

## REVIEW

# Experimental animal models of pulmonary hypertension: Development and challenges

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## Abstract

Pulmonary hypertension (PH) is clinically divided into 5 major types, characterized by elevation in pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR), finally leading to right heart failure and death. The pathogenesis of this arteriopathy remains unclear, leaving it impossible to target pulmonary vascular remodeling and reverse the deterioration of right ventricular (RV) function. Different animal models have been designed to reflect the complex mechanistic origins and pathology of PH, roughly divided into 4 categories according to the modeling methods: non-invasive models in vivo, invasive models in vivo, gene editing models, and multi-means joint modeling. Though each model shares some molecular and pathological changes with different classes of human PH, in most cases the molecular etiology of human PH is poorly known. The appropriate use of classic and novel PH animal models is essential for the hunt of molecular targets to reverse severe phenotypes.

## KEYWORDS

animal models, BMPR2, chronic hypoxia, monocrotaline, pulmonary hypertension, Sugen 5416

## 1 | INTRODUCTION

Pulmonary hypertension (PH) is a severe, progressive vascular disorder characterized by elevation in pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR), finally leading to right heart failure and death. During the rise in PAP and PVR, vascular remodeling is accompanied by accumulation of pulmonary artery smooth muscle cells (PASMCs), endothelial cells (ECs), fibroblasts, myofibroblasts and pericytes in pulmonary arterial wall, which leads to thickening of the inner and outer linings of blood vessels, loss and

obstructive remodeling of the pulmonary vascular bed and perivascular inflammation.<sup>1-3</sup>

Associated with multiple primary causes, PH encompasses a group of clinical entities. According to the latest classification proposed by the Sixth World Symposium on PH, the disease can develop due to pulmonary arterial disorder, left heart disease, lung disease or hypoxia, chronic thromboembolic disease and other unclear or multifactorial mechanisms.<sup>4</sup> Though current treatments can improve clinical symptoms and hemodynamic abnormality to some extent, it is difficult to target pulmonary vascular remodeling and

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reverse the deterioration of right ventricular (RV) function, and to this end animal models are indispensable tools for mechanism research.<sup>5</sup> However, at present there is no single animal model that can fully replicate all the characteristics of human PH evolution, including hemodynamic changes and pathological remodeling,<sup>6,7</sup> and therefore a wide variety of experimental PH models have been derived, which can be roughly divided into 4 categories according to the modeling types: non-invasive models *in vivo*, invasive models *in vivo*, gene editing models, and multi-means joint modeling.<sup>8-10</sup> In the following sections, we will briefly summarize some widely accepted PH animal models and discuss the challenges of their applications.

## 2 | NON-INVASIVE MODELS IN VIVO

### 2.1 | Chronic hypoxia

The chronic hypoxia-induced PH (CHPH) animal model is a classic experimental PH model with good predictability and repeatability.<sup>11</sup> Preclinical models of CHPH share some common pathologies while also displaying differences due to species and age. Rats and mice are the most commonly used hypoxic animals.<sup>7</sup> Although in humans, hypobaric hypoxia leads to more severe hypoxemia, hypocapnia, blood alkalosis and a lower O<sub>2</sub> arterial saturation compared with normobaric hypoxia, few differences were seen in rats during these two types of hypoxia stress.<sup>12,13</sup>

Mice and rats are generally exposed to chronic hypoxia (CH) for 3-4 weeks for PH development, although both can survive longer at 10% oxygen.<sup>14</sup> Simple hypoxic modeling rarely causes a significant increase in pulmonary venous pressure of rats, but vascular remodeling, dysfunction and apoptosis of endothelial cells can be found in both small pulmonary arteries (PA) and pulmonary veins (PV).<sup>15</sup> Mice exposed to CH also develop pulmonary vascular remodeling and an increase in right ventricular systolic pressure (RVSP), usually milder than in rats.<sup>16</sup> Most of these changes are reversible once the normoxic environment is restored, which makes the CHPH model highly suitable for investigation of less severe forms of PH, like those associated with chronic lung diseases.<sup>17</sup>

