Chapter 7
Animal Models with Pulmonary Hypertension

Kohtaro Abe

Abstract Pulmonary arterial hypertension (PAH) comprises a multifactorial group of pulmonary vascular disorders that frequently lead to right heart failure and premature death. Histologically, patients with severe PAH have combinations of small pulmonary arterial medial and adventitial thickening, occlusive neointima, and complex plexiform lesions. Despite recent advances in treatments targeting those remodeled pulmonary arteries, the mortality in severe PAH is still high. To explore the novel treatment for severe PAH, better understandings of the pathogenesis of these lesions are needed. Numerous studies to investigate the pathogenic cellular and molecular mechanisms have been done using conventional animal models (i.e., chronically hypoxic and monocrotaline-injected rats) of pulmonary hypertension (PH). Although these animal models have contributed to provide important mechanistic insights for the development of the treatments, they do not develop the histological hallmarks of PAH, plexiform lesions. This chapter provides an overview of the histological characteristics observed in humans with pulmonary hypertension and preclinical models and discusses the better model to be used for investigating the pathogenesis of PAH and preclinical drug evaluations.

Keywords Pulmonary hypertension • Animal model • Vascular endothelial growth factor (VEGF) • Plexiform lesions

7.1 Pathological Findings of Pulmonary Vascular Lesions in Human PAH

Pulmonary arterial hypertension (PAH: World Health Organization Group 1 pulmonary hypertension) is frequently severe and leads to right heart failure and death [1]. The major contributing factors to increase pulmonary arterial resistance and pressure include sustained vasoconstriction, in situ thrombus, and progressive vascular

K. Abe (✉)
Department of Cardiovascular Medicine, Kyushu University Graduate School of Medicine, 3-1-1, Maidashi, Higashi-ku 812-8582, Fukuoka, Japan
e-mail: koabe@cardiol.med.kyushu-u.ac.jp

Y. Fukumoto (ed.), *Diagnosis and Treatment of Pulmonary Hypertension*,
DOI 10.1007/978-981-287-840-3_7

Table 7.1 Histological features of grades of hypertensive pulmonary vascular lesions (Heath-Edwards classification)

	Grades of hypertensive pulmonary vascular lesions					
	1	2	3	4	5	6
Types of media of arteries/arterioles	Hypertrophied →					
			Some generalized dilatation →			
					Local dilatation lesions →	
						NA →
Types of intimal reaction	None →					
		Cellular →				
			Fibrous and fibroclastic →			
					Plexiform lesion →	

NA necrotizing arteritis

remodeling [2, 3]. Particularly, vascular remodeling is thought to be a major player to narrow small pulmonary arteries in patients with advanced PAH. In 1958, Heath and Edwards classified six grades of hypertensive pulmonary arterial lesions according to histological features in media and intimal areas in 69 patients primarily with congenital heart disease-associated pulmonary hypertension (previously known as secondary pulmonary hypertension). [4]. As shown in Table 7.1, a medial muscular pulmonary artery is hypertrophied toward vascular lumen (grade1) at the early stage of PAH. As advance in pulmonary hypertension, proliferation of intimal cells in arterioles and small muscular arteries raises a fibrous thickening from a cellular thickening (grades 2 and 3). At the late stage, the plexiform lesions (grade 4) appear with neointimal lesions. Of note, this study also indicated the indistinguishable lesions in two patients with primary pulmonary hypertension (currently known as idiopathic PAH). These remodeled pulmonary arteries obviously contribute to increases in pulmonary arterial resistance and pressure in PAH. Numerous studies using lung tissue samples from patients have been done for better understandings of the pathogenesis and developing novel treatments of PAH. However, these clinical studies include the following critical limitations. First, it is essentially impossible to obtain the lung samples at the early-stage patients, because, in most cases, only a single lung specimen becomes available at the time of transplant or at autopsy. Second, it is also impossible to obtain serial lung tissue samples for detailed assessment of the lung vascular morphology/pathobiology because of its invasiveness. Third, it is impossible to exclude the effects of treatments (i.e., a high dose of intravenous epoprostenol) on the cellular and molecular signaling, because most of the end-stage patients have received various treatments [5]. Therefore, a novel experimental model that precisely simulates the clinical PAH phenotype is needed to solve these problems.

7.2 Conventional Animal Models with Pulmonary Hypertension

In order to investigate the pathogenesis of PAH, several animal models with pulmonary hypertension have been utilized [6]. The most frequently used animal models are the chronic hypoxia- and monocrotaline-exposed rodents. It is true that these conventional models have contributed to the better understanding of the pathogenesis of PAH and provided valuable information to the subsequent clinical studies. However, they do not adequately replicate human PAH.

