Redirecting TGF-β signaling pathways: advancing targeted therapy for PAH and FOP

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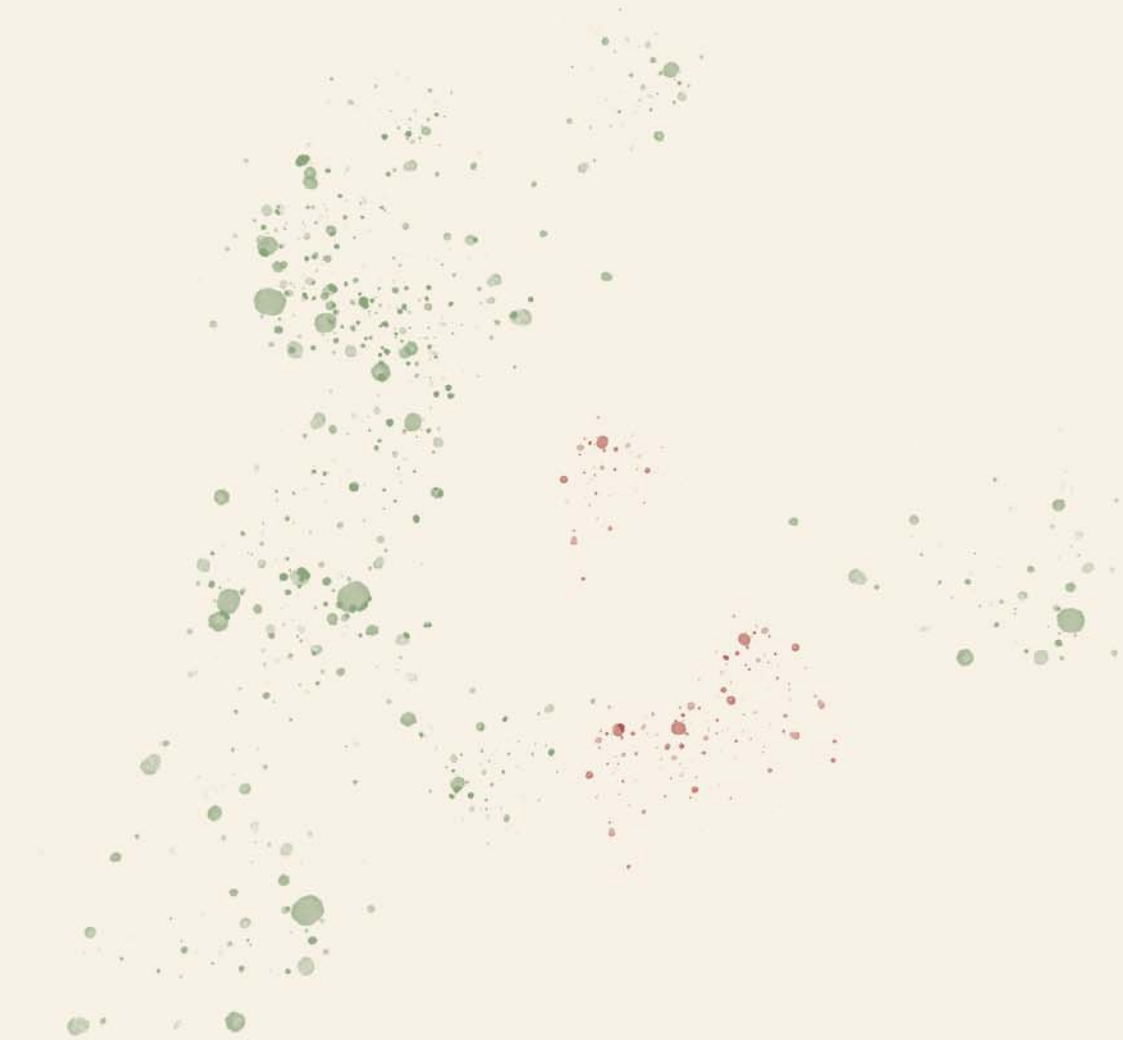
Chapter 1



General introduction

Preface

Tissue homeostasis relies on an intricate balance of extracellular biochemical and physical stimuli that regulate cellular processes like proliferation, differentiation, migration, apoptosis and survival.[1] These regulatory signals are transmitted through the cell via a process known as signal transduction, which can be initiated by upstream ligands like hormones, growth factors, and cytokines. Upon binding to specific receptor kinases, these ligands elicit downstream intracellular biochemical signaling responses through second messengers. Receptor-mediated signaling pathways can be stratified by the phosphorylation of their target substrates, primarily tyrosine and serine/threonine residues. Tyrosine receptor kinases (TRKs) are activated by ligands such as epithelial growth factor (EGF) or vascular endothelial growth factor (VEGF).[2] In contrast, serine/threonine kinase receptors are predominantly represented by members of the Transforming growth factor-β (TGF-β) superfamily.[3]

These signaling pathways orchestrate subcellular processes, including gene transcription, translation, and post-translational modifications, ultimately regulating cellular behavior. Dysregulated signal transduction is a hallmark of the pathogenesis of many, if not all, human diseases.[1,4,5] Understanding these signaling mechanisms in a context-dependent manner is therefore essential to identify strategies to restore tissue homeostasis and treat disease. In this thesis, we will primarily focus on TGF-β signaling and its role in tissue homeostasis and disease, while also exploring complementary signaling pathways where relevant.

The TGF-β signaling pathway

TGF-β signaling regulates pleiotropic biological processes during development and tissue homeostasis.[6] The mammalian TGF-β superfamily consists of more than 33 ligand-encoding genes including TGF-βs, Activins, Inhibins, Nodal, Bone morphogenetic proteins (BMPs), Growth differentiation factors (GDFs), and Anti-Müllerian Hormone (AMH).[7–13] Most TGF-β superfamily ligands, with BMPs being the exception,[14] are secreted as an inactive precursor and bound within a latent complex.[15] The active form is stabilized by latency-associated peptides (LAPs) and latent TGF-β binding proteins (LTBPs), which shield the ligand from premature activation.[16] Latent complexes are often sequestered at the plasma membrane or integrated into the extracellular matrix (ECM),[17] building a reservoir of latent inactive TGF-β. Activation occurs through mechanic or enzymatic cleavage, releasing the active ligands, and allowing a quick functional and localized response on demand.[15] Additionally, some TGF-β ligands are secreted in their active form, facilitating autocrine, paracrine or endocrine signaling. TGF-β ligands can present as monomers or homo/hetero-dimeric complexes.[18,19] As such, circulating levels of e.g. BMP9 (in picogram)[20] and Activin A (in pico to nanogram)[21] can be measured.

