Published in final edited form as:

Drug Discov Today. 2014 August ; 19(8): 1270–1276. doi:10.1016/j.drudis.2014.06.014.

Targeting the Wnt signaling pathways in pulmonary arterial hypertension

Vinicio de Jesus Perez¹, Ke Yuan¹, Tero-Pekka Alastalo^{2,3}, Edda Spiekerkoetter¹, and Marlene Rabinovitch⁴

¹Division of Pulmonary and Critical Care Medicine, Stanford University Medical Center, 300 Pasteur Drive Grant S140B, Stanford, CA 94305, USA ²Children's Hospital Helsinki, Tukholmankatu 8, FI-00290 Helsinki, Finland ³Biomedicum Helsinki, Tukholmankatu 8, FI-00290 Helsinki, Finland ⁴Pediatric Cardiology, Stanford University Medical Center, 300 Pasteur Drive Grant S140B, Stanford, CA 94305, USA

Abstract

Pulmonary arterial hypertension (PAH) is a life-threatening disorder that is associated with elevated pulmonary pressures and right heart failure resulting from progressive loss and thickening of small pulmonary arteries. Despite their ability to improve symptoms, current therapies fail to prevent disease progression, leaving lung transplantation as the only therapy in end-stage PAH. To overcome the limitations of current therapies, there is an active search for disease-modifying agents capable of altering the natural history of, and improving clinical outcomes in, PAH. The Wnt signaling pathways have emerged as attractive treatment targets in PAH given their role in the preservation of pulmonary vascular homeostasis and the recent development of Wnt-specific compounds and biological therapies capable of modulating pathway activity. In this review, we summarize the literature describing the role of Wnt signaling in the pulmonary circulation and discuss promising advances in the field of Wnt therapeutics that could lead to novel clinical therapies capable of preventing and/or reversing pulmonary vascular pathology in patients with this devastating disease.

Introduction

Following their discovery in 1981 [1,2], the Wnt signaling pathways have been the subject of intense investigation by both physicians and scientist alike because of the range of developmental events and biological processes that they control. Given the influence of Wnt signaling on the preservation of cell and tissue homeostasis [3], it is not surprising that mutations that alter Wnt pathway activation can lead to the development of disease states. To date, more than 20 clinical diseases have been linked to abnormal Wnt signaling, including developmental anomalies (e.g. spina bifida [4]), degenerative conditions (e.g. Alzheimer's disease [5]), malignancies (e.g. colon cancer [6]) and chronic diseases (e.g. atherosclerosis [7]). Given the range of clinical disorders associated with abnormal Wnt

signaling, there has been tremendous interest in developing therapeutic approaches that could restore Wnt pathway activity to physiological levels. In recent years, there have been numerous advances in the development of Wnt pathway modulators, some of which have shown great promise and are currently being evaluated for clinical use in phase 2 clinical trials. Given the rapid growth of this field, it is expected that Wnt-based therapeutics will become part of the management of many acute and chronic conditions in the near future.

PAH is a rare but devastating disorder associated with progressive increase in pulmonary pressures that, if untreated, leads to right heart failure and death [8]. The pathology of PAH is characterized by progressive loss of small vessels and wall thickening from increased hypertrophy and proliferation of smooth muscle in the medial layer, resulting in luminal obliteration and increase in pulmonary vascular resistance [9]. To date, none of the available therapies have been shown to promote angiogenesis or reverse established medial thickening, thus resulting in disease progression and eventual failure of therapy. Given the known role of Wnt signaling in regulating angiogenesis and cell growth, it is tempting to speculate that Wnt-signaling modulation could have a role in the development of disease-modifying agents to treat this condition. In recent years, evidence has been gathered to support a key role for the Wnt signaling pathways in PAH pathobiology and potential targets have been identified that could be amenable for the development of novel Wnt-based therapeutics. This review provides a summary of this evidence to date and a bird's eye view of the state of the field of Wnt-based therapeutics, along with speculations concerning specific approaches that could be relevant for delivery of these agents to the pulmonary circulation.

