Decoding tissue-specific TGF-β signaling in pulmonary arterial hypertension: from genetic predisposition to mechanobiology

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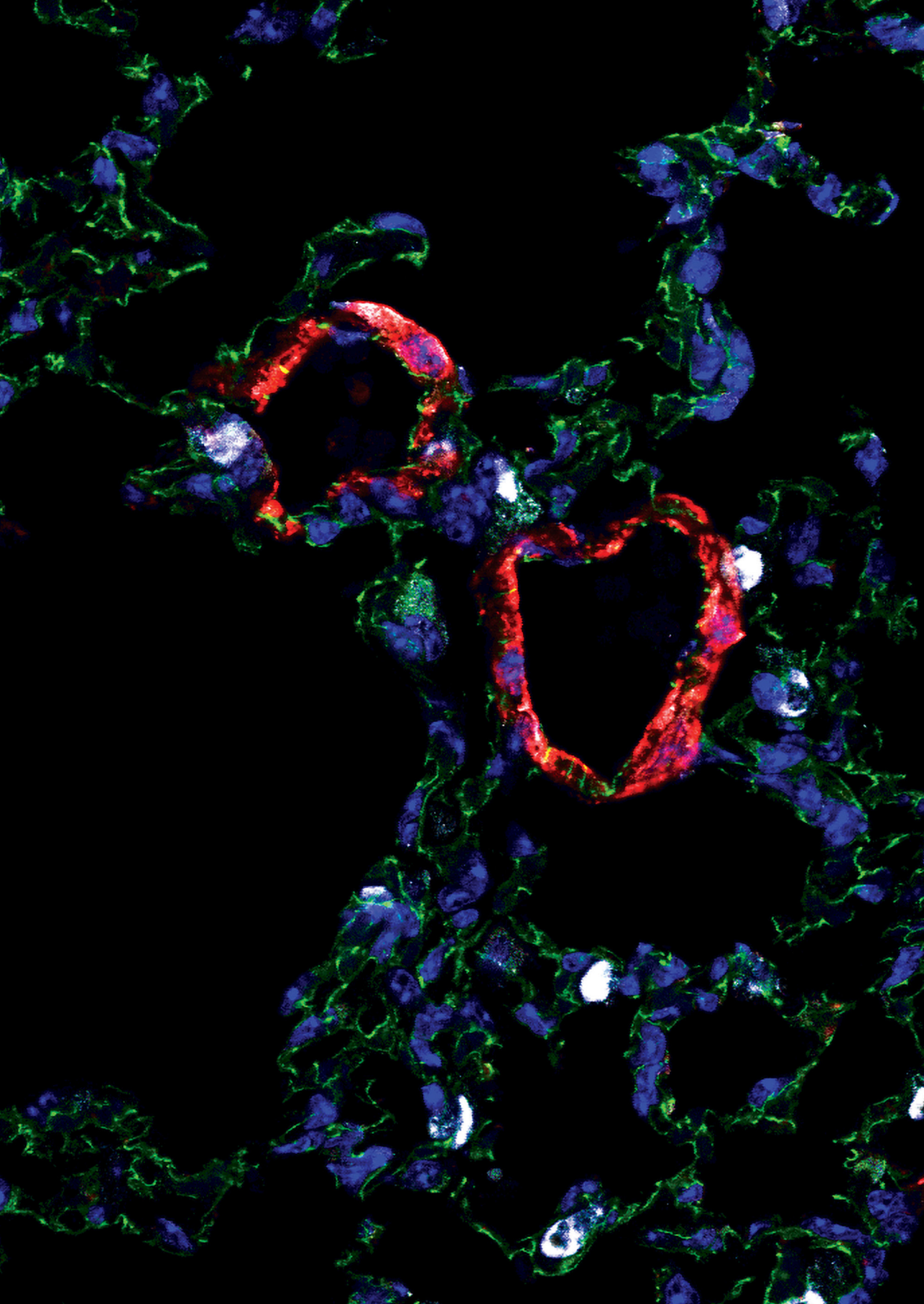