Active TGF-β ligands bind their respective plasma-membrane receptors guided by binding affinity and receptor availability (Figure 1).[3] The TGF-β receptors consist of seven different type I receptors (referred to as Activin receptor-like kinase (ALK) 1-7) and five different type II receptors, including TGFβR2, Activin receptor type 2A (ACTR2A), ACTR2B, BMPR2, and AMHR2.[22] Upon binding of a ligand dimer, the receptors form tetra-heteromeric complexes consisting of at least two type II and two type I receptors.[23] Ligand binding preferences differ

within the superfamily: TGF-β ligands bind their type II receptors with high affinity before recruiting their type I receptors, whereas BMP ligands favour type I receptor binding first.[23,24] Certain ligands, including TGF-β2, exhibit low affinity for both type I and II receptors and depend on TGF-β type III co-receptors like Betaglycan (TGFβR3) and Endoglin for signaling.[23,25] Type III co-receptors therefore fine-tune ligand-receptor complex formation.[26–28] When cleaved from the membrane, soluble forms of these co-receptors can act as ligand traps and reduce ligand availability.[29] Also, other extracellular ligands like Noggin, Gremlin and Follistatin modulate TGF-β signaling by functioning as agonists or antagonists (Figure 1).[30] Upon complex receptor oligomerization, the type II receptor will phosphorylate the type I receptor on its glycine-serine (GS)-rich motif N-terminally located to the kinase domain, initiating intracellular signaling.[3]

The active type I receptor then phosphorylates its downstream effectors including the receptor-regulated small mothers against decapentaplegic (R-SMADs). The SMAD family contains 8 different SMADs, categorized into R-SMADs (SMAD1-5 and -8), a common partner-SMAD (SMAD4 or co-SMAD), and inhibitory (I-)SMADs (SMAD6 and -7).[31] Phosphorylation of SMAD2/3 occurs through complexes containing ALK5/TGFβR2 by TGF-βs or ALK4/ACTRII by Activins. BMPs and GDFs phosphorylate SMAD1/5/8 via ALK1/2/3/6 and type II containing complexes. The ligand-specific receptor complexes are depicted in *Figure 1*. Negative feedback is mediated by I-SMADs, which inhibit signaling by competing with SMAD4[32] and promoting TGF-β receptor degradation by recruiting E3 ubiquitin ligases such as SMURF1/2.[33–35]

Upon activation, R-SMADs form a trimeric complex with co-SMAD4 in the cytoplasm and translocate to the nucleus, where they act as nuclear transcription factors. Binding to gene regulatory elements is motif-specific where SMAD2/3 recognizes CAGA-rich sequences, and SMAD1/5/8 interact with BMP responsive elements (BRE).[36–38] SMADs have relatively low intrinsic DNA-binding affinity and require context-dependent co-factors to enhance transcription.[39] Additionally, SMADs can also function as co-factors for other transcription factors, adding another layer of regulatory complexity. Canonical downstream targets of SMAD2/3 include connective tissue growth factor (*CTGF*) and plasminogen activator inhibitor-1 (*PAI1*)/*Serpine*, whereas SMAD1/5/8 drive the expression of inhibitor of differentiation *(ID)-1* and *ID-3*, among others. These distinct transcriptional outputs underscore the distinct cellular outcomes mediated by TGF-β and BMP signaling pathways. Therefore, a balanced interplay between SMAD2/3 and SMAD1/5/8 signaling is essential for regulating diverse biological processes and maintaining appropriate cellular responses.

While SMAD-dependent signaling is recognized as the canonical TGF-β pathway, TGF-β receptors can also activate non-SMAD signaling cascades.[40,41] These include phosphatidylinositol 3-kinase (PI3K)/AKT, Rho-associated small GTPases, and mitogen-activated protein kinases (MAPKs) pathways, including JNK, p38 isoforms and ERK.[41] Mechanistically, the processes underlying TGF-β-induced non-SMAD signaling are less well understood. While type I receptors are crucial for SMAD-dependent signaling, it is hypothesized that type II receptors may play a more central role in non-SMAD signaling.[40,42] Moreover, although TGF-β receptors are primarily serine/threonine kinases, they can phosphorylate tyrosine substrates, classifying them as dual-specificity kinases.[43] Non-SMAD

pathway activation by TGF-β superfamily receptors is highly context-dependent.[44] For example, TGF-β directly phosphorylates ShcA, which activates ERK in mouse fibroblasts, regulating cellular proliferation and apoptosis.[43] In vascular biology, TGF-β-mediated inhibition of vascular smooth muscle cell proliferation depends on p38 signaling.[45] Non-SMAD signaling downstream of BMP ligands and receptors are even less well-studied than TGF-β-induced effects.[40,46–49] This thesis aims to provide further insights into context-dependent activation and functional outcomes of non-SMAD signaling pathways.

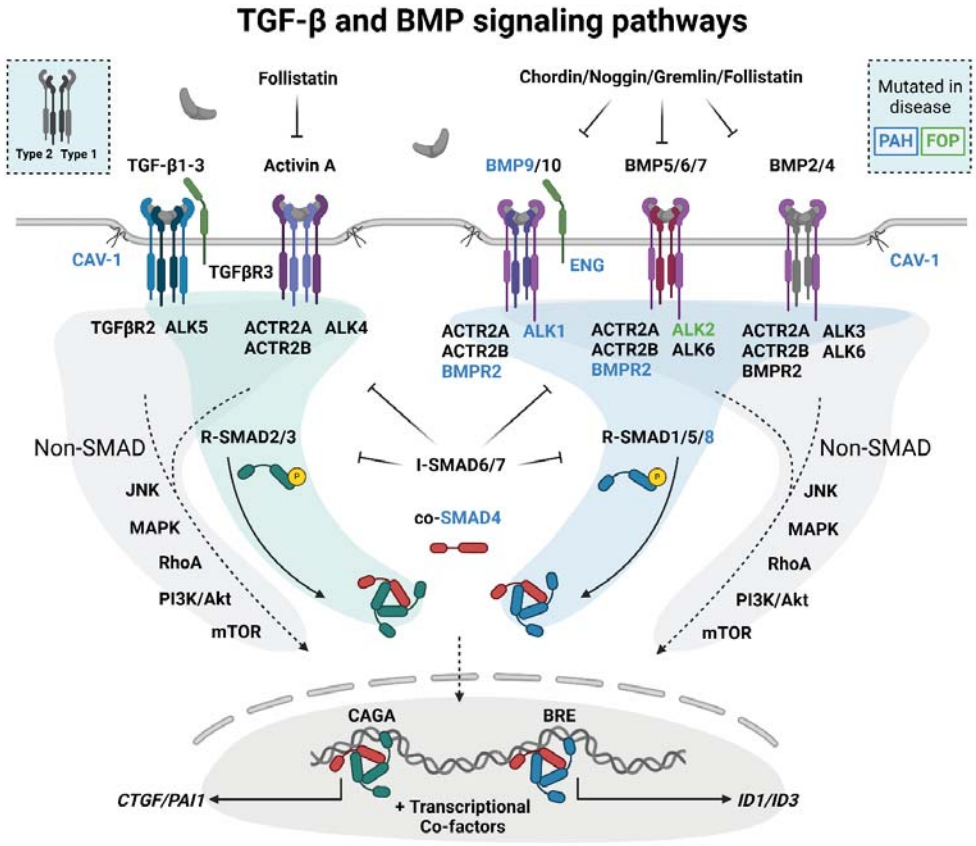


Figure 1. Schematic depiction of TGF-β and BMP signaling pathways. TGF-β superfamily ligands bind their respective type II and type I receptors. Following oligomerization, the type I receptors phosphorylate downstream R-SMADs and form a trimer with co-SMAD4. Active SMAD complexes translocate to the nucleus and function as transcription factors partnered with other transcriptional co-factors, binding to CAGA- (R-SMAD2/3 responsive) or BRE-elements (R-SMAD1/5/8 responsive). Canonical transcriptional targets of TGF-β include CTGF/PAI1 while BMPs typically induce ID1/3 expression. Negative regulation involves antagonists like Follistatin, binding competition of I-SMAD6/7, and proteasomal degradation of TGF-β receptors mediated through these inhibitory SMADs. Besides this SMAD-dependent signaling, non-SMAD signaling pathways (e.g. MAPK, RhoA, PI3K) may be activated through TGF-β or BMP receptors. Mutations in these signaling pathways can cause the

Figure 1. *(continued)*
development of pulmonary arterial hypertension (PAH, shown in blue) or fibrodysplasia ossificans progressiva (FOP, shown in green). Abbreviations are explained in the text.