Role of the Wnt signaling pathways in preservation of pulmonary vascular homeostasis

The best characterized of the Wnt signaling pathways is the Wnt/ β -catenin (bC) pathway, whose chief downstream effector is bC, a highly dynamic cytoplasmic protein that can translocate to the nucleus to selectively alter gene expression (Fig. 1a). In the normal steady state, bC is targeted for degradation by a cytoplasmic protein complex comprising Axin, adenomatous polyposis coli and glycogen synthase kinase 3 β (GSK3 β). Once bound to this complex, bC is phosphorylated by GSK3 β and targeted for subsequent ubiquitination and proteasomal degradation [10]. Wnt ligands trigger Wnt/bC pathway activation by first binding to a receptor complex comprising a member of the frizzled (FZD) family of seven pass membrane receptors and low-density lipoprotein receptor-related protein (LRP) 5/6, followed by activation of disheveled (Dvl), a cytoplasmic protein that is ultimately responsible for the inactivation of the bC degradation complex. Upon release from the degradation complex, bC translocates to the nucleus, where it binds a transcriptional complex comprising lymphoid-enhancing factor (LEF) and T cell factor (TCF), resulting in the transcription of genes involved in cell fate, proliferation, survival and differentiation [11].

In addition to the Wnt/bC pathway, there are other Wnt pathways that have more specialized biological functions and whose activation is independent of bC accumulation. Among these, Wnt/ planar cell polarity (PCP) signaling is a highly conserved pathway that regulates cell

and tissue polarity along the planar axis of epithelial or mesenchymal cell sheets. In mammals, activation of Wnt/PCP is necessary for neural tube closure, and mutations that disrupt this event result in neural tube defects, such as craniorachischisis and spina bifida. Whereas Wnt/PCP activation also follows the binding of a Wnt ligand with a member of the FZD receptor family, recruitment of other co-receptors, such as receptor tyrosine kinase-like orphan receptor 2 (ROR2), is necessary to activate the pathway (Fig. 1b). Once the Wnt/PCP receptor complex is activated, Dvl is once again responsible for initiating the signaling cascade that culminates in the activation of the small GTPases Rac, Rho and cdc42. Once activated, these proteins trigger cytoskeletal rearrangements that allow cells to navigate their immediate environment and synchronize their movements to that of other cells within a specific tissue plane [12,13].

Relevance of Wnt signaling to PAH pathogenesis was first suggested in a study looking at the expression profile of laser-microdissected plexiform lesions, which demonstrated significant upregulation of PCP pathway genes, such as those encoding Wnt11, Rho kinase, Disheveled and Daam1 [14]. Around this time, it was reported for the first time that the protective effects of bone morphogenetic protein (BMP) on the pulmonary endothelium were dependent on simultaneous activation of both Wnt/bC and Wnt/PCP [15]. Following stimulation with BMP2, pulmonary artery endothelial cells (PAECs) demonstrated an increase in bC transcriptional activity that correlated with an increase in genes involved in PAEC proliferation and survival. Intriguingly, it was found that BMP2 can also trigger pulmonary angiogenesis in a bC-independent manner by activating Wnt/PCP in PAECs. In response to Wnt/PCP activation, PAECs can organize into functional vascular networks that can deliver both oxygen and nutrients to active tissues. Thus, it was concluded that concomitant activation of both Wnt signaling pathways is necessary for preservation of pulmonary vascular homeostasis and that loss of function in either Wnt pathway could contribute to PAH by limiting the extent of vascular regeneration in response to injury.

One of the main pathological features of PAH is vascular obstruction caused by accumulation of pulmonary smooth muscle cells (PASMCs) within the wall of small pulmonary arteries. Previous studies showed that signaling through BMP2 can facilitate pulmonary artery PASMC motility [16] and suppress proliferation induced by platelet-derived growth factor (PDGF)-BB [17], but whether this is also dependent on BMP-mediated recruitment of the Wnt signaling pathways was at that stage unknown. Using a similar experimental approach as the one used in the PAEC studies, researchers found that BMP2 could also activate both Wnt/bC and Wnt/PCP in PASMCs [15], but the timing for each signaling event was different mutations that interfered with sequential Wnt pathway activation resulted in increased PASMC growth in response to PDGF stimulation. The authors went on to show that BMP signaling could induce tandem and independent recruitment of the Wnt/bC and the Wnt/PCP pathways to suppress proliferation and induce motility respectively as following: BMP2 can induce production and release fibronectin (FN) in PASMCs in a bC-dependent fashion [18]. A key component of the vascular extracellular matrix, FN, can activate Wnt/PCP in the absence of Wnt ligands by triggering Dvl-mediated RhoA and Rac1 activation via integrin-linked kinase (ILK) 1. The binding of ILK-1 to Dvl is a key event that results in exclusive activation of Wnt/PCP while simultaneously suppressing Wnt/bC. The final outcome of this chain of events is that, in