### 2.2 | Sugen 5416

A modification of the CHPH model has been developed with the addition of the vascular endothelial growth factor receptor (VEGFR)-2 antagonist Sugen 5416 (SU5416; semaxanib) to mice or rats, which results in increased remodeling and development of plexiform lesions even after normoxia recovery.<sup>18,19</sup> In 2001, Taraseviciene-Stewart et al. first combined SU5416 with chronic hypoxia for 3 weeks and then re-exposed models to Denver altitude conditions for an additional 3 weeks, causing severe PH and low hematocrit in adult male Sprague Dawley (SD) rats.<sup>20</sup> Pathologically, PH in the rats manifested as the closure of the anterior capillary artery lumen caused by the excessive proliferation of pulmonary arterial endothelial cells

(PAECs) and PSMCs, and the appearance of pulmonary plexiform lesions, similar to that of pulmonary arterial hypertension (PAH) patients.<sup>18,21</sup> Re-exposure to normoxia leads to rapid reversal of polycythemia and partial decrease in PAP, with persisting hypertension and pulmonary vascular remodeling characterized by progressive intima-obstruction.<sup>18,20,22</sup> Another study showed that a single dose subcutaneous injection of SU5416 (20 mg/kg) in 1-day-old newborn rats could impair pulmonary vascular growth and postnatal alveolarization, leading to long-term PH and abnormal lung structure persisting even into adulthood.<sup>23</sup>

With a half-life of only 30 minutes, SU5416 is rapidly cleared *in vivo* and does not accumulate in major organs, especially in lungs. However, SU5416 can specifically bind to ECs, enriched in the membrane and thence slowly released into the cytoplasm, which ensures long-term inhibition of VEGFR.<sup>24</sup> Loss of EC growth signal, inflammation and oxidative stress due to inhibition of VEGFR, TGF- $\beta$ /BMP/Smad, Hif-1 $\alpha$ , mitogen-activated protein kinase (MAPK), aryl hydrocarbon receptor (AhR) and other signaling pathways are involved in the regulation of the SU5416/hypoxic (SuHx) PH model.<sup>21,25,26</sup> Sharing other hallmarks (concentric laminar and plexiform lesions) of human PAH, the rodent SuHx PH model is regarded as a more appropriate preclinical model of occlusive PAH than the CHPH models, particularly for genetic modification or long-term intervention.<sup>27-29</sup>

Rodent strains and gender differences affect the SuHx modeling. "Typical" SD rats have good survival in the SuHx model and are suitable for the study of novel therapies of established PAH, while Fischer rats reflect a strain-specific defect in RV adaptation in response to the increased hemodynamic load, allowing the study of the mechanisms of RV remodeling; Lewis rats are not suitable for the SuHx model of PH, showing only a marginal response.<sup>30</sup> As for gender, a supplement of female sex hormones given before SU5416 challenge (without hypoxia) markedly reduced susceptibility to the severe PAH phenotype in a hyperresponsive rat strain (SDHR) due to a decline in SU5416-induced EC apoptosis, but could not reverse established severe PAH.<sup>31</sup> This would suggest that sex hormones mainly contribute to the initiation of pulmonary vascular remodeling. More investigations on the precise protective role of sex hormones in the disease setting would provide novel therapeutic approaches for the early stage of PH.