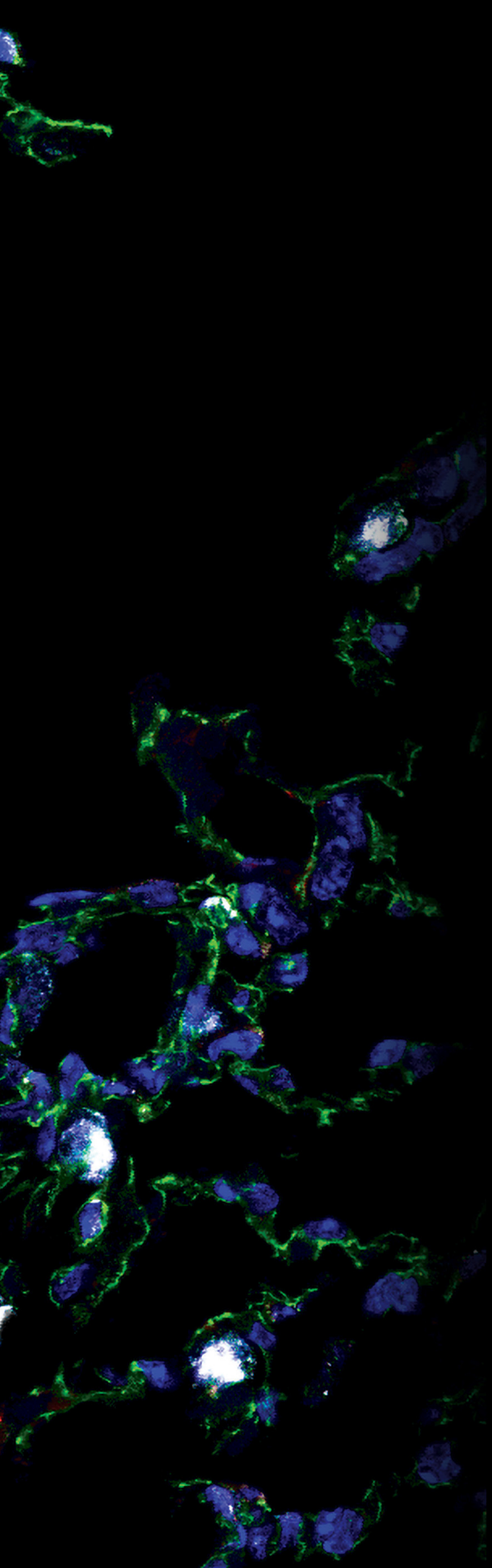

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General Discussion

From Central Dogma to Clinical Dilemma

The Transforming growth factor (TGF)-β superfamily of cytokines plays a pivotal role in regulating diverse cellular processes, including embryonic development, immune system activation, angiogenesis, and tissue homeostasis[1]. In the context of Pulmonary Arterial Hypertension (PAH), alterations in TGF-β signaling have long been implicated, with a particular focus on germline mutations in the *BMPR2* gene. However, the findings presented in this thesis, alongside emerging literature, underscore that PAH pathogenesis cannot be explained by a BMPR2-centric view alone. One that is shaped not only by mutations, but also by the dynamic interplay between ligand activity, receptor availability, environmental cues, and secondary stressors converging on the endothelium[2]. While *BMPR2* mutations represent the most common genetic predisposition, their incomplete penetrance highlights the importance of additional factors in disease initiation and progression. This "multi-hit" model is a recurring theme in this thesis and underpins the link between molecular signaling, biomechanical forces, and inflammation. Thus, we demonstrate that inflammatory signals, mechanical stress, and altered ligand availability interact with genetic risk to disrupt vascular homeostasis. Understanding these interacting triggers is critical for identifying new opportunities for intervention. Despite the recognized importance of the TGF-β superfamily, a persistent and fundamental challenge remains: how can we safely and precisely target components of this complex signaling network for therapeutic purposes? The therapeutic potential of this pathway is clear, but its pleiotropy and context-dependence raise significant concerns. Can we define ligand-, cell type–, or context-specific therapeutic strategies that restore balance without triggering adverse remodeling elsewhere? These questions drive much of the experimental work presented in this thesis (Chapters 3-6).

The context-dependent nature of TGF-β signaling is particularly evident when examining how specific ligands and receptor configurations produce opposing outcomes across different cellular environments. Nowhere is this complexity more apparent than in endothelial cells (ECs), where the balance between two type I receptors (ALK1 and ALK5) determines whether TGF-β acts as a promoter or suppressor of angiogenesis[3]. ALK1 activation primarily leads to SMAD1/5 activation, supporting EC proliferation and migration, and promoting the early phases of angiogenesis. Conversely, ALK5 signals through SMAD2/3 and regulates growth arrest, the stabilization of the vessel wall, and the production of extracellular matrix. Endoglin, a co-receptor highly expressed in angiogenic ECs, shifts the balance toward ALK1 signaling[4]. Importantly,