response to a vascular insult, PASMC can effectively mobilize to reach the damage zone and aid in tissue repair while preventing FN accumulation via suppression of bC-dependent growth. The consequences of interfering with this protective signaling mechanism were shown using an in vivo carotid stent model where a mutant Dvl construct lacking ILK-1 binding domains was found to result in neointima formation and medial thickening from excessive PASMC accumulation.

In conclusion, the above evidence enables us to propose a model whereby the Wnt signaling pathways has a major role in preserving pulmonary vascular homeostasis by promoting healing after injury (Fig. 2a). However, alterations in Wnt pathway activation could lead to PAH by preventing proper small vessel regeneration and allowing excessive PASMC growth (Fig. 2b). Despite the seeming complexity of these pathways, it is possible to point to some key signaling components that could serve as therapeutic targets for drug development. In the next sections, we focus on relevant developments in Wnt therapeutics that can address these targets and provide avenues to help manipulate the Wnt pathways to treat vascular pathology in PAH.

Targeting Wnt signaling in PAH

Given the key role of Wnt signaling pathways in pulmonary angiogenesis and vascular remodeling in PAH, it is possible to predict that Wnt modulators could be useful as disease-modifying therapies. However, the development of potential Wnt modulators must take in consideration the range of cellular functions and molecular targets regulated by Wnt pathways across the body to minimize the potential for off-target effects and systemic toxicity. Previously, the success of identifying clinically useful Wnt modulators has been limited because of the complexity of the Wnt signaling pathways and limited access to high throughput screening (HTS) methods capable of screening large numbers of candidate compounds at a given time. In addition to screening small molecule libraries, recent advances in understanding of Wnt pathway regulation have led to the development of highly specific biological therapies capable of targeting Wnt ligands, receptors and intracellular components within specific compartments in the body. To date, there are at least ten clinical trials investigating the impact of Wnt modulators in the treatment of malignancies and chronic conditions, such as inflammatory bowel disease, Alzheimer's disease and psoriasis.

Below, we discuss some of the strategies that can help identify potentially useful Wnt modulators for use in PAH. In addition, we briefly discuss some promising developments in nanomedicine that could allow targeted delivery of Wnt modulators to the pulmonary circulation.

Screening FDA approved and small molecule libraries for Wnt modulators

One approach to accelerate the discovery of novel Wnt mediators is using high throughput methods to screen large libraries of US Food and Drug Administration (FDA) approved and novel small molecules. An advantage of screening libraries of FDA-approved compounds is that the safety profile of these agents in humans is well known and approval for a new indication could be accomplished over a shorter amount of time compared with that of novel compounds [19–21]. To date, there are several FDA-approved drug classes that have been

shown to have potential as Wnt modulators. For instance, nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac and sulindac, have been shown to inhibit bC activation in patients with familial adenomatous polyposis (FAP), a genetic condition associated with abnormal activation of Wnt/ bC signaling in the colon leading to polyp formation and high risk of colon cancer [22–24]. The mechanism for NSAID-mediated inhibition of Wnt/bC appears to be facilitated by preventing the nuclear translocation and transcriptional activity of bC, which results in reduced transcription of genes involved in proliferation and survival of cancer cells [22]. Likewise, cyclooxygenase 2 (COX2) inhibitors have been FDA approved for the treatment of patients with FAP because of their ability to suppress polyp growth via inhibition of Wnt/bC activity [25,26]. In the pulmonary circulation, COX2 induces production of thromboxane A2, a powerful vasoconstrictor that is upregulated during hypoxia and appears to be directly linked to the vascular remodeling observed in the setting chronic hypoxia. In an effort to test whether COX2 inhibition could prevent hypoxia-induced pulmonary vasoconstriction (HPV), Kylhammar *et al.* treated 18 anesthetized pigs with a nimesulide infusion during normoxia and hypoxia for 45 min [27]. Hypoxic pigs treated with nimesulide demonstrated a 10–11% reduction in pulmonary artery pressures, suggesting benefit in HPV. However, use of celecoxib in rats exposed to chronic hypoxia demonstrated exacerbation of pulmonary pressures, with an increase in serum thromboxane A1 level raising concerns regarding the safety of this therapy in the pulmonary circulation [28]. Although none of these studies looked at Wnt/bC activation in the pulmonary circulation, the present results must be considered carefully before using these therapies in patients with PAH.