Disruption of the delicate balance between TGF-β and BMP signaling, for example through gene mutations in receptors or altered secretion of TGF-β ligands, can contribute to the development of various diseases including cancer[4,50], fibrosis[51], cardiovascular disease[52,53] and musculoskeletal disorders[54]. This thesis specifically explores two rare genetic disorders linked to dysregulated TGF-β signaling: pulmonary arterial hypertension (PAH) and fibrodysplasia ossificans progressiva (FOP).

Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a rare cardiovascular disorder characterized by pulmonary vascular remodeling, which increases vascular resistance and pressure, resulting in right ventricle hypertrophy, dilatation and ultimately leading to right heart failure (RHF). Clinically, PAH is classified within WHO group I of pulmonary hypertension and can be further stratified into hereditary PAH (HPAH) and idiopathic PAH (IPAH). HPAH encompasses cases with familial or genetic origins, while IPAH refers to non-familial sporadic cases. Pre-capillary PH, including PAH, is defined by a mean pulmonary arterial pressure (mPAP) > 20 mmHg, a pulmonary arterial wedge pressure (PAWP) of ≤ 15 mmHg, and pulmonary vascular resistance (PVR) of ≥ 2 wood units.[55] With an incidence of about ~6 cases per million adults, PAH is considered a rare disorder.[55] Recent registries reveal an increasing diagnosis of older PAH patients with a balanced sex distribution, often associated with underlying cardiovascular comorbidities.[56] In contrast, younger PAH patients, predominantly those with HPAH, show a marked female predominance. Notably, male patients present with more severe phenotypes. **Chapter 4** of this thesis explores sex-related differences in PAH in more detail.[57]

The pathophysiology of PAH involves a combination of vasoconstriction and remodeling of the pulmonary vasculature, which leads to remodeling of the right heart as an adaptive response. Vascular remodeling is characterized by thickening of all three arterial layers due to cellular proliferation, resistance to apoptosis, inflammatory infiltration, increased migration and endothelial-to-mesenchymal transition (EndMT). This process of remodeling involves multiple cell types within the vascular bed, including endothelial cells, smooth muscle cells, pericytes, and immune cells.[58] Advanced vascular remodeling can result in plexiform lesion formation and increased thrombosis, ultimately obstructing the pulmonary arteries.[59] Remodeling of the right heart in PH begins with ventricular hypertrophy and progresses to dilatation. Cardiac myofibroblasts play a central role by driving extracellular matrix (ECM) production, which contributes to fibrosis and stiffening of the ventricle.[60,61] The heart's adaptive capacity is a critical determinant of disease severity and mortality. Triggers for PAH development include inflammation, hypoxia, sex(-hormones), genetic predisposition and dysregulated TGF-β signaling.[57,58,62]

Loss-of-function mutations in approximately 17 genes contributes to PAH development,[62] many of which are linked to the TGF-β signaling pathway involving BMP signaling. As such, *BMPR2* mutations are found in 80% of all HPAH cases and up to 20% in IPAH patients.[63] However, the disease penetrance of BMPR2 mutation carriers is low (~30%) with a clear sex-

bias, with 41% of female carriers developing the disease compared to 14% of males.[64] It is therefore accepted that secondary triggers influence BMPR2 signaling, directly or indirectly. Indeed, factors such as hypoxia[65], inflammation[66,67] and sex-hormones[57,68] have been shown to regulate the BMPR2 signaling pathway. Although more than 650 *BMPR2* mutations have been described so far, most lead to functional loss or even haploinsufficiency.[69,70] Interestingly, in PAH patients without an identified genetic cause, reduced BMP signaling has been observed alongside increased TGF-β signaling in the lung vasculature.[71–73] This imbalance is further supported by elevated circulating levels of TGF-β1, GDF8/11, Activin A and Follistatin in patient blood samples.[21,73–75] Consequently, enhanced TGF-β/SMAD2/3 signaling promotes endothelial dysfunction, EndMT and pro-proliferative pathways in ECs and smooth muscle cells,[76–78] whereas BMP signaling supports vascular quiescence.[79] In conclusion, the disbalanced TGF-β signaling pathway underlies vascular dysfunction and PAH pathogenesis.

At diagnosis, PAH patients are typically prescribed vasodilators targeting the guanylate cyclase, endothelin, or prostacyclin pathways. Current guidelines recommend triple therapy for severe disease, which has significantly improved patient outcomes and delayed progression to severe RHF.[55,56] In these advanced cases, patients may be eligible for lung transplantation. Although no curative treatment exists, targeting the underlying aberrant TGF-β signaling offers a promising therapeutic approach.[75] This thesis aims to identify more effective and selective therapeutics targeting the TGF-β signaling family, which could significantly enhance clinical outcomes for PAH patients.

Fibrodysplasia ossificans progressiva

Fibrodysplasia ossificans progressiva (FOP) is an ultra-rare autosomal dominant genetic disorder with an estimated prevalence of 1 in a million people.[80,81] This musculoskeletal disorder is characterized by the abnormal formation of heterotopic bone, through endochondral ossification, in soft connective tissues including tendons, ligaments, and skeletal muscle. While heterotopic ossification (HO) is the hallmark and most burdensome symptom causing pain and severe immobility, FOP patients may also develop arthritis[82], osteochondromas[83] or (small) skeletal/joint dysplasias[82,84]. HO is caused by random or injury-induced episodic painful inflammatory events known as 'flare-ups'. Upon injury and related inflammatory triggers, these flare-ups activate fibro-adipogenic progenitors (FAPs), which initiate endochondral ossification instead of the normal muscle regeneration process.[85,86] The underlying cause of FOP was identified in 2006 as a mutation in the *ACVR1* gene, encoding the ALK2 receptor.[87] Further genetic screening showed that almost all patients carry the specific gain-of-function mutation *ACVR1* c.617G>A which encodes the ALK2 p.R206H variant.

Being a BMP type I receptor, ALK2 induces SMAD1/5/8 signaling, driving osteochondrogenic pathways in mesenchymal cells.[49,88] Its high-affinity ligands are BMP6, BMP7 and Activins, with BMPs acting as agonists and Activins as antagonists for the wild-type ALK2. The p.R206H mutation occurs in the receptor's GS domain, a crucial regulatory region that mediates kinase activity and is typically phosphorylated by type II receptors upon oligomerization.[87] Studies have shown that artificial mutations like ALK2 p.Q207D (also located in the GS domain) lead to constitutive receptor activation.[88] Initially, also the p.R206H mutation was hypothesized to confer constitutive activity.[88] However, research since 2015 has revealed that the mutant

ALK2^{R206H} receptor exhibits a neofunctional response to Activin A binding.[89] Recent studies also highlight that Activin B, AB and AC can also activate ALK2^{R206H} signaling. Other mechanisms of oligomerizing mutated ALK2 receptor complexes, such as ALK2 antibody binding[90] or experimental methods like optogenetics[91], also resulted in downstream pathway activation. This demonstrates that the oligomerization of ALK2^{R206H} receptor complexes alone is sufficient to activate signaling, whereas ALK2^{WT} receptors remain unresponsive. Nevertheless, the formation of functional signaling complexes still relies on the presence of a structural, though not necessarily functional, type II receptor within the complex.[91] In summary, the FOP mutation renders ALK2 active upon Activin (A, B, AB, and AC isoforms) binding, an effect that would normally be inhibitory in wild-type cells. Thus, this aberrant overactivation of BMP signaling likely underlies the osteochondrogenic symptoms observed in FOP.