ALK1 and ALK5 are co-expressed in the same cell, and their antagonistic signaling must be tightly balanced to maintain vascular homeostasis. The behavior of BMP9, a high-affinity ALK1 ligand, further complicates this balance. BMP9 is typically described as a factor that promotes vascular quiescence, inhibiting EC proliferation and migration, thereby supporting vessel stability under physiological conditions[5]. However, our work and other studies highlight its sometimes paradoxical roles. Under certain experimental conditions, especially at elevated concentrations, BMP9 can also exhibit pro-angiogenic properties. This shift is thought to result from receptor saturation and promiscuity: while BMP9 predominantly signals through the high-affinity ALK1–BMPR2 complex, increasing ligand levels can lead to engagement of lower-affinity type I receptors, such as ALK2, as well as alternative type II receptors, like ACVR2A and ACVR2B. These receptor configurations may trigger non-canonical or even inflammatory signaling responses, particularly when co-receptors, such as endoglin, are differentially expressed[6–8]. Thus, the same ligand can either promote vascular integrity or drive pathological remodeling, depending on concentration, receptor availability, and cellular state. These insights reinforce a key argument of this thesis: therapeutic modulation of this pathway must move beyond ligand inhibition or activation alone and instead consider the contextual determinants that shape functional outcomes. In this light, even small perturbations in receptor expression or inflammatory context can tip the balance toward maladaptation[9]. *BMPR2* deficiency is a central hallmark in heritable and idiopathic PAH, and its consequences extend beyond a simple loss of canonical BMP signaling. Prior studies have shown that ECs lacking *BMPR2* exhibit heightened responsiveness to Activin A. This altered sensitivity leads to aberrant phosphorylation of both SMAD2/3 and SMAD1/5, suggesting the formation of non-canonical receptor complexes composed of BMPR1, TGFβR1, and TGFβR2. Notably, Activin A-induced SMAD1/5 signaling is absent in the healthy endothelium and becomes pronounced in the context of *BMPR2* loss, indicating an essential shift in utilization of receptors and signaling dynamics[10]. To understand how these altered signaling patterns translate into pathogenic phenotypes, we conducted transcriptomic profiling of *BMPR2*-deficient ECs under defined biochemical and mechanical conditions (Chapter 5). RNA sequencing of a newly engineered *BMPR2*-deficient EC line stimulated with Activin A under static conditions revealed a distinct transcriptional signature enriched for PAH-associated genes. These data suggest that Activin A, in the context of *BMPR2* loss, can actively drive pathogenic gene expression, contributing to endothelial dysfunction and reinforcing the concept of a multi-hit model of PAH pathogenesis. Activin A functions as a key driver of disease progression in genetically susceptible individuals, positioning

it as a target for both diagnostic refinement and therapeutic intervention, as explored in this thesis (Chapters 4 and 5).

Because cell type–specific, ligand-specific, and context-dependent signaling must all be considered when evaluating the therapeutic potential of TGF-β superfamily modulation, strategies that overlook this complexity risk off-target effects or limited efficacy. Effective treatment of PAH will therefore require precision modulation that goes beyond generic inhibition of activation of selected pathway components, but context-aware intervention that accounts for the cellular environment, receptor landscape, and competing cues. Recent advances, such as ligand traps and isoform-engineered Fc fusion proteins, aim to restore signaling balance; for example, by shifting activity away from pro-proliferative Activins toward more vasculoprotective BMP responses[11]. Findings from Chapter 5 of this thesis provide direct experimental evidence supporting this need for contextual precision. Using a doxycycline-inducible *BMPR2* knockdown system in ECs, we systematically interrogated how genetic susceptibility (*BMPR2* loss), shear stress, and ligand exposure (Activin A) interact to shape endothelial gene expression. RNA sequencing revealed that *BMPR2* deficiency alone was insufficient to induce a disease-like transcriptional program. However, the combination of *BMPR2* loss and Activin A exposure under static conditions led to a distinct transcriptional signature enriched for PAH-associated genes. Moreover, the addition of laminar shear stress reduced the expression of several genes associated with vascular dysfunction, suggesting that physiological flow can partially restore endothelial stability even in *BMPR2*-deficient cells exposed to Activin A. These flow-suppressed genes include regulators of inflammation, endothelial barrier function, and remodeling. Therefore, laminar shear stress may actively protect ECs against pathological reprogramming.

