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EXPERIMENTAL MODELS OF PULMONARY HYPERTENSION

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ABSTRACT

Pulmonary arterial hypertension (PAH), is highly ruinous condition that targets the endothelial cells of pulmonary arteries resulting in vasoconstriction and vascular remodeling which has become rampant and perpetuating. Pulmonary hypertension is also a predictor of various adverse effects. Subsequently, preclinical experimental settings are organized to investigate the PAH in animal models and new strategies for patient with PAH are needed. The pathogenesis of the disease is poorly understood due to its complicated structure. Animal models have improved current understanding of the complex pathophysiology of the disease and contributed to understand its underlying mechanisms which help in the development of therapeutic targets. Chronic hypoxia and the monocrotaline-induced rat models are the most widely used animal models of pulmonary arterial hypertension. It is amazing that rodent models, such as monocrotaline and pneumonectomy and left pneumonectomy plus Su5416 yield more severe PH than single stimuli models which is similar to human pathophysiology. There are also new models such as endothelin B receptor deficiency, bleomycin induced pulmonary hypertension and some other models are used which are based on endothelial cell proliferation due to some growth factors. Ren2 model is also a new model which is based on renin angiotensin system. However the cumulative literature for animal models for pulmonary hypertension is inadequately available. In the present review, we have discussed

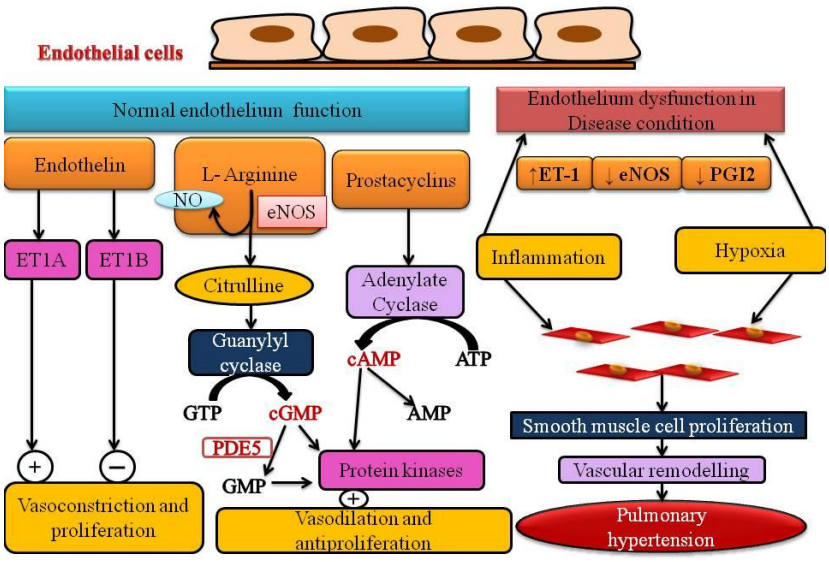
various experimental models of pulmonary hypertension.

KEYWORDS: Pulmonary hypertension; chronic hypoxia; endothelium dysfunction; vascular remodeling; rat models; Vascular remodeling.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is defined as a mean pressure in the pulmonary artery (mPAP) is more than 25 mmHg.[1] There are many different clinical reasons that can cause pulmonary hypertension in different ways such as congenital heart defects, chronic lung disease, autoimmune disease, HIV infection, hepatic disorders, thrombotic, pulmonary vasculitis, longstanding left ventricular failure, mitral valve stenosis/insufficiency and drugs, etc. There are five distinct group of classification of Pulmonary hypertension recognizes by WHO describing a mean pulmonary artery pressure > 25mmHg.[2] These groups have therapeutic implication and shared pathophysiological, haemodynamic and, histological features. The main advantage of using different kinds of models is to understand the pathophysiology of human PH economically and safely. Most of the characteristic features of human PH are relevant to the animal models such as increased proliferation, decreased apoptosis and hypertrophy of pulmonary artery smooth muscle cells (PASMC), increased apoptosis of endothelial cells and vasoconstriction are responsible for obstructive pulmonary vascular remodeling, with narrowing and obliteration of the vascular lumen.[3] In addition there is distal muscularisation and medial hypertrophy of small PAs, adventitial thickening, oedema and haemosiderosis, distal extension of PASMC and medial hypertrophy of small PAs, interstitial fibrosis, emphysema etc.[5]

