

A brief overview of mouse models of pulmonary arterial hypertension: problems and prospects

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Gomez-Arroyo J, Saleem SJ, Mizuno S, Syed AA, Bogaard HJ, Abbate A, Taraseviciene-Stewart L, Sung Y, Kraskauskas D, Farkas D, Conrad DH, Nicolls MR, Voelkel NF. A brief overview of mouse models of pulmonary arterial hypertension: problems and prospects. *Am J Physiol Lung Cell Mol Physiol* 302: L977–L991, 2012. First published February 3, 2012; doi:10.1152/ajplung.00362.2011.—Many chronic pulmonary diseases are associated with pulmonary hypertension (PH) and pulmonary vascular remodeling, which is a term that continues to be used to describe a wide spectrum of vascular abnormalities. Pulmonary vascular structural changes frequently increase pulmonary vascular resistance, causing PH and right heart failure. Although rat models had been standard models of PH research, in more recent years the availability of genetically engineered mice has made this species attractive for many investigators. Here we review a large amount of data derived from experimental PH reports published since 1996. These studies using wild-type and genetically designed mice illustrate the challenges and opportunities provided by these models. Hemodynamic measurements are difficult to obtain in mice, and right heart failure has not been investigated in mice. Anatomical, cellular, and genetic differences distinguish mice and rats, and pharmacogenomics may explain the degree of PH and the particular mode of pulmonary vascular adaptation and also the response of the right ventricle.

right ventricle failure; transgenic; vascular remodeling; Sugen; SU5416

“The mice, which helplessly find themselves between the cat’s teeth, acquire no merit from their enforced sacrifice.”

—Mahatma Gandhi (1869–1948)

THE DISCOVERY OF HYPOXIC PULMONARY vasoconstriction by von Euler and Liljestrand (24) brought attention to the “lesser circulation” and triggered the first series of systematic studies of the lung circulation and of pulmonary hypertension in both animals and normal people, at sea level and at high altitude (37, 38, 105, 119, 121). Later on, pulmonary hypertension (PH) was described in patients with a wide variety of diseases, ranging from chronic lung diseases (chronic obstructive pulmonary disease, collagen vascular diseases), infections (human immunodeficiency virus/acquired immunodeficiency syndrome, schistosomiasis) and heart diseases (congenital and acquired), to idiopathic and hereditary forms of PH. For recent reviews of the classification of PH and diseases associated with PH, see Refs. 3, 22, 26, 62, 87, 88. The epidemic of severe PH in three European countries associated with the intake of the amphetamine-related anorexigen aminorex fumarate generated an

even greater interest in the pathogenesis of human PH diseases (2). This epidemic prompted the first World Health Organization meeting on PH and promoted intense research activities aimed to build animal models of drug-induced PH (65). Early studies conducted at the Cardiovascular Pulmonary Research Laboratory in Denver, Colorado, described species-specific differences in the response of the lung circulation to chronic hypoxia and presented the concept of “responders and nonresponders.” For example: cattle develop very severe PH when exposed to chronic hypoxia whereas sheep do not (38). Conversely, commercially bred laboratory rats, when not afflicted by bacterial or viral infections (36), develop moderate PH associated with significant pulmonary vascular remodeling (mainly media muscularization) and right ventricular hypertrophy (RVH) when exposed to chronic hypoxia (41, 45, 114). As a consequence, the species *rattus*, and several strains of this species (23, 41, 48, 49, 77), became the preferred animal for mechanistic studies of PH and preclinical drug trials (78, 84, 93, 100, 131, 132) for more than 50 years (95).