The Endothelium as a Gatekeeper of Vascular Integrity in PAH

ECs play a central role in maintaining vascular homeostasis, functioning as gatekeepers that preserve vessel integrity through the regulation of barrier function, anti-inflammatory signaling, and vasoactive balance[12]. This quiescent phenotype is actively maintained by growth factors, such as BMP9 signaling via BMPR2. Once disrupted, ECs shift from preserving vascular stability to driving pathogenic remodeling. In PAH, such disruption arises from the convergence of multiple interacting stressors,

including genetic mutations, disturbed shear stress, and inflammation[13]. Loss of BMPR2 heightens sensitivity to pro-inflammatory and pro-mesenchymal stimuli, compromising endothelial integrity and facilitating processes such as Endothelial-to-mesenchymal transition (EndMT)[14]. One example explored in Chapter 3 is the role of interleukin (IL)-33 as a trigger of endothelial dysfunction. IL-33 was significantly upregulated in PAH patient-derived ECs, as well as in lungs from Su/Hx mice and human PAH patients, and promoted EndMT in pulmonary arterial ECs (PAECs). BMP9 counteracted IL-33-induced EndMT by inducing sST2, a décor receptor that neutralizes IL-33 signaling. This protective axis complements prior findings on IL-6, where BMP9 responses were instead exacerbated in PAH ECs[15]. Together, these studies illustrate that BMP9's effects can be protective or pathogenic depending on the inflammatory milieu and endothelial state; reinforcing the context-dependence introduced earlier.

As we demonstrate in Chapters 3–5, the balance between vascular stability and remodeling depends not only on genetic predisposition but also on how ECs interpret inflammatory, biochemical, and mechanical inputs. Once endothelial dysfunction is established, ECs contribute to disease progression by promoting leukocyte recruitment, smooth muscle proliferation, and pathological angiogenesis[2]. This transformation, partly driven by imbalanced TGF-β, Activin, and BMP signaling, shifts the endothelium from a quiescent barrier to a driver of vascular pathology. Findings across multiple chapters, including AP-1–regulated transcription (Chapter 5), IL-33–induced EndMT (Chapter 3), and Activin A-mediated reprogramming (Chapters 4–5), support a model in which restoring endothelial stability represents a viable therapeutic strategy in PAH. Targeting this gatekeeper function may not only halt disease progression but also enable earlier intervention. In PAH, this means targeting the Activin-GDF axis while preserving or restoring BMP signaling. This approach continues to evolve as we gain a deeper understanding of the interplay between different members of this complex superfamily. Future therapies will likely require not only greater selectivity but also context-aware delivery systems to maximize efficacy and minimize adverse effects. This rethinking of the therapeutic objective sets the stage for exploring Activin A both as a therapeutic target and a potential biomarker for disease activity and progression.

Dual Role of Activin A in PAH: Biomarker of Risk and Target for Therapy

Activin A has emerged as both a mechanistic driver and potential biomarker in PAH [16]. Its dual role offers a unique opportunity to link molecular mechanisms with clinical translation, especially in the context of pulmonary vascular remodeling in PAH. As demonstrated in Chapter 4, serum levels of Activin A increased stepwise from healthy controls to carriers of pathogenic *BMPR2* variants, and PAH patients, highlighting its potential in risk stratification. In contrast, other family members, such as Activin B and Activin C, showed inconsistent or non-significant patterns. Mechanistically, *BMPR2* deficiency enhances Activin A signaling by shifting the balance away from SMAD1/5-mediated signaling toward SMAD2/3 activation via ALK4/5. This rewiring of receptor usage sensitizes ECs to Activin A, potentially promoting its expression and establishing a feed-forward loop. Although the precise cellular source of circulating Activin A in early disease remains unresolved, several observations suggest both endothelial and immune contributions[10]. *BMPR2*-deficient ECs exhibit impaired barrier function, elevated ICAM-1 and VCAM-1, and increased monocyte adhesion and transmigration, even in mutation carriers without clinical disease[17, 18]. These changes suggest a pre-inflamed endothelial phenotype that may drive the upregulation of autocrine or paracrine Activin A. Additionally, Activin A can directly promote BMPR2 downregulation through receptor internalization and degradation, further amplifying this loop. Its elevation in genetically at-risk individuals further strengthens its case as a candidate for targeted intervention. However, its utility as a single biomarker may be limited due to overlapping levels with healthy populations. Activin A might be best interpreted in combination with other indicators, such as inflammatory cytokines (e.g., IL-6, TNF-α, IL-1β), and cardiac imaging features (e.g., right atrial volume, strain metrics). Nonetheless, these mechanistic insights help explain why sotatercept, a ligand trap for Activin-class ligands, has shown meaningful clinical benefit. Its success underscores the therapeutic potential of modulating TGF-β superfamily signaling, while also revealing the challenges of broadly targeting multiple ligands in a pathway whose effects are highly context-dependent. The findings in this thesis highlight the need for more selective, mechanism-based approaches that build on early molecular insights to guide future therapeutic development.