Fig. 1. Pathology of the pulmonary hypertension



ANIMAL MODELS

MONOCROTALINE INDUCED PULMONARY HYPERTENSION

MCT is derived from the seeds of the plant of Crotalaria spectabilis. MCT fed rats induces a pulmonary vascular injury, pulmonary vasculitis, RV hypertrophy and endothelial cell apoptosis only after converted to monocrotaline pyrrole in liver (6-8). However, the response is variable among species, strains, and even animals to MCT because of differences in the hepatic metabolism by cytochrome P-450. Currently, the rat is the preferred species for the study of monocrotaline-induced PH.[9] It is the most widely used animal model of Group I pulmonary hypertension considered by WHO. Early evidence suggested monocrotaline involved alterations of cellular metabolism and oxygen consumption with the systemic toxicity.[10] Guarnieri and Muscari recognized the monocrotaline toxicity towards cardiac mitochondria in 1988, which found reduction in complex I, II, and IV activities as well as increased superoxide production in cardiac mitochondria following high-dose (105 mg/kg) monocrotaline treatment.[11] Monocrotaline has been shown to upregulate aerobic glycolysis in cardiomyocytes and in hepatocytes.[12-14] Compounds thought to inhibit fatty acid oxidation and restore more normal glucose oxidation (e.g., trimetazidine) have been shown to be beneficial in monocrotaline induced PAH, similar to pyruvate dyhydrogenase kinase inhibitor (e.g., Dichloroacetate). Monocrotaline is most accurately described as a model of pulmonary multiorgan system toxicity along with pulmonary fibrosis, hepatic injury, immunotoxicty and DNA cross linking.[15] It has been suggested that several different metabolic modulators are present which may be beneficial in human PAH, induced by a large number of agents that have not been beneficial in human disease.[16-19]

CHRONIC HYPOXIA

Chronic hypoxia is one of the animal models which have been used to induce PH in rats**.** It can be achieved to high altitude (Group 3.6 PH) by placing rodents either in hypobaric or normobaric hypoxia chambers. Normally, 10% or 12% O2 is used, for three to five weeks. This is reliable to increase in RVSP of roughly in rats, with right ventricular hypertrophy and pulmonary vasculature remodelling. The main advantage to hypoxia is its simplicity and reliability. According to WHO it is often used in for all forms of PH, for many of which it is probably molecularly irrelevant. Further, because it is dependent on not just vasoreactivity, but also inflammation, metabolism, and proliferation.[20, 21]

MONOCROTALINE AND PNEUMONECTOMY MODEL OF PULMONARY HYPERTENSION

Monocrotaline plus pneumonectomy creates a medial hypertrophy being accompanied by neointimal lesions.[22, 23] Left pneumonectomy[24] when combined with MCTP-induced injury, the changes in blood flow and minimal increases in pressures caused by the pneumonectomy were found to be sufficient to induce intimal remodeling in the distal pulmonary arteries. It had been suggested that shear stress, rather than stretch or distention, was responsible for the neointimal formation in the presence of MCTP injury. Many new drugs treatment has been tested by the investigators in this model. Importantly, simvastatin has been found to attenuate the development of PH and to reverse established disease and promote survival in this model.[25-26]

CHRONIC HYPOXIA + SUGEN 5416

SUGEN 5416 (SU-5416) and chronic hypoxic was used by Taraseviciene-Stewart et al. for the development of the model of PAH in rats.[27] Originally, adult male Sprague–Dawley rats were injected subcutaneously with 200 mg/kg of SU-5416 three times per week for 3-weeks along with chronic hypoxia. Progression of PAH occurred even after return to normoxia. The PH in this model is severe, with RVSP of 96 mmHg.[28] These rats had severe PH and developed a plexiform arteriopathy that was indistinguishable from human PAH. In this model rats developed pulmonary hypertension with right ventricular systolic pressure 100 mm Hg and severe pulmonary arteriopathy, including concentric neointimal and complex plexiform-like lesions. Immunohistochemical analyses showed that these structures closely resembling human plexiform lesions. This model provides a new and rigorous approach for investigating the genetic abnormalities, hemodynamic effects, and reversibility of plexiform and other occlusive lesions in pulmonary arterial hypertension.[29]