7.2.1 Monocrotaline

Monocrotaline is a toxic pyrrolizidine alkaloid made from the plant *Crotalaria spectabilis* [7, 8]. Rats are most commonly used since mice cannot convert monocrotaline into the monocrotaline pyrrole due to the lack of the hepatic metabolism by cytochrome *P-450*. Generally, an adult male rat is administered with a single injection of monocrotaline (typically 60 mg/kg subcutaneously or intraperitoneally), then leading to high pulmonary arterial pressures within 3 weeks and a death of unknown cause. Although the mechanisms that monocrotaline causes pulmonary hypertension have not been fully understood, it is widely thought that endothelial injury and accumulation of inflammatory cells caused by monocrotaline may play an important role in the development of vascular remodeling [6, 7, 8]. However, this model presents only increased vascular wall thickness medial wall thickness (grade 1) but not neointimal lesions, which are frequently observed in the late stage of human PAH. In addition, monocrotaline directly causes myocarditis with significant liver and kidney damages, suggesting that the multiple organ failure is a possible cause of death in this model [9, 10]. Thus, monocrotaline-exposed rats do not seem to replicate idiopathic PAH in human, even though they have severe pulmonary hypertension.

7.2.2 Chronic Hypoxia

Various types of animals develop mild to moderate pulmonary hypertension after a couple of weeks under hypoxic (typically 10 % O_2) or hypobaric conditions [6, 11]. Generally, rats and mice exposed to 3-week hypoxia present only increased vascular wall thickness (grade 1). However, there is no evidence of right heart failure in chronic hypoxia-exposed animals [6]. Thus, this model does not also seem to adequately replicate severe PAH.

7.3 Other Animal Models with Pulmonary Hypertension

7.3.1 Fawn-Hooded Rats

Fawn-hooded rats spontaneously present severe pulmonary hypertension and extent of medial hypertrophy, which are further developed in the mild hypoxia of Denver's high altitude [12, 13]. However, they develop not only pulmonary but also systemic hypertension, which is not typical in human PAH.

7.3.2 Mice Overexpressing S100A4/Mts1

S100A4/Mts1, a member of the S100 family of calcium-binding proteins, was initially found in metastatic mouse mammary adenocarcinoma cells [14]. Transgenic mice overexpressing S100A4/Mts1 develop pulmonary arterial changes resembling human neointimal lesions leading to occlusion of the arterial lumen [15]. The plexiform-like lesions were also observed in this mice model. However, surprisingly, there was no evidence of medical wall thickness and severe pulmonary hypertension.

7.3.3 Mice Overexpressing IL-6

Several reports have indicated that serum levels of interleukin-6 (IL-6) were elevated and that the expression in the lungs was increased in patients with PAH [16]. Mice, which overexpress IL-6, were reported to develop pulmonary hypertension, which was enhanced by chronic exposure to hypoxia [17]. Of note, this animal model demonstrates not only medical wall thickness but also intimal occlusive lesions. However, there was no evidence of plexiform lesion formations.

7.3.4 Murine Models with BMPR2 Mutations

Bone morphogenetic protein receptor type 2 (BMPR2] mutations are found in approximately 80 % of cases of heritable PAH and approximately 20 % of idiopathic PAH patients [18, 19]. Since the function of the BMPR2 receptor serves as an internal brake against TGF-β signaling, loss of BMPR2 function activate TGF-β signaling, resulting in various proliferative and inflammatory responses. Compared to wild-type mice, transgenic mice with heterozygous BMPR2 mutations develop increased wall thickness with mild pulmonary hypertension under normoxic condition [20]. When BMPR2 deficiency mice are exposed to hypoxia for 3–5 weeks, they showed further

increases in RV systolic pressure and medial thickness of small pulmonary arteries without intimal lesions [20, 21]. These results suggest that the BMPR2 mutation alone may be insufficient to generate severe pulmonary hypertension.