Twenty years after the first report of the SuHx model, it is regarded as an important preclinical model of PAH, with many of its features replicated by numerous research groups. However, limitations still exist.<sup>32</sup> Some investigators insist that the model cannot represent all the important features of the severe forms of human PAH, and thus genetically engineered animals are better tools for future pulmonary vascular research.<sup>33</sup> Moreover, injecting SU5416 in mice does not completely recapitulate the pathological features in rats, perhaps due to differences in cytochrome P450 genes between the two rodent species.<sup>34</sup> How SU5416 and hypoxia serve as two hits interacting on a cellular and molecular level during the pathogenesis of PAH is still uncertain, and the SD-hyperresponsive type that develops severe PAH with mono SU5416 may be helpful in addressing such questions.<sup>30</sup>

Novel PH models combining SU5416 with other second hits have also been emerging. In athymic (T cell-deficient) rats, a single dose of SU5416 (10 mg/kg) is sufficient to induce severe and progressive PH even under normoxic conditions, with macrophages accumulating around occluded small to mid-sized pulmonary arterioles.<sup>35</sup> The combination of abnormal pulmonary blood flow by left pneumonectomy and SU5416 can also induce severe PH of rats.<sup>36</sup> Ovalbumin immunized male SD rats treated with SU5416 result in severe angio-obliterative PAH, accompanied by increased IL-6 expression in the lungs.<sup>37</sup> Besides, a single subcutaneous injection of SU5416 (20 mg/kg) and intraperitoneal injections of morphine (10 mg/kg, daily) for 35 days can induce PAH in male SD rats that is similar to human PAH.<sup>38</sup>

## 2.3 | Monocrotaline

Monocrotaline (MCT) is an 11-membered macrocyclic pyrrolizidine alkaloid derived from the seeds of the *Crotalaria spectabilis* plant. Lalich et al. first reported pulmonary arteritis produced in rat by feeding *Crotalaria spectabilis* in 1961, and in 1967 *Crotalaria spectabilis* was first reported to establish MCT-induced PH in rats, together with RV hypertrophy and an increase in the medial thickness of pulmonary arteries.<sup>39,40</sup> Since then, the MCT-induced PH model has been widely used for nearly 60 years due to its simplicity, reproducibility and low cost.<sup>8,41,42</sup> MCT should be metabolized into the toxic metabolite MCT pyrrole (MCTP) by liver cytochrome P450 3A4 (CYP3A4), which in turn leads to vascular EC damage and inflammation.<sup>8,9</sup> MCT-induced PH has been described mainly in rats with a single subcutaneous injection of MCT (50–80 mg/kg), and it is difficult to obtain the PH phenotype in mice even with a direct injection of MCTP.<sup>8,43,44</sup>

With its broad toxicity, MCT induces a syndrome characterized by hepatic venous occlusive disease, myocarditis, interstitial pulmonary fibrosis, acute lung injury, necrotizing pulmonary arteritis, and renal insufficiency.<sup>44–47</sup> Therefore, although MCT is commonly used to induce a PH model, the pathogenic characteristics of this model are not totally in line with PAH in humans.

Pulmonary toxicity of MCT differs at different periods of lung development, related to alveolar growth and growth of the pulmonary vascular bed.<sup>48</sup> Recently, a novel MCT rat model was reported to be a good mimic of chronic neonatal PH characterized by reduced pulmonary microvascular growth, increased arterial muscularization, elevated RV systolic pressure, and RV hypertrophy.<sup>49</sup> An influence of gender on PH severity was seen in obese MCT-injected Zucker rats, and differences in MCT-induced developmental toxicity and fetal hepatotoxicity may contribute to the selective CYP3A induction in female fetuses.<sup>50,51</sup> Barrier-disruptive mechanisms may act as an earlier event in the MCT-PH model, increasing lung permeability related to early progression of PH in male rats than females.<sup>52</sup>

SuHx- and MCT-induced PH models are the two main models used in PH with different pathophysiological characteristics:

MCT-PH is associated with endothelial toxicity and marked by lung inflammation, while SuHx-PH is characterized by pulmonary vascular proliferative lesions such as the formation of neointimal plexiform lesions. Single-cell RNA sequencing was leveraged to determine and prioritize dysregulated genes, pathways, and cell types in lungs of SuHx and MCT PH rat models, demonstrating their relevance to human PAH and utility for drug repositioning.<sup>53</sup>