ENDOTHELIN

Endothelin-1 (ET1, EDN1) is a 21 amino acid peptide, expressed primarily in endothelial cells by. It acts as a potent vasoconstrictor, through endothelin A (ET-A) and endothelin B (ET-B). Bosentan, a current treatment for PAH, is an endothelin receptor blocker. Knockout mice for both receptor ETA and ETB have been die of respiratory failure at birth, with additional craniofacial developmental abnormalities.[30] Although, overexpression of ET1 in transgenic mice does leads to hypertrophy, increased oxidant stress,[31] and vascular inflammation.[32] However, both overexpression and deletion still have important role in

regulating development, angiogenesis and inflammation which further improves vascular tone.[33, 34]

BLEOMYCIN INDUCED LUNG FIBROSIS

Bleomycin, is an antibiotic obtained from Streptomyces verticillus. Bleomycin has been used as an animal model of pulmonary hypertension through inhibition of ROS generation and RhoA/Rho kinase activation.[35, 36] A single dose of bleomycin (3.75 U/kg) was given to wistar rat intratracheally to induce lung injury, increases pulmonary arterial pressure and pulmonary vascular resistance. BH4 (20mg/kg) or vehicle (control) was administered orally once a day, during 21 days. The last day of the treatment period, jugular vein was cannulated to reach the pulmonary artery through the right ventricle. Rats were sacrificed and plasma, lungs and heart were removed. Plasmatic BH4 concentration was measured by high performance liquid chromatography. RV/LV + S ratio was calculated to provide an index of right ventricular hypertrophy, by dissecting the right ventricular (RV) wall of the heart and weighed along with the left ventricle wall plus septum (LV + S). The wall thickness was calculated by the external perimeter. TGF-β1 and ET-1 gene and protein expression were measured in lung homogenates as pulmonary vascular remodeling markers by real time-PCR and western blotting. Bleomycin reduced ~2.3-fold the BH4 plasmatic levels, augmented the pulmonary hypertension and RV/LV + S ratio 1,7-fold and 1,3-fold respectively and increased the ET-1 and TGF-β1 gene expression to ~2-fold and ~6-fold versus control respectively. Oral BH4 suppressed pulmonary hypertension and right ventricular hypertrophy. BH4 supplementation improved wall thickness and reduced the ET-1 and TGF-β1 gene and protein expression to control levels.[37]

ANGIOPOIETIN 1 OVEREXPRESSION MODEL

Angiopoietin 1 is a protein which play important role in angiogenesis. Ang-1 is critical for vessel maturation, adhesion, migration, and survival.[38, 39] It has been documented that over-expression of Ang-1 protein developed PH, including hypertrophy and hyperplasia of the arteries. The right ventricular systolic pressure (RVSP) in high levels Ang-1 rats increased i.e. from 27 mmHg to 48 mmHg. Du et al. found there was an upregulation of expression of angiopoietin-1 in the lungs of PH patients and phosphorylation of TIE2, its endothelial-specific receptor. They concluded that disease severity is directly correlated with the Ang-1 expression.[40] Furthermore, Kugathasan et al. noted that transfer of Ang-1 gene improved PAH.[41] A rat model of PH has been developed by injecting 2·1010 genomic particles of

adeno-associated virus-angiopoietin-1 (AAV-Ang-1) into the RV outflow tract of anaesthetized, 12-week old Fischer rats.[42] The animals are sacrificed 1–2 months after infection. Survival was decreases in rat having high levels of angiopoietin 1 as compared to control rats after injection (i.e. 5.2 months v/s 12 months).[41]

PULMONARY HYPERTENSION IN ATHYMIC RATS

Athymic nude rnu/rnu rats are T-cell lacking rats that exhibit less inflammation and have only a mild elevation in pulmonary artery pressure. These animals was not required to develop severe pulmonary hypertension but developed significantly worse pulmonary vascular disease to the extent that exposing them to hypoxic conditions. Inflammation was worse in these athymic animals; this inflammation is mainly consisting of macrophages, B cells, and evidence of anti- endothelial antibodies.[43] It has been known for decades that severe PAH is also occurs in a relatively high incidence in virally-infected individuals such as in patients with HIV/AIDS.[44] In these clinical PAH conditions and in the Su5416/hypoxia model of pulmonary hypertension, pulmonary inflammation is particularly prominent. Subsequently, it had been investigated with the help of Su5416 model whether this inflammation was directly contributing to pulmonary hypertension pathogenesis. In this model inbred euthymic (T-cell replete) and athymic rats were used for checking the anti-inflammmatory Treg activity in athymic rat and it has been found that T cell deficient rats develop inflammation in response to Su5416 due to the lack of anti- inflammatory treg activity and pulmonary hypertension occurs in T-cell-deficient and and not T- cell replete rats.[45] It was found that animals lacking T-cells would actually exhibit less inflammation and therefore have only a mild elevation in pulmonary artery pressures. Anti- inflammatory activity was most strongly exhibited in CD4+T-cells. Immune reconstitution of athymic rats with CD4+CD25^{hi} T-cells that were also strongly forkhead box P3 (FOXP3) positive (i.e.,"classic" Tregs), effectively attenuated the development of pulmonary hypertension. CD4+CD25-cells injected into athymic rats also excerpted regulatory activity in preventing disease. The presence of Tregs in animals being treated with Su5416 is associated with the limitation of peri-arteriolar inflammation and endothelial apoptosis, as well as the upregulation of vascular bone morphogenetic protein receptor-2 (BMPR2). The clinical significance of these findings is that it provided an explanation of why patients with autoimmune diseases or viral infections, who also have abnormal Treg activity and immune dysregulation, are susceptible to producing PAH following vascular injury.[46]