7.4 What Do We Need to Establish the Preclinical Model Resembling Human Pathologic Findings?

As summarized in Table 7.2, there are no models that replicate human severe PAH with occlusive intimal and complex plexiform lesions. Therefore, some investigators attempted to modify these models to develop pulmonary vascular lesions. Tanaka et al. have reported that monocrotaline-exposed rats with a subclavian to pulmonary artery anastomosis develop neointimal changes [22]. White et al. have also demonstrated that monocrotaline given to left pneumonectomized young rats caused severe pulmonary hypertension accompanied by development of occlusive intimal lesions [23]. These results suggest that at least two "hits" (monocrotaline pulse excessive shear stress) are necessary for the development of intimal lesions of small pulmonary arteries. Interestingly, the model reported by White presented perivascular plexiform-like lesions. The majority of cells in these lesions, which are comprised by α-smooth muscle actin- and vascular endothelial growth factor (VEGF)-positive cells, are highly proliferative. Also, these lesions include disorganized vascular channels lined by von Willebrand factor (an endothelial marker)-positive cells. The similar features of these lesions are observed in vascular lesions of human PAH. On the other hand, there is a major difference in time course of lesion formation between White's model and human PAH. In contrast to the development of plexiform lesions in advanced and severe stages of human PAH, the plexiform-like lesion in pneumonectomized rats develops very early (within 1 week) before the establishment of severe pulmonary hypertension.

Table 7.2 Histological characteristics in conventional animal models with pulmonary hypertension

Models	Species	Pulmonary hypertension	Medial hypertrophy	Intimal lesions	Plexiform lesions
Chronic hypoxia	R, M, D, G, P, S	Low	Yes	No	No
MCT	R, D, S	High	Yes	No	No
MCT/pneumonectomy	R, D, S	High	Yes	Yes	Yes or no
Fawn-hooded	R	High	Yes	No	No
S100A4/Mts4 overexpressing	M	Low	Yes	Yes	No
IL-6 overexpressing	M	Low	Yes	Yes	No
$BMPR2^{+/-}$	M	Low	Yes	No	No
Systemic shunt	R, D, P, S	High	Yes	Yes	Yes or no

Grade is characterized MCT, monocrotaline; SERT, serotonin transporter; BMPR2, bone morphogenetic protein receptor type 2; "yes" characteristic is present; "no" characteristic is not present
R rats, *M* mice, *D* dos, *G* guinea pigs, *P* pigs, *S* sheep

7.5 Preclinical Animal Model with Severe PH and Complex Occlusive Lesion Formation

In 2001, Tuder et al. have reported that VEGF and VEGF receptor-2 are overexpressed in both intimal and plexiform lesions in human PAH [24]. They initially hypothesized that the blockade of VEGF signaling by VEGF receptor blocker Sugen(SU)5416, semaxinib, might attenuate the development of pulmonary hypertension in chronic hypoxia-exposed rats (Sugen5416/hypoxia/normoxia-exposed rats) [25]. However, unexpectedly, this "two-hit" rats present severe pulmonary hypertension and occlusive intimal lesion 3 weeks after SU5416 injection, unlike chronic hypoxia-exposed alone rats [25]. Although it is not totally clear how VEGF blockade accelerates pulmonary hypertension and occlusive intimal lesion in chronic hypoxia-exposed rats, Taraseviciene-Stewart et al. proposed the possible mechanisms as the following [25, 26]. The initial blockade of VEGF by SU5416 causes endothelial cell apoptosis in pulmonary arteries, and then majority of cells are killed. However, survived apoptosis-resistant endothelial cells are proliferated to occlude the vascular lumen under hypoxic condition, which is assumed to increase shear stress on the surface of inner cells. Interestingly, high pulmonary arterial pressure induced by the combination of SU5416 and hypoxia is sustained even after return to normoxia. They concluded that by 5 weeks (3 weeks of chronic hypoxia and 2 weeks of reexposure to normoxia) after the SU5416 injection, this "two-hit" model, but neither SU5416 nor hypoxia alone, develops severe progressive PAH associated with formation of occlusive neointimal lesions in small pulmonary arteries and arterioles. However, the plexiform lesions characteristic of human severe PAH have not been observed 5-week time point after SU5416 injection [26, 27].