Mechanobiology and Modeling – Reframing the Endothelial Standard

While biochemical ligands are central to PAH pathobiology, our work in Chapter 6 shows that mechanical forces are equally critical in determining endothelial fate. ECs are dynamic mechano-sensors that continuously interpret the physical forces in their environment to regulate vascular function. Their phenotype, gene expression, and behavior are shaped by a variety of mechanical stimuli, including shear stress from blood flow, loading, substrate stiffness, and matrix composition. These forces are sensed by mechanosensitive ion channels, such as Piezo1, as well as apical features like the glycocalyx and primary cilia, cell–cell junction proteins like PECAM-1 and VE-cadherin, and integrins at the cell–matrix interface. Together, these inputs are integrated through the cytoskeleton and nucleus to activate transcriptional programs that regulate endothelial phenotype[19, 20]. Mechano-transduction pathways activated by these forces influence key endothelial functions, including alignment, nitric oxide production, inflammatory signaling, and extracellular matrix remodeling. While laminar flow promotes endothelial quiescence and vascular homeostasis, disturbed or oscillatory flow can induce proliferation, inflammation, and permeability - hallmarks of endothelial dysfunction in diseases like PAH[21]. One could argue that the mechanical microenvironment acts as a trigger, interpreted by ECs to decide whether to maintain homeostasis or initiate remodeling. Importantly, these mechanical stimuli can indirectly initiate key second messenger cascades, including TGF-β signaling. For instance, integrin-mediated pulling forces can release latent TGF-β from extracellular matrix depots, initiating downstream SMAD signaling. Moreover, disturbed flow or increased substrate stiffness can shift the balance toward pro-fibrotic, SMAD2/3-driven transcriptional programs, even in the absence of elevated ligand levels[21].

Despite the clear impact of biomechanical cues on EC function, much of EC research continues to rely on static culture conditions that poorly mimic the physiological or pathological environments *in vivo*. Alternative platforms now enable a more accurate replication of vascular biomechanics, mimicking both substrate-derived cues (such as stiffness, curvature, and topography) and fluid-derived stresses, including laminar and disturbed shear flow. For example, grooved or fibrous substrates enable direct cell alignment and elongation, mimicking the anisotropic organization found in blood vessels. Hydrogels with tunable stiffness can recreate the compliance of healthy or diseased vasculature, influencing cell behavior such as spreading, traction forces, and

differentiation. Similarly, 3D microvessel-on-chip platforms allow ECs to be cultured under flow within physiologically curved, perfused channels, more closely reflecting native vessel architecture[19]. Flow-based systems that reproduce pulsatile, oscillatory, or laminar shear stress can be a useful tool for studying inflammatory responses, gene regulation, or mechano-transduction under dynamic conditions. However, such platforms often remain inaccessible due to the high cost of commercially available systems or the technical complexity of building custom setups, posing significant financial and logistical barriers for many academic labs. To address this gap, we developed a PDMS-based Nylon Vessel-on-a-chip (NVoC) system, offering a more accessible and scalable alternative (Chapter 6). Fabricated using reusable 3D-printed molds and compatible with standard lab workflows, the NVoC enables parallel flow conditions across multiple channels simultaneously and supports physiological and pathological shear stress modeling at low cost. It reliably induces shear-responsive gene expression and endothelial alignment comparable to commercial systems, while significantly reducing material costs and technical complexity. In doing so, it provides a practical and inclusive solution for bringing flow-based experimentation into routine vascular research. While animal models have been crucial in studying the molecular mechanisms of PAH and evaluating therapeutic strategies, they do not adequately reflect the complex nature of human disease. Species-specific differences, technical demands, and limited translational success highlight the need for complementary human-relevant models[22]. In this context, organ-on-a-chip (OOC) systems have emerged as powerful tools to study PAH pathobiology in a controlled and physiologically relevant setting. These micro-engineered platforms allow precise manipulation of biomechanical and biochemical cues, such as shear stress, hypoxia, and inflammation, while incorporating co-cultured patient-derived cells to capture disease heterogeneity. Notably, OOC models have successfully recapitulated essential features of PAH, including smooth muscle proliferation, sex disparities, and drug responsiveness, offering mechanistic insights beyond traditional *in vitro* systems[23-25]. Integration of these systems with transcriptomic analysis has proven central to the findings in Chapter 5, offering a deeper insight into how defined mechanical and biochemical conditions shape endothelial gene expression. Our findings from Chapter 5 support the "second hit" hypothesis, where environmental cues are required to unmask the pathological consequences of genetic vulnerability. Importantly, we found that physiological laminar shear stress exerts a protective effect, even in the context of BMPR2 deficiency. Flow exposure suppressed pro-inflammatory transcription factors such as FOSB and cJUN, and induced a broader set of shear-responsive genes that preserved homeostasis. This buffering effect was lost under static conditions, where