Given its dynamic nature, bC has been shown to interact with a variety of nuclear receptors that are currently targets for several FDA-approved therapies. It was recently shown that BMP stimulation in PAECs can trigger formation of a protein complex between bC and PPAR γ , a target of FDA-approved antidiabetic agents, such as pioglitazone and rosiglitazone [29]. It was found that the angiogenic properties of BMP were lost when PAECs were treated with either peroxisome proliferator-activated receptor gamma (PPAR γ) agonists (i.e. GW9662) or antagonists (i.e. rosiglitazone). This resulted from disruption of the bC-PPAR γ complex by either drug class that led to significant changes in the gene expression profile of PAECs in response to BMP stimulation. Using CHiP-chip analysis, a list of gene-specific promoters were identified with evidence of binding by both PPAR γ and bC, suggesting that the simultaneous presence of these two agents is necessary for proper gene expression. Among the genes regulated by both bC and PPAR γ , researchers focused on apelin given prior reports demonstrating its link to PAH [30,31]. Given that loss of either PPAR γ or bC was associated with reduced apelin production, researchers tested whether exogenous apelin replacement could prevent PAH in endothelial-specific *TIE2CrePPAR $\gamma^{fl/fl}$* mice. Genetically modified mice offer a unique advantage for the study of human diseases because they can be used to manipulate the expression level of any gene to study its function and reveal their contribution to specific disease processes. However, when compared with rats and neonatal calves, the severity of PAH seen in mice is mild, a fact that must be kept in mind when extrapolating data to patients. As predicted, it was found that apelin administration prevented PAH development in *TIE2CrePPAR $\gamma^{fl/fl}$* mice, leading to the

conclusion that disrupting the BMP-mediated bC-PPAR γ complex can lead to severe vessel damage and development of PAH.

On the basis of this study, it might seem that the use of PPAR γ agonists might not be beneficial in PAH. However, it is important to point out that PPAR γ has protective effects in the pulmonary circulation that are independent of bC. Studies in *Apo*^{-/-} mice demonstrated that development of PAH in mice exposed to a high-fat diet could be prevented by treatment with rosiglitazone [17]. This preclinical study, along with published reports demonstrating a link between insulin resistance, BMPR2 mutations and PAH development [32–34], led to a clinical trial testing the utility of pioglitazone in treating patients with PAH. Therefore, given the biological complexity of these agents, it is possible that its adverse effects on bC could be overcome by benefits in other areas that in the end might add up to a clear clinical benefit.

Despite a large number of reports in the literature describing the use of FDA-approved drugs in cell and animal models of disease, testing each individual compound in PAH would be time consuming and would delay the discovery of clinically relevant Wnt modulators. In addition, even though there might be more than one study looking at a specific compound for a given indication, reproducibility of results is difficult given the different conditions used across studies leading to conflicting reports and a perceived lack of confidence on the clinical potential of the compound. To circumvent these limitations, HTS strategies make use of libraries of known compounds that can be tested simultaneously under controlled experimental conditions, leading to reproducible and reliable readouts and fast identification of biologically active compounds. To screen these libraries for Wnt modulators, it is necessary to have a reporter system that can reliably provide readout of change in Wnt signaling activity in the presence of potential activators or inhibitors. For this purpose, there are now cell lines stably transfected with bC luciferase reporters (i.e. Super TOP flash) that can produce increased amounts of luciferase in response to agents that trigger transcriptional activity of bC [35]. Although the reporter line is well tailored to document Wnt/bC activation, it can also be used to screen for pathway inhibitors. Given that Wnt modulators that stimulate or inhibit bC activation in these reporter lines can be acting at any level of the Wnt/bC pathway (i.e. ligand mimetics, GSK3 β inhibition or TCF/bC activators), these screens could lead to the discovery of candidate compounds with different mechanisms of action across the entire pathway.