We have previously reported that the later stages (i.e., 13–14 weeks after the SU5416 injection) of the SU5416/hypoxia/normoxia-exposed rats would develop plexiform lesions with progressive pulmonary hypertension (Fig. 7.1a) [28]. As shown in Fig. 7.1b, RV systolic pressure (RVSP, a marker of systolic pulmonary arterial pressure) initially increased time dependently, and it appeared to reach its maximum (>100 mmHg) around 5 weeks after SU5416 injection and stayed at about the same high level thereafter. At the 3- to 5-week time point, cardiac index (CI) decreased to approximately 50 % of normal and tended to further decrease at the 8- and 13-week time points. Reflecting the increases in RVSP and the reductions in CI, estimated total pulmonary resistance index (TPRI) estimated by dividing RVSP by CI showed a progressive increase from 1 to 13 weeks (Fig. 7.1c) [29]. There was no significant elevation in systemic arterial pressure. Serial histological examination revealed that SU5416/hypoxia/normoxia-exposed rats time dependently demonstrated various forms of vascular remodeling without lung parenchymal abnormalities (Fig. 7.2a). Following Heath-Edwards classification, medial wall hypertrophy (grade 1) and neointimal thickening (grades 2 and 3) appeared from 3- to 5-week time points after the SU5416 injection. At 13–14 weeks after the

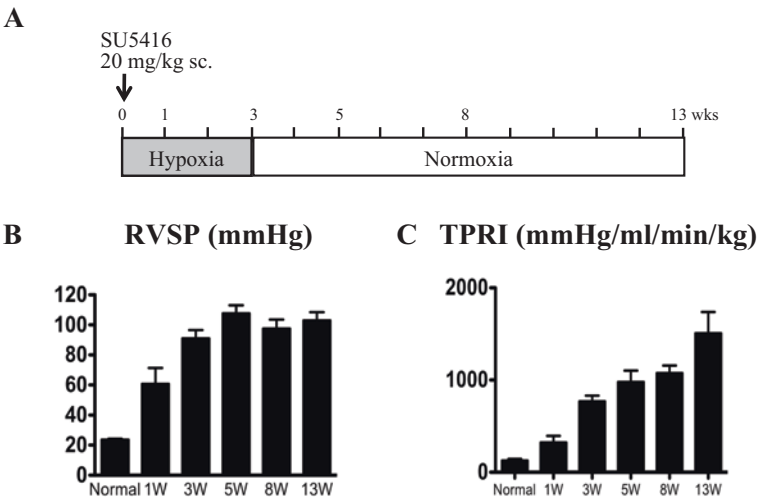
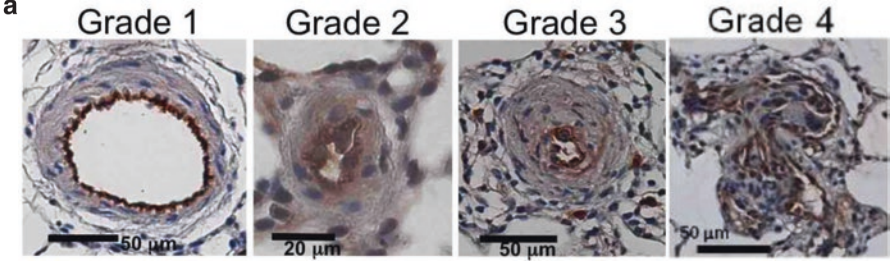


Fig. 7.1 SU5416/normoxia/hypoxia-exposed PAH rats. Protocol of SU5416/normoxia/hypoxia model (**a**), temporal changes in right ventricular systolic pressure (RVSP) (**b**) and total pulmonary resistance index (TPVRI) (**c**) in Sugen5416/hypoxia/normoxia-exposed rats at 1, 3, 5, 8, and 13 weeks after the Sugen5416 injection (Modified from Abe [28] and Toba [29])

SU5416 injection, rats developed severe PAH accompanied by concentric laminar neointimal and complex plexiform lesions (grade 4) strikingly similar to that observed in human severe PAH. Unfortunately, mice with the combination of SU5416 and hypoxia failed to develop severe pulmonary hypertension and complex plexiform lesion [30]. The reason for the difference between the rat and murine models is unknown.

The mortality rate of SU5416/hypoxia/normoxia-exposed SD rats was low despite of low cardiac function. This result is uncommon in human PAH with RV dysfunction. However, this mortality rate was significantly worsening in Fischer344 with the combination of SU5416 and hypoxia, even though the severities of both pulmonary hypertension and RV function are similar between SD and Fischer 344 rats [31, 32]. In addition, the mortality rate in SU5416/hypoxia/normoxia-exposed SD rats increases after treadmill exercise [33], probably because complete resting state may prevent the death associated with RV heart failure in this model. Thus, some modifications of this model are needed to replicate time course of human PAH.

a



b

Weeks after SU5416	0	5	8	13.14
Histological Grade				
1	-	+	+	+
2	-	+	+	+
3	-	-	+	+
4	-	-	-	+