Noteworthy, despite ALK2^{R206H} being ubiquitously expressed, FOP manifests in specific tissues, suggesting that Activin-induced SMAD1/5/8 phosphorylation may not be the only mechanism causing FOP. This thesis further delves into the abnormal signaling in FOP by focusing on non-SMAD signaling pathways. As described in section 1.1., TGF-β and BMP signaling receptors can also activate non-SMAD signaling pathways[41], which are often highly context-dependent and may function in a cell-type and/or tissue-specific manner. In FOP, several studies have identified alterations in non-SMAD signaling pathways, including mTOR[92], PI3K[93], and YAP1/RhoA GTPase[94] signaling. To comprehensively characterize non-SMAD pathways in FOP, in **chapter 5**, we used non-biased multi-omics profiling in an ALK2^{R206H} background.

Currently, treatment options for FOP patients remain limited, primarily focusing on injury prevention and the use of high-dose anti-inflammatory medications (e.g., prednisone, NSAIDs) during flare-ups.[95] Given the extreme pain associated with these flare-ups, the use of analgesics may also be warranted. However, no curative therapies exist for FOP yet. Given the importance of altered TGF-β signaling as a driver for HO[86] and the tissue-specificity in disease development, this thesis aims to identify novel therapeutics targeting TGF-β signaling including context-dependent non-SMAD signaling routes to treat FOP.

Since the start of this thesis, the field of TGF-β signaling modulators for clinical use (Figure 2) has gained significant momentum, of which the recent developments are extensively discussed in **chapter 7**. The recurrent problem in testing drugs directly targeting TGF-β signaling lies in the high degree of similarity among its receptors, often leading to drug development failures. The methodologies used for testing such drugs might be optimized to identify specific and effective drug candidates. Consequently, we will examine the current in vitro techniques employed to measure TGF-β signaling.

Conventional in vitro methods to detect TGF-β signaling and -modulators

To understand aberrant TGF-β signaling and identify novel therapeutics for TGF-β-associated disorders, highly selective and potent in vitro high-throughput (HTP) methods are indispensable. These methods should be particularly valuable for evaluating potential therapeutics such as small kinase inhibitors, allosteric molecules, antibodies, and fusion ligand traps (Figure 2). Commonly used assays include cross-linking-based systems, transcriptional reporters, and western blotting. Cross-linking assays can measure receptor-specific interactions but require radioactive isotopes, specific antibodies, and labor-intensive protocols.[96] Transcriptional reporter assays (i.e. CAGA- and BRE-luciferase) measure downstream canonical pathway activation,[97,98] while western blotting detects (phosphorylated) protein levels affected by the drug. However, these assays lack receptor specificity, are typically low- to medium-throughput and require cell lysis. Other approaches include in vitro kinase assays, surface plasmon resonance (SPR), and protein arrays.[99,100] In vitro kinase assays and SPR rely on purified recombinant proteins, making them scalable but physiologically limited. Protein arrays, such as sandwich-ELISAs or dot blots, accommodate complex cellular systems and allow medium throughput.[101] Yet, these rely on antibody availability and involve cell lysis, preventing real-time kinetic measurements crucial for understanding cellular physiology and pharmacokinetics.

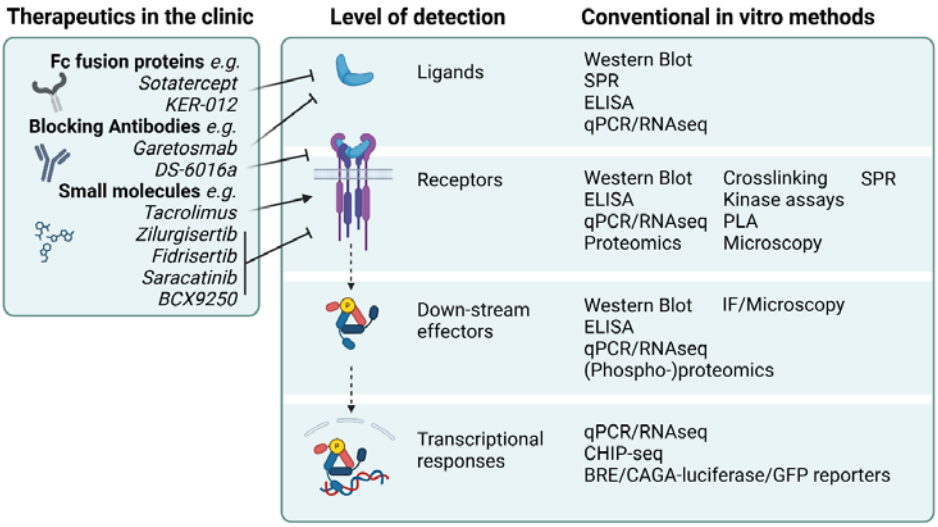


Figure 2. Clinically tested therapeutics directly targeting the TGF-β signaling pathway for PAH/FOP and conventional methods used to detect the corresponding signaling level. There are three generally studied groups of therapeutics directly targeting TGF-β signaling: 1) Fc-fusion proteins, 2) blocking antibodies, and 3) small molecules. The clinical therapeutics exemplified were mostly developed since the start of this thesis and are discussed in the general discussion found in chapter 7. These drugs often target a certain signaling level, ranging from circulating ligands to transcriptional responses. There are various conventional in vitro methods used to detect different signaling levels. Abbreviations not described in the main text; ELISA: enzyme-linked immunosorbent-

Figure 2. *(continued)*
assay, IF: immunofluorescence microscopy, qPCR: quantitative polymerase chain reaction, PLA: proximity ligation assay and CHIP-seq: Chromatin Immunoprecipitation-sequencing.

Despite their utility, conventional assays share key limitations. Many lack physiological relevance as they rely on recombinant proteins, artificially high ATP levels, or overly simplified experimental conditions. Most assays are incompatible with live-cell kinetic readouts because they involve cell lysis without high-throughput capacity. These shortcomings impede the ability to study drug effects on cellular physiology, pharmacokinetics, and complex cellular environments, such as compound diffusion and stability, in real time and high-throughput settings. Addressing these challenges is crucial for developing HTP screening methods that reflect cellular complexity and facilitate the identification of effective TGF-β signaling modulators. In this thesis, we investigate innovative approaches to overcome these limitations, advancing the development of novel therapeutics targeting TGF-β signaling.

Thesis outline

The central hypothesis of this thesis is that novel therapeutics targeting TGF-β-associated signaling pathways can be identified and investigated for their potential in treating PAH and FOP. To achieve this, we combined method development with drug screening while simultaneously exploring relevant downstream signaling pathways and therapeutic targets. Accordingly, this thesis is structured into two main parts: (I) development of molecular tools (**chapter 2-3**): Establishing innovative approaches to identify modulators of TGF-β signaling, and (II) molecular mechanisms and target discovery (**chapter 4-6**): Investigating the molecular underpinnings of PAH and FOP to discover novel therapeutic targets.

This integrated approach aims to advance both the methodological framework and the therapeutic landscape for TGF-β associated disorders (Figure 3).