Several groups studying Wnt/bC in several contexts have successfully used HTS to discover novel Wnt modulators. Recently, one group combined a biological *Xenopus* egg-based assay with HTS to uncover a role for pyrvinium, a potent FDA-approved antihelminthic drug that works as a casein kinase 1 inhibitor to prevent bC accumulation by increasing GSK3 β activity [36]. Use of pyrvinium in a mouse model of myocardial infarction (MI) showed that this drug can reduce the size of the infarct, leading the authors to conclude that bC suppression could help preserve myocardial function in post-MI patients. Another group performing a HTS with a HEK293-based reporter cell system identified XAV939, a novel compound that inhibits bC activation by stabilizing the bC degradation complex [37]. Although most of the work has focused on identifying Wnt inhibitors for use in the treatment of cancer or inflammatory conditions, HTS can also be designed to screen

compound libraries for potent Wnt activators by using either Wnt reporter lines [38] or fluorescence microscopy [39]. Thus, implementation of a HTS-based strategy can help identify candidate Wnt modulators that can be further validated in preclinical studies to determine their potential as novel PAH therapies.

A study was recently published describing a HTS approach to screen a >3500 FDA compound library against a cell line engineered to express a luciferase reporter to document BMP signaling activity. Using this tool, the authors were able to show that FK506, a known immunosuppressive agent, could strongly induce BMP signaling activity in the reporter line, leading the authors to propose that this FDA-approved compound could be used to restore BMP signaling in PAH [40]. The efficacy of low-dose FK506 has been confirmed to improve angiogenesis and restore normal growth of PAH endothelial cells, and its therapeutic benefit in preventing vascular remodeling indifferent animal models of PAH has also been shown. Given these findings, the researchers have now moved to phase 2 clinical studies to test the safety and efficacy of low-dose FK506 in patients with PAH. The success of this strategy led to additional studies screening compound libraries using a Wnt/bC reporter line to identify potentially relevant FDA-approved and novel compounds that can be tested in pre-clinical studies. Beyond testing these compounds in cell systems, it will be imperative to use animal models to determine carefully the clinical dose range required to minimize off-target effects outside the pulmonary vascular compartment.

Use of biological therapies

As indicated above, Wnt signaling pathways appear to have cell-specific effects in the pulmonary circulation that must be taken into account when planning HTS studies. Thus, although activation of bC appears to promote angiogenesis, it can also lead to increased SMC growth and might contribute to worsened vascular remodeling. In addition, some pathway components might have other cellular functions in addition to their involvement in Wnt signaling that could be affected if they are targeted with small compounds. For instance, APC inhibition could result in small vessel loss, given the role of this molecule in establishment of endothelial adhesions and cell survival [41]. One way to narrow the biological impact of a given Wnt therapy is to target a specific Wnt signaling component that is associated with abnormal cellular function in all pulmonary vascular types. Similar to BMPR2 mutations, ideal Wnt pathway targets would be associated with abnormal cellular phenotype in all pulmonary vascular cell and restoration of their level or function should result in restoration of homeostasis. To achieve this, a comprehensive profile of the genotype and expression patterns of all Wnt components should be performed in well-characterized healthy individuals and patients with PAH. With the advent of next-generation sequencing, it is feasible to study the genome of patients for potential disease-causing variants in Wnt genes using whole-exome or genome sequencing followed by validation studies using cells isolated from lungs of patients with PAH undergoing transplant or at autopsy, and preclinical models of PAH.

In recent years, there have been some encouraging developments using biological agents that either mimic or block activity of endogenous Wnt components in a highly specific fashion. Despite the availability of recombinant Wnt ligands that can activate Wnt signaling

in vitro and *in vivo*, it is difficult to predict the biological effect of any Wnt, given that most Wnts have cell-specific effects that could lead to adverse consequences. However, in certain diseases, significant alterations in specific Wnts can help design specific therapies tailored to that particular Wnt. For example, various forms of cancer shown to over express Wnt1 demonstrated decreased size and survival when treated with a Wnt1-blocking antibody [42,43]. Similarly, sarcomas that over express the FZD10 receptor have shown significant regression in response to FZD10 antibodies bound to the radioisotope Yttrium-90 [44]. Other strategies being used to target a broader range of Wnts in malignancies that display marked alteration in numerous Wnt ligands include the use of a decoy FZD receptor to bind Wnts before they can trigger Wnt signaling in cells. For example, use of a soluble form of the cysteine-binding domain of the FZD8 receptor (OMP-54F28) has been shown to have antineoplastic activity against tumors [45], which has led to a currently active open-label phase 1 clinical trial (NCT01608867).

