Journal Pre-proofs

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Please cite this article as: M. Tiezzi, H. Deng, N. Baeyens, Endothelial mechanosensing: a forgotten target to treat vascular remodeling in hypertension?, *Biochemical Pharmacology* (2022), doi: https://doi.org/10.1016/j.bcp. 2022.115290

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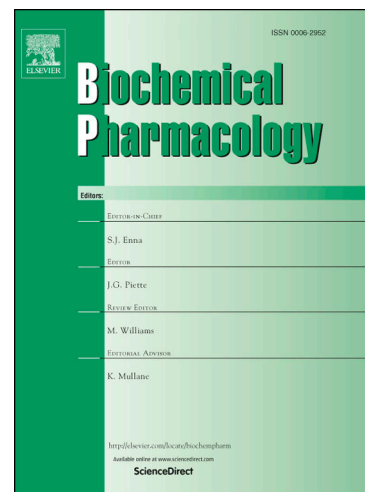
Endothelial mechanosensing: a forgotten target to treat vascular remodeling in hypertension?

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Abstract

The endothelium is a mechanosensitive organ whose pleiotropic actions regulate vessel structure to adjust tissue perfusion. To do so, it possesses ion channels, receptor complexes, and signaling pathways responding to blood flow, whose activation will either maintain vascular integrity and quiescence or, on the contrary, remodel the vessel's structure in both health and disease. Recent studies have demonstrated the crucial role of endothelial inflammation, endothelial to mesenchymal transition (EndMT), and perturbed hemodynamics in the progression of pulmonary arterial hypertension and essential hypertension. These two distinct diseases share some common mechanistic cues, pointing towards new potential therapeutic approaches to treat them. In this review, we summarize these common mechanisms to map future drug development strategies targeting flow sensing mechanisms and vascular remodeling.

Keywords: mechanotransduction, blood flow, shear stress, vascular remodeling, pulmonary hypertension, systemic hypertension, EndMT, TGF-beta signaling, BMP9

List of abbreviations:

ACVRL1: activin A receptor type II-like 1
ATII: angiotensin II
ATP: adenosine triphosphate
BMP: bone morphogenetic protein
BMPR: BMP receptor
BP: blood pressure
CCM: cerebral cavernous malformations
CHD: congenital heart disease
CSA: cross-sectional area
CTD: connective tissue disease
CTEPH: chronic thromboembolic pulmonary heart
ECM: extracellular matrix
ECs: endothelial cells
EDHF: endothelial-derived hyperopolarizing factor
EndMT: endothelial to mesenchymal transition
eNOS: inducible nitric oxide synthase

ERA: endothelin-1 Receptor antagonists
ET-1: endothelin-1
FSS: fluid shear stress
GDF2: growth/differentiation factor 2
GMP: guanosine monophosphate
GPCRs: G protein-coupled receptors
HHT: haemorrhagic telangiectasia
HSP: heat shock protein
HT: systemic hypertension
ICAM-1: intercellular adhesion molecule 1
KLF: Kurppel-like factor
LSS: laminar shear stress
MCP-1: monocyte chemoattractant protein-1
MEKK: mitogen-activated protein kinase/extracellular signal-regulated kinase kinase
mPAP: mean pulmonary arterial pressure
MRI: magnetic resonance
NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells
NO: nitric oxide
NRP-1: neuropilin 1
OSS: oscillatory shear stress
PAH: pulmonary arterial hypertension
PDE5-i: phosphodiesterase 5-inhibitors (PDE5-i)
PH: pulmonary hypertension
PECAM-1: platelet endothelial cell adhesion molecule
PGI2: prostacyclin
PVOD: pulmonary veno-occlusive disease
SMA: smooth muscle actin
SNP: sodium nitroprusside
TGFβ: transforming Growth Factor beta
TGFBR: TGFβ receptor
TXA2: thromboxane A2
VEGFR3: vascular endothelial growth factor receptor 3
VSMCs: vascular smooth muscle cells
WSS: wall shear stress.

1. Introduction

Systemic hypertension and pulmonary arterial hypertension (PAH) are two distinct diseases developing in networks characterized by different physiology, different pressure conditions (high pressures in the systemic circuit and low pressures in the pulmonary circuit), and different vessel compliance. In clinical practice, these two entities, predominantly found in distinct patient populations, rarely overlap, as their aetiologies differ. However, recent evidence from fundamental research studies points to common mechanisms triggering abnormal vascular remodeling events in these respective vascular networks. These recent advancements map out a path to navigate the field of vascular remodeling and endothelial dysfunction to unleash new therapeutic approaches.

This review aims to shed light on the mechanisms responsible for vascular remodeling and endothelial flow sensing alterations and their contribution to the development of systemic and pulmonary hypertension. We will pay particular attention to shared biomolecular mechanisms as prospective targets for future therapies.

2. Normal endothelial function and vascular homeostasis

The endothelium is the layer of cells covering the inner part of blood vessels (arteries, capillaries, veins) and lymphatic vessels. This squamous monolayer is composed of mononucleated elongated cells, called endothelial cells (ECs), that align towards the direction of blood (or lymphatic) flow and are the interface between the bloodstream and the vessel wall, which makes them capable of perceiving signals, integrating them and of adapting their response accordingly. Far from being only a passive barrier, the endothelium is now considered a dynamic organ capable of interacting with the environment and preserving vessels' homeostasis through a coherent response to damage and the capability to modulate and contain this response. The endothelium orchestrates vessel tone and structure, coagulation, local inflammation, and even lipids deposition. To do so, endothelial cells produce several mediators that display pleiotropic actions. The most important factors that influence endothelial responses are Nitric Oxide (NO)[1], generated by eNOS, but also ATP[2], Prostacyclin (PGI2)[3], Endothelial-Derived Hyperpolarizing Factor (EDHF)[4], Endothelin-1 (ET-1)[5], Thromboxane A2 (TXA2)[6] and Angiotensin II (AT-II)[7]. Table 1 summarizes the function of these mediators.

Mediator	Effect
NO	Vasodilation Anti-inflammatory Anti-coagulant
ATP	Vasodilation
PGI2	Vasodilation
EDHF	Vasodilation
ET-1	Vasoconstriction Pro-inflammatory Pro-coagulant
TXA2	Vasoconstriction Platelets aggregation
AT-II	Vasoconstriction Pro-inflammatory Pro-coagulant

Table 1: primary mediators produced by endothelial cells and their actions. NO: Nitric Oxyde; PGI2: Prostacyclin; EDHF: Endothelial-Derived Hyperpolarizing Factor; ET-1: Endothelin-1; TXA2: Thromboxane A2; AT-II: Angiotensin II. Different mediators can be present simultaneously but at different concentrations, depending on the type and entity of the stimulus.

The normal endothelial function requires ECs to ensure basal NO release, resulting in a chronic low-grade stimulus that promotes vasodilation and lowers local inflammation. The main effectors of vasoconstriction and vasodilation are vascular smooth muscle cells (VSMCs), localized in the vessels' media, right behind the endothelial cell layer and the basement membrane. The vasodilation can happen either via the action of mediators produced by endothelial cells (the so-called *endothelium-dependent vasodilation*) (see Table 1) or via exogenous mediators like sodium nitroprusside (SNP) or nitroglycerin which are not produced by the human body but are used in clinical practice as potent vasodilators. These exogenous factors act directly on VSMCs to promote vasodilation, regardless of the presence of a functional endothelium (the so-called *endothelium-independent vasodilation*).

3. Fluid shear stress sensing and vessel homeostasis

The endothelium is continuously exposed to blood flow, which profoundly influences ECs behaviour[8,9]. In steady state conditions, blood exerts on ECs both a frictional force parallel to the vessels' wall and secondary to blood flow (called *fluid shear stress* or *wall shear stress, FSS or WSS*) and another force perpendicular to this wall and secondary to the pressure exerted by blood. FSS magnitude is determined by flow velocity and

blood viscosity and is inversely correlated to vessel diameter. Recent evidence shows that any changes in FSS will translate into a local endothelial response to adapt to new flow conditions. Indeed, high throughput, genome-wide gene expression studies demonstrated that FSS directly regulates the transcription of thousands of genes in ECs, pointing to a central regulatory role of FSS in endothelial function and homeostasis[10]. Another example of this flow-induced plasticity is capillary remodeling, where pruning of capillary branches is observed in areas of decreased flow, while capillary splitting is seen in regions of increased blood flow[11]. Also, endothelial cells directly control vasodilation, proportionally to the magnitude of FSS in blood vessels[12-14].

FSS parameters vary within different regions in the cardiovascular system: ECs are exposed to laminar shear stress (LSS) in straight segments of vessels where the flow is unidirectional; in contrast, ECs are exposed to oscillatory shear stress (OSS) in vessel branches, bifurcations, and curvatures, with the flow usually being low and multidirectional. Physiologic, unidirectional, laminar flow suppresses cell cycle progression[15] and promotes the expression of anti-inflammatory and anti-oxidative genes[16,17]. On the other hand, low and disturbed flow stimulates cell proliferation and turnover and promotes the expression of inflammatory and oxidative genes[18,19]. These subtle regulations ensure sufficient oxygen and nutrient distribution to organs in healthy conditions. Disturbed flow promotes drastic changes in endothelial phenotype and vessel architecture. Thus, laminar, unidirectional flow with physiological values of shear stress intensities is atheroprotective. In contrast, low flow combined with the multidirectional flow is involved in the onset and progression of atherosclerosis and neointima proliferation[20] and is often labeled as "atheroprone"[21].