Activin A stimulation amplified pathogenic transcriptional responses. These context-dependent transcriptional shifts further underscore the interplay between mechanical and biochemical cues in shaping the fate of ECs. Several genes stood out for their regulation across conditions and known relevance to endothelial dysfunction. *ANGPTL4* (angiopoietin-like 4), for instance, was strongly induced by Activin A but repressed under HSS, consistent with its proposed role in promoting endothelial activation[26]. Notably, the function of *ANGPTL4* is highly context-dependent, with studies reporting both protective and disruptive effects on endothelial integrity and angiogenesis[27-29]. Another hit, *EPHA2*, a receptor tyrosine kinase predominantly expressed in lung ECs, was downregulated under laminar shear stress in our model, suggesting a role in flow-sensitive endothelial activation. *EPHA2* is well established as a key regulator of vascular permeability and inflammation, with ligand (ephrin-A1) engagement leading to the disruption of tight and adherent junctions, activation of NF-κB signaling, and induction of proinflammatory chemokines and adhesion molecules[30-32]. Conversely, *SOX7*, which is classically involved in maintaining endothelial identity and promoting VE-cadherin expression, was upregulated by Activin A in *BMPR2*-deficient cells, yet suppressed by flow. This pattern contrasts with its well-characterized role in supporting endothelial identity, possibly reflecting a stress-adaptive or developmental reprogramming [33-35]. Similarly, *TGM2* or *TG2* (transglutaminase 2), a calcium-dependent ECM crosslinker and pro-fibrotic enzyme, was induced by Activin A and *BMPR2* knockdown, and suppressed under shear. Given its roles in vascular stiffening, ECM remodeling, and smooth muscle proliferation, its regulation in our system supports the anti-remodeling effects of physiological flow[36-38]. Together, these findings support a model (Figure 1) in which endothelial responses to mechanical cues are susceptible to both the magnitude and pattern of shear stress. Genes respond differentially to laminar flow versus pathological conditions, illustrating how even moderate shifts in hemodynamic force can tip the endothelial transcriptome toward protection or pathology. As the field progresses toward precision medicine, platforms capable of simulating physiological flow will be indispensable for studying therapeutic responses under dynamic, disease-relevant conditions.

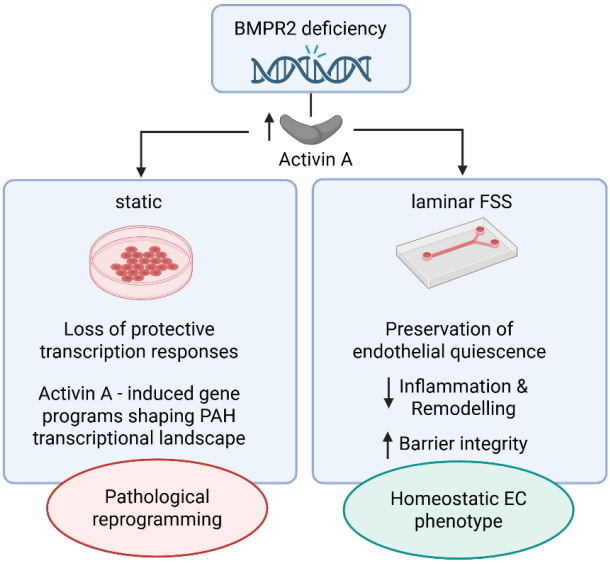

Figure 1. Mechanistic modulation of endothelial phenotype in *BMPR2*-deficient cells. *BMPR2* deficiency sensitizes endothelial cells (ECs) to Activin A signaling, resulting in divergent outcomes that depend on the mechanical microenvironment. Under static or disturbed flow conditions (*speculative*), protective shear-responsive gene expression is lost, enabling Activin A–induced transcriptional programs associated with inflammation, remodeling, and PAH progression. In contrast, laminar fluid shear stress (FSS) preserves endothelial quiescence by suppressing inflammatory pathways while maintaining barrier integrity and promoting a homeostatic EC phenotype.

The Future of PAH Research

The mechanistic insights presented in this thesis set the stage for a shift from symptom management to disease modification in PAH. Most approved therapies focus on vasodilation rather than targeting the underlying drivers of disease. While such treatments can delay progression, they are not curative and offer limited benefit for many patients, particularly those with advanced or non-vasoresponsive disease. A new research era is emerging, one that seeks not only to manage symptoms but to modify the disease itself by addressing the root causes of vascular remodeling and right ventricular dysfunction. This paradigm shift is already reflected in the development of sotatercept, a first-in-class ligand trap that rebalances TGF-β superfamily signaling by targeting multiple Activin-class ligands. Sotatercept binds Activin A, Activin B, GDF8, and GDF11, redirecting signaling from the pro-proliferative SMAD2/3 axis toward the anti-proliferative SMAD1/5/9