The advent and development of genetically modified mice, as tools to dissect cellular and molecular signaling pathways, created an understandable desire to generate mouse models for PH studies. Possibly the first experiments reporting the use of transgenic mice for PH research were conducted in 5-lipoxy-

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genase (5-LO) knockout (KO) mice (5-LO^{-/-}) exposed to chronic hypoxia (112). The 5-LO KO mice were introduced into the study protocol to test whether arachidonic acid metabolites, via the 5-lipoxygenase pathway, contributed to the development of chronic hypoxia-induced PH, and supported the pharmacological experimental use of the 5-LO inhibitor A866. These early studies demonstrated two main points: 1) the degree of hypoxia-induced PH and pulmonary vascular remodeling was significantly less in mice compared with rats; and 2) the relatively small degree of PH (compared with rats) and the extent of lung vessel structure changes were even less prominent in the mice lacking 5-LO, making a case for inflammation in the pathogenesis of chronic hypoxia-induced PH (112). The observed robust differences in the response to chronic hypoxia between mice and rats motivated a comparative mouse/rat study, evaluating the gene expression changes induced in the lung tissues following exposure to an identical degree and duration of chronic hypoxia (45). The microarray results showed a categorically different gene expression pattern between mouse and rat lung tissues. The expression of a number of genes was increased in the rat lungs but decreased in the mouse lungs (45). Chronic hypoxia finds the mouse lung circulation relatively unresponsive.

In recent years a considerable number of transgenic mice have been generated to investigate mechanisms of pulmonary hypertension (Table 1). In particular, the report of associations between mutations in the bone morphogenetic protein receptor 2 (*BMPR2*) gene with both familiar and idiopathic forms of pulmonary arterial hypertension (PAH) (46, 59, 70, 97) led to multiple attempts to generate novel animal models of PAH. In addition to *BMPR2* KO mice, researchers have targeted other genes suspected to be involved in the pathogenesis of PAH (Table 1). Indeed, the transgenic mouse studies have strongly influenced the way we think of the pathogenesis of PAH and helped shift the early mechanical concepts of pressure (and flow) dependency toward disease models built on cell-to-cell interactions, immune dysregulation, metabolic changes, and abnormal cell phenotypes (19, 67, 75, 76, 101, 115, 123).

Here we review many of the published studies of mouse models of PH and provide an overview of the hemodynamic- and tissue analysis-derived data for normal and hypoxia-challenged wild-type (WT) and transgenic mice. In addition, we report our own data derived from experiments designed to generate a mouse model of predictable and severe PH, guided by our own experience with the rat model of SU5416-induced VEGF receptor blockade and chronic hypoxia (100). Our experimental data obtained with different mouse strains and genetically engineered mice illustrate both the problems and opportunities associated with mouse models of chronic PH. Here, we evaluate the published data, identify several potential problems related to data acquisition and interpretation, and point out gaps that might explain divergent results. With this review, we also intend to contrast mouse models of PH with informative data derived from rat PH models, and we try to put the murine models in a perspective with the clinical and translational reality of severe human PAH.

Overview of Mouse Models of Pulmonary Hypertension

In the early studies of PH in mice, the animals were challenged with chronic hypoxia because monocrotaline had been

found to be ineffective. This circumstance had been explained by the inability of mice to metabolize monocrotaline to its active metabolite (dehydromonocrotaline), which requires a CYP3A isoenzyme lacking in the mouse liver (35, 50, 120). Although the degree of hypoxia-induced PH developed in mice is rather small (98), precise measurements of cardiac output and right ventricular and central pulmonary arterial elastance can be made (104, 108). In 2004, West et al. (116) tested the hypothesis that the loss of *BMPR2* signaling in smooth muscle cells was sufficient to cause PH in transgenic mice. The authors constructed a smooth muscle-specific transgenic mouse expressing a dominant-negative *BMPRII* variant, under the control of a tetracycline-gene switch. These mice were studied under anesthesia (heart rates 260 ± 40 bpm), and right ventricular systolic pressure (RVSP) was measured with a Millar transducer-tipped catheter. The RVSP ranged from 40 to 70 mmHg at Denver altitude. However, although the RVSP was increased, these transgenic mice mainly developed muscularization of the media but not severe intimal cell proliferation, which is the hallmark of human plexogenic PAH (106). In 2005, Song et al. (91) hypothesized that *BMPRII* haploinsufficiency required an additional hit to trigger vascular remodeling: in this particular case, an inflammatory hit (“two-hit hypothesis”). By means of an adenoviral vector, Song et al. overexpressed 5-lipoxygenase in the lungs of heterozygous mutant *BMPR*^{+/-} mice and demonstrated a small increase in RVSP with modest pulmonary vascular changes. In contrast to the findings reported by West et al., Song’s study reported that mice heterozygous for a *BMPR2*-null allele, which have a genetic defect similar to that of some patients with familiar and idiopathic PAH, do not develop PH spontaneously (91).