Vascular remodeling is defined by the spectrum of modifications of vessel structure[22] occurring because of biochemical, genetic, and morphologic alterations in vessel walls. It is a complex process involving changes in the structure of the intima, the elastic lamina, and/or the media and adventitia of the vessel. Vessel remodeling contributes to the progression of various cardiovascular diseases, such as hypertension, atherosclerosis, and aneurysms. It is also an essential physiological mechanism, where remodeling allows blood vessels to meet the demand of tissues to modify their structure/organization to receive appropriate perfusion with maximal efficiency, such as adaptation to exercise[23]. There are two main types of vascular remodeling: *inward* and *outward remodeling,* characterized by a reduction or increased vessel caliber. Inward remodeling describes non-dilated, stiffer vessels with VSMCs and matrix rearrangement around a narrowed lumen. Outward remodeling sees media rearrangements directed outwards, thus dilating vessels and their lumen. Both remodeling types can be divided into hypertrophic, eutrophic, or hypertrophic, depending on the intima's or the media's proliferation. Depending on the kind of insult, flow conditions in different vessels' districts, and vessels' wall characteristics, these alterations can be found in the same patient at distinct locations and various stages of the disease.

The way vessels sense and react to flow alterations is the object of ongoing scientific research identifying cellular structures, receptor complexes, ion channels, and signaling pathways responding to blood flow. An important player in the flow sensing process is the $P2Y_2$ receptor, located on the endothelial cell surface and coupled to G_q and G_{11} proteins. This receptor – activated by physiological values of flow – mediates an intracellular calcium influx and further activates two distinct pathways that converge to the endothelial nitric oxide synthase (eNOS) phosphorylation[24]. These pathways are represented by PECAM-1/mTORC and PKN2, and result in the phosphorylation of eNOS at different sites[25], directly controlling NO production. Importantly, PECAM-1 is a member of an endothelial-specific junctional complex together with VEGF receptors and VE-cadherin, and it is formed following PECAM-1 phosphorylation induced by laminar flow[26]. Interestingly, PECAM-1 and VE-cadherin are bearing force through their association with the cytoskeleton, and upon application of flow, tension across junctional VE-cadherin decreases, while tension across PECAM-1 increases[27], linking directly mechanical force transduction to this receptor complex. This junctional complex directly modulates cell-to-cell junctions, vasodilation but also local inflammation, as it was also shown to be involved in generating inflammatory signals and atherosclerosis through NF-kB stimulation while exposed to

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disturbed flow[28]. Flow sensing, cell adhesion and inflammation are deeply linked cellular responses, mainly when blood flow is disturbed, and explain the association between altered shear stress, inflammation, and vascular remodeling.

Vessel homeostasis is tuned by a FSS "set point", represented by FSS values within a physiological range, that contribute to the maintenance of typical vessel architecture and function. Typical values of FSS in healthy humans range from highly pulsatile 1-4 Pascals (Pa; 10-40 dynes/cm2) in arteries, 0.1-0.6 Pa (1-6 dynes/cm2) with low pulsatility in veins, and even lower values in lymphatic vessels. Within these values, ECs align in the flow direction, and there is active repression of inflammatory NFkB signaling and activation of Smad1 signaling, contributing directly to vessel quiescence (Figure 1A-B). On the other hand, changes in FSS magnitude outside this set point trigger vessel remodeling to restore initial levels of FSS by altering lumen diameter[29-31]. An impressive example of vessel remodeling in response to a sustained increase in FSS magnitude is autologous arteriovenous fistulas (AVFs), a surgical procedure connecting an artery to a vein, which is used to create an artificial peripheral shunt, providing a useful vascular access with increased blood supply for hemodialysis. In AVFs, the blood flowing in the artery – much faster and circulating at higher pressures than in the veins - triggers a consistent physiological outward remodeling of the vessel, associated with increased blood supply[32,33]. Sometimes, this remodeling does not resolve on the venous side of AVFs and is one of the leading causes of AVFs occlusion over time, a frequent phenomenon in chronically hemodialyzed patients, leading to further re-interventions. An important article by Alan Dardik's group showed that whenever we create AVFs that minimize vessel deformation and create a more stable increase in FSS than the standard procedure (i.e. RADAR procedure), this is associated with greater success, reduced neo-intima formation, increased eNOS activation, and Kruppel-like factor (KLF2) transcription[34], meaning that FSS sensation and mechanotransduction are required for adequate vessel remodeling. Contrastingly, improper flow characteristics lead to abnormal remodeling, consecutive to the activation of pathological flow sensing pathways.

Of interest, we have linked this FSS set point to the expression of vascular endothelial growth factor receptor 3 (VEGFR3)[30], a member of a mechanosensory junctional complex comprising PECAM-1, VEGFRs and VE-Cadherin[26,35]. Vascular endothelial growth factor (VEGF) and VEGF receptors are traditionally associated both with angiogenesis (the physiological development of new blood vessels) and neoangiogenesis (pathological vessels' formation in cancer). This could suggest their role as positive contributors to vascular homeostasis and flow sensing, as supported by the evidence that their pharmacological inhibitors (used in cancer treatment) are known to provoke hypertension and trigger inward remodeling in treated patients[36]. Although endothelial dysfunction is often highlighted as a probable cause for this secondary effect, few authors consider the likelihood that the main alteration could indeed be altered steady-state FSS sensing. This would lead to an imbalance in NO release and transcriptional changes in ECs, triggering increased blood pressure and pathological remodeling. Indeed, when vessels display decreased sensitivity to FSS, this would lead to inward remodeling to increase FSS so that flow can be sensed by the endothelial cells. This adverse effect is dose-dependent and reversible upon treatment arrest. Similarly, the abovementioned mechanosensory junctional complex (PECAM-1, VEGFR3 and VE-Cadherin), located at the junctions between endothelial cells, has demonstrated an essential role in sensing directly FSS and to contribute to the progressive NO release in response to increased FSS[37]. As VEGFR3 expression is much higher in vessels with low flow values, such as lymphatic vessels, we propose that they allow for greater sensitivity and lower set points in the lymphatic system. Thus, the FSS set point is not a constant but depends on local flow properties and the expression of mechanosensors in the ECs.

Flow sensing also directly contributes to the architecture of capillaries, notably by pruning under perfused branches of the network[38]. That is, ECs migrate away from areas of low FSS to areas of higher FSS, where they polarize in flow direction and stabilize the correctly perfused network[39].

Thus, the endothelium is a mechanosensitive organ fine-tuning a ballet of cellular responses to modulate vascular perfusion according to tissue needs.

3.1 BMP signaling: a master regulator a flow-dependent vessel remodeling

Transforming Growth Factor beta (TGFβ) is the prototypical member of a vast family of cytokines and growth factors that promote various cell and tissue responses, transduced via receptors complexes present at the cell surface and through the activation of Smad transcription factors[40]. TGFβ receptor 1 (TGFBR1), also known as Alk5, is the primary type I receptor and it is associated to TGFBRII, a type II receptor which directly binds TGFβ and phosphorylates Alk5. Alk5 is a serine/threonine-kinase receptor that further activates the phosphorylation of Smad2 and Smad3 in response to TGFβ, which translocate in the nucleus after association with a co-Smad partner, Smad4, where they act as transcription factors. TGFβ actions are broad and tissue-dependent and vary from extracellular matrix production and tissue fibrosis, cell growth, differentiation and development, to cell arrest and apoptosis[40]. (Figure 2)

In vascular cell homeostasis, TGFβ has been reported to act both via Alk5 and Alk1-endoglin, eliciting opposite effects in a highly context-dependent manner[41]. Even if current evidence points out the discordant effect of TGFβ signaling on the endothelium, its global and broad impact on ECs could be summarized as helping to keep the balance between ECs proliferation and quiescence.

Bone morphogenetic protein 9 and 10 (BMP9 and BMP10) are two secreted cytokines belonging to the TGFβ superfamily. Discovered initially as mediators promoting bone and cartilage formation, they are synthesized as inactive precursors by the liver and right atrium and are critical players in vascular homeostasis[42]. These cytokines are organized in the form of dimers and can associate as homo or heterodimers[43]. The primary receptor complex identified for BMP9 and BMP10 signaling is formed by Alk1, BMPR2 and endoglin (Figure 2). Mutations in the ligands, the receptors or Smad4 have been associated with severe congenital vascular disorders: pulmonary hypertension[44,45], haemorrhagic telangiectasia (HHT)[46-49] and patent ductus arteriosus [50]. These aetiologies are vastly different but share a common feature: they are abnormal vascular remodeling events, highlighting the essential role of this signaling pathway in vascular homeostasis.

Given their action on cells that are located at the interface with blood, it is not surprising that BMP9/10 signaling was shown to be associated with flow sensing and flow-mediated vascular adaptations. First, Smad1 activity was measured as being maximal in response to physiological values of flow associated with the FSS set point[30] (Figure 1B). Further, we showed that flow induces Alk1 and endoglin association, increasing BMP9 signalling potency. This was shown to be essential to inhibiting endothelial cell proliferation and promoting vascular stability and quiescence when the flow is within a normal range[51]. That is, very high or low shear stress would impair this pathway in an unknown way, leading to ECs proliferation and altered remodeling that are tightly linked to atherogenesis or vascular malformations. An important observation was described by Holger Gerhardt's group: Smad1 signaling can be activated by low shear stress if and only if the endothelial cell arbors a primary cilium[52]. In this specific case, a different receptor complex would be formed, allowing for this essential stabilization pathway to be activated to promote capillary vessel stabilization even if lowly perfused. This observation demonstrates that cell surface receptor complexes induced by flow are critical to understanding the wide variety of signaling responses in ECs. This also raise an important concern for drug development strategies based on testing on endothelial cells in dishes, without flow: are the outcomes of those screenings relevant to human physiology?