## 2.4 | Schistosome-related pah

Schistosome-related PAH (Sch-PAH) is a fatal complication of chronic schistosomiasis infection with unknown mechanism, and a leading cause of PAH-related morbidity and mortality worldwide.<sup>54,55</sup> Current evidence suggests that mechanical obstruction of lung vasculature by embolized eggs, systemic inflammation, pulmonary vascular remodeling, and underlying liver disease might be the most relevant pathogenic mechanisms for Sch-PAH.<sup>56</sup> Female C57/BL6 mice can develop severe vascular remodeling from 12 weeks onwards after transcutaneous injection with a low dose (approximately 30 cercariae) of *Schistosoma mansoni*, characterized by perivascular inflammation, marked medial thickening and the formation of plexiform-like lesions without significant PH.<sup>57</sup> Female mice manifested a heavier worm burden than males during chronic infection, as the presence of testosterone results in decreased schistosome survival and increased host survival in experimental infections, and the extent of pulmonary vascular remodeling correlated with levels of inflammatory cytokines and lung egg burden.<sup>58–60</sup> The complement systems of mice and rats are different, and rats have natural immunity to schistosomiasis infection, which suggests that the complement signaling system may play a critical role in regulating proinflammatory and pro-proliferative processes in the initiation of PH.<sup>61,62</sup>

Praziquantel can prevent development of Sch-PAH in female mice and reverse established pulmonary vascular remodeling, with significantly reduced mRNA expression of IL-13, IL-8, and IL-4 in lungs.<sup>63</sup> A bioinformatics study of Sch-PAH biomarkers based on mouse whole lung tissue RNA-Seq data found that Smad9, BMPR2 (bone morphogenetic protein type 2 receptor), endoglin, and IL4, as well as signal transduction, signal sensor activity, and immune system processes may play important roles in Sch-PAH, and VEGF signaling is necessary for the immune-stimulated vascular remodeling induced by *Schistosoma* egg exposure in mice.<sup>64,65</sup>

Pulmonary vascular remodeling in PH is reported to be dependent on increased TGF- $\beta$  signaling.<sup>66</sup> *Il4<sup>-/-</sup>/Il13<sup>-/-</sup>* mice were protected from Sch-PAH, suggested that a combined deficiency of IL-4 and IL-13 is required for protection against TGF- $\beta$ -induced pulmonary vascular disease after *Schistosoma* exposure.<sup>67</sup> In future, studies involving inhibition of these pathways might lead to potential novel therapeutic approaches for patients with Sch-PAH.

## 2.5 | Bleomycin

Bleomycin is used as a chemotherapeutic agent to treat a variety of cancers, but it has pulmonary toxicity, mainly driving pulmonary fibrosis and further leading to PH. Hence, intratracheal instillation of bleomycin in rodents is considered as a method to generate pulmonary fibrosis, creating a secondary PH animal model.<sup>73,74</sup> The bleomycin-PH models show significant increases in RVSP, evident medial thickening and vessel muscularization, filling the gap in PH associated with parenchymal lung disease research animal models.<sup>68,69</sup> Bleomycin-induced downregulation of BMP9/BMPR2/Smad signaling in the PA endothelium may act as a trigger to induce PH, suggesting novel therapeutic strategies for the treatment of this subtype of PH.<sup>63</sup>

## 2.6 | Mitomycin-c-induced pulmonary veno-occlusive disease

Pulmonary veno-occlusive disease (PVOD) is a rare form of PH caused by progressive blockage of the small veins in the lungs, with a poor prognosis. Chemotherapy is one of the risk factors for PVOD,<sup>70</sup> and the mitomycin-C(MMC)-induced PVOD rat is a successful model for human PVOD. In Wistar rats, PVOD develops with a single dose intraperitoneal administration of MMC at days 21–35, and is characterized by marked thickening and occlusion in small pulmonary veins and significantly elevated RV pressure.<sup>71</sup> MMC-induced PVOD is associated with arterial and microvascular remodeling secondary to severe venous remodeling, suggesting that precapillary and capillary alterations may be initial events in PVOD.<sup>72</sup> Female rats seem to be more sensitive to the toxicity of MMC.<sup>71</sup>