SEVERE PULMONARY HYPERTENSION IN THE SU5416/OVALBUMIN MODEL

Daley et al.[47] demonstrated that repeated immunization of mice with ovalbumin causes a striking muscularization of pulmonary arterioles which is T-cell-dependent. However the mice do not develop an elevation in pulmonary artery pressures, right ventricular hypertrophy, or endothelial cell-driven lumen obliteration. OVA immunization has been shown to increase the lung levels of HIF-1α protein similar to hypoxia.[48] Mice treated with Su5416 and immunized with OVA did not develop pulmonary hypertension (unpublished data). It has been hypothesized that a combination of OVA immunization with VEGFR blockade-induced lung cell apoptosis would produce severe angio-obliterative pulmonary hypertension. As noted above, the issues and problems of mouse models of pulmonary hypertension have been recently reviewed.[49] However when wild type Sprague-Dawley rats were subjected to combine Su5416/OVA treatment, severe pulmonary hypertension developed, and 20% of the animals died after eight weeks from right ventricular failure.[50]. The lung tissue expressed high levels of IL-6, especially in the endothelial cells of the pulmonary arteriolar lesions. Unexpectedly, depletion of B cells using an anti-rat CD20 antibody prevented increase in pulmonary artery pressure, indicating that B cells are involved in the development of pulmonary hypertension in this model.

SEVERE PULMONARY HYPERTENSION IN A MODEL OF SU5416 COMBINED WITH LUNG TISSUE OVEREXPRESSION OF TGF-B

When the (adenovirus) AdTGF-β1 model of lung fibrosis and pulmonary hypertension was combined with a single injection of the VEGFR inhibitor Su5416, the results were ruination of small pulmonary arteries with von Willebrand Factor+cells, a further reduction of lung capillarization, and a much more severe increase in pulmonary artery pressure.[51,52] However, elevated pulmonary artery pressures and changes of lung vascularization have been show in human idiopathic pulmonary fibrosis; additionally moderate pulmonary hypertension associated with endothelial cell apoptosis, vascular rarefaction, and increased pulmonary artery muscularization is present in AdTGF-β1 related experimental lung fibrosis.[53] Even though anti-angiogenic tyrosine kinase inhibitors are currently investigated as a potential therapy for idiopathic pulmonary fibrosis, the combination of lung tissue over expression of active TGF-β1 and Su5416 did not prevent the development of lung fibrosis, but preferably increased the fibrotic activity and pulmonary angioproliferation found in this AdTGF-β1/Su5416 model. The degree of pressure overload is usually mild in pulmonary hypertension patient with idiopathic pulmonary fibrosis, and angioproliferative lesions are

rarely seen in these patients. The AdTGF-β1/Su5416 may be used to model the disease of this subdivision of severe pulmonary hypertension and PH with idiopathic pulmonary fibrosis.