In a recent study, we showed that Smad2/3 nuclear translocation is maximal under low FSS (1-5 dynes/cm2) and then decreases significantly as FSS reaches the physiological range (>12 dynes/cm2). This low fluid shear stress-mediated Smad2/3 activation directly induces inward arterial remodeling [53] (Figure 1). Low shear

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stress activates Smad2/3 through transforming growth factor (TGF)-β type Ⅰ receptor Alk5 and co-receptor Neuropilin-1 (Nrp1), leading to inward remodelling and, surprisingly, this required BMP9 but not BMP10. This contrasts with the redundant role of BMP9 and BMP10 in activation of the Alk1-endoglin complex and might be explained by a different affinity for hetero or homodimers of BMPs for each receptor complex, as BMP9-BMP10 heterodimers are the most abundant dimers circulating in healthy individuals' plasma. Nonetheless, recent research showed that selective BMP9 inhibition had a beneficial impact in preclinical models of pulmonary hypertension[54], hinting for a crucial need to characterize the exact nature of the circulating dimers in health and disease.

The dichotomy between the Alk1/Smad1,5,9 and Alk5/Smad2,3 signaling with respect to flow intensity and remodeling is explained by the existence of a negative feedback loop targeting directly smad2,3[53,55]. Physiological values of flow are known to activate the junctional mechanosensory complex and other mechanosensors such as Piezo1 and G protein-coupled receptors (GPCRs) to control vasoreactivity through NO production [14]. A flow-specific signaling cascade has been identified subsequent to the activation of the junctional complex and Piezo1, leading to the activation of a MAPK cascade[56]. Evidence showed that the anti-inflammatory effect of physiological laminar flow is mediated through activation of ERK5[57-59]. ERK5 subsequently contributes to the transcription of two atheroprotective genes, KLF2 and KLF4. Kruppel-like factors (KLF) are a family composed of 18 different factors that are zinc-finger proteins largely expressed in different tissues and organs. KLF2 (and later, KLF4) has been identified as a key player in vascular homeostasis and fluid shear sensing. FSS induces KLF2 in a dose-dependent fashion [60-62], and this induction further promotes the expression of genes involved in governing vascular homeostasis and, more prominently, eNOS[63].

Physiological values of FSS significantly reduce smad2,3 activation, and we demonstrated that this could be explained by controlling the phosphorylation of the linker region of those Smads. Indeed, when phosphorylated, this region prevents nuclear translocation of Smad2/3 and inhibits their activation, thus resulting in vessel homeostasis. This specific phosphorylation is promoted by CDK2, whose activity is directly controlled by a MEKK3/ERK5/KLF2 axis. Inhibition of CDK2 with flavopiridol reversed the inhibition of smad2/3 translocation in the nucleus at physiological values of shear stress 4 hours after administration. Chronic in vivo administration of this compound in healthy animals leads to the spontaneous development of pulmonary hypertension after three weeks, supporting the protective role of CDK2 and the major contribution of flow-induced Smad2/3 signaling in the development of pulmonary hypertension[53]. Endothelial-specific deletion of MEKK3, a kinase localized upstream of ERK5 and CDK2, released the inhibition of Smad2/3 and led to Smad2/3 nuclear translocation, promoting inward remodeling in both pulmonary and arterial circuits and resulting in spontaneous pulmonary arterial hypertension and systemic hypertension, confirming perfectly our initial results[64]. Therefore, a direct association exists between low flow-mediated activation of Smad2/3 signaling and the development of both aetiologies.

Activation of Smad2/3 signaling triggers Endothelial to Mesenchymal Transition (EndMT). This phenotype transition refers to metabolic and phenotypic reprogramming of endothelial cells towards a mesenchymal phenotype. When exposed to different stimuli (TGFβ, FGF), endothelial cells can lose endothelial cell markers and acquire mesenchymal characteristics helpful to respond to an insult[65]. This results in several pathological consequences of considerable clinical significance in diseases ranging from atherosclerosis to Cerebral Cavernous Malformations (CCM) or pulmonary arterial hypertension (PAH)[66-68]. Undergoing EndMT, endothelial cells acquire properties of mesenchymal cells (fibroblasts, smooth muscle cells), including enhanced expression of mesenchymal markers (fibronectin, N-Cadherin, collagen) and smooth muscle markers (SMA, SM22α, SM-Calponin), as well as increased expression of various leukocyte adhesion molecules. In doing so, they also alter their cell-to-cell interactions, detach from the endothelial monolayer and migrate from intima to media acquiring the capability to infiltrate the vessel wall and induce tissue

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fibrosis and stiffening of the vessel. Both forms of hypertension reviewed in this article have been associated with EndMT, underlying a possible shared pathway leading to vascular abnormalities.

3.2 YAP/TAZ: flow-dependent transcriptional coactivator

Other mediators for mechanosensing are represented by Yes-associated protein (YAP) and its ortholog transcriptional coactivator with a PDZ-binding domain (TAZ), known as final effector proteins of Hippo signaling pathway[69,70]. YAP/TAZ shuttles between cytoplasm and nucleus, where it binds to transcription factors, mainly transcriptional enhancer factor domain (TEAD), to regulate cell proliferation and regeneration, wound healing, cell lineage fate, and mechanosensing. YAP/TAZ can exert such a wide spread of actions through the alternance of cytoplasmic or nuclear translocation and its phosphorylated or unphosphorylated forms[71]. Endothelial YAP/TAZ responses to shear stress and its localization within the cell are differentially regulated by shear stress patterns. Oscillatory shear stress promotes YAP/TAZ nuclear translocation, while physiological laminar shear stress moves YAP/TAZ out of the nucleus[72]. YAP/TAZ-dependent mechanotransduction induces pulmonary vascular proliferation and remodeling, alters glutaminolysis, and finally contributes to pulmonary hypertension[73]. A recent study also reported that YAP/TAZ-TEAD pathway promotes EC growth and proliferation by increasing nutrient uptake and usage[74].

3.3 Piezo1: a Dr Jekyll/Mr Hyde mechanosensor in vascular remodeling?

Piezo1 is a recently described mechanosensitive ion channel and its discovery as a mechanoreceptor for touch sensation has been awarded the 2021 Nobel Prize in Physiology and Medicine[75,76]. It is activated by mechanical stretch of the plasma membrane and mediates a cations influx within the stretched cell. In endothelial cells, Piezo1 is a fluid shear stress mechanosensor, required during early vascular development[77,78] but also involved in various pathologies[79,80]. Global knockout of Piezo1 in mice is embryonically lethal because of impaired development of blood vessels[77,78]. Recent evidence hints at a role of Piezo1 both in HT and PAH: endothelial Piezo1 activity is required for flow-induced adenosine triphosphate (ATP) release and vasodilation and, consequently, inducible endothelial-specific deficiency of Piezo1 in mice leads to spontaneous systemic hypertension[79]. On the other hand, two recent studies found that Piezo1 expression is upregulated in pulmonary arterial endothelial cells (PAECs) from patients and animal models with idiopathic pulmonary arterial hypertension[81,82]. This upregulation is associated with a change of the ECs phenotype, with increased expression of mesenchymatous markers. These two conflicting results somehow highlight that Piezo1 activity is finely regulated and, while playing a role in the maintenance of vascular homeostasis, its overactivation would open the gates for global endothelial dysfunction. This could be explained by the very fast inactivation of the channel[75,76,83], which most likely prevents a massive entry of cations in the cell in response to physiological stimuli. Although highly attractive, pharmacological modulation of such an essential mechanosensor would most likely be too risky for the patients as both inhibition or overactivation would lead to extensive vascular remodeling and possible toxicity.

3.4 PKN2: a new mediator for flow-induced NO release and blood pressure

Protein kinase N2 (PKN2) is a serine/threonine kinase with an important role in cell cycle progression, actin cytoskeleton assembly, cell migration, cell adhesion, and has been recently reported that it can be phosphorylated and activated by physiological shear stress through Piezo1 and G_q/G_{11}-mediated signaling[25]. Activated PKN2 promotes endothelial NOS (eNOS) phosphorylation and increases eNOS activity and NO release. More interestingly, mice with endothelial-specific PKN2 knockout develop systemic hypertension with reduced eNOS activation[25], revealing a new mechanism how flow shear stress regulates eNOS and blood pressure[84].

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4. Vascular remodelling in the context of pulmonary hypertension and systemic hypertension

Different types of flow alterations can result in the activation of different biochemical pathways and different remodeling patterns (see Figure 1, Figure 3), possibly giving the rationale for future targeted therapies. In this chapter, we will try to summarize recent evidence of vessel remodeling both in systemic and pulmonary hypertension and provide clinical relevance for the mechanistic insights discussed above.

4.1 Systemic hypertension

Systemic hypertension (HT) is a chronic, highly prevalent disease defined by the presence of systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg at rest [85]. The prevalence of HT worldwide is estimated at 1.28 billion adults (https://www.who.int), and it represents a major cause of death. If non-controlled, HT progresses silently towards overt atherosclerosis, cardiovascular complications, and target organ damage, especially if other cardiovascular risk factors are present, which is often the case.

Hypertension is usually divided into "essential" hypertension and "secondary" hypertension. While in the first group hypertension is the expression of altered vascular remodeling, and the primary insult is directly in the vessels (often via the combination of cardiovascular risk factors), in "secondary" hypertension, there is always an identified and treatable upstream cause leading to increased blood pressure (hormones overproduction, renal disease, aortic coarctation, etc). Thus, vascular alterations and remodeling usually differ from "essential" hypertension [85,86]. Essential hypertension represents nearly 90% of the cases of systemic hypertension, and its cause is unknown. While approaching the review of scientific evidence on vascular remodeling and flow alterations in HT, it is hard not to mix studies on HT and atherosclerosis. This chapter aims to concentrate on the available scientific evidence of HT alone.