Besides being able to identify relevant molecular targets within the Wnt pathways, we must also consider the most effective means to deliver these to the pulmonary circulation while minimizing systemic adverse effects. In addition, use of biological Wnt modulators, such as recombinant Wnts, will also require carriers that help preserve their biological activity. Nanoparticles have emerged as an exciting alternative to accommodate the requirements for delivering active Wnts to the lungs. Given their small size, aerosolized nanoparticles can be delivered to the distal airspaces effectively and to release encapsulated compounds locally with limited systemic effects [46]. Although no studies have yet been done looking at Wnt delivery to the lung, there have been reports demonstrating that Wnt3a packaged in liposomal particles has intact biological activity and can activate Wnt/bC signaling, as evidenced by luciferase production in cell lines transfected with bC luciferase reporter [47]. Likewise, liposomal Wnt3a has been shown to accelerate bone formation around prosthetic implants inserted during bone surgery [48]. Future studies testing the nano-particle-based Wnt modulators targeted to the pulmonary circulation could serve to exploit this method of drug delivery in patients with PAH.

Concluding remarks

Studies to date have shown that Wnt signaling pathways has a crucial role in the regulation of pulmonary angiogenesis and vascular remodeling, and therapies that modulate Wnt pathway activity could be of use in patients with PAH. However, because of the complexity of pathway signaling, the extent of cross-talk with other signaling pathways and the large number of signaling components, it has been difficult to identify safe and effective therapies that specifically target Wnt signaling in disease. Nevertheless, recent advances using HTS and biologic compounds have moved us closer to identifying potentially useful therapies to be tested in clinical trials. The future of Wnt therapeutics in PAH will depend on identifying specific abnormalities within the Wnt pathways that could be amenable to correction by supplementation of either mimetics or inhibitors specific for a given factor deficiency. Furthermore, attempts at modulating Wnt signaling have to be tempered by the possibility of opposite effects of Wnt activation in different cell types within the vascular tissue. Finally, given the range of biological processes under the influence of Wnt signaling, use of delivery methods that can bypass systemic exposure to Wnt modulators and deliver the agents

specifically to the lung will be instrumental to increase the chances of success when introducing these therapies to patients with PAH.