REN2 MODEL OF PAH

This model of PAH was developed in the Ren2 which is a derivative of the Sprague-Dawley (SD) rat. This type of rats expresses the mouse renin gene in renal and extra renal sites resulting in increased synthesis of Ang II via the local RAS in tissues, that result in PAH in the Ren2 due in part to oxidative stress. It was reported that lung expresses mouse renin and other RAS components in 8 to 9 week old male Ren2 rats. It also showed that there is increase in intrapulmonary NADPH oxidase activity, superoxide, RVSP, and resistance of the pulmonary arterioles due to increase in the synthesis of Ang II.[54] It is well documented that Ang II stimulates NADPH oxidase-generated ROS in the vasculature.[55,56], via protein kinase C[59] and more prolonged stimulation via growth factors transactivation.[57,58] Further, it causes redox- sensitive XO activation, eNOS uncoupling in vascular tissue and increases in superoxide levels.[59, 60] The superoxide dismutase/catalase mimetic, tempol, reverses PAH and pulmonary vascular remodeling. Lastly, it showed that PAH developed prior to the onset of LV dysfunction and was not due to hypoxemia.[61] These studies supported the concept that activation of renin- angiotensin system (RAS) may be responsible for PAH as a result of oxidative stress induced by NADPH oxidase. With the help of this concept, other laboratories demonstrated the potential efficacy of gene therapy targeting the RAS for treatment of PAH.[62,63] It is likely that therapies specifically targeting the RAS will reduce the oxidative stress in the pulmonary vasculature system, as well as in the RV.[54, 61]

Table 1.

Experimental model	Pathological similarity to PAH
Monocrotaline (MCT)	Pulmonary vascular remodeling and elevated PAP, right ventricular hypertrophy, vascular lesions
Chronic hypoxia (CH)	Pulmonary artery muscularization, medial and adventitial thickening
Monocrotaline and pneumonectomy	Pathological changes same as MCT plus, Medial hypertrophy accompanied by neointimal lesions.
Chronic hypoxia + sugen 5416	As for CH plus severe pulmonary arteriopathy, including concentric neointimal and complex plexiform-like lesions
Endothelin B (ETB) receptor deficiency	Exacerbate CH induced PH when ETB deficient rats exposing to CH.With MCT it produces PH with Neointimal lesions similar to those observed in humans and mean PAP increases
Bleomycin induced lung fibrosis	Right ventricular hypertrophy, vascular remodelling and arterial wall thickness
Angiopoietin 1 overexpression model	complex plexiform lesions resulting from intimal hyperplasia which leads to luminal occlusion and arteriolar pruning, including medial

	hypertrophy and muscular hyperplasia of the arteries
Pulmonary hypertension in athymic rats	Pulmonary inflammation
Su5416/ovalbumin model	Pulmonary artery muscularization, right ventricular failure, pulmonary arteriolar lesions, pulmonary vascular remodeling and an increase in pulmonary artery pressure
Su5416 combined with lung tissue overexpression of tgf- β	obliteration of small pulmonary arteries reduction of lung capillarization, severe increase in pulmonary artery pressure
Ren2 model of PAH	Increase in right ventricular systolic pressure, and medial layer thickening of pulmonary resistance arterioles

CONCLUSION

It has been concluded that animal models are employed to facilitate a better understanding of the pathophysiology of different form of pulmonary arterial hypertension. In the present review it is more apparent that animal models have provided, and will continue to provide the several mechanisms involved in the structural and functional abnormalities, that contribute to the development of pulmonary hypertensive disease process. On the other hand, it is also expected that a preclinical model of human PAH will develop which reproduces complete spectrum of changes observed in PH patient. Use of these several types of animal models will aided to continue to test new assumption about pathogenesis and also assess the ability of newly developed agents to clinically prevent the reverse established disease. It has been concluded that animal model of pulmonary hypertension shares many features which are universal to human pulmonary hypertension. However, these models are not reproduces the full spectrum of changes observed in PH patients, but they are excellent tools to study pulmonary hypertension. This review has also focused on the new models which employing recent advances in pathophysiology of pulmonary hypertension. Hopefully, current investigations with these developing models can be used to better discriminate particular mechanisms of disease which are usually applicable to different form of clinical PAH.

REFERENCES

1. Montani D, Gunther S, Dorfmüller P, Perros F, Girerd B, Garcia G, Jaïs X, Savale L, Artaud- Macari E, Price LC, Humbert M, Simonneau G and Sitbon O, et al. Pulmonary arterial hypertension. Orphanet J Rare Dis, 2013; 8: 97.
2. Archer SL, Weir EK, Wilkins MR et al. Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. Circulation, 2010; 121: 2045–66.
3. Ryan J, Bloch K, Archer SL et al. Rodent models of pulmonary hypertension: harmonisation with the world health organisation's categorisation of human PH. Int J Clin

Pract suppl, 2011; 172: 15–34.