Fig. 7.2 Pulmonary arterial lesions in SU5416/normoxia/hypoxia-exposed PAH rats. Representative photomicrograph showing sequential changes of pulmonary vascular lesions following Heath-Edwards classification in various stages of SU5416/normoxia/hypoxia-exposed PAH rats (**a**) and timing in sequential appearances of difference grades of pulmonary arterial lesions (**b**). High-magnification photomicrograph showing medial wall thickness (grade 1), cellular intimal reaction/proliferation (grade 2), concentric laminar neointimal lesion (grade 3), and plexiform lesion (grade 4). von Willebrand factor stained (Modified from Abe [28])

7.6 Conclusion

This chapter reviewed the similarities and differences among various models and human PAH from the point of view of pathophysiological findings. Numerous animal models have been investigated since the monocrotaline-exposed rat was firstly described in 1967 [7]. It is true that these animal models have provided variable mechanisms of the development and maintenance of PAH. However, there is no perfect animal PAH models mimicking human severe PAH. We should understand this fact, when we plan to translate preclinical data to clinical.

Acknowledgment and Funding We thank Dr. Masahiko Oka for helpful editorial comments. This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion Science (20588107).

References

1. Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, et al. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2009;54:S43–54.
2. Stacher E, Graham BB, Hunt JM, Gandjeva A, Groshong SD, McLaughlin VV, et al. Modern age pathology of pulmonary arterial hypertension. Am J Respir Crit Care Med. 2012;186:261–72.
3. Wagenvoort CA. Plexogenic arteriopathy. Thorax. 1994;49:S39–45.
4. Heath D, Edwards JE. The pathology of hypertensive pulmonary vascular disease: a description of six grades of structural changes in the pulmonary arteries with special reference to congenital cardiac septal defects. Circulation. 1958;18:533–47.
5. Galiè N, Corris PA, Frost A, Girgis RE, Granton J, Jing ZC, et al. Updated treatment algorithm of pulmonary arterial hypertension. J Am Coll Cardiol. 2013;62(25 Suppl):D60–72.
6. Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF. Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. Am J Physiol Lung Cell Mol Physiol. 2009;297:L1013–32.
7. Kay JM, Harris P, Heath D. Pulmonary hypertension produced in rats by ingestion of *Crotalaria spectabilis* seeds. Thorax. 1967;22(2):176–9.
8. Wilson DW, Segall HJ, Pan LC, Lamé MW, Estep JE, Morin D. Mechanisms and pathology of monocrotaline pulmonary toxicity. Crit Rev Toxicol. 1992;22(5–6):307–25.
9. Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskauskas D, Voelkel NF, Bogaard HJ. The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol. 2012;302(4):L363–9.
10. Kurozumi T, Tanaka K, Kido M, Shoyama Y. Monocrotaline-induced renal lesions. Mol Pathol. 1983;39(3):377–86.
11. Herget J, Suggett AJ, Leach E, Barer GR. Resolution of pulmonary hypertension and other features induced by chronic hypoxia in rats during complete and intermittent normoxia. Thorax. 1978;33(4):468–73.
12. Sato K, Webb S, Tucker A, Rabinovitch M, O'Brien RF, McMurtry IF, Stelzner TJ. Factors influencing the idiopathic development of pulmonary hypertension in the fawn hooded rat. Am Rev Respir Dis. 1992;145(4 Pt 1):793–7.
13. Nagaoka T, Gebb SA, Karoor V, Homma N, Morris KG, McMurtry IF, Oka M. Involvement of RhoA/Rho kinase signaling in pulmonary hypertension of the fawn-hooded rat. J Appl Physiol. 2006;100(3):996–1002.