It was not until 2008 that another group of researchers reported a *BMPR2*-KO mouse that developed spontaneous PH with evidence of vascular obliteration. Hong et al. (42) conditionally ablated the *BMPRII* gene specifically in pulmonary vascular endothelial cells, utilizing a novel endothelial *Alk1-Cre* transgenic mouse. This report was relevant given the fact that complete deletion of the *BMPR2* gene had not been reported before, because *BMPR2*-null mice are embryonically lethal (*BMPR2* is required for septation of the outflow tract of the mammalian heart and embryonic gastrulation) (8, 21). Hong et al. demonstrated that complete loss of *BMPR2* in endothelial cells was sufficient to generate pulmonary vascular remodeling and spontaneous PH. However, there were two important findings: 1) There was a high variability within the transgenic mice (RVSP ranged from 20 to 56 mmHg), with only a third of the animals developing PH. 2) Even after organizing the mice into groups with and without PH, PH mice developed only a modest degree of right ventricular (RV) hypertrophy, even when the RVSP was elevated (Table 1). Although it has been reported that human plexiform lesions are characterized by a significant decrease in the expression of *BMPR2* (in idiopathic, familiar, and some associated PAH forms) (4), Hong et al.’s study (42) exemplifies how, even after complete ablation, *BMPR2* dysfunction is neither sufficient nor required for the development of complex vascular lesions or for the development of right ventricular failure (RVF).

The large variability in the RVSP and pulmonary vascular remodeling in *BMPR2*-KO mouse models complicates the evaluation of preclinical drug trials. To our knowledge there are only two studies that attempted an interventional preclinical

Table 1. Pulmonary hypertension and pulmonary vasculature remodeling in mouse models published from 1996 to 2011