4. Lewis GD, Shah R, Shahzad K et al. Sildenafil improves exercise capacity and quality of life in patients with systolic heart failure and secondary pulmonary hypertension. Circulation, 2007; 116: 1555–62.
5. Sweiss NJ, Hushaw L, Thenappan T et al. Diagnosis and management of pulmonary hypertension in systemic sclerosis. Curr Rheumatol Rep, 2010; 12: 8–18.
6. Gomez-Arroyo JG , Farkas L, Alhussaini A A, Farkas D, Kraskauskas D, Voelkel NF, and Bogaard HJ et al. The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol, 2012; 302: L363–L369.
7. Das M, Fessel J, Tang H, West J et al. A process-based review of mouse models of pulmonary hypertension. Pulm Circ, 2012 Oct; 2(4): 415-33.
8. Tamosiuniene R, Tian W, Dhillon G, Wang L, Sung, YK, et al. Regulatory T cells limit vascular endothelial injury and prevent pulmonary hypertension. Circ Res, 2011; 109: 867–879.
9. Bras G, Jelliffe DB, Stuart KL et al. Veno-occlusive disease of liver with nonportal type of cirrhosis, occurring in Jamaica. AMA Arch Pathol 1954; 57: 285–300.
10. Meyrick B, Gamble W, Reid L et al. Development of Crotalaria pulmonary hypertension: Hemodynamic and structural study. Am J Physiol, 1980; 239: H692-702.
11. Guarnieri C, Muscari C. Beneficial effects of trimetazidine on mitochondrial function and superoxide production in the cardiac muscle of monocrotaline-treated rats. Biochem Pharmacol, 1988; 37: 4685-8.
12. Piao L, Fang YH, Cadete VJ, Wietholt C, Urboniene D, Toth PT, et al. The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: Resuscitating the hibernating right ventricle. J Mol Med (Berl), 2010; 88: 47-60.
13. Piao L, Marsboom G, Archer SL et al. Mitochondrial metabolic adaptation in right ventricular hypertrophy and failure. J Mol Med (Berl), 2010; 88: 1011-20.
14. Mingatto FE, Maioli MA, Bracht A, Ishii-Iwamoto EL et al. Effects of monocrotaline on energy metabolism in the rat liver. Toxicol Lett, 2008; 182: 115-20.
15. Ezzat T, van den Broek MA, Davies N, Dejong CH, Bast A, Malago M, et al. The flavonoid monomer prevents monocrotaline-induced hepatic sinusoidal injury in rats. J Surg Oncol, 2012; 106: 72-8.
16. Li XL, Guan RJ, Li JJ et al. Attenuation of monocrotaline-induced pulmonary arterial hypertension in rats by rosuvastatin. J Cardiovasc Pharmacol, 2012; 60: 219-26.

17. Han DD, Wang Y, Zhang XH, Liu JR, Wang HL et al. Fluoxetine protects against monocrotaline-induced pulmonary arterial remodeling by inhibition of hypoxia-inducible factor- 1alpha and vascular endothelial growth factor. Can J Physiol Pharmacol, 2012; 90: 445-54.
18. Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskauskas D, Voelkel NF, et al. The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol, 2012; 302: L363-9.
19. Zuckerbraun BS, Chin BY, Wegiel B, Billiar TR, Czsimadia E, Rao J, et al. Carbon monoxide reverses established pulmonary hypertension. J Exp Med, 2006; 203: 2109-19.
20. Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF et al. 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.
21. Taraseviciene-Stewart L, Kasahara Y et al. 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(2): 427-38.
22. Strobl M, Schreiber C, Panzenbock A, Winter MP, Bergmeister H, Jakowitsch J, Mascherbauer J, Lang IM, Wexberg P, Bonderman D et al. Exhaled nitric oxide measurement to monitor pulmonary hypertension in a pneumonectomy- monocrotaline rat model. Am J Physiol Lung Cell Mol Physiol, 2013; 305(7): L485-90.
23. Tanaka Y, Schuster DP, Davis EC, Patterson GA, Botney MD et al. The role of vascular injury and hemodynamics in rat pulmonary artery remodeling. J Clin Invest, 1996; 98: 434–442.
24. Okada K, Tanaka Y, Bernstein M, Zhang W, Patterson GA, Botney MD, et al. Pulmonary hemodynamics modify the rat pulmonary artery response to injury. A neointimal model of pulmonary hypertension. Am J Pathol, 1997; 151: 1019–1025.
25. Nishimura T, Faul JL, Berry GJ, Vaszar LT, Qiu D, Pearl RG, Kao PN, et al. Simvastatin attenuates smooth muscle neointimal proliferation and pulmonary hypertension in rats. Am J Respir Crit Care Med, 2002; 166: 1403–1408.
26. Nishimura T, Vaszar LT, Faul JL, Zhao G, Berry GJ, Shi L, Qiu D, Benson G, Pearl RG, Kao PN, et al. Simvastatin rescues rats from fatal pulmonary hypertension by inducing apoptosis of neointimal smooth muscle cells Circulation, 2003; 108: 1640–1645.
27. Kurt R. Stenmark, Barbara Meyrick, Nazzareno Galie, Wolter J. Mooi, and Ivan F. McMurtry et al. Animal models of pulmonary arterial hypertension: the hope for etiological. Am J Physiol Lung Cell Mol Physiol, 2009; 297: L1013– L1032