While previously considered a functional disease, where peripheral reduction in vascular caliber was mostly a result of a chronic adrenergic stimulus (vasoconstriction) and not of structural changes, current abundant evidence points out endothelial dysfunction and altered vascular remodeling as major players in the evolution of essential hypertension. Endothelial dysfunction is undoubtedly present in HT patients, and it translates into an impaired expression of constitutive endothelial nitric oxide synthase (eNOS)[87] and altered flow sensing. Diabetes, dyslipidemia, obesity, and active smoking represent confounding elements in the analysis of HT-induced endothelial dysfunction, as they all contribute to endothelial impairment and amplify the damage. In normal conditions, vasodilation is mediated by eNOS activation. Such activation occurs via two phosphorylation sites on the enzyme: serine 633, serine 1177 and – more recently discovered – serine 1179[25]. Different biochemical pathways regulate these phosphorylations: while the first is mediated mostly by protein kinase A (PKA) pathway, the latter is induced mostly by Akt (also called protein kinase B) and PECAM-1/VE-cadherin/PI3K/mTORC pathway (see Figure 4). It is currently unknown how and to which extent these respective pathways are impaired in systemic hypertension. However, animal studies selectively blocking these enzymes have shown the development of a hypertensive phenotype in mice via decreased NO biovailability[88]. But diminished vasodilation is not the unique feature in HT; vessels also show deep remodeling both in proximal and distal arteries. Main histological alterations in HT vessels are represented by: *inward eutrophic remodeling of arterioles*, *outward remodeling*, capillary rarefaction, large arteries remodeling (with increased intima-media thickness and enlarged lumen), and changes in distensibility mediated by calcium and extracellular matrix (ECM) deposition within the wall. All these alterations most likely contribute to the persistence of the disease, even when treated. *Inward eutrophic remodeling* represents an adaptive mechanism where the vessel re-arranges its media by repositioning vascular smooth muscle cells (VSMCs) through cytoskeletal modifications in response to prolonged low flow or persistent inflammation. Thus, the artery doesn't dilate (the cross-sectional area is preserved) but shows a decreased

lumen diameter and increased media-to-lumen ratio[89]. This remodeling is not observed in "secondary" forms of hypertension and most likely contributes to the "irreversible" nature of the disease.

On the other hand, *outward remodeling* can also occur as a response to elevated blood pressure whenever the vessel tries to minimize pressure overload. This type of remodeling consists of an increased cross-section of the vessel that could be (or not) associated with VSMCs hypertrophia/ hyperplasia and an increase in extracellular matrix (ECM) production. (Figure 3)**.** Depending on the type of insult (hypertension, plaques, aneurysm, dissection) and its duration, the vessels can respond either way. A hypertensive patient could thus display inward and outward remodeling simultaneously, usually in different vascular districts. Together with alteration in resistance vessels, essential HT also displays a reduction in vascular density in the capillary district, the so-called *capillary rarefaction* [90,91]. Capillary rarefaction consists of a decrease in the number of branches in the capillary network due to vascular changes and remodeling, as observed during vascular pruning[92]. This phenomenon starts with a reduction in capillary perfusion (*functional* rarefaction). It can evolve towards *structural* rarefaction with decreased number of distal microvessels and a further increase of total vascular resistance [93,94] (Figure 5)**.** It is a process whose timing and etiology are unclear but present in the early and late phases of the disease [95]. In vivo studies suggest an inverse correlation between capillary density, systolic blood pressure, and endothelial dysfunction, meaning that the lower the number of capillaries, the higher the systolic blood pressure (because of increased vascular resistances) and the endothelial dysfunction[90,96].

Flow studies in essential hypertension are not abundant. Still, given HT pathogenesis, where multiple risk factors induce arterial remodeling prior to the onset of increased systemic pressures, it is not surprising that some evidence describe decreased flow essential hypertension patients, associated with impaired endothelial-dependent vasodilation[97]. Globally, it seems that HT is primarily related to endothelial dysfunction, which leads to altered flow sensing and decreased shear stress because of vessel remodeling. The reduced shear stress is linked to the development of an atherogenic profile, vessel deformation and atherosclerosis, very often associated with HT and worsening systemic pressures. In particular, in the HT population, low FSS in the carotid arteries is associated with increased pathological remodeling[98], confirming that flow alterations are a key player in the self-sustaining vicious circle of HT and atherosclerosis. In fact, HT patients are very often affected with multiple cardiovascular risk factors linked to endothelial dysfunction, and they are more prone to vascular abnormalities and subsequently altered flow (in bifurcation zones, atherosclerotic lesions, narrow segments etc). Up to now, it is not clear whether flow alterations precede HT onset, sustain systemic hypertension after its onset, or both. But the link between shear stress alterations and systemic hypertension can be explored through the emerging scientific evidence on cellular mediators of flow sensing, obtained essentially by *in vitro* experiences or animal models. For example, when stimulated by physiological flow, the mechanosensor Piezo1 was shown to induce vasodilation through eNOS phosphorylation[14], while its selective inhibition induces HT in animal models[79], showing its role in intracellular transmission of disturbed flow, and the link of these alterations with the genesis and maintain of HT. Similarly, PKN2 (a serine threonine kinase described above and stimulated upstream by Piezo1) has recently shown a key role in modulating flow-induced cellular responses (*in vitro* study): in fact, in conditions of laminar flow, PKN2 directly promotes eNOS phosphorylation (serine 1177 and 1179 sites) via Akt pathway and, when inhibited, reduces NO production in an *in vitro* model on aortic endothelial cells, emerging as a crucial protein where different pathways converge to induce NO production in response to flow[25].

While endothelial to mesenchymal transition has been put in evidence in PAH (see next chapter), there is lacking evidence of a direct link between EndMT and essential hypertension. However, EndMT is a landmark feature in atherosclerosis, a condition often associated to HT. In particular, the exposure of cultured human endothelial cells to inflammatory cytokines and oscillatory shear stress results in the induction of TGFbeta pathway with concomitant loss of fibroblast growth factor (FGF) signalling, an alteration linked to atherosclerosis progression and to the transition of endothelial cells to a mesenchymal phenotype (i.e.

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EndMT)[68]. Further research is needed to characterize endothelial phenotype in different vascular districts of HT patients, precise the proteins involved and explore its link with altered flow and altered flow sensing.

4.2 Pulmonary hypertension (pre-capillary, Groupe 1)

Pulmonary hypertension is a progressive disease of the pulmonary circulation characterized by an increase in mean pulmonary arterial pressure (mPAP) > 20 mmHg at rest assessed by right heart catheterization[99]. This increase of mPAP is classically considered secondary to a remodeling process of distal pulmonary arteries, an obstruction at different levels of pulmonary arterial circulation or a consequence of a backward transmission of increased left heart pressures (as seen in left heart disease; even if this is recently a matter of debate[100]). Pulmonary hypertension is thus divided in "precapillary" or "postcapillary" forms, depending on the physiopathological mechanism leading to pressure increase in the pulmonary circulation. Based on the aetiology of increased mPAP, the European Society of Cardiology (ESC) guidelines divide pulmonary hypertension into 5 groups, all sharing increased pulmonary pressures but characterized by vastly different physiopathology, different treatment options, and different prognoses [85].

We will focus the object of this review to Groupe 1 pulmonary hypertension, which is commonly referred to as pulmonary arterial hypertension (PAH). This pathology is characterized by an important and irreversible remodeling of distal pulmonary arteries represented by plexiform lesions, and progressively responsible for distal stenosis and obstruction, leading to an increase of pulmonary pressure on the arterial side of pulmonary circulation ("pre-capillary" pulmonary hypertension). This form of PH is either idiopathic (iPAH, representing more than 50% of PAH cases), heritable (linked to Bone Morphogenetic Protein Receptor 2 mutations in 75% of the cases of the heritable form), drug or toxin-induced, or associated to other clinical entities like congenital heart disease (CHD), HIV infection, connective tissue disease (CTD), schistosomiasis or portal hypertension. In all these cases, the exact pathogenesis is often unclear, but sustained hypoxia, inflammation as well as genetic mutations and flow alterations seem to play a crucial role. Untreated PAH naturally evolves in right heart pressure overload with progressive congestion and ultimately global heart failure, representing – despite the low incidence – a disabling disease with a poor short-term prognosis that needs to be recognized and promptly treated.

Table 2 summarizes the different forms of pulmonary arterial hypertension (Group 1) as it is the main focus of this review compared to other forms of the disease. Given the lack of abundant evidence about vascular remodeling for each single subgroup of PAH, this review will put together available evidence for all the below-mentioned sub-types of Group 1 pulmonary hypertension.