Mouse Strain	Genetic Modification	RVSP, mmHg		RV/LV+S		Histology		Author
		Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	
C57BL6/J	Wild type/Normal	13	18	N/A	N/A	N/A	↑ musc.	Vanderpool et al. (108)
C57BL6/J	Wild type/Normal	10–20	14–26	0.24	0.31	N/A	N/A	Tabima et al. (98)
C57BL6/J	5-LO KO	N/A	N/A	0.25	0.32	Normal	↓ musc.	Voelkel et al. (112)
C57BL6/J	PGI ₂ synthase OE	30	30	N/A	N/A	Normal	↓ musc.	Geraci et al. (33)
C57BL6/J	HO-1 KO	12	19	N/A	N/A	N/A	modest musc.	Yu et al. (129)
FVB/N	SMC BMPR2 dom. neg	44	N/A	0.315	N/A	↑ musc.	N/A	West et al. (116)
C57BL6/J	BMPR2 [±]	12	N/A	N/A	N/A	N/A	N/A	Song et al. (91)
	BMPR2 [±] with Ad5LO OE	23	N/A	N/A	N/A	N/A	↑ musc.	
C57BL6/J	5-HTT OE	33	N/A	0.37	N/A	↑ musc.	N/A	Guignabert et al. (39)
C57BL6/J	VIP KO	29.5	N/A	0.34	N/A	↑ musc.	↑ musc.	Said et al. (80)
R26R	PVEC BMPR ^{-/-} KO	27	N/A	0.27	N/A	↑ musc.	N/A	Hong et al. (42)
						Occlusion		
C57BL6/J	SMC PPAR-γ ^{-/-} KO	29	N/A	0.45	N/A	↑ musc.	N/A	Hansmann et al. (40)
C57BL6/J	SMC BMPR2 [±]	24	N/A	N/A	N/A	↑ musc.	N/A	Song et al. (90)
	SMC BMPR2 [±] with MCT + Ad5LO	38	N/A	N/A	N/A	↑ musc.	N/A	
						Inflamm.		
C57BL/6	Eln ^{-/-}	80	N/A	↑ RV hyp.	N/A	↑ musc.	N/A	Shifren et al. (86)
FVB/N	SMC BMPR2 ^{R899X}	39	N/A	N/A	N/A	↑ musc.	N/A	West et al. (117)
C57BL/6	Wildtype with OVA exposure	25	32	0.20	N/A	↑↑↑ musc.	↑↑↑ musc.	Daley et al. (19)
C57BL6/J	TPH1 ^{-/-} KO with dexfenflur.	15	N/A	0.23	N/A	Normal	N/A	Dempsey et al. (20)
	HumanSERT ⁺ with dexfenflur	40	24	0.27	0.30	↑ musc.	↑ musc.	
	Wild type with dexfenflur	30	N/A	0.21	N/A	↑ musc.	N/A	
C57BL6/J	Notch ^{-/-} KO	20	20	0.20	0.22	N/A	↑ musc.	Li et al. (56)
C57BL6/J	IL-6 OE	35	63	0.36	0.69	N/A	↑ musc. Occlusion	Steiner et al. (94)
						Inflamm.		
C57BL6/J	SMC ID-1 ^{-/-} KO	20	28	0.258	0.36	Normal	↑ musc.	Lowery et al. (57)
C57BL6/J	Nogo ^{-/-}	15*	15*	0.16	0.23	N/A	↓ musc.	Sutendra et al. (96)
C57BL6/J	A _{2A} R ^{-/-}	39	45	0.26	N/A	↑ musc.	N/A	Xu et al. (122)
C57BL6/J	Wild type with imatinib	23	25	0.29	0.34		↓ musc.	Gambaryan et al. (31)
	N/A CTGF OE	22	N/A	0.42		↑ musc.	N/A	Chen et al. (15)
C57BL6/J	HumanMMP9 OE	29	N/A	N/A	N/A	N/A	N/A	George et al. (32)
	HumanMMP9 OE with MCT	90	N/A	N/A	N/A	↑ musc.	N/A	
						Inflamm		
C57BL6/J	RKIP ^{-/-}	25	35	0.28	0.35	N/A	N/A	Morecroft et al. (66)
FVB/N	SMC BMPR2 ^{R899X}	43	N/A	0.34	N/A	↑ musc.	N/A	Yasuda et al. (124)
	SMC BMPR2 ^{R899X} with fasudil	37	N/A	0.28	N/A	↓ musc.	N/A	
C57BL6/J	COX-2 ^{-/-} KO	13	N/A	N/A	N/A	N/A	N/A	Seta et al. (85)
	COX-2 ^{-/-} KO with MCT	17	N/A	N/A	N/A	Perivascular edema	N/A	
FVB	HumanHO-1 OE	20	21	0.21	0.22	Normal	↓ musc.	Vergadi et al. (109)
SV129	Apelin KO	25	33	N/A	N/A	N/A	↑ musc.	Chandra et al. (14)
C57BL6/J	Wild type/Normal	22	35	0.20	0.45	N/A	↑ musc.	Yu et al. (130)
C57BL6/J	P53 KO	18	35	0.21	0.35	Normal	↑ musc.	Mizuno et al. (64)
C57BL6/J	Wild type/Normal with SU5416	27	50	0.20	0.35	Normal	↑ musc. occlusion	Ciuclan et al. (16)
R26R	PVEC BMPR ^{-/-} KO	37	N/A	0.23	N/A	↑ musc.	N/A	Burton et al. (13)
	PVEC BMPR ^{-/-} KO with SCH527123	26	N/A	0.19	N/A	↓ musc.	N/A	
CD1-B6.Cg 5A _{mc} /J	Calpain-4 KO	18	21	0.19	0.20	Normal	↓ musc.	Ma et al. (58)
C57BL6	OX40L OE	30	N/A	0.23	N/A	↑ musc of large vessels	N/A	Rabieyousefi et al. (74)
C57BL6	<i>S. Mansoni</i> parasitic infection	31	N/A	0.25	N/A	↑ musc.	N/A	Crosby et al. (18)