PVOD caused by MMC is associated with dose-dependent depletion of pulmonary GCN2 levels and decreased Smad1/5/8 signaling in rats, which can be restored by amifostine.<sup>71</sup> Recently, Zhang's group found that MMC could activate pulmonary vascular endothelial-to-mesenchymal transition and PVOD via a Smad3-dependent pathway in rats.<sup>73</sup> Treatment with a selective Smad3 inhibitor markedly prevented the pathogenesis of MMC-induced PVOD in rats. All these studies suggest novel strategies for PVOD therapy.

## 3 | INVASIVE MODELS IN VIVO

Surgical invasive methods for PH models are not widely used because of the operative difficulty, mainly including pneumonectomy, pulmonary shunt and pulmonary artery binding (PAB), and associated high mortality. Pneumonectomy and vascular shunt lead to increased blood flow in the remaining pulmonary arteries, which plays a key role in the development of PH. Moderate and sustained PH of SD rats after right lung pneumonectomy alone was reported recently, with a perioperative mortality of <10%.<sup>74</sup> In order to reproduce more human PAH phenotype, pneumonectomy is frequently

combined with MCT or SU5416 for “two-hit model”.<sup>75,76</sup> The systemic artery-pulmonary artery shunt and the arteriovenous shunt are commonly used in left-to-right shunt-type PH models, of which the former uses the pressure difference between systematic and pulmonary arteries to establish a shunt, effectively shunting the blood of the systemic circulation to the pulmonary circulation, and the latter increases the return blood volume through the arteriovenous shunt, increasing the pulmonary circulation blood flow and promoting the formation of PAH.<sup>77,78</sup>

Accumulating evidence shows that RV function is a robust indicator of prognosis in PH.<sup>79,80</sup> PAB is an ideal model for evaluating RV overload or failure, avoiding potential confounding effects of hypoxia, VEGF inhibition and MCT toxicity on RV.<sup>81,82</sup> By changing the diameter of constriction, PAB can simulate adaptive and decompensated RV failure based on demand.<sup>83</sup> Sophisticated technical skill is needed to replicate the position of the constriction on the pulmonary trunk. One limitation of PAB is the inability to observe gradual increases of PVR in PH, which may be solved by using juvenile animals, since the constricting band steadily tightens with age to increase afterload.<sup>83,84</sup> All the invasive and non-invasive methods for creating rodent PH models are summarized in [Table 1](#).

## 4 | GENE EDITING MODELS

The past two decades have witnessed major advances in the field of genetic susceptibility to PH. A variety of genes such as *BMPR2*, *KCNK3*, *SMAD9* have been identified as predisposing genes of familial pulmonary arterial hypertension (FPAH) and idiopathic pulmonary arterial hypertension (IPAH).<sup>85,86</sup> The initial damage to the pulmonary artery in IPAH or FPAH may result from a combination of genetic susceptibility and environmental impact or epigenetic modulation.<sup>87,88</sup> In addition to genetic predisposing genes, there are a variety of molecules with important effects on the development of PH, such as interleukin (IL)-6, 5-hydroxytryptaphane (HT) transporters, hypoxia-inducible factor (HIF) and prolyl hydroxylase domain enzymes (PHDs), giving rise to a group of associated gene-editing animals.