Pulmonary arterial hypertension, PAH (Group"1)"pre-capillary" pulmonary hypertension	
1.1 Idiopathic	
1.2 Heritable	1.2.1 BMPR2 mutation 1.2.2 Other mutations (ACVRL1, Endoglin, GDF2)
1.3 Drug / toxins - induced	Anorexigenic drugs, fenfluramine, metamphetamines, etc
1.4 Associated with:	1.4.1 Connective Tissue Disease 1.4.2 HIV infection 1.4.3 Portal hypertension 1.4.4 Congenital Heart Disease

	1.4.5 Schistosomiasis
1.5 PAH with features of venous/capillary (PVOD/PCH) involvement	
1.6 Persistent PH of the newborn	

Table *2: PAH classification (Group 1), adapted from the 2015 European Society of Cardiology (ESC) guidelines[99].* *Pulmonary veno-occlusive disease, pulmonary capillary haemangiomatosis and persistent pulmonary hypertension of the newborn have been omitted for clarity.*

PAH is a disease of the totality of the pulmonary vascular tree, capable of altering all the layers of the vessel wall (intima, media, and adventitia) and promoting an *in situ* pro-inflammatory and pro-coagulant pattern that worsens vascular function and pulmonary pressures. Main peripheral histological alterations include media hypertrophy, arteriolar muscularization, intimal growth, fibrosis, and plexiform lesions. Such alterations occur mainly distally in pulmonary circulation, even if recent evidence underlines that even bigger arterial vessels and pulmonary veins/venules display vascular abnormalities. The common denominator between them is an endothelial dysfunction with ECs proliferation and the presence of hyperproliferative VSMCs capable of infiltrating the entire vessel wall, recruiting fibroblasts that differentiate into smooth muscle cells and contribute to intima fibrosis. An abundant amount of *in vivo* data exists on circulating levels of endothelial biomarkers or the enumeration of circulating endothelial cells in PAH. These studies, despite being conducted in different pulmonary hypertension populations and with different methodologies, confirm the presence of a shared endothelial involvement in PH, especially in pre-capillary forms, and link these alterations with worsening hemodynamics parameters and bad prognosis for the patient[101,102 103].The global spectrum of alterations of ECs through the different stages of the disease goes from endothelial damage and apoptosis in the early phases to increased ECs proliferation and apoptosis-resistance in later stages, until endothelial senescence in the last phases of the disease[104]. This is associated with increased *in situ* coagulability leading to pulmonary microthrombosis and vessel obstruction, globally witnessing of a dysfunctional endothelium with aberrant crosstalk between ECs, VSMCs, pericytes and fibroblasts in distal vessels[105]. The evolution of ECs behaviour (from injured to hyperproliferative and then senescent) could be explained by the fact that the damaged endothelium contributes to the increase in pulmonary arterial pressures (promoting vasoconstriction) and that the low FSS secondary to this increase amplifies the endothelial dysfunction to the point that in the late stages of the disease such vascular remodeling is not reversible anymore, as witnessed by increased number of apoptosis-resistant ECs in lungs of patients with irreversible PAH[106]. The endothelial disorganization is a hallmark of plexiform lesions, that are the typical histological alteration found in PAH. These lesions consist in dysregulated intima proliferation to constitute clusters of endothelial cells and inflammatory infiltration, with progressive obliteration of vascular lumen. They are the result of the hyperproliferative phase of ECs and are localized in distal arterioles, and seem to stand apart from other lesions, showing no significant variations between different forms of PAH, and seem to have unique characteristics and genetic signature as compared to similar lesions found in some types of cancer [107]. In particular, plexiform lesions were shown *ex vivo* to display different features as compared to glioblastoma lesions, especially the upregulation of key genes that promote angiogenesis and endothelial cells sprouting, such as hypoxia-inducible factor α (HIF α), TGFβ, VEGF, VEGFR and NOTCH[107]. Globally, PAH vascular remodeling seems to differ from the one described in HT histologically. While essential hypertension displays inward and outward remodeling as two simultaneous expressions of the disease (described above), PAH shows what we can call a global dysorganization of vessels' structure, with inward remodeling and excessive ECs and VSMCs proliferation being the main characteristics, with a contribution of excessive peripheral vasoconstriction, especially when sustained hypoxia is present[108].

More recently, two additional important features of PAH have been recognized. An increased vascular extracellular matrix (ECM) stiffness is observed, with early dysregulation of elastin and collagen production.

Secondly, there is a metabolic shift of endothelial cells and VSMCs from glycolysis alone to glutaminolysis, meeting the metabolic demands of hyperproliferative cellular populations. Scientific evidence shows how matrix rearrangements occur even in the early stages of the disease and in different types of pulmonary cells. Such ECM dysregulation is mediated by the abovementioned YAP/TAZ[109]. The demonstration of the role of YAP/TAZ in promoting the metabolic shift to glutaminolysis both in pulmonary endothelial cells and pulmonary vascular smooth muscle cells *in vitro*, could give the rationale for acting on it to elaborate new pharmacological treatments for PAH[73]. Moreover, as previously described, altered flow induces YAP/TAZ activation and its nuclear translocation in an *in vitro* model of atherosclerosis, leading to expression of genes involved in remodeling and ECM synthesis thus to atherosclerotic lesions[72]. This could confirm that indeed disturbed flow acts as a key player in both PAH and HT pathogenesis and could also suggest a common target to modify or reverse vascular remodeling.

Another hint on pathogenetic mechanisms of vascular remodeling in PAH is offered by heritable forms of the disease, that is associated to Bone Morphogenetic Protein Receptor 2 (BMPR2) mutations in 70 to 80% of the cases[44,110]. Alk1/BMPRII are strong mediators in vessels homeostasis and survival; the mutation of this co-receptor, leading to its dysfunction or inactivation, provokes altered endothelial permeability and pathological remodeling, together with impaired ECs metabolism, increased local inflammation and disrupted DNA repair[111,112]. The Alk1/BMPRII receptor complex, alongside with endoglin, is also a crucial player in flow sensing, as it is stimulated by physiological shear stress and can thus mediate vascular homeostasis. Thus, it is not surprising that its mutation leads to PAH, where vascular remodeling and altered flow sensing are driving the development of the disease. Of note, other different mutations have been identified as cause of heritable PAH, and they are directly linked to this signalling cascade and endothelial quiescence as well (ACVRL1, Endoglin, GDF2) [113,114]. However, heritable forms of PH display low penetrance, so it is likely that a "second hit" would be necessary to develop the disease, for example hypoxia, inflammation, somatic mutations, or local changes in hemodynamics. For example, studies on animal models point out hypoxia as a mediator of vascular alterations and pulmonary pressures via the induction of Hypoxia-Induced Factor 1α (HIF-1α), a transcription factor responsible for pulmonary vascular muscle cell proliferation, which most likely contributes to media hyperplasia[115].

In PAH, vascular remodeling is also aggravated – like in systemic hypertension - by the presence of chronic inflammation. Indeed, PAH patients have been shown to display systemic and local inflammation within the lung. The classical animal model of PAH uses the administration of potent inflammatory solutions such as monocrotaline in the respiratory system to reproduce the increase in pulmonary pressure and the histological lesions[116]. This is witnessed by histological studies showing the presence of several T and B lymphocyte infiltrates in the perivascular area and by the correlation of these infiltrates with vascular remodeling[117]. However, those models do not recapitulate fully the human disease as there is no obstructive remodeling observed, and it is also associated with severe myocarditis. This model is often referred to as an acute toxic model, which manages to be cured by almost any agent trialed so far[118], calling for caution when using it as a preclinical model.

Chronic inflammation is both a cause and a consequence of endothelial dysregulation, as it may be the *primum movens* leading to endothelial damage but also the result of dysfunctional ECs that fail to maintain low levels of vascular inflammatory mediators. In the same fashion, damaged ECs fail to regulate local coagulation, contributing to vascular microthrombosis in the pulmonary bed. Inflammation within the lung is also associated to altered mechanosensing: in fact, low laminar and oscillatory shear stress leads to activation of inflammatory pathways such as NF-κB and Smad2/3, increasing leukocyte adhesion molecule expression and local inflammation. The recruited immune cells secrete cytokines and chemokines in the proximity of the inflamed endothelium, generating further inflammation in a positive feedback loop. Low and oscillatory shear stress also synergize with cytokine-induced Smad2/3 activation, while physiological shear stress inhibits such activation[55], hinting at an essential gatekeeper role of physiological mechanosensing.

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Local inflammation, sustained hypoxia and vascular alterations contribute to the reduction of distal functional blood vessels, which leads to worsen pulmonary pressures and to secondary dilation of proximal vasculature in response to pressure increase. This *vessels rarefaction,* especially in the gas-exchange areas, mimics the rarefaction found in systemic hypertension, but it has been mainly studied in animal models and up to now is considered a peculiar reaction of the lung to sustained hypoxia (opposite to systemic circulation): while the hypoxic stimulus leads to angiogenesis in systemic vessels, the same condition provokes vascular loss in the pulmonary bed[119]. Up to now, why PAH patients display rarefaction despite an overexpression of angiogenesis markers is still a matter of debate[105], but as low shear stress can stimulate vessels' remodeling with the aim of restoring the physiological set point (Smad 2/3 translocation described above), we can hypothesize that rarefaction is a late expression of PAH, where flow mechanosensing is already altered and vascular remodeling is pathological secondary to a chronic low flow condition.

But PAH is not only a small vessels' disease: even if historically alterations in distal vessels have been considered the hallmark of PAH, more recently, it became evident that this disease could, in fact, begin distally but evolve also through proximal and distal alterations, both of them amplifying vascular remodeling and mechanosignaling alterations (Figure 6). With respect to pulmonary vascular bead in its totality, together with remodelling of resistance vessels (*plexiform lesions*, arteriolar muscularization, intimal fibrosis) and the decrease of peripheral blood vessels (*rarefaction*), we can also find secondary dilation and increased stiffness of proximal pulmonary vessels [119].