RVSP, right ventricular systolic pressure; RV, right ventricle; LV, left ventricle; S, interventricular septum; N/A, not analyzed; KO, knockout; OE, overexpression; SMC, smooth muscle cell; PVEC, pulmonary vascular endothelial cell; 5-LO, 5-lipoxygenase; PGI₂, prostacyclin; HO, heme oxygenase; BMPR2, bone morphogenetic protein receptor type 2; Ad5LO, adenoviral vector overexpressing 5-lipoxygenase; 5-HTT, 5-hydroxytryptamine transporter; VIP, vasoactive intestinal peptide; PPAR, peroxisome proliferator-activated receptor; MCT, monocrotaline; Eln, elastin; OVA, ovalbumin; TPH1, tryptophan hydroxylase-1; dexfenflur, dexfenfluramine; SERT, human serotonin receptor; IL-6, interleukin 6; ID-1, inhibitors of differentiation; A_{2A}R, adenosine A2 receptor; CTGF, connective tissue growth factor; MMP, matrix metalloproteinase-9; RKIP, Raf-1 kinase inhibitor protein; COX-2, cyclooxygenase 2. *, mean pulmonary artery pressure.

trial in genetically engineered mice. Yasuda, West, and colleagues (124) treated mice expressing a dominant-negative BMPRII gene (with a termination mutation at amino acid 899) specific for smooth muscle cells (BM $PR2^{R899X}$), with fasudil, a Rho kinase inhibitor. These mice developed spontaneous PH (RVSP of 43 mmHg) with mild RV hypertrophy. Fourteen days of treatment with fasudil ameliorated PH. However, the authors could not show a decrease in Rho kinase activation nor modified Smad protein phosphorylation (BM $PR2$ downstream signaling proteins) in treated animals compared with vehicle controls. Furthermore, the drug was not administered after PH had been established, which somewhat diminished the translational potential. The second study by Burton and collaborators (13) utilized the same endothelial cell restricted BM $PR^{-/-}$ mice published by Hong, Oh, and colleagues (42). Mice were treated with the CXCR1/2 antagonist SCH527123, with the rationale that CXCR1-2-mediated leukocyte migration plays a role in the development of pulmonary hypertension. SCH527123 treatment resulted in decreased muscularization of pulmonary arteries, decreased RVSP, and increased (systemic) cardiac output (13). Interestingly, in contrast to what had been published originally by Hong et al., Burton and collaborators did not report pulmonary angioblation.