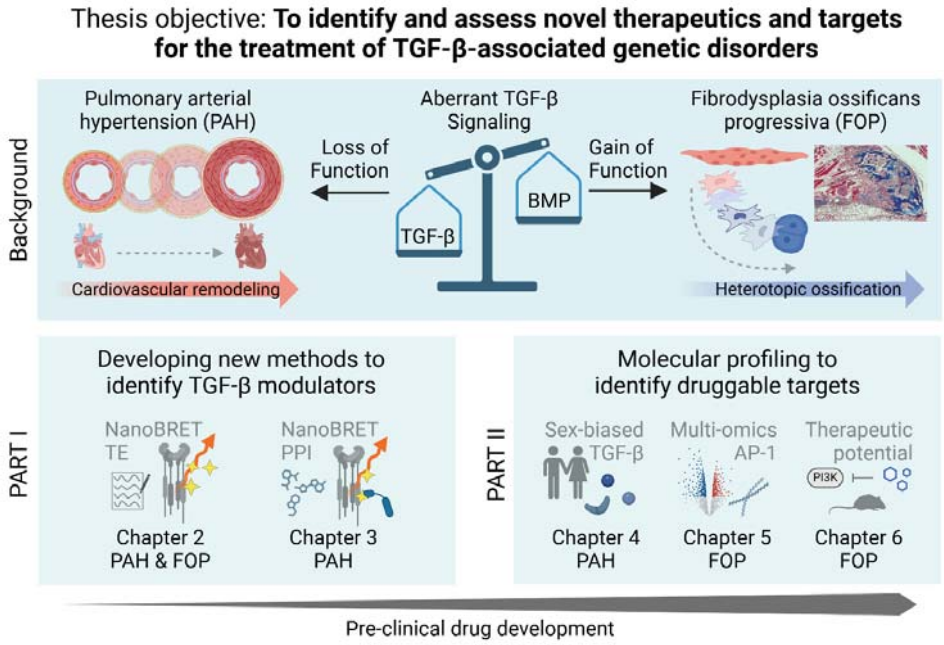


Figure 3. An overview of the central objective and thesis outline. The overall objective of this thesis is to identify and test novel therapeutics and targets for the treatment of the TGF-β-associated genetic disorders PAH and FOP. The thesis is stratified in two parts: 1) to develop new methods to identify new TGF-β modulators and 2) to profile molecular mechanisms in disease to find and study druggable targets. Part I includes chapter 2-3 while part II involves chapter 4-6. The chapters are generally arranged at increasing stages of pre-clinical drug development. All abbreviations are described in the main text.

In **chapter 2** we introduce a comprehensive toolkit to study TGF-β receptor-selective compound target engagement (TE) in live cells. The toolkit includes a combination of established and newly developed constructs, tracers, cell lines and protocols. The study focused on optimizing and characterizing the current nanoBRET TE methodology as a high-

throughput screening method. This improved protocol stands out from other methods for its TGF-β receptor-specific, live cell (and kinetic), high-throughput, and antibody-independent readout.

While the nanoBRET TE assay proved valuable for studying kinase inhibitors, it is unsuitable to quantify ligand-inducible TGF-β receptor activation. As such, tracer-based NanoBRET TE is limited to small molecules that compete with the tracer's interaction capacity. However, a broader range of drugs, including ligand (ant)agonists, allosteric modulators, ligand traps, and antibodies (Figure 2), can target TGF-β signaling. Therefore, in **chapter 3**, we further developed our nanoBRET toolkit to measure the protein-protein interaction (PPI) of TGF-β receptors with either downstream SMAD effectors or a type II TGF-β receptor. We focused on interactions relevant in PAH to ultimately screen for a potential drug to treat this disease. This proof-of-principle study demonstrated that nanoBRET-based PPI systems can detect ligand-induced TGF-β receptor-specific interactions. Unfortunately, the nanoBRET-based PPI assays proved unsuitable for large-scale experiments, hence, we performed a drug screen using nanoBRET TE and an experimental kinase inhibitor library to identify novel potential PAH therapeutics (see **chapter 3**).

In part II of this thesis, we shifted to exploring and assessing novel TGF-β signaling-associated molecular mechanisms to find targets for treating PAH or FOP. As such, **chapter 4** introduces context-dependent cross-talk of sex determinants on TGF-β signaling in PAH. In this literature review, we assessed the relationship of sex-hormones and -genetics on TGF-β superfamily members. Following this work, we may better explain the increased female predominance and a more severe male phenotype observed in PAH. The findings reviewed here also led to the hypothesis that sex-hormones, including estrogen and dehydroepiandrosterone (DHEA), might be a valid (tissue-specific) therapeutic target dependent on the sex of the patient.

While canonical SMAD signaling has been extensively studied in TGF-β/BMP genetic diseases like PAH and FOP, **chapter 5** focuses on exploring which non-SMAD signaling pathways are over-activated using FOP as an experimental model. Combining phosphoproteomics with transcriptomics unveiled multiple non-SMAD signaling routes differentially regulated in $ALK2^{R206H}$ expressing human mesenchymal stem cells (hMSCs). Downstream differential regulation of Activator Protein-1 (AP-1) was identified and showed a promising therapeutic target to reduce endochondral ossification in vitro. Consistent with our multi-omics findings, in **chapter 6**, we investigated the molecular and cellular effects of targeting PI3Kα through BYL719 (Alpelisib) repurposing in FOP. BYL719's mechanism of action and target selectivity were determined, alongside the optimization of drug administration protocols, using human in vitro and mouse in vivo models.

This thesis integrates molecular, cellular and animal studies to redirect TGF-β superfamily-induced SMAD and non-SMAD signaling pathways. While these studies were conducted in the context of PAH and FOP, many findings in this thesis may very well be extended to other research fields including cancer, cardiovascular disease, musculoskeletal disorders and fibrosis.