Similarly to endothelial dysfunction and altered remodeling, multiple studies have established the emerging role of flow and flow sensing in PAH, starting from experiences on animal models[120]. These studies globally show that at the beginning of the disease, increased shear stress might induce molecular alterations and adhesion molecules rearrangements (i.e. PECAM-cleavage) capable to alter flow sensing, alter cellular adhesions and induce shear-induced injury and remodeling of pulmonary arteries (see Table 3). An example of this mechanism is represented by congenital heart diseases, in which atrial or ventricular septal defects provoke a volume overload in the right heart (in case of left-to-right shunts) that leads to increased shear stress in the pulmonary vascular bed; if not corrected, such high shear stress leads to vascular remodeling, to a point that PAH is irreversible and it becomes responsible of decreased shear stress as a result of vascular remodeling and increases pressures[121]. Interestingly, a newly described mechanosensory, Piezo1, stimulated by increased shear stress in cultured pulmonary endothelial cells (PAECs), was shown to induce the remodeling pathways like NOTCH[81], providing the rationale for flow-induced vascular remodeling in PAH. Even if the first phases of the disease seem to be caracterised by increased shear stress, when PAH is settled, evidence shows the presence of low shear stress especially in the proximal branches of arterial circulation, associated with increased wall stiffness. This low shear stress observed in the proximal district is capable to mediate and amplify vascular remodeling both in proximal and distal vessels[122]: recent *in vivo* studies, coupling imaging and invasive techniques in patients with PAH, confirmed the presence of low FSS in the proximal branches of pulmonary circulation at the later stages of the disease and its association with worsen hemodynamics and increased vascular stiffness[123]. Moreover, endothelial cells from PH patients show delayed flow adaptations when exposed in vitro to high shear stress, indicating that normal flow sensing is, indeed, impaired[124]. This picture of distal-proximal interaction in PAH is further aggravated by the presence of an increased pulsatile flow in pulmonary circulation, resulting from dilated and stiffer pulmonary arteries with less compliance. In fact, vessels exposed to pulsatile flow not only increase their pathological remodeling in response to altered flow but also activate inflammatory and proliferative signaling within pulmonary microvascular endothelial cells, linking flow alterations with the severity of distal vascular lesions in a self-sustaining positive feedback loop [125].

Authors	Year	Population	Methods	Results

Tang et al [122]	2012	PAH patients N = 5 Controls N = 5	Wall shear stress (WSS) assessment via MRI and computational fluid dynamics in the pulmonary circulation.	Significant reduction in WSS in PAH patients.
Li et al [125]	2009	Pulmonary microvascular endothelial cells cultures (in vitro study)	Effects of pathological flow on endothelial mediators	Pathological shear stress reduces vasodilators release and promotes vasoconstrictors release
Li et al [126]	2009	Pulmonary microvascular endothelial cells (in vitro study)	Analysis of gene expression of adhesion molecules (ICAM-1, E-selectin), chemokines (MCP-1) and growth factor/receptor after exposure to altered flow.	Increased pulsatile flow induces an increase in ICAM-1, E-selectin, MCP-1, VEGF, and leukocyte adhesion.
Truong et al [127]	2013	PAH patients N= 25 Controls N = 4 Pediatric population	Flow assessment via phase contrast imaging of the right pulmonary artery by MRI	WSS is significantly decreased in pulmonary arteries of PAH patients
Schäfer et al [123]	2016	PH patients N = 17 Controls N = 5	Flow assessment by cardiac magnetic resonance and hemodynamic assessment by right heart catheterization	Significant reduction in WSS in PH patients. Correlation between WSS and stiffness indexes and with worse hemodynamics.
Szulcek et al [124]	2016	Biopsies from PAH patients (mostly idiopathic PAH) N = 7 (ex vivo study)	Lung microvascular endothelial cells and pulmonary arterial endothelial cells exposed to high shear stress	Significantly delayed shear adaptation in PH microvascular endothelial cells, with PECAM-1 significantly decreased.

Table 3. scientific evidence about pulmonary hypertension and shear stress (human models). PAH: pulmonary arterial hypertension.

An important feature of PAH is the accumulation of proliferating alpha-smooth muscle actin (αSMA) positive cells in distal vascular lesions. Given the large amount of evidence pointing to endothelial dysfunction and the notion that endothelial cells can indeed transform into mesenchymal cells, it is increasingly accepted that

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EndMT contributes to these αSMA positive cells, thus playing an important role in PAH. This hypothesis has previously been suggested by experiments on animal models[128,129] and is recently supported by *ex vivo* studies on human lung samples from PH patients, where endothelial cells were shown to migrate and acquire mesenchymal markers (αSMA and phospho-vimentin), possibly secondarily to loss of BMPR2 signalling[67]. Further experiments performed by Good and colleagues demonstrated the induction of EndMT by inflammatory cytokines both in vivo and in vitro, and the capability of EndMT cells to secrete inflammatory mediators, contributing to the sustained inflammation of the endothelium[130]. Thus, recent evidence supports EndMT as a key player in PAH pathogenesis and evolution. It is likely that this severe, irreversible form of inflammation alters the gatekeeping role of physiological flow sensing, unleashing dramatic positive feedback leading to unresolved vascular remodeling of the lung vasculature.

Up to now, different animal models of PAH exist, but as previously described, they often reproduce forms of the disease that do not cover the isolated idiopathic PAH, and are secondary to lung damage (monocrotaline), genetic mutations (for example BMPR2 knockout rats) or congenital heart disease (monocrotaline + aortocaval shunt)[131]. Also, given the complexity of the disease, often multifactorial, no animal model can mimic the human disease. These elements must be considered when analyzing the results of preclinical research in PAH.

5. Discussion and perspectives

Systemic and pulmonary hypertension (Group 1, PAH) are two different diseases, each of them with peculiar characteristics and treatment, occurring in two vascular districts organized in series, thus affecting one another basically through hemodynamic interactions (load conditions, volemia, cardiac performance). In humans, these two conditions are rarely overlapping. They are associated with different clinical outcomes and are treated differently, targeting VSMCs directly in systemic hypertension, or ECs mediators acting on VSMCs in PAH. However, recent discoveries on the mechanisms of vascular remodeling and flow sensing can not be underestimated and targeting such mechanisms must be taken into account for future studies as well as for the understanding of the disease.

While acknowledging the role of inflammation and multiple external factors contributing to the evolution of both HT and PAH (such as hypoxia, increased adrenergic tone or systemic conditions and risk factors), and the fact that the control of such factors is sometimes difficult to reach especially in the pre-symptomatic phase of the disease, a few proteins belonging to flow-sensing pathways stand apart in the pathogenesis of vascular remodeling and can represent targets for future research.

BMP and BMP receptors and TGFbeta signaling are key players in vessel homeostasis. In particular, BMP9/Alk5/Neuropilin1 complex emerges as a crucial structure that translates disturbed flow in intracellular messages promoting inward remodeling and endothelial-to-mesenchymal transition, possibly leading to vascular alterations; on the other side, BMP10/Alk1/Endoglin induces endothelial stabilization. The relevance of these mediators in vascular alterations is well represented by hereditary forms of PAH, where a mutation on BMPR2 (belonging to the same BMPR family and thus acting through Smad phosphorylation) represents one of the main aetiologic factors. When mutated, this receptor displays a loss-of-function that clinically translates into altered vessels' homeostasis and ultimately PAH, showing that the balance between the different BMP signalling pathways is lost. Recent evidence starts to point out such mediators and their balance as potential subject of study, especially in PAH[132]. Further research could be conducted on BMP signaling alterations in HT and/or target Alk5/ Neuropilin1 in animal models to explore to which extent such selective blockage prevents the development of pathological vascular alterations. These data will need to be confirmed in the respective human populations, and their interactions with currently approved therapies should also be investigated.

To improve selective targeting of the BMP signalling cascade, new scientific evidence is required especially on the action of CDK2. Such intracytoplasmic mediator – stimulated by physiological shear stress via Kruppel-like factor - is known to phosphorylate Smad 2/3 in a way that inhibits its nuclear translocation contrasting pathological remodeling. Stimulating the production or increasing the concentration of CDK2 can be a future research domain both in HT and PAH, where vascular remodeling determines most of the hemodynamic changes sustaining the disease. After all, when a balance is unbalanced, another way to reach back the equilibrium is to load the underloaded plate.

But more effective than blocking a pathway with such a broad spectrum of actions – contributing to pathological but also physiological responses to flow – discovering other proteins of the mechanosignalling cascade and acting more distally on specific flow-sensing mechanisms could be more of interest for future research. On the other hand, without underscoring the importance of mechanotransduction pathways and their emerging role in flow sensing, two considerations must be done: 1) that both HT and PAH are multifactorial diseases, and thus that a multi-aetiologic approach is required both in diagnosis and therapy (i.e. targeting a pathway can be insufficient to cure the disease but could help in reducing its vascular complications); and 2) that future research could be focalized on shedding light on the possible different expression and activation of such pathways in different cellular populations (endothelial cells of different vascular districts, VSMCs etc) and in both diseases, with further confirmation in human models.

Endothelial dysfunction and pathological remodeling are common features of two diseases that – from a clinical point of view – have different expressions and therapies. Indeed, treatment strategies for HT often are not the same used in PAH: ACE-inhibitors have an important effect on systemic pressures, but they do not act on pulmonary circulation; in the same way, mediators acting on NO release work better in PAH than in HT, and this without inducing significant reduction in systemic blood pressure. However, the available scientific evidence – until now realized mostly *in vitro* or on animal models – indicates that common features of these diseases are the disruption of endothelial homeostasis, with endothelial cells proliferation, altered mechanosensing, cytoskeletal rearrangements within the vessels' wall, local inflammation, and also vascular, metabolic alterations. In both, the whole vascular tree is affected and displays different degrees of endothelial dysfunction, possibly distributed with a "gradient" or a pattern between arteries, arterioles, capillaries and veins, and whose extension represents a matter for future research. Until now, drug therapy for both conditions has targeted vasodilation via several drugs represented for example by non-selective beta-blockers and ACE-inhibitors for HT, and endothelin-receptor antagonists (ERA) and phosphodiesterase 5-inhibitors (PDE5-i) for PAH; but looking at these clinical entities from the point of view of vascular remodeling, we can hypothesize that altered vasodilation drives the first phases of the disease, while pathological remodeling is responsible for advanced stages of both HT and PAH, thus becoming an interesting pharmacological target. Indeed, recent research efforts in PAH treatment focus on drugs that do not act on vasodilation but instead on TGFβ signaling or Tyrosin-kinases inhibition, and even the forms of PH classified as "secondary", i.e. due to left heart disease or pulmonary disease, have shown altered vascular homeostasis and sometimes display a severe and disproportionate elevation of pulmonary pressures possibly secondary to altered vascular remodeling[100,133]; in the same way, clinicians are often confronted to severe forms of systemic hypertension, where the control of blood pressure is difficult to obtain even when patients are treated with multiple drugs. All these arguments translate a lack of knowledge in the pathogenesis and evolution of the two conditions, which could indeed be partially filled with increasing research on vascular flow sensing and vascular remodeling mechanisms.