### 4.1 | BMPR2

*BMPR2* was the first PAH disease-associated gene to be identified, and thus is currently the most dominant PAH disease gene. In FPAH and IPAH patients, the frequency of *BMPR2* mutation is 70–80% and 15–20%, respectively.<sup>89</sup> The pathogenic mechanisms of *BMPR2* gene mutations mainly include dominant negative effects and haploinsufficiency, so that patients cannot maintain normal physiological functions, resulting in the appearance of disease phenotypes.<sup>90,91</sup> Due to the loss of exons 4 and 5, *BMPR2*<sup>ΔEx4–5/+</sup> mice show mild PH under normoxia, with impaired ability of the pulmonary vascular system to remodel under long-term hypoxia.<sup>92</sup> *BMPR2*<sup>ΔEx2/+</sup> mice also show high responsiveness to hypoxic stimulation.<sup>93</sup> Mouse strains

TABLE 1 Common preclinical rodent animal models of pulmonary hypertension

Model	Species	Dosage	Duration	Strengthen	Limitation
Chronic hypoxia	Rats/mice	10% O <sub>2</sub> <sup>11-17</sup>	3-5 weeks <sup>11-17</sup>	Good predictability and repeatability	Mild pulmonary hypertension
Monocrotaline	Rats	A single subcutaneous injection of MCT (40–80 mg/kg) <sup>8,39,40,48</sup>	3-4 weeks	Simplicity, reproducibility and low cost, severe pulmonary hypertension	Systemic toxicity, not thought to closely replicate PAH in humans
Sugen+Hypoxia	Rats/mice	Rats: a single subcutaneous injection of Sugen (20 mg/kg) <sup>18,19,20,22,29</sup> Mice: subcutaneous injection of Sugen (20 mg/kg) once a week, for 3–4 weeks <sup>21,27,28</sup>	3 weeks of hypoxia (10% O <sub>2</sub> ) followed by 1–4 weeks of normoxia (21% O <sub>2</sub> )	The appearance of concentric laminar and plexiform lesions similar to PAH patient	Strains and gender differences
Schistosome	Mice	Injection transcutaneously with a low dose (approximately 30 cercariae) of <i>Schistosoma mansoni</i> <sup>57-60</sup>	12 weeks	Pulmonary vascular remodeling correlated with levels of inflammatory cytokines and lung egg burden, close pathogenic mechanisms for Sch-PAH	Species and gender differences
Bleomycin	Rats/mice	Intratracheal injection at a dose of 4–5 mg/kg dissolved in saline <sup>63,68,69</sup>	21–35 days	Mainly manifested by causing pulmonary fibrosis leading to PH	
Mitomycin C	Rats	4 mg/kg dissolved in saline <sup>72</sup>	7 weeks	Important model for human PVOD	Not commonly used
Pneumonectomy	Rats/mice	Left/right pneumonectomy <sup>74,76</sup>		Proper model for evaluating cardioprotective molecules when RV failure	High operation difficulty and mortality
Pulmonary shunt	Rats/mice	The systemic artery-pulmonary artery shunt the arteriovenous shunt		Mimics pathophysiology of congenital shunts	High operation difficulty and mortality
Pulmonary artery banding	Rats/mice	Simulate adaptive banding based on demand <sup>81-83</sup>	7-8 weeks	Adjustable, adaptive and decompensated RV failure based on demand	High operation difficulty and mortality

expressing inducible, smooth muscle cell (SMC)-specific dominant negative alleles of the *BMPR2* gene are more robust PAH models than knockout models. Using the rtTA system under the control of a SMC-specific promoter, different *BMPR2* mutations are inducible specifically in SMC. SM22-rtTA×TetO7-*BMPR2*<sup>R899X</sup> mice exhibit dropout of small vessels, perivascular inflammation and partial RVSP increase after 8 weeks induction, while SM22-rtTA×TRE-*Bmpr2*<sup>Δx4+</sup> mice develop significant elevated RVSP without full pulmonary vascular inflammation or remodeling.<sup>101,102</sup>

*BMPR2* mutant rats have recently been generated and show greater susceptibility to PH than mice. Hautefort et al. created the first rat line carrying a monoallelic mutation in *BMPR2* and observed age-dependent spontaneous PH in partial rats, with high susceptibility to chronic hypoxia and RV dysfunction.<sup>94</sup> The RV dysfunction was notably characterized by reduced cardiomyocyte diameter, altered calcium transportation, decreased calcium sensitivity and shortened action potential duration.