The role of inflammation and immune dysfunction in the pathogenesis of pulmonary hypertension has been increasingly discussed in recent years, but whether inflammation is a cause or consequence of pulmonary vascular remodeling continues to be debated (71, 101, 111). It has now been 15 years since the report that patients with idiopathic PAH have increased plasma levels of interleukins 1 β and 6 (47), and it has been recently reported that high levels of IL-6 predict mortality in idiopathic PAH patients (92). Other proinflammatory conditions associated with PAH are also characterized by increased levels of IL-6, such as systemic lupus erythematosus (128) and Castleman's disease (73). In 2009, Steiner et al. (94) generated a transgenic mouse using the Clara cell 10-kDa promoter to constitutively drive lung-specific expression of IL-6 (IL6-OE). They tested the hypothesis that IL-6 overexpression would be sufficient to generate pulmonary hypertension in mice. Under baseline conditions IL6-OE mice did not develop pulmonary hypertension. However, upon chronic hypoxia exposure, the IL6-OE mice developed severe pulmonary hypertension (average RVSP of 60 mmHg), neointimal proliferation, and robust RV hypertrophy. Furthermore, Steiner and collaborators demonstrated a significantly higher number of fully and partially occluded vessels compared with controls. The vascular lesions present in this transgenic model reproduced some molecular features of human plexiform lesions (76, 106), including increased expression of the angiogenic factors VEGF and PDGF, increased expression of prosurvival proteins such as survivin and Bcl-2, and increased phosphorylation of ERK. Most importantly, Steiner's study was the first to quantify the number of occluded vessels in a transgenic mouse model. Unfortunately, the authors did not evaluate whether this model of angioblation pulmonary hypertension was reversible upon return to normoxia. Reversibility is particularly important for the design of preclinical drug trials, since most patients with PAH with specific therapy do not experience a large drop in the pulmonary artery pressure (30) and the complex vascular lesions do not regress. Spontaneous regression of pulmonary hypertension becomes an impediment for PAH animal models

and should be investigated. For example, Ciucan et al. (16) reported a mouse model of PH utilizing a VEGF receptor blocker (SU5416) and chronic hypoxia in WT C57BL6 mice (Fig. 1A), based on the rat protocol originally described by Taraseviciene-Stewart in 2001 (100). This mouse model is particularly attractive because it does not require genetic manipulations. After three doses of SU5416 (as opposed to a single dose in the rat model) and 3 wk of exposure to 10% hypoxia, these mice develop PH (average RVSP 49 mmHg) with modest RV hypertrophy (RV/LV+S 0.35, where LV+S is left ventricle plus septum), at least, compared with the IL6-OE mouse model (RV/LV+S 0.69)(94) or the original SU5416/hypoxia rat model (RV/LV+S 0.77) (9, 11) (Fig. 1, B and D). The SU5416/hypoxia mouse model develops muscularization and, although not quantified, various degrees of concentric neointimal thickening. The main disadvantage of this model is that, upon return to normoxia, the vascular remodeling, pulmonary hypertension, and RV hypertrophy return to normal. In contrast, the relevance of the SU5416/hypoxia rat model is illustrated mainly by two aspects: 1) the high reproducibility of severe PAH is accompanied by angioblation lesions and RV failure (1, 100); and 2) akin to human disease, PAH in the SU5416/hypoxia rat model is progressive, nonreversible, fatal, and unresponsive to conventional PAH drug treatment (12).

As severe human pulmonary arterial hypertension is characterized by complex pulmonary vascular lesions, it is surprising that vascular obliteration has not been the focus of experimental studies. Surprisingly, relatively few of the reported studies have systematically quantified the pulmonary vascular changes, with only three (16, 42, 94) studies reporting vaso-occlusive changes and only one (94) measuring the percentage of fully and partially occluded lesions (Table 1).

As shown in Table 1, baseline RVSP measurements in unchallenged WT mice can vary from 10–20 mmHg up to 22 mmHg (98, 130), and up to 35 after chronic hypoxia exposure (16). From the 40 studies selected for this review, 30 of these reported PH, 10 of 30 studies reported an average RVSP higher than 30 mmHg, and only in 8 studies had mice been exposed to chronic hypoxia. Challenging the animals with chronic hypoxia can unmask pulmonary vascular hyperreactivity (57, 66, 122), trigger neointimal formation (16, 94, 100), or even render a paradoxical decrease in pressure. For example, Dempsey et al. (20) described that mice overexpressing a human variant of the serotonin transporter treated with the anorexigen dexfenfluramine developed PH (RVSP 40 mmHg). However, upon exposure to chronic hypoxia, the pulmonary artery pressure decreased resembling that of the unchallenged WT values (mean RVSP 24 mmHg).