Regarding pulmonary hypertension, while plexiform lesions are mainly shared between the majority of PAH (Group 1) subgroups, venular involvement emerges as a possible shared aspect between the different disease groups of PH. Patients with pre-capillary forms of PH and systemic scleroderma were shown to display venular remodeling mimicking the one found in another kind of PH secondary to pulmonary veno-occlusive disease (PVOD)[134-136], and this has been proposed as a possible explanation for their poor prognosis and their

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suboptimal response to conventional PAH treatments[137]; venular alterations were also described in Groupe 2 PH patients (PH associated to left heart disease) and were linked to a worse hemodynamic profile[133]. This evidence suggests the key role of vascular remodeling on pathogenesis and prognosis, remembering that it involves the totality of the vasculature without being restricted only to the arterial side of pulmonary circulation.

In conclusion, flow sensing mechanisms, endothelial dysfunction, and vascular remodeling are indeed underscored by clinicians and often considered important only when normal flow conditions are already altered, as this is a well-known cause of vascular alterations (dilation, stenosis). But they are indeed tightly linked entities that seem to have an influence also on cellular metabolism and local inflammation as well, amplifying the damage. The conventional idea of controlling blood pressure by treating only the "muscular" component of the vasculature seems now out of date both in HT and in PAH. An in-depth study of the pathways acting on endothelial cells, cytoskeleton, extracellular matrix, and VSMCs within the vessel wall and their respective cross-talk might be of interest not only to treat but also to anticipate vascular alterations and to have more control over the pathogenesis of these diseases that still are responsible for great morbidity and mortality, leading to cardiovascular complications, heart failure, and even the necessity for a lung transplant for PAH patients.

Acknowledgments

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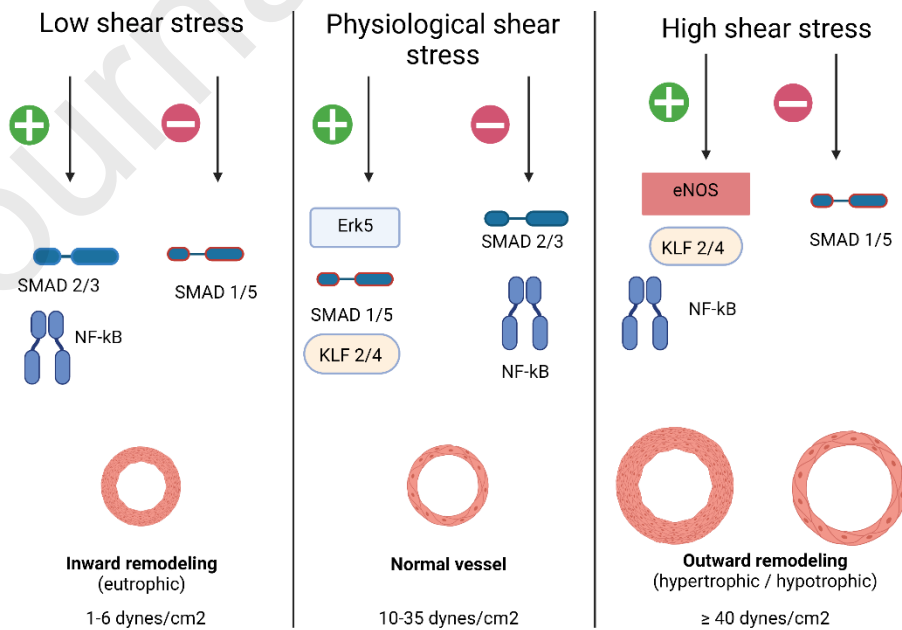
Credit author statement:

Margherita Tiezzi: Litterature review, writing, conceptualization and preparation of the figures on Biorender.com, **Hanqiang Deng:** Litterature review, writing, conceptualization of the figures, **Nicolas Baeyens:** Supervision, literature review, writing and conceptualization of the figures
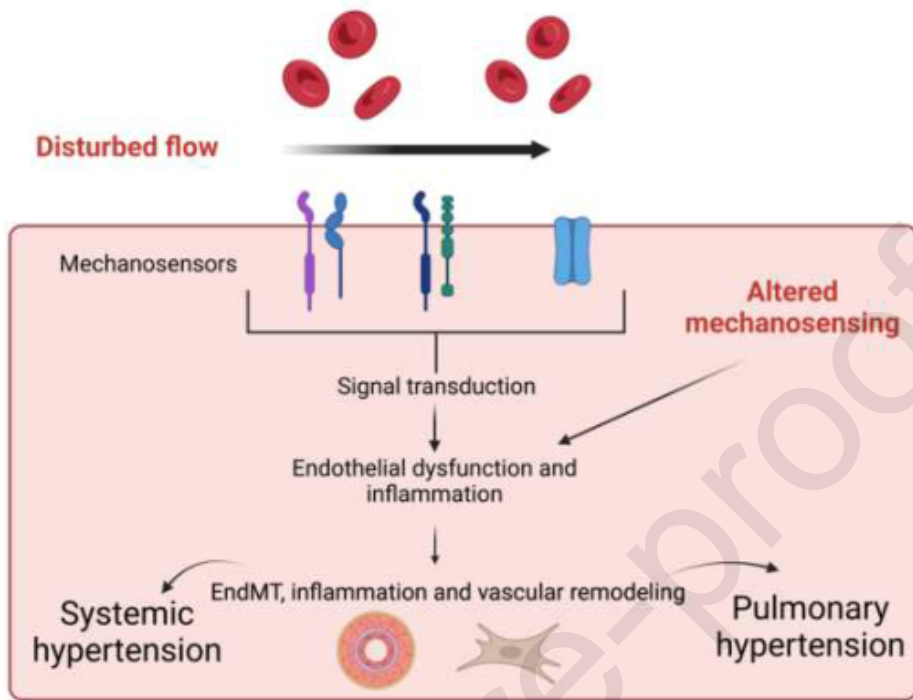
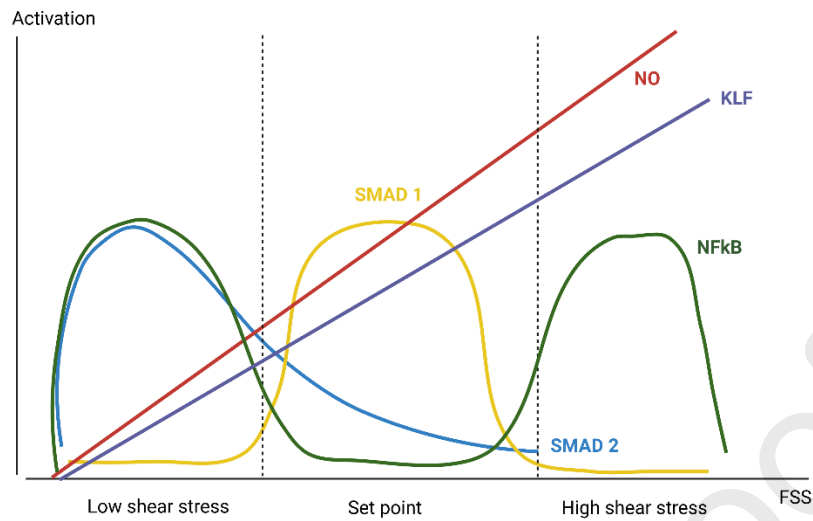# Figures and legends:

A

B

Figure 1: *Biochemical pathways and mediators involved in flow-dependent vascular remodeling. Fluid shear stress values within a physiological range (set point) maintains vessel homeostasis with activation of Smad1 and the NO pathway. FSS values below this physiological range (low shear stress) trigger inward remodeling of the vessels, mainly by activating smad2/3 and NFkB. FSS intensities reaching beyond the set point (High shear stress) will further activate the NO pathway and NFkB while repressing Smad1 activation, triggering outward remodeling of the vessel. (Created with BioRender.com)*

Figure 2: *BMP signaling in response to FSS: an overview of the biochemical pathways involved in controlling vascular quiescence and inward remodeling. Low shear stress activates Smad2/3 through TGF-β type Ⅰ receptor Alk5 and co-receptor Nrp1, inducing Endothelial-to-Mesenchymal Transition (EndMT) and inward remodeling of the vessel, as observed in hypertension and atherosclerosis. Physiological shear stress activates MEKK3-KLF2-CDK2 pathway, which inhibits Smad2/3 activation by phosphorylating its linker region, actively repressing this deleterious signaling pathway.*