References

1. Nusse R, Varmus HE. Wnt genes. *Cell*. 1992; 69:1073–1087. [PubMed: 1617723]
2. Nusse R, et al. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. *Nature*. 1984; 307:131–136. [PubMed: 6318122]
3. Lim X, Nusse R. Wnt signaling in skin development, homeostasis, and disease. *Cold Spring Harb Perspect Biol*. 2013; 5:a008029. [PubMed: 23209129]
4. Ueno N, Greene ND. Planar cell polarity genes and neural tube closure. *Birth Defects Res C*. 2003; 69:318–324.
5. De Ferrari GV, Inestrosa NC. Wnt signaling function in Alzheimer's disease. *Brain Res*. 2000; 33:1–12.
6. Burgess AW, et al. Wnt signaling and colon tumorigenesis: a view from the periphery. *Exp Cell Res*. 2011; 317:2748–2758. [PubMed: 21884696]
7. Marinou K, et al. Wnt signaling in cardiovascular physiology. *Trends Endocrinol Metab*. 2012; 23:628–636. [PubMed: 22902904]
8. McLaughlin VV, et al. ACCF/AHA 2009 expert consensus document on pulmonary hypertension: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. *J Am Coll Cardiol*. 2009; 53:1573–1619. [PubMed: 19389575]
9. Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest*. 2012; 122:4306–4313. [PubMed: 23202738]
10. Munemitsu S, et al. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc Natl Acad Sci U S A*. 1995; 92:3046–3050. [PubMed: 7708772]
11. Papkoff J, et al. Wnt-1 regulates free pools of catenins and stabilizes APC-catenin complexes. *Mol Cell Biol*. 1996; 16:2128–2134. [PubMed: 8628279]
12. Shulman JM, et al. Frizzled signaling and the developmental control of cell polarity. *Trends Genet*. 1998; 14:452–458. [PubMed: 9825673]
13. Veeman MT, et al. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev Cell*. 2003; 5:367–377. [PubMed: 12967557]
14. Laumanns IP, et al. The noncanonical WNT pathway is operative in idiopathic pulmonary arterial hypertension. *Am J Respir Cell Mol Biol*. 2009; 40:683–691. [PubMed: 19029018]
15. de Jesus Perez VA, et al. Bone morphogenetic protein 2 induces pulmonary angiogenesis via Wnt-beta-catenin and Wnt-RhoA-Rac1 pathways. *J Cell Biol*. 2009; 184:83–99. [PubMed: 19139264]
16. Spiekeroetter E, et al. S100A4 and bone morphogenetic protein-2 codependently induce vascular smooth muscle cell migration via phosphoextracellular signal-regulated kinase and chloride intracellular channel 4. *Circ Res*. 2009; 105:639–647. [PubMed: 19713532]
17. Hansmann G, et al. An antiproliferative BMP-2/PPARgamma/apoE axis in human and murine SMCs and its role in pulmonary hypertension. *J Clin Invest*. 2008; 118:1846–1857. [PubMed: 18382765]
18. Perez VA, et al. BMP promotes motility and represses growth of smooth muscle cells by activation of tandem Wnt pathways. *J Cell Biol*. 2011; 192:171–188. [PubMed: 21220513]
19. Rodriguez-Devora JI, et al. Direct assembling methodologies for high-throughput bioscreening. *Biotechnol J*. 2011; 6:1454–1465. [PubMed: 22021162]
20. Renner S, et al. Recent trends and observations in the design of high-quality screening collections. *Future Med Chem*. 2011; 3:751–766. [PubMed: 21554080]
21. Prummer M. Hypothesis testing in high-throughput screening for drug discovery. *J Biomol Screen*. 2012; 17:519–529. [PubMed: 22233646]

22. Boon EM, et al. Sulindac targets nuclear beta-catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines. *Br J Cancer*. 2004; 90:224–229. [PubMed: 14710233]
23. Marotta A, et al. Dysregulation of integrin-linked kinase (ILK) signaling in colonic polyposis. *Oncogene*. 2001; 20:6250–6257. [PubMed: 11593435]
24. Koornstra JJ, et al. Sulindac inhibits beta-catenin expression in normal-appearing colon of hereditary nonpolyposis colorectal cancer and familial adenomatous polyposis patients. *Cancer Epidemiol Biomarkers Prev*. 2005; 14:1608–1612. [PubMed: 16030090]
25. Greenspan EJ, et al. Ibuprofen inhibits activation of nuclear {beta}-catenin in human colon adenomas and induces the phosphorylation of GSK-3{beta}. *Cancer Prev Res*. 2011; 4:161–171.
26. Wu WK, et al. Cyclooxygenase-2 in tumorigenesis of gastrointestinal cancers: an update on the molecular mechanisms. *Cancer Lett*. 2010; 295:7–16. [PubMed: 20381235]
27. Kylhammar D, Radegran G. Cyclooxygenase-2 inhibition and thromboxane A(2) receptor antagonism attenuate hypoxic pulmonary vasoconstriction in a porcine model. *Acta Physiol*. 2012; 205:507–519.
28. Zeng HH, et al. Effect of celecoxib on pulmonary hypertension of chronic hypoxia and hypercapnic rats. *Zhongguo Ying Yong Sheng Li Xue Za Zhi (Chin J Appl Physiol)*. 2011; 27:29–32.
29. Alastalo TP, et al. Disruption of PPARgamma/beta-catenin-mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. *J Clin Invest*. 2011; 121:3735–3746. [PubMed: 21821917]
30. Chandra SM, et al. Disruption of the apelin-APJ system worsens hypoxia-induced pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2011; 31:814–820. [PubMed: 21233449]
31. Andersen CU, et al. Pulmonary apelin levels and effects in rats with hypoxic pulmonary hypertension. *Respir Med*. 2009; 103:1663–1671.
32. Zamanian RT, et al. Insulin resistance in pulmonary arterial hypertension. *Eur Respir J*. 2009; 33:318–324.
33. West J, et al. A potential role for insulin resistance in experimental pulmonary hypertension. *Eur Respir J*. 2013; 41:861–871.
34. Pugh ME, et al. Unrecognized glucose intolerance is common in pulmonary arterial hypertension. *J Heart Lung Transplant*. 2011; 30:904–911. [PubMed: 21493097]
35. Biechele TL, et al. Transcription-based reporters of Wnt/beta-catenin signaling. *Cold Spring Harb Protoc* 2009. 2009 p.doi:10.1101/2009.09.01.2223.
36. Thorne CA, et al. Small-molecule inhibition of Wnt signaling through activation of casein kinase 1alpha. *Nat Chem Biol*. 2010; 6:829–836. [PubMed: 20890287]
37. Bao R, Attisano, et al. Inhibition of tankyrases induces Axin stabilization and blocks Wnt signalling in breast cancer cells. *PLoS One*. 2012; 7:e48670. [PubMed: 23144924]
38. Bodine PV, et al. A small molecule inhibitor of the Wnt antagonist secreted frizzled-related protein-1 stimulates bone formation. *Bone*. 2009; 44:1063–1068. [PubMed: 19254787]
39. Borchert KM, et al. Screening for activators of the wingless type/Frizzled pathway by automated fluorescent microscopy. *Methods Enzymol*. 2006; 414:140–150. [PubMed: 17110191]
40. Spiekeroetter E, et al. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest*. 2013; 123:3600–3613. [PubMed: 23867624]
41. de Jesus Perez VA, et al. Loss of adenomatous polyposis coli-alpha3 integrin interaction promotes endothelial apoptosis in mice and humans. *Circ Res*. 2012; 111:1551–1564. [PubMed: 23011394]
42. Wei W, et al. Blockade of Wnt-1 signaling leads to anti-tumor effects in hepatocellular carcinoma cells. *Mol Cancer*. 2009; 8:76. [PubMed: 19778454]
43. You L, et al. Wnt-1 signal as a potential cancer therapeutic target. *Drug News Perspect*. 2006; 19:27–31. [PubMed: 16550254]
44. Hanaoka H, et al. Radio immunotherapy of solid tumors targeting a cell-surface protein, FZD10: therapeutic efficacy largely depends on radio sensitivity. *Ann Nucl Med*. 2009; 23:479–485. [PubMed: 19412654]

