Cooke JP. Murine model of hindlimb ischemia. *Journal of visualized experiments : JoVE*. 2009. doi: 10.3791/1035
27. Yan J, Tie G, Park B, Yan Y, Nowicki PT, Messina LM. Recovery from hind limb ischemia is less effective in type 2 than in type 1 diabetic mice: roles of endothelial nitric oxide synthase and endothelial progenitor cells. *Journal of vascular surgery*. 2009;50:1412-1422. doi: 10.1016/j.jvs.2009.08.007
28. Deng H, Min E, Baeyens N, Coon BG, Hu R, Zhuang ZW, Chen M, Huang B, Afolabi T, Zarkada G, et al. Activation of Smad2/3 signaling by low fluid shear stress mediates artery inward remodeling. *Proceedings of the National Academy of Sciences of the United States of America*. 2021;118. doi: 10.1073/pnas.2105339118
29. Boon RA, Horrevoets AJ. Key transcriptional regulators of the vasoprotective effects of shear stress. *Hamostaseologie*. 2009;29:39-40, 41-33.
30. Kelley R, Ren R, Pi X, Wu Y, Moreno I, Willis M, Moser M, Ross M, Podkowa M, Attisano L, et al. A concentration-dependent endocytic trap and sink mechanism converts Bmper from an activator to an inhibitor of Bmp signaling. *The Journal of cell biology*. 2009;184:597-609. doi: 10.1083/jcb.200808064
31. Villarreal G, Jr., Zhang Y, Larman HB, Gracia-Sancho J, Koo A, Garcia-Cardena G. Defining the regulation of KLF4 expression and its downstream transcriptional targets in vascular endothelial cells. *Biochem Biophys Res Commun*. 2010;391:984-989. doi: 10.1016/j.bbrc.2009.12.002
32. Moonen JR, Chappell J, Shi M, Shinohara T, Li D, Mumbach MR, Zhang F, Nair RV, Nasser J, Mai DH, et al. KLF4 recruits SWI/SNF to increase chromatin accessibility and reprogram the endothelial enhancer landscape under laminar shear stress. *Nature communications*. 2022;13:4941. doi: 10.1038/s41467-022-32566-9

33. Sissaoui S, Yu J, Yan A, Li R, Yukselen O, Kucukural A, Zhu LJ, Lawson ND. Genomic Characterization of Endothelial Enhancers Reveals a Multifunctional Role for NR2F2 in Regulation of Arteriovenous Gene Expression. *Circulation research*. 2020;126:875-888. doi: 10.1161/CIRCRESAHA.119.316075
34. Ola R, Dubrac A, Han J, Zhang F, Fang JS, Larrivee B, Lee M, Urarte AA, Kraehling JR, Genet G, et al. PI3 kinase inhibition improves vascular malformations in mouse models of hereditary haemorrhagic telangiectasia. *Nature communications*. 2016;7:13650. doi: 10.1038/ncomms13650
35. Ruiz S, Zhao H, Chandakkar P, Papoin J, Choi H, Nomura-Kitabayashi A, Patel R, Gillen M, Diao L, Chatterjee PK, et al. Correcting Smad1/5/8, mTOR, and VEGFR2 treats pathology in hereditary hemorrhagic telangiectasia models. *The Journal of clinical investigation*. 2020;130:942-957. doi: 10.1172/JCI127425
36. Bidart M, Ricard N, Levet S, Samson M, Mallet C, David L, Subileau M, Tillet E, Feige JJ, Bailly S. BMP9 is produced by hepatocytes and circulates mainly in an active mature form complexed to its prodomain. *Cell Mol Life Sci*. 2012;69:313-324. doi: 10.1007/s00018-011-0751-1
37. Neuhaus H, Rosen V, Thies RS. Heart specific expression of mouse BMP-10 a novel member of the TGF-beta superfamily. *Mech Dev*. 1999;80:181-184. doi: 10.1016/s0925-4773(98)00221-4
38. Hazarika S, Dokun AO, Li Y, Popel AS, Kontos CD, Annex BH. Impaired angiogenesis after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth factor receptor 1 and soluble vascular endothelial growth factor receptor 1. *Circulation research*. 2007;101:948-956. doi: 10.1161/CIRCRESAHA.107.160630
39. Ola R, Kunzel SH, Zhang F, Genet G, Chakraborty R, Pibouin-Fragner L, Martin K, Sessa W, Dubrac A, Eichmann A. SMAD4 Prevents Flow Induced Arteriovenous Malformations by Inhibiting Casein Kinase 2. *Circulation*. 2018;138:2379-2394. doi: 10.1161/CIRCULATIONAHA.118.033842
40. Banerjee K, Lin Y, Gahn J, Cordero J, Gupta P, Mohamed I, Graupera M, Dobreva G, Schwartz MA, Ola R. SMAD4 maintains the fluid shear stress set point to protect against arterial-venous malformations. *The Journal of clinical investigation*. 2023;133. doi: 10.1172/JCI168352
41. Bernabeu C, Bayrak-Toydemir P, McDonald J, Letarte M. Potential Second-Hits in Hereditary Hemorrhagic Telangiectasia. *J Clin Med*. 2020;9. doi: 10.3390/jcm9113571
42. Korshunov VA, Schwartz SM, Berk BC. Vascular remodeling: hemodynamic and biochemical mechanisms underlying Glagov's phenomenon. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:1722-1728. doi: 10.1161/ATVBAHA.106.129254
43. Schaper W. Collateral circulation: past and present. *Basic Res Cardiol*. 2009;104:5-21. doi: 10.1007/s00395-008-0760-x
44. Simons M, Eichmann A. Molecular controls of arterial morphogenesis. *Circulation research*. 2015;116:1712-1724. doi: 10.1161/CIRCRESAHA.116.302953
45. Chen H, Brady Ridgway J, Sai T, Lai J, Warming S, Chen H, Roose-Girma M, Zhang G, Shou W, Yan M. Context-dependent signaling defines roles of BMP9 and BMP10 in embryonic and postnatal development. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110:11887-11892. doi: 10.1073/pnas.1306074110
46. Fisher OS, Deng H, Liu D, Zhang Y, Wei R, Deng Y, Zhang F, Louvi A, Turk BE, Boggon TJ, et al. Structure and vascular function of MEKK3-cerebral cavernous malformations 2 complex. *Nature communications*. 2015;6:7937. doi: 10.1038/ncomms8937
47. Deng H, Xu Y, Hu X, Zhuang ZW, Chang Y, Wang Y, Ntokou A, Schwartz MA, Su B, Simons M. MEKK3-TGFbeta crosstalk regulates inward arterial remodeling. *Proceedings of the National Academy of Sciences of the United States of America*. 2021;118. doi: 10.1073/pnas.2112625118
48. Deng H, Schwartz MA. High Fluid Shear Stress Inhibits Cytokine-Driven Smad2/3 Activation in Vascular Endothelial Cells. *Journal of the American Heart Association*. 2022;11:e025337. doi: 10.1161/JAHA.121.025337