References

1. Valls PO, Esposito A. Signalling dynamics, cell decisions, and homeostatic control in health and disease. *Curr Opin Cell Biol*. 2022;75. doi:10.1016/j.ceb.2022.01.011
2. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2010;141(7):1117-1134. doi:10.1016/j.cell.2010.06.011
3. Derynck R. TGF-β-receptor-mediated signaling. *Trends Biochem Sci*. 1994;19(1992):168-194. doi:10.1093/oxfordhb/9780199215362.013.8
4. Massagué J, Blain SW, Lo RS. TGFβ signaling in growth control, cancer, and heritable disorders. *Cell*. 2000;103(2):295-309. doi:10.1016/S0092-8674(00)00121-5
5. Garcia M, Mulvagh SL, Merz CNB, Buring JE, Manson JAE. Cardiovascular disease in women: Clinical perspectives. *Circ Res*. 2016;118(8):1273-1293. doi:10.1161/CIRCRESAHA.116.307547
6. Massagué J, Sheppard D. TGF-β signaling in health and disease. *Cell*. 2023;186(19):4007-4037. doi:10.1016/j.cell.2023.07.036
7. Kingsley DM. The TGF-β superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes Dev*. 1994;8(2):133-146. doi:10.1101/gad.8.2.133
8. Javelaud D, Mauviel A. Mammalian transforming growth factor-βs: Smad signaling and physio-pathological roles. *International Journal of Biochemistry and Cell Biology*. 2004;36(7):1161-1165. doi:10.1016/S1357-2725(03)00255-3
9. Sanchez-Duffhues G, Williams E, Goumans MJ, Heldin CH, ten Dijke P. Bone morphogenetic protein receptors: Structure, function and targeting by selective small molecule kinase inhibitors. *Bone*. 2020;138(April):1-13. doi:10.1016/j.bone.2020.115472
10. Shen MM. Nodal signaling: developmental roles and regulation. *Development*. 2007;1034:1023-1034. doi:10.1242/dev.000166
11. Namwanje M, Brown CW. Activins and Inhibins: Roles in Development, Physiology, and Disease. *Cold Spring Harb Perspect Biol*. 2016;8(7):1-55.
12. Chang H, Brown CW, Matzuk MM. Genetic analysis of the mammalian transforming growth factor-β superfamily. *Endocr Rev*. 2002;23(6):787-823. doi:10.1210/er.2002-0003
13. Visser JA. AMH signaling: from receptor to target gene. *Mol Cell Endocrinol*. 2003;211:65-73. doi:10.1016/j.mce.2003.09.012
14. Jiang H, Salmon RM, Upton PD, et al. The prodomain-bound form of bone morphogenetic protein 10 is biologically active on endothelial cells. *Journal of Biological Chemistry*. 2016;291(6):2954-2966. doi:10.1074/jbc.M115.683292
15. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFβ activation. *J Cell Sci*. 2003;116(2):217-224. doi:10.1242/jcs.00229
16. Constam DB. Regulation of TGFβ and related signals by precursor processing. *Semin Cell Dev Biol*. 2014;32:85-97. doi:10.1016/j.semcdb.2014.01.008
17. Stockis J, Dedobbeleer O, Lucas S. Role of GARP in the activation of latent TGF-β1. *Mol Biosyst*. 2017;13(10):1925-1935. doi:10.1039/c7mb00251c
18. Valera E, Isaacs MJ, Kawakami Y, Belmonte JCI, Choe S. BMP-2/6 heterodimer is more effective than BMP-2 or BMP-6 homodimers as inductor of differentiation of human embryonic stem cells. *PLoS One*. 2010;5(6). doi:10.1371/journal.pone.0011167
19. Hinck AP, Mueller TD, Springer TA. Structural biology and evolution of the TGF-β family. *Cold Spring Harb Perspect Biol*. 2016;8(12):1-51. doi:10.1101/cshperspect.a022103
20. Bidart M, Ricard N, Levet S, et al. BMP9 is produced by hepatocytes and circulates mainly in an active mature form complexed to its prodomain. *Cellular and Molecular Life Sciences*. 2012;69(2):313-324. doi:10.1007/s00018-011-0751-1
21. Guignabert C, Savale L, Boucly A, et al. Serum and Pulmonary Expression Profiles of the Activin Signaling System in Pulmonary Arterial Hypertension. *Circulation*. 2023;147:1-13. doi:10.1161/CIRCULATIONAHA.122.061501

22. Heldin CH, Moustakas A. Signaling receptors for TGF-β family members. *Cold Spring Harb Perspect Biol*. 2016;8(8):1-33. doi:10.1101/cshperspect.a022053
23. Ehrlich M, Gutman O, Knaus P, Henis YI. Oligomeric interactions of TGF-β and BMP receptors. *FEBS Lett*. 2012;586(14):1885-1896. doi:10.1016/j.febslet.2012.01.040
24. Aykul S, Martinez-Hackert E. Transforming Growth Factor-β Family Ligands Can Function as Antagonists by Competing for Type II Receptor Binding. *J Biol Chem*. 2016;291(20):10792-10804. doi:10.1074/jbc.M115.713487
25. Cheifetz S, Hernandez H, Laiho M, Ten Dijke P, Iwata KK, Massague J. Distinct Transforming Growth Factor-β (TGF-β) Receptor Subsets as Determinants of Cellular Responsiveness to Three TGF-β Isoforms. *J Biol Chem*. 1990;265(33):20533-20538.
26. Lebrin F, Goumans MJ, Jonker L, et al. Endoglin promotes endothelial cell proliferation and TGF-β/ALK1 signal transduction. *EMBO Journal*. 2004;23(20):4018-4028. doi:10.1038/sj.emboj.7600386
27. Velasco S, Alvarez-Muñoz P, Pericacho M, et al. L- and S-endoglin differentially modulate TGFβ1 signaling mediated by ALK1 and ALK5 in L6E9 myoblasts. *J Cell Sci*. 2008;121(6):913-919. doi:10.1242/jcs.023283
28. López-Casillas F, Wrana JL, Massagué J. Betaglycan presents ligand to the TGFβ signaling receptor. *Cell*. 1993;73(7):1435-1444. doi:10.1016/0092-8674(93)90368-Z
29. Nickel J, Ten Dijke P, Mueller TD. TGF-β family co-receptor function and signaling. *Acta Biochim Biophys Sin (Shanghai)*. 2018;50(1):12-36. doi:10.1093/abbs/gmx126
30. Chang C. Agonists and antagonists of TGF-β family ligands. *Cold Spring Harb Perspect Biol*. 2016;8(8):1-51. doi:10.1101/cshperspect.a021923
31. Miyazono K, Ten Dijke P, Heldin CH. TGF-β signaling by Smad proteins. *Adv Immunol*. 2000;75:115-157. doi:10.1016/s0065-2776(00)75003-6
32. Hata A, Lagna G, Massagué J, Hemmati-Brivanlou A. Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. *Genes Dev*. 1998;12:186-197. www.genesdev.org
33. Miyazawa K, Miyazono K. Regulation of TGF- b Family Signaling by Inhibitory Smads. *Cold Spring Harb Perspect Biol*. 2017;9:1-25.
34. Murakami G, Chiba T, Tanaka K. Smurf1 Interacts with Transforming Growth Factor-b Type I Receptor through Smad7 and Induces Receptor Degradation. *Journal of Biological Chemistry*. 2001;276(16):12477-12480. doi:10.1074/jbc.C100008200
35. Hayashi H, Abdollah S, Qiu Y, et al. The MAD-related protein Smad7 associates with the TGFβ receptor and functions as an antagonist of TGFβ signaling. *Cell*. 1997;89(7):1165-1173. doi:10.1016/S0092-8674(00)80303-7
36. Itoh Y, Koinuma D, Omata C, et al. A comparative analysis of Smad-responsive motifs identifies multiple regulatory inputs for TGF-β transcriptional activation. *Journal of Biological Chemistry*. 2019;294(42):15466-15479. doi:10.1074/jbc.RA119.009877
37. Katagiri T, Imada M, Yanai T, Suda T, Takahashi N, Kamijo R. Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis. *Genes to Cells*. 2002;7(9):949-960. doi:10.1046/j.1365-2443.2002.00573.x
38. López-Rovira T, Chalaux E, Massagué J, Rosa JL, Ventura F. Direct binding of Smad1 and Smad4 to two distinct motifs mediates bone morphogenetic protein-specific transcriptional activation of Id1 gene. *Journal of Biological Chemistry*. 2002;277(5):3176-3185. doi:10.1074/jbc.M106826200
39. Massagué J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev*. 2005;19(23):2783-2810. doi:10.1101/gad.1350705
40. Zhang YE. Non-Smad pathways in TGF-β signaling. *Cell Res*. 2009;19(1):128-139. doi:10.1038/cr.2008.328
41. Zhang YE. Non-Smad signaling pathways of the TGF-β family. *Cold Spring Harb Perspect Biol*. 2017;9(2). doi:10.1101/cshperspect.a022129