45. Hendaoui I, et al. Inhibition of Wnt/beta-catenin signaling by a soluble collagen-derived frizzled domain interacting with Wnt3a and the receptors frizzled 1 and 8. PLoS One. 2012; 7:e30601. [PubMed: 22303445]
46. Da Silva AL, et al. Nanoparticle-based therapy for respiratory diseases. Anais Acad Brasil Cien. 2013; 85:137–146.
47. Morrell NT, et al. Liposomal packaging generates Wnt protein with in vivo biological activity. PLoS One. 2008; 3:e2930. [PubMed: 18698373]
48. Minear S, et al. Wnt proteins promote bone regeneration. Sci Translat Med. 2010; 2:29ra30.

Figure 1.

The Wnt canonical and non-canonical pathways. (a) The Wnt/β-catenin(bC) pathway. In the normal steady state, bC is targeted for degradation by a cytoplasmic APC/Axin/GSK-3β protein complex. Once bound to this complex, bC is targeted by coordinated phosphorylation by CK1/APC/Axin/GSK-3β-complex leading to its ubiquitination and proteasomal degradation. However, when the Wnt ligand binds to Frizzled/LRP5/6 co-receptors, subsequently triggers activation of Dvl followed by releasing bC from an inhibitory APC/Axin/GSK-3β-complex. bC translocates into the nucleus and activates genes involved in cell proliferation, survival and differentiation. (b). The Wnt/PCP pathway. When the Wnt ligand binds to Wnt/PCP receptor complex, Dvl is responsible for initiating the signaling cascade that culminates in the activation of the small GTPases Rac, Rho and cdc42. Once activated, these proteins trigger cytoskeletal rearrangements which regulate cell motility and polarity.

Figure 2. Proposed models. (a). After vascular injury, Wnt/ β C regulates cell proliferation/regeneration while Wnt/PCP regulates cell migration on the injury sites. Wnt signaling pathways play a major role in preserving pulmonary vascular homeostasis. (b) In PAH, altered Wnt pathway activation could lead to progressive loss of small vessels and allow excessive PASMC growth.