#### 4.2 | KCNK3

Mutations in the *KCNK3* gene encoding an outward rectifier K<sup>+</sup> channel is associated with development of an aggressive form of PAH.<sup>95</sup> *KCNK3* does not form a functional channel in mouse PSMCs, but in contrast is highly expressed in rat pulmonary vasculature.<sup>96,97</sup> The first *KCNK3*-mutated rat model was established by Lambert's group, with mild spontaneous RVSP elevation and pulmonary vascular remodeling, highlighting *KCNK3* loss of function as a determinant in pulmonary vascular remodeling.<sup>98</sup> Heterozygous-*Kcnk3*<sup>Δ94ex1/+</sup> rats are characterized by PA remodeling and peri-vascular collagen crosslinking while homozygous rats show PA remodeling and PA vasoconstriction. This model provides new opportunities for developing therapeutic targets against PH.<sup>98</sup>

#### 4.3 | IL-6

Proinflammatory factor IL-6, the best studied cytokine that induces PH, is correlated with disease severity.<sup>99,100</sup> Injection of mice with recombinant murine IL-6 caused mild PH in normoxia, and exacerbated CHPH, whereas rats receiving recombinant IL-6 presented PH associated with increased thrombi, occlusion of small muscular arteries, and localized hemorrhage.<sup>101,102</sup> Knockout of IL-6 has a protective effect on disease progression of chronic hypoxic mice.<sup>103</sup> IL-6 overexpressing mice are typical inflammation driven PH models, with RV hypertrophy, pulmonary vascular muscularization and occlusive neointimal angioproliferative lesions composed of ECs and T lymphocytes.<sup>104</sup> Arterial lesion induced by IL-6 is accompanied by activation of proangiogenic factor (VEGF), proproliferative kinase extracellular signal-regulated kinase, proproliferative transcription factors (c-MYC and MAX) and antiapoptotic proteins (survivin and Bcl-2), as well as downregulation of growth inhibitor (transforming growth factor-β) and pro-apoptotic kinases (JNK and p38),

suggesting that IL-6 promotes the occurrence and progression of PAH via pro-proliferation and anti-apoptotic mechanisms.<sup>104</sup>

#### 4.4 | 5-HTT

5-HT is a pulmonary vasoconstrictor taken up by the 5-HT transporter (5-HTT). Overexpression of 5-HTT leads to excessive proliferation of PSMCs and pulmonary vasoconstriction, while 5-HTT knockout in mice provides a mildly protective function against CHPH.<sup>105,106</sup> MacLean et al. generated a 5-HTT overexpressing mouse model by transfecting a yeast chromosome with the human 5-HTT gene, which exhibited evident RVSP elevation both in normoxic and hypoxic conditions compared with wild-type mice.<sup>107</sup> Subsequently, Guignabert et al. established a SMC-specific 5-HTT overexpression (SM22-5-HTT [+]) mouse model.<sup>108</sup> Eight-week-old mice exhibited PH with marked increases in RVSP and RV hypertrophy, but no changes in systemic arterial pressure. The phenotypes were more severe when the mice were exposed to chronic hypoxia or after MCT challenge, consistent with the proposal that the level of 5-HTT expression is an important modifier of PH despite etiology.