42. Galliher AJ, Schiemann WP. Src phosphorylates Tyr284 in TGF-β type II receptor and regulates TGF-β stimulation of p38 MAPK during breast cancer cell proliferation and invasion. *Cancer Res*. 2007;67(8):3752-3758. doi:10.1158/0008-5472.CAN-06-3851
43. Lee MK, Pardoux C, Hall MC, et al. TGF-β activates Erk MAP kinase signalling through direct phosphorylation of ShcA. *EMBO Journal*. 2007;26(17):3957-3967. doi:10.1038/sj.emboj.7601818
44. David CJ, Massagué J. Contextual Determinants of TGF-β Action in Development, Immunity and Cancer. *Nat Rev Mol Cell Biol*. 2018;19(7):419-435. doi:10.1038/s41580-018-0007-0.Contextual
45. Seay U, Sedding D, Krick S, Hecker M, Seeger W, Eickelberg O. Transforming growth factor-β-dependent growth inhibition in primary vascular smooth muscle cells is p38-dependent. *Journal of Pharmacology and Experimental Therapeutics*. 2005;315(3):1005-1012. doi:10.1124/jpet.105.091249
46. Guzman A, Zelman-Femiak M, Boergermann JH, et al. SMAD versus non-SMAD signaling is determined by lateral mobility of Bone Morphogenetic Protein (BMP) receptors. *Journal of Biological Chemistry*. 2012;287(47):39492-39504. doi:10.1074/jbc.M112.387639
47. Boergermann JH, Kopf J, Yu PB, Knaus P. Dorsomorphin and LDN-193189 inhibit BMP-mediated Smad, p38 and Akt signalling in C2C12 cells. *International Journal of Biochemistry and Cell Biology*. 2010;42(11):1802-1807. doi:10.1016/j.biocel.2010.07.018
48. Ghosh-Choudhury N, Abboud SL, Nishimura R, Celeste A, Mahimainathan L, Choudhury GG. Requirement of BMP-2-induced phosphatidylinositol 3-kinase and Akt serine/threonine kinase in osteoblast differentiation and Smad-dependent BMP-2 gene transcription. *Journal of Biological Chemistry*. 2002;277(36):33361-33368. doi:10.1074/jbc.M205053200
49. Rigueur D, Brugger S, Anbarchian T, Kim JK, Lee Y, Lyons KM. The type I BMP receptor ACVR1/ALK2 is required for chondrogenesis during development. *Journal of Bone and Mineral Research*. 2015;30(4):733-741. doi:10.1002/jbmr.2385
50. Bierie B, Moses HL. Tumour microenvironment - TGFB: The molecular Jekyll and Hyde of cancer. *Nat Rev Cancer*. 2006;6(7):506-520. doi:10.1038/nrc1926
51. Biernacka A, Dobaczewski M, Frangogiannis NG. TGF-β signaling in fibrosis. *Growth Factors*. 2011;29(5):196-202. doi:10.3109/08977194.2011.595714.TGF-
52. Goumans MJ, ten Dijke P. TGF-β signaling in control of cardiovascular function. *Cold Spring Harb Perspect Biol*. 2018;10(2):1-40. doi:10.1101/cshperspect.a022210
53. Goumans MJ, Liu Z, Ten Dijke P. TGF-β signaling in vascular biology and dysfunction. *Cell Res*. 2009;19(1):116-127. doi:10.1038/cr.2008.326
54. Kaplan FS, Xu M, Seemann P, et al. Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. *Hum Mutat*. 2009;30(3):379-390. doi:10.1002/humu.20868
55. Humbert M, Kovacs G, Hoeper MM, et al. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J*. 2022;43(38):3618-3731. doi:10.1093/eurheartj/ehac237
56. Lau EMT, Giannoulatou E, Celermajer DS, Humbert M. Epidemiology and treatment of pulmonary arterial hypertension. *Nat Rev Cardiol*. 2017;14(10):603-614. doi:10.1038/nrcardio.2017.84
57. Wits M, Becher C, De Man F, Sanchez-Duffhues G, Goumans MJ. Sex-biased TGFβ signalling in pulmonary arterial hypertension. *Cardiovasc Res*. 2023;119(13):2262-2277. doi:10.1093/cvr/cvad129
58. Hemnes AR, Humbert M. Pathobiology of pulmonary arterial hypertension: Understanding the roads less travelled. *European Respiratory Review*. 2017;26(146). doi:10.1183/16000617.0093-2017
59. Humbert M, Guignabert C, Bonnet S, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J*. 2019;53(1). doi:10.1183/13993003.01887-2018

60. Naeije R, Manes A. The right ventricle in pulmonary arterial hypertension. *European Respiratory Review*. 2014;23(134):476-487. doi:10.1183/09059180.00007414
61. Bekedam FT, Goumans MJ, Bogaard HJ, de Man FS, Llucià-Valldeperas A. Molecular mechanisms and targets of right ventricular fibrosis in pulmonary hypertension. *Pharmacol Ther*. 2023;244. doi:10.1016/j.pharmthera.2023.108389
62. Morrell NW, Aldred MA, Chung WK, et al. Genetics and genomics of pulmonary arterial hypertension. *Eur Respir J*. 2019;53(1). doi:10.1183/13993003.01899-2018
63. Evans JDW, Girerd B, Montani D, et al. BMPR2 mutations and survival in pulmonary arterial hypertension: An individual participant data meta-analysis. *Lancet Respir Med*. 2016;4(2):129-137. doi:10.1016/S2213-2600(15)00544-5
64. Larkin EK, Newman JH, Austin ED, et al. Longitudinal analysis casts doubt on the presence of genetic anticipation in heritable pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2012;186(9):892-896. doi:10.1164/rccm.201205-0886OC
65. Frank DB, Lowery J, Anderson L, Brink M, Reese J, de Caestecker M. Increased susceptibility to hypoxic pulmonary hypertension in Bmpr2 mutant mice is associated with endothelial dysfunction in the pulmonary vasculature. *Am J Physiol Lung Cell Mol Physiol*. 2008;294:98-109. doi:10.1152/ajplung.00034.2007.-Patients
66. Hurst LA, Dunmore BJ, Long L, et al. TNFα drives pulmonary arterial hypertension by suppressing the BMP type-II receptor and altering NOTCH signalling. *Nat Commun*. 2017;8. doi:10.1038/ncomms14079
67. Sánchez-Duffhues G, García de Vinuesa A, van de Pol V, et al. Inflammation induces endothelial-to-mesenchymal transition and promotes vascular calcification through downregulation of BMPR2. *Journal of Pathology*. 2019;247(3):333-346. doi:10.1002/path.5193
68. Austin ED, Hamid R, Hemnes AR, et al. BMPR2 expression is suppressed by signaling through the estrogen receptor. *Biol Sex Differ*. 2012;3(1):6. doi:10.1186/2042-6410-3-6
69. Machado RD, Southgate L, Eichstaedt CA, et al. Pulmonary Arterial Hypertension: A Current Perspective on Established and Emerging Molecular Genetic Defects. *Hum Mol Genet*. 2015;36(12):1113-1127. doi:10.1002/humu.22904
70. Machado RD, Aldred MA, James V, et al. Mutations of the TGF-β Type II Receptor BMPR2 in Pulmonary Arterial Hypertension. *Human Mutation HGVS*. 2006;27(May 2005):1-6. doi:10.1002/humu
71. Happé C, Kurakula K, Sun XQ, et al. The BMP Receptor 2 in Pulmonary Arterial Hypertension: When and Where the Animal Model Matches the Patient. *Cells*. 2020;9(6). doi:10.3390/cells9061422
72. Gore B, Izikki M, Mercier O, et al. Key role of the endothelial TGF-β/ALK1/endoglin signaling pathway in humans and rodents pulmonary hypertension. *PLoS One*. 2014;9(6). doi:10.1371/journal.pone.0100310
73. Selimovic N, Bergh CH, Andersson B, Sakiniene E, Carlsten H, Rundqvist B. Growth factors and interleukin-6 across the lung circulation in pulmonary hypertension. *European Respiratory Journal*. 2009;34(3):662-668. doi:10.1183/09031936.00174908
74. Yndestad A, Larsen KO, Øie E, et al. Elevated levels of activin A in clinical and experimental pulmonary hypertension. *J Appl Physiol*. 2009;106(4):1356-1364. doi:10.1152/japplphysiol.90719.2008
75. Yung LM, Yang P, Joshi S, et al. ACTRIIA-Fc rebalances activin/GDF versus BMP signaling in pulmonary hypertension. *Sci Transl Med*. 2020;12(543). doi:10.1126/scitranslmed.aaz5660
76. Cunha SI, Magnusson PU, Dejana E, Lampugnani MG. Deregulated TGF-β/BMP signaling in vascular malformations. *Circ Res*. 2017;121(8):981-999. doi:10.1161/CIRCRESAHA.117.309930
77. Ma J, Sanchez-Duffhues G, Goumans MJ, ten Dijke P. TGF-β-Induced Endothelial to Mesenchymal Transition in Disease and Tissue Engineering. *Front Cell Dev Biol*. 2020;8(April):1-14. doi:10.3389/fcell.2020.00260
78. Dejana E, Hirschi KK, Simons M. The molecular basis of endothelial cell plasticity. *Nat Commun*. 2017;8. doi:10.1038/ncomms14361