There is one mouse model that perfectly exemplifies the complexity of vascular remodeling and PH. Daley and collaborators (19) described in 2008 a mouse model of PH induced by repeated antigen exposure, via a Th2 immune response. Wild-type C57BL6 mice were immunized with an intermittent exposure to intranasal *Aspergillus fumigatus* antigen. These mice developed severe thickening of the walls of small- and medium-caliber pulmonary arteries. Histologically, smooth muscle cells multilayered within the thickened wall of the remodeled pulmonary arteries, whereas von Willebrand factor-positive endothelial cells remained in a single-cell layer (Fig. 1, E and F). Strikingly, although pulmonary vascular remodeling

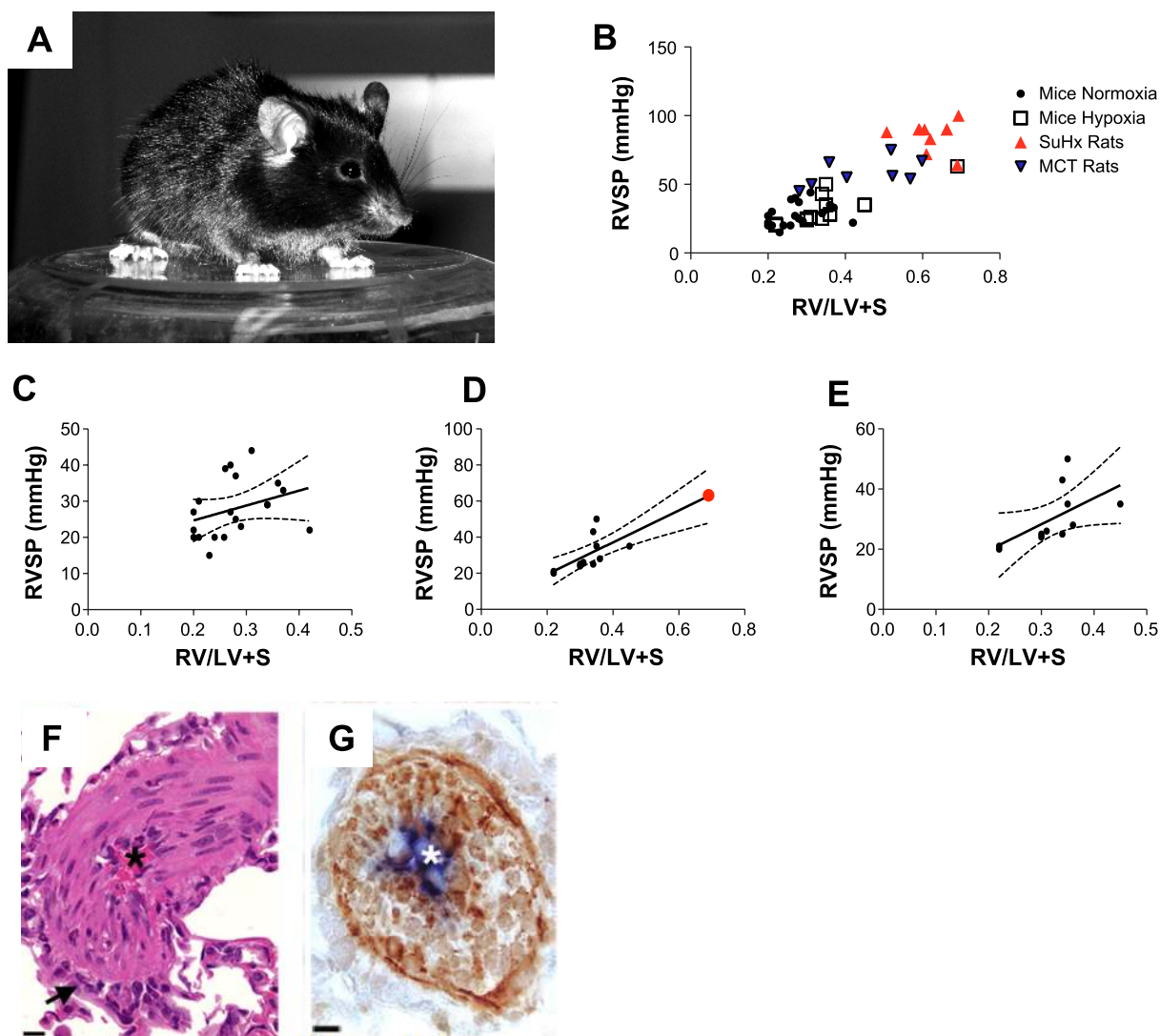


Fig. 1. *A*: C57BL6 mouse. *B*: correlation between right ventricular systolic pressure (RVSP) and right ventricle (RV) to left ventricle (LV) plus septum (S) ratio (RV/LV+S) in mouse pulmonary hypertension (PH) models exposed to normoxia and hypoxia, compared with the combination of SU5416 and chronic hypoxia (SuHx) and monocrotaline (MCT)-injured rat models. *C*: correlation between RVSP and RV/LV+S in mouse models exposed to normoxia. Pearson coefficient 0.32, $P = 0.150$. *D*: correlation between RVSP and RV/LV+S in mouse models exposed to hypoxia. If all mouse models are included, the Pearson coefficient is 0.82, $P = 0.0008$. However, the correlation seems to be driven by an outlier, the IL6-OE mice (red dot). Thus, if the latter is not included in the statistical calculations, the coefficient becomes nonsignificant ($r = 0.58$, $P = 0.057$). *E*: hematoxylin and eosin (HE)-stained lung section of a pulmonary vessel obtained from wild-type C57BL6 mice chronically exposed to ovalbumin. The mice develop severe pulmonary arterial muscularization without pulmonary hypertension. The vascular remodeling does not involve endothelial cell proliferation (*G*; brown staining is smooth muscle actin and blue is von Willebrand factor). *F* and *G* are reprinted from Daley et al. (2008), doi:10.1084/jem.20071008.