28. Abe K, Toba M, Alzoubi A. Formation of plexiform lesions in experimental severe pulmonary arterial hypertension. Circulation, 2010; 121: 2747–54.
29. Oka M, Homma N, Taraseviciene-Stewart L et al. Rho kinase-mediated vasoconstriction is important in severe occlusive pulmonary arterial hypertension in rats Circ Res 2007; 100: 923–9.
30. Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, et al. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature 1994; 368: 703-10.
31. Amiri F, Virdis A, Neves MF, Iglarz M, Seidah NG, Touyz RM, et al. Endothelium-restricted overexpression of human endothelin-1 causes vascular remodeling and endothelial dysfunction. Circulation, 2004; 110: 2233-40.
32. Amiri F, Paradis P, Reudelhuber TL, Schiffrin EL. Vascular inflammation in absence of blood pressure elevation in transgenic murine model overexpressing endothelin-1 in endothelial cells. J Hypertens, 2008; 26: 1102-9.
33. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, et al.
34. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. Development, 1998; 125: 813-24.
35. Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, et al. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. Cell, 1994; 79: 1267-76.
36. Chua F, Gauldie J, Laurent GJ et al. Pulmonary fibrosis: searching for model answers. Am J Respir Cell Mol Biol, 2005; 33: 9–13.
37. Champion HC, Bivalacqua TJ, D'Souza FM, Ortiz LA, Jeter JR, Toyoda K, Heistad DD, Hyman AL, Kadowitz PJ, et al. Gene transfer of endothelial nitric oxide synthase to the lung of the mouse in vivo. Effect on agonist-induced and flow-mediated vascular responses. Circ Res, 1999; 84: 1422–1432.
38. Gilbert Scott F. Developmental Biology (Loose Leaf). Sinauer Associates Incorporated. ISBN, 2010; 978-0-87893-558-1.
39. Fagiani E, Christofori G. Angiopoietins in angiogenesis. CancerLett 2013; 328(1): 18–26. Doi:10. 1016/j.canlet.2012.08.018. PMID 22922303.
40. Du L, Sullivan CC, Chu D et al. Signaling molecules in nonfamilial pulmonary hypertension. N Engl J Med, 2003; 348: 500–9.
41. Kugathasan L, Dutly AE, Zhao YD et al. Role of angiopoietin-1 in experimental and

human pulmonary arterial hypertension. Chest, 2005; 128: 633S–42S.