14. Ebralidze A, Tulchinsky E, Grigorian M, Afanasyeva A, Senin V, Revazova E, Lukanidin E. Isolation and characterization of a gene specifically expressed in different metastatic cells and whose deduced gene product has a high degree of homology to a Ca2+−binding protein family. Genes Dev. 1989;3:1086–93.
15. Greenway S, van Suylen RJ, Du Marchie Sarvaas G, Kwan E, Ambartsumian N, Lukanidin E, Rabinovitch M. S100A4/Mts1 produces murine pulmonary artery changes resembling plexogenic arteriopathy and is increased in human plexogenic arteriopathy. Am J Pathol. 2004;164:253–62.
16. Humbert M, Monti G, Brenot F, Sitbon O, Portier A, Grangeot-Keros L, Duroux P, Galanaud P, Simonneau G, Emilie D. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. Am J Respir Crit Care Med. 1995;151(5):1628–31.
17. Steiner MK, Syrkina OL, Kolliputi N, Mark EJ, Hales CA, Waxman AB. Interleukin-6 overexpression induces pulmonary hypertension. Circ Res. 2009;104(2):236–44.
18. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, Hodge SE, Knowles JA. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. Am J Hum Genet. 2000;67(3):737–44.
19. Elliott CG. Genetics of pulmonary arterial hypertension: current and future implications. Semin Respir Crit Care Med. 2005;26(4):365–71.
20. Beppu H, Ichinose F, Kawai N, Jones RC, Yu PB, Zapol WM, Miyazono K, Li E, Bloch KD. BMPR-II heterozygous mice have mild pulmonary hypertension and an impaired pulmonary vascular remodeling response to prolonged hypoxia. Am J Physiol Lung Cell Mol Physiol. 2004;287(6):L1241–7.
21. 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(1):L98–109.
22. Tanaka Y, Schuster DP, Davis EC, Patterson GA, Botney MD. The role of vascular injury and hemodynamics in rat pulmonary artery remodeling. J Clin Invest. 1996;98(2):434–42.
23. White RJ, Meoli DF, Swarthout RF, Kallop DY, Galaria II, Harvey JL, Miller CM, Blaxall BC, Hall CM, Pierce RA, Cool CD, Taubman MB. Plexiform-like lesions and increased tissue factor expression in a rat model of severe pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol. 2007;293:L583–90.
24. Tuder RM, Chacon M, Alger L, Wang J, Taraseviciene-Stewart L, Kasahara Y, Cool CD, Bishop AE, Geraci M, Semenza GL, Yacoub M, Polak JM, Voelkel NF. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. J Pathol. 2001;195(3):367–74.
25. Taraseviciene-Stewart L, Kasahara Y, Alger L, Hirth P, Mc Mahon G, Waltenberger J, Voelkel NF, Tuder RM. Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. FASEB J. 2001;15:427–38.
26. Sakao S, Taraseviciene-Stewart L, Lee JD, Wood K, Cool CD, Voelkel NF. Initial apoptosis is followed by increased proliferation of apoptosis-resistant endothelial cells. FASEB J. 2005;19(9):1178–80.
27. Oka M, Homma N, Taraseviciene-Stewart L, Morris KG, Kraskauskas D, Burns N, Voelkel NF, McMurtry IF. Rho kinase-mediated vasoconstriction is important in severe occlusive pulmonary arterial hypertension in rats. Circ Res. 2007;100:923–9.
28. Abe K, Toba M, Alzoubi A, Ito M, Fagan KA, Cool CD, Voelkel NF, McMurtry IF, Oka M. Formation of plexiform lesions in experimental severe pulmonary arterial hypertension. Circulation. 2010;121:2747–54.
29. Toba M, Alzoubi A, O'Neill KD, Gairhe S, Matsumoto Y, Oshima K, Abe K, Oka M, McMurtry IF. Temporal hemodynamic and histological progression in Sugen5416/hypoxia/normoxia-exposed pulmonary arterial hypertensive rats. Am J Physiol Heart Circ Physiol. 2014;306(2):H243–50.

30. Ciuclan L, Bonneau O, Hussey M, Duggan N, Holmes AM, Good R, Stringer R, Jones P, Morrell NW, Jarai G, Walker C, Westwick J, Thomas M. A novel murine model of severe pulmonary arterial hypertension. Am J Respir Crit Care Med. 2011;184(10):1171–82.
31. Jiang B, Deng Y, Suen C, Taha M, Chaudhary KR, Courtman DW, Stewart DJ. Marked strain-specific differences in the SU5416 rat model of severe pulmonary arterial hypertension. Am J Respir Cell Mol Biol. 2016;54(4):461–8.
32. Alzoubi A, Almalouf P, Toba M, O'Neill K, Qian X, Francis M, Taylor MS, Alexeyev M, McMurtry IF, Oka M, Stevens T. TRPC4 inactivation confers a survival benefit in severe pulmonary arterial hypertension. Am J Pathol. 2013;183(6):1779–88.
33. Bogaard HJ, Natarajan R, Mizuno S, Abbate A, Chang PJ, Chau VQ, Hoke NN, Kraskauskas D, Kasper M, Salloum FN, Voelkel NF. Adrenergic receptor blockade reverses right heart remodeling and dysfunction in pulmonary hypertensive rats. Am J Respir Crit Care Med. 2010;182:652–60.