pathway[39]. Its clinical efficacy has been demonstrated in multiple Phase 2 and 3 trials (e.g., STELLAR, ZENITH, SPECTRA), where it significantly reduced mortality, transplantation risk, and PAH-related hospitalizations. Additionally, it promoted reverse remodeling of the right ventricle, suggesting a disease-modifying potential[39, 40]. However, its mechanism remains incompletely understood. Recent proteomic analyses have revealed that sotatercept also lowers circulating BMP9 and BMP10 levels (ligands critical for endothelial homeostasis), raising concerns about potential off-target effects. Side effects, such as telangiectasia, associated with BMP9/10 deficiency, highlight the risk of broadly targeting multiple ligands simultaneously[41]. Rather than exemplifying precision medicine, sotatercept illustrates both the promise and pitfalls of global pathway modulation. New investigational therapies now aim to slow down or even reverse the proliferative, fibrotic, and inflammatory changes that drive PAH. These include BMPR2 pathway modulators, kinase inhibitors, metabolic modulators, anti-inflammatory agents, and novel prostacyclin formulations, among others. Some, like seralutinib (an inhaled PDGFR/CSF1R inhibitor), are designed to act locally within the lungs to minimize systemic toxicity. Others, such as dichloroacetate or metformin, target metabolic dysfunction and may be repurposed based on patient-specific metabolic phenotypes[42, 43]. These efforts reflect a broader shift from managing symptoms to actively reversing structural and molecular abnormalities. Significantly, there is also growing recognition of the need to develop therapies that directly support right ventricular (RV) function, a significant determinant of prognosis in PAH. Despite the central role of RV adaptation in survival, current treatments largely neglect this aspect[2]. Additionally, PAH is a highly heterogeneous disease, with variable genetic, epigenetic, inflammatory, and mechanical drivers. Not all patients respond equally to the same therapeutic strategy, and while randomized clinical trials are foundational, they often fail to capture the heterogeneity of treatment response[44]. To address this, future research must focus on refining patient subgroups more precisely. Biomarkers such as Activin A, inflammatory cytokines, or NT-proBNP, when integrated with cardiac imaging and hemodynamic data, can help stratify patients by risk of clinical worsening and responsiveness to therapy. Genetic profiling may further identify those more likely to respond to specific therapies, for instance, carriers of pathogenic *BMPR2* variants may benefit from TGF-β superfamily modulation.

Emerging multi-omics technologies (transcriptomics, proteomics, metabolomics, and epigenomics) offer powerful tools to unravel the complex cellular interactions and signaling networks underpinning PAH. By integrating these data, clinicians can classify patients into distinct molecular subgroups defined by distinct pathogenic mechanisms, offering a

path toward more personalized treatment strategies. Single-cell RNA sequencing further enhances this precision by identifying disease-driving cell populations and their gene expression profiles[45, 46]. These insights are also leading to advances in drug discovery. Multi-omics-based analyses have already enabled the prediction and repurposing of drugs tailored to specific disease subtypes. One such study found second-generation phosphodiesterase-V (PDE5) inhibitors to be more effective in a hypoxia-associated PAH subgroup, suggesting new directions for therapeutic refinement[45].

Machine learning (ML) is becoming essential in translating these discoveries into clinical practice. ML algorithms can detect PAH earlier by recognizing diagnostic patterns in electronic health records, and they outperform traditional models in predicting disease progression. ML also supports the discovery of novel molecular sub-phenotypes that align more closely with disease biology than conventional clinical classifications. Importantly, supervised ML can forecast treatment response and guide individualized therapies to those most likely to benefit[46]. Finally, ML could improve clinical trial design by using real-time data to adjust for safety and effectiveness (Figure 2). Traditional trial designs are increasingly inadequate for rare, complex diseases like PAH. Flexible formats, such as event-driven, platform, or adaptive trials, can accelerate therapeutic evaluations. However, for real progress, we need to include more diverse patient groups, as current data are mostly limited to sex, demographic, and geographic representation[43]. Personalized medicine cannot succeed if it is only validated in a subset of patients.

An increasingly important focus in PAH research is the preclinical phase, where growing evidence suggests that carriers of pathogenic *BMPR2* variants exhibit subclinical vascular dysfunction well before a clinical diagnosis is made. Our own findings, such as the elevation of Activin A in asymptomatic individuals (Chapter 4), suggest that mechanical forces and genetic risk interact early to shape disease susceptibility. Can we detect pheno-conversion before irreversible remodeling occurs? If so, how early is early enough to intervene? Addressing these questions lies at the heart of the future research agenda and will require longitudinal biomarker studies, high-resolution imaging, and, perhaps most importantly, carefully considered ethical guidelines to support preventive treatments in asymptomatic high-risk individuals.