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Physiological shear stress activates Smad1/5 through Alk1 and Endoglin to promote vessel stabilization. Created with Biorender.com.
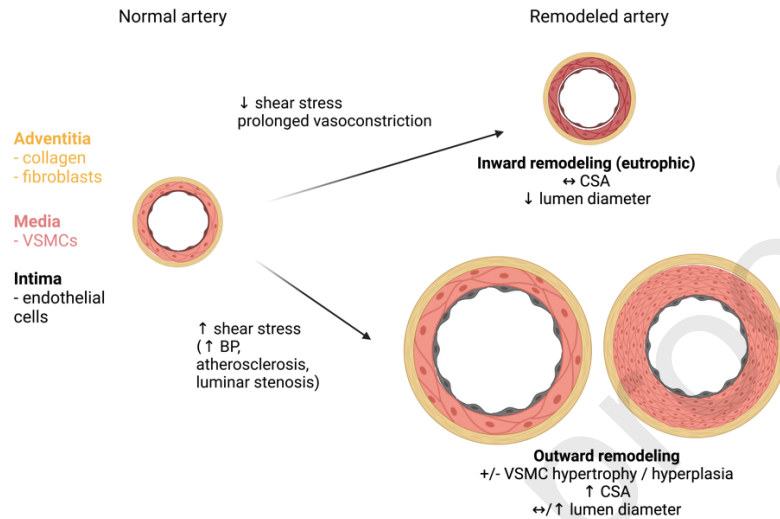

Figure 3*: **Types of remodeling frequently seen in essential hypertension.** In inward remodeling, resistance vessels have a smaller lumen diameter but do not change their cross-sectional area and become stiffer as a result of VSMCs and matrix rearrangements. In outward remodeling, resistance vessels increase their cross sectional area to decrease wall tension; this can be associated with VSMCs hyperplasia and <mark>hypertrophy</mark> or not. VSMC: vascular smooth muscle cells, CSA: cross sectional area, BP: blood pressure. Created with Biorender.com.*
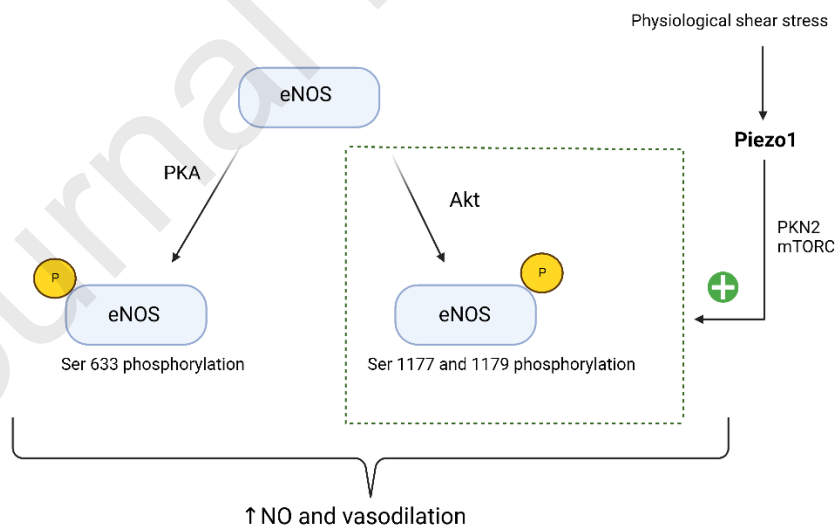

***Figure 4:** eNOS phosphorylation is a central regulator of NO synthesis, mediating vascular smooth muscle relaxation and vessel dilation. Fluid shear stress activates several mechanosensors, including Piezo1, which transduces eNOS activation through PKN2 and Akt. Created with Biorender.com.*
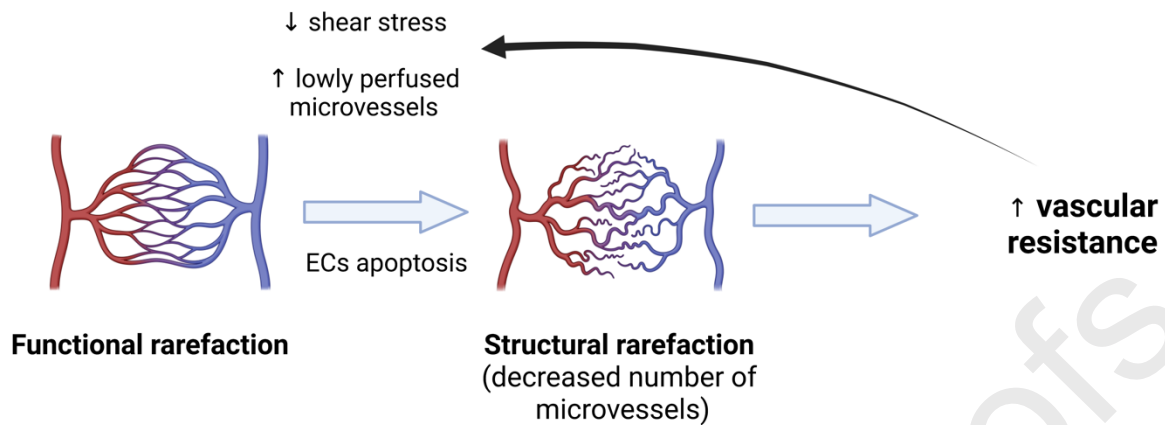
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Figure 5: Schematic representation of the evolution of capillary rarefaction in "essential" hypertension. *Scientific evidence suggests that functional rarefaction is the first alteration in HT patients. If flow alterations or endothelial dysfunction persist, the number of capillaries progressively decreases, leading to a further increase in blood pressure (structural rarefaction). ECs: endothelial cells. Created with Biorender.com.*
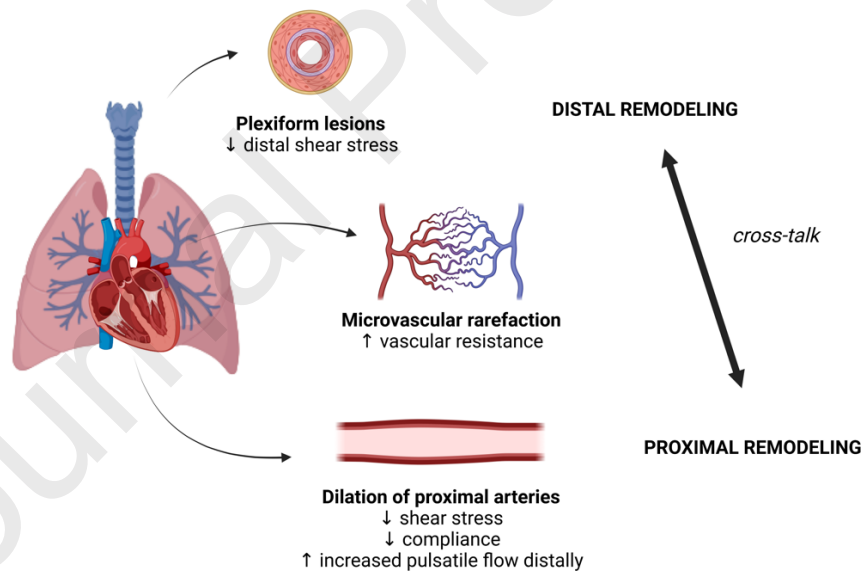

Figure 6: *Schematic resume of the main alterations observed in vascular remodeling and flow patterns in pulmonary arterial hypertension. Created with Biorender.com.*

Figure 1: Biochemical pathways and mediators involved in flow-dependent vascular remodeling. Fluid shear stress values within a physiological range (set point) maintains vessel homeostasis with activation of Smad1 and the NO pathway. FSS values below this physiological range (low shear stress) trigger inward remodeling of the vessels, mainly by activating smad2/3 and NFkB. FSS intensities reaching beyond the set point (High shear stress) will further activate the NO pathway and NFkB while repressing Smad1 activation, triggering outward remodeling of the vessel. Created with BioRender.com.

Figure 2: BMP signaling in response to FSS: an overview of the biochemical pathways involved in controlling vascular quiescence and inward remodeling. Low shear stress activates Smad2/3 through TGF-β type Ⅰ receptor Alk5 and co-receptor Nrp1, inducing Endothelial-to-Mesenchymal Transition (EndMT) and inward remodeling of the vessel, as observed in hypertension and atherosclerosis. Physiological shear stress activates MEKK3-KLF2-CDK2 pathway, which inhibits Smad2/3 activation by phosphorylating its linker region, actively repressing this deleterious signaling pathway. Physiological shear stress activates Smad1/5 through Alk1 and Endoglin to promote vessel stabilization. Created with Biorender.com.

Figure 3: Types of remodeling frequently seen in essential hypertension. In inward remodeling, resistance vessels have a smaller lumen diameter but do not change their cross-sectional area and become stiffer due to VSMCs and matrix rearrangements. In outward remodeling, resistance vessels increase their cross-sectional area to decrease wall tension; this can be associated with VSMCs hyperplasia and hypertrophy or not. VSMC: vascular smooth muscle cells, CSA: cross-sectional area, BP: blood pressure. Created with Biorender.com.

Figure 4: Control of vasodilation through eNOS. eNOS phosphorylation is a central regulator of NO synthesis, mediating vascular smooth muscle relaxation and vessel dilation. Fluid shear stress activates several mechanosensors, including Piezo1, which transduces eNOS activation through PKN2 and Akt. Created with Biorender.com.

Figure 5: Schematic representation of the evolution of capillary rarefaction in "essential" hypertension. Scientific evidence suggests that functional rarefaction is the first alteration in HT patients. If flow alterations or endothelial dysfunction persist, the number of capillaries progressively decreases, leading to a further increase in blood pressure (structural rarefaction). ECs: endothelial cells. Created with Biorender.com.

Figure 6: Schematic resume of the main alterations observed in vascular remodeling and flow patterns in pulmonary arterial hypertension. Created with Biorender.com.