#### 4.5 | S100A4/Mts1

S100A4/Mts1 is involved in metastasis activities like angiogenesis, inflammation and motility, thus S100A4/Mts1 transgenic mice were initially created for studying metastatic tumor biology. However, this model presented mild increases in RVSP, pulmonary vascular remodeling and periarterial inflammatory responses, including the development of plexiform-like lesions in a subset of mice.<sup>109</sup> The specific mechanism by which S100A4 participates in plexiform lesion development remains unclear. Furthermore, S100A4/Mts1 overexpressing mice showed severe PH and RV hypertrophy under chronic hypoxia, which could not be reversed in normoxia.<sup>110</sup> Both Mts1 and its receptor, receptor for advanced glycation end-products (RAGE), have been implicated in the pathogenesis of human PH.<sup>111,112</sup>

### 5 | MULTI-MEANS JOINT MODELING

Compared with single factor induced models, PH models induced by multiple factors have a better correlation with more phenotypic features and severe human PH. In PH modality, invasive and non-invasive means in vivo and gene editing means are often used in combination, for example, in MCT/pneumonectomy rats, SU5416/pneumonectomy rats, SuHx models, IL-6Tg+/hypoxia mice etc.<sup>111,113,114,115</sup> Multi-means joint modeling increases the rate of development of PH, but also increases the mortality of the animals. As PH patients usually have multiple severe clinical manifestations, multi-factor induction models are more beneficial for the discovery of relevant therapeutic targets for hemodynamic and structural changes in the pulmonary circulation.

## 6 | CONCLUSIONS

While it is clear that a perfect preclinical model of human PH is not yet available, there is no doubt that animal models have provided useful insights into the occurrence and development of PH. The developmental history of PH models can be roughly divided into three stages in chronological order. The first stage focused on reproducing the non-specific intima, adventitia thickening of the pulmonary artery and elevated RVSP as well as PAP, using simple methods, mainly including classical chronic hypoxia and MCT models. The MCT rat model is also a typical inflammation mediated PH model, with pronounced medial hypertrophy. The second stage was to study the occurrence of plexiform lesions, vascular occlusion, and progressive PAP, and so the surgical shunt models, embolization models, genetic modification models, SuHx models etc. came into being. The third stage has been to focus on the adaptive remodeling of animal models to the development of RV failure, represented by RV high-afterload models such as PAB, since a growing body of evidence shows that RV failure has important effects on the prognosis of PH. These three stages cannot be completely separated. An interplay of metabolism, inflammation and oxidative damage also contributes to the development of RV failure, via as-yet unclear mechanisms.

It is critical to select an appropriate model for PH investigation, considering not only the pathophysiological mechanism of the method itself, but also the different responses to the modeling method of different animal species and genders. The fawn-hooded rat (FHR) is the only rat model that develops spontaneous PAH, evident in 68% of PAH offspring.<sup>116</sup> Cattle are more sensitive to hypoxia than rats, but their use in CHPH models is greatly cost limited.<sup>117</sup> Female animals are generally less susceptible in hypoxia- or MCT-induced PH, but in mutant and transgenic rodents, sex-based differences in PH are occasionally complex and contradictory. Some models, such as S100A4/Mts1 overexpression mice, display a female bias with high penetrance and severity. Others may show the opposite; for example, female mice lacking apolipoprotein E (ApoE) develop a much less severe PH phenotype than the males.<sup>118,119</sup>

In general, PH is cardiopulmonary condition due to a variety of factors rather than one disease. Although there are similarities in the pulmonary vasculature alterations caused by different factors, the various triggers pinpoint the distinct lesion origin driving PH development. Given the fact that right heart failure is regarded as a main consequence of PH and will ultimately lead to death, it is critical and necessary to seek molecular targets against existing pulmonary vascular remodeling and RV dysfunction using the most appropriate experimental animal models. The selection of animal model should be based on the research purpose and may involve a combination of both classic and novel modeling means.

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### CONFLICT OF INTEREST

The authors have declared no conflict of interest.

### AUTHOR CONTRIBUTIONS

X. -H.W. and J.-L.M. contributed to the review's design, writing and manuscript preparation; D.D., Y.-J.M. and Y.-P.W. proofed and advised on the content of the manuscript; Z.-C.J. conceived the review and revised the manuscript. All authors contributed to the article and approved the submitted version.

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