42. Chu D, Sullivan CC, Du L. A new animal model for pulmonary hypertension based on the overexpression of a single gene, angiopoietin-1. Ann Thorac Surg, 2004; 77: 449–56.
43. Taraseviciene-Stewart L, Nicolls MR, Kraskauskas D, Scerbavicius R, Burns N, Cool C, et al. Absence of T Cells Confers Increased Pulmonary Arterial Hypertension and Vascular Remodeling. Am J Respir Crit Care Med, 2007; 175: 1280-9.
44. Nicolls MR, Taraseviciene-Stewart L, Rai PR, Badesch DB, Voelkel NF et al. Autoimmunity and pulmonary hypertension: A perspective. Eur Respir J, 2005; 26: 1110-8.
45. Tamosiuniene R, Tian W, Dhillon G, Wang L, Sung YK, Gera L, et al. Regulatory T Cells Limit Vascular Endothelial Injury and Prevent Pulmonary Hypertension. Circ Res, 2011; 109: 867-79.
46. Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskauskas D, Voelkel NF, and Bogaard HJ et al, The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol, 2012; 302: L363-9.
47. Tamosiuniene R, Nicolls MR, et al. Regulatory T cells and pulmonary hypertension. Trends Cardiovasc Med, 2011; 21: 166-71.
48. Daley E, Emson C, Guignabert C, de Waal Malefyt R, Louten J, Kurup VP, et al. Pulmonary arterial remodeling induced by a Th2 immune response. J Exp Med, 2008; 205: 361-72.
49. Lee KS, Kim SR, Park HS, Park SJ, Min KH, Lee KY, et al. A novel thiol compound, N-acetylcysteine amide, attenuates allergic airway disease by regulating activation of NF-kappa B and hypoxia-inducible factor-1 alpha. Exp Mol Med, 2007; 39: 756-68.
50. Gomez-Arroyo J, Saleem SJ, Mizuno S, Syed AA, Bogaard HJ, Abbate A, et al. A Brief Overview of Mouse Models of Pulmonary Arterial Hypertension: Problems and Prospects. Am J Physiol Lung Cell Mol Physiol, 2012; 302: L977-91.
51. Mizuno S, Farkas L, Alhussaini A, Farkas D, Gomez-Arroyo J, Kraskauskas D, et al. Severe Pulmonary arterial hypertension induced by SU5416 and ovalbumin immunization. Am J Respir Cell Mol Biol, 2012; 47(5): 679-87.
52. Farkas L, Farkas D, Ask K, Möller A, Gauldie J, Margetts P, et al. VEGF ameliorates pulmonary hypertension through inhibition of endothelial apoptosis in experimental lung fibrosis in rats. J Clin Invest, 2009; 119: 1298-311.
53. Farkas L, Farkas D, Kraskauskas D, Gauldie J, Kolb M, Voelkel N, et al. Caspase inhibition reduces severe pulmonary hypertension in the AdTGF-1β/SU5416 model of

angioproliferative pulmonary hypertension and lung fibrosis. Eur Respir J, 2011; 38: 594s.

54. Farkas L. Vegf receptor inhibition in experimental lung fibrosis results in severe angioproliferative pulmonary hypertension and increased fibrogenic activity. Am J Respir Crit Care Med, 2010; 181: A6315.
55. DeMarco VG, Habibi J, Whaley-Connell AT, Schneider RI, Heller RL, Bosanquet JP, Hayden MR, Delcour K, Cooper SA, Andresen BT, Sowers JR, Dellsperger KC, et al. Oxidative stress contributes to pulmonary hypertension in the transgenic (mRen2)27 rat. Am J Physiol Heart Circ Physiol, 2008; 294: H2659-H2668.
56. Griendling KK, Sorescu D, Lassègue B, Ushio-Fukai M. Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. Arterioscler Thromb Vasc Biol, 2000; 20: 2175-2183.
57. Hanna IR, Taniyama Y, Szöcs K, Rocic P, Griendling KK. NAD(P)H oxidase-derived reactive oxygen species as mediators of angiotensin II signaling. Antioxid Redox Signal, 2002; 4: 899-914.
58. Wei Y, Whaley-Connell AT, Chen K, Habibi J, Uptergrove GM, Clark SE, Stump CS, Ferrario CM, Sowers JR, et al. NADPH oxidase contributes to vascular inflammation, insulin resistance, and remodeling in the transgenic (mRen2) rat. Hypertension, 2007; 50: 384-391.
59. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res, 1994; 74: 1141-1148.
60. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, Griendling KK, et al. Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. Circ Res, 2002; 91: 406-413.
61. Ushio-Fukai M, Griendling KK, Becker PL, Hilenski L, Halleran S, Alexander RW, et al.
62. Epidermal growth factor receptor transactivation by angiotensin II requires reactive oxygen species in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol, 2001; 21: 489-495.
63. Griendling KK, Ushio-Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. Regul Pept, 2000; 91: 21-27.

64. Landmesser U, Spiekermann S, Preuss C, Sorrentino S, Fischer D, Manes C, Mueller M, Drexler H, et al. Angiotensin II induces endothelial xanthine oxidase activation: role for endothelial dysfunction in patients with coronary disease. Arterioscler Thromb Vasc Biol 2007; 27: 943-948.c
65. DeMarco VG, Habibi J, Whaley-Connell AT, Schneider RI, Sowers JR, Andresen BT, Gutweiler AA, Ma L, Johnson MS, Ferrario CM, Dellsperger KC, et al. Rosuvastatin ameliorates the development of pulmonary arterial hypertension in the transgenic (mRen2)27 rat. Am J Physiol Heart Circ Physiol 2009; 297: H1128-H1139.