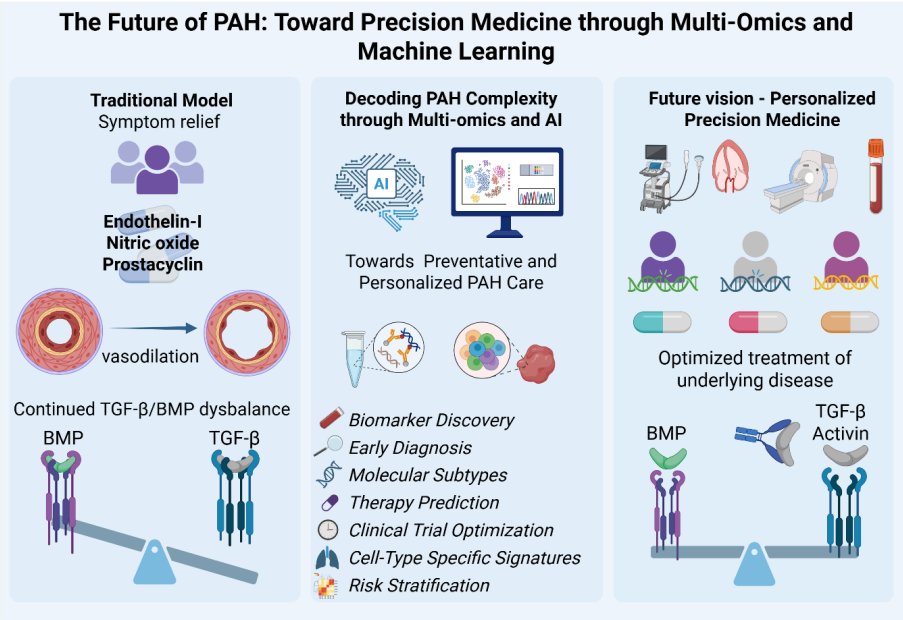

Figure 2. The Future of PAH: Towards Precision Medicine through Multi-Omics and Machine Learning. This schematic outlines a potential future of PAH management and precision approaches enabled by multi-omics and artificial intelligence (AI). Left panel**:** The traditional model focuses on symptom relief through vasodilators, without addressing the underlying TGF-β/BMP signaling imbalance that is central to disease progression. Middle panel: Integration of multi-omics technologies and AI allows for a deeper understanding of PAH heterogeneity. This approach allows the discovery of biomarkers, early diagnosis, molecular subtype classification, and personalized therapy prediction, laying the groundwork for preventative and individualized care. Right panel: The envisioned future of PAH research emphasizes the development of tailored treatment strategies based on genetic, molecular, and cellular profiling. This includes targeting specific TGF-β superfamily components (e.g., BMP, Activins) to restore signaling balance and optimize treatment of the underlying disease.

Conclusions

The work presented in this thesis supports a shift in how we conceptualize TGF-β superfamily signaling in PAH. Our findings highlight the dynamic nature of this pathway, where cellular context, environmental cues, and tissue specificity shape its effects. ECs are at the heart of this regulatory network. They integrate genetic risk, mechanical forces, and inflammatory signals into distinct transcriptional outputs that either maintain vascular homeostasis or initiate pathological remodeling. This contextual

sensitivity has long complicated therapeutic targeting. Rather than attempting to silence a multifunctional pathway, we propose to restore its physiological balance by modulating the specific ligands, receptors, and downstream effectors that are dysregulated in diseases like PAH. The emergence of Activin A as a biomarker and a therapeutic target, along with the clinical success of sotatercept, marks a turning point in this strategy. These advances illustrate that it is possible to intervene within the TGF-β superfamily; a nuanced understanding of timing, cell type, and signaling dynamics guides it. Equally important is the realization that modeling endothelial biology *in vitro* must reflect the physical reality in which these cells operate. Shear stress is not a secondary variable; it is a primary determinant of endothelial behavior. We argue that flow exposure should be the default condition in experimental design, and we offer an accessible platform to support this transition. Looking forward, the goal is not to define a single target or therapy, but to build a framework for understanding how different cues intersect to drive endothelial decisions. Such a framework can guide more precise interventions, better biomarker panels, and ultimately, more personalized approaches for treating and preventing PAH.

In this sense, the broader question that opened this discussion, whether the TGF-β superfamily can ever be safely and effectively targeted, leads us not to a definitive answer, but to a deeper understanding of what is required to do so. Therapeutic success will not come from simplifying the biology, but from embracing its complexity.

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