79. Ricard N, Bailly S, Guignabert C, Simons M. The quiescent endothelium: signalling pathways regulating organ-specific endothelial normalcy. *Nat Rev Cardiol*. 2021;18(8):565-580. doi:10.1038/s41569-021-00517-4
80. Liljesthröm M, Pignolo R, Kaplan F. Epidemiology of the Global Fibrodysplasia Ossificans Progressiva (FOP) Community. *J Rare Dis Res Treat*. 2020;5(2):31-36. doi:10.29245/2572-9411/2020/2.1196
81. Baujat G, Choquet R, Bouée S, et al. Prevalence of fibrodysplasia ossificans progressiva (FOP) in France: An estimate based on a record linkage of two national databases. *Orphanet J Rare Dis*. 2017;12(1). doi:10.1186/s13023-017-0674-5
82. Towler OW, Shore EM, Kaplan FS. Skeletal malformations and developmental arthropathy in individuals who have fibrodysplasia ossificans progressiva. *Bone*. 2020;130. doi:10.1016/j.bone.2019.115116
83. Deirmengian GK, Hebela NM, O'Connell M, Glaser DL, Shore EM, Kaplan FS. Proximal tibial osteochondromas in patients with fibrodysplasia ossificans progressiva. *Journal of Bone and Joint Surgery*. 2008;90(2):366-374. doi:10.2106/JBJS.G.00774
84. Kaplan FS, Al Mukaddam M, Stanley A, Towler OW, Shore EM. Fibrodysplasia ossificans progressiva (FOP): A disorder of osteochondrogenesis. *Bone*. 2020;140. doi:10.1016/j.bone.2020.115539
85. Joe AWB, Yi L, Natarajan A, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat Cell Biol*. 2010;12(2):153-163. doi:10.1038/ncb2015
86. Lees-Shepard JB, Yamamoto M, Biswas AA, et al. Activin-dependent signaling in fibro/adipogenic progenitors causes fibrodysplasia ossificans progressiva. *Nat Commun*. 2018;9(1). doi:10.1038/s41467-018-02872-2
87. Shore EM, Xu M, Feldman GJ, et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nat Genet*. 2006;38(5):525-528. doi:10.1038/ng1783
88. Van Dinther M, Visser N, De Gorter DJJ, et al. ALK2 R206H mutation linked to fibrodysplasia ossificans progressiva confers constitutive activity to the BMP type I receptor and sensitizes mesenchymal cells to BMP-induced osteoblast differentiation and bone formation. *Journal of Bone and Mineral Research*. 2010;25(6):1208-1215. doi:10.1002/jbmr.091110
89. Hino K, Ikeya M, Horigome K, et al. Neofunction of ACVR1 in fibrodysplasia ossificans progressiva. *Proc Natl Acad Sci U S A*. 2015;112(50):15438-15443. doi:10.1073/pnas.1510540112
90. Aykul S, Huang L, Wang L, et al. Anti-ACVR1 antibodies exacerbate heterotopic ossification in fibrodysplasia ossificans progressiva (FOP) by activating FOP-mutant ACVR1. *J Clin Invest*. 2022;132(12):1-13.
91. Ramachandran A, Mehić M, Wasim L, et al. Pathogenic ACVR1 R206H activation by Activin A-induced receptor clustering and autophosphorylation. *EMBO J*. 2021;14(40):1-22. doi:10.15252/embj.2020106317
92. Hino K, Horigome K, Nishio M, et al. Activin-A enhances mTOR signaling to promote aberrant chondrogenesis in fibrodysplasia ossificans progressiva. *Journal of Clinical Investigation*. 2017;127(9):3339-3352. doi:10.1172/JCI93521
93. Valer JA, Sánchez-de-Diego C, Gámez B, Mishina Y, Rosa JL, Ventura F. Inhibition of phosphatidylinositol 3-kinase α (PI3Kα) prevents heterotopic ossification. *EMBO Mol Med*. 2019;11(9):1-13. doi:10.15252/emmm.201910567
94. Stanley A, Heo S jin, Mauck RL, Mourkioti F, Shore EM. Elevated BMP and Mechanical Signaling Through YAP1/RhoA Poises FOP Mesenchymal Progenitors for Osteogenesis. *Journal of Bone and Mineral Research*. 2019;34(10):1894-1909. doi:10.1002/jbmr.3760
95. Pignolo RJ, Shore EM, Kaplan FS. Fibrodysplasia ossificans progressiva: Clinical and genetic aspects. *Orphanet J Rare Dis*. 2011;6(1):80. doi:10.1186/1750-1172-6-80
96. Cheifetzs S, Like B, Massague J. Cellular Distribution of Type I and Type II Receptors for Transforming Growth Factor-β. *J Biol Chem*. 1986;261(21):9972-9978.

97. Korchynskyi O, Ten Dijke P. Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. *Journal of Biological Chemistry*. 2002;277(7):4883-4891. doi:10.1074/jbc.M111023200
98. Dennler S, Itoh S, Vivien D, Ten Dijke P, Phane Huet S, Gauthier JM. Direct binding of Smad3 and Smad4 to critical TGFβ-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J*. 1998;17(11):3091-3100. https://www.embopress.org
99. Ma H, Deacon S, Horiuchi K. The challenge of selecting protein kinase assays for lead discovery optimization. *Expert Opin Drug Discov*. 2008;3(6):607-621. doi:10.1517/17460441.3.6.607
100. De Mol NJ, Fischer MJE. Surface Plasmon Resonance Methods and Protocols. *Methods in Molecular Biology*. 2010;627. www.springer.com/series/7651
101. Martiny-Baron G, Haasen D, D'Dorazio D, Voshol J, Fabbro D. Characterization of Kinase Inhibitors Using Reverse Phase Protein Arrays. *Methods in Molecular Biology*. 2011;785:79-107. doi:10.1007/978-1-61779-286-1_7