was ubiquitous and severe, the RVSP was not elevated (range 21–29 mmHg) and there were no signs of right heart hypertrophy. Thus, at least in the mouse, severe pulmonary vascular remodeling does not necessarily cause severe pulmonary arterial hypertension.

Right Ventricular Failure in Transgenic Mouse Models of Pulmonary Hypertension

RVF is the leading cause of death and the main determinant of survival in patients with PAH (7, 83). Intriguingly, whereas the mechanisms of pulmonary vascular remodeling have been extensively studied in both mouse and rat models of PAH, the mechanisms underlying RVF remain largely elusive (9, 110). RV hypertrophy is easy to measure by separating the RV free

wall from the rest of the heart (28). In rats, RVSP strongly correlates with the RV/LV+S (72), suggesting that the RV responds to an increased afterload with a corresponding degree of hypertrophy. Rat models of PAH, such as the SU5416/hypoxia and monocrotaline-injury models, generate robust RV hypertrophy (mean RV/LV+S 0.67–0.76) (11, 35). In contrast to rat models, only the IL6-OE mice seem to develop a comparable degree of hypertrophy (average RV/LV+S 0.69) and PH (94). When plotting data extracted from the reviewed mouse PH studies (Fig. 1, *B–D*), there seems to be an “uncoupling” between RVSP and RV/LV+S for the majority of PH mouse studies, even after hypoxia exposure. For example, Xu et al. (122) demonstrated that mice lacking the adenosine A2A receptor develop spontaneous PH (RVSP 39 mmHg); however,

the RV/LV+S was only 0.26, a value that is within normal limits for C57BL6/J WT mice (98). In contrast, Chen and collaborators (15) reported that mice overexpressing connective tissue growth factor generate modest PH (average RVSP 22 mmHg vs. 10 mmHg in WT controls) but develop significant RV hypertrophy (RV/LV+S 0.42).

Another important point to make is that right ventricular hypertrophy does not necessarily imply RVF. For instance, patients with Eisenmenger’s syndrome (PAH associated with a congenital systemic-to-pulmonary shunt) develop severe PH and right ventricular hypertrophy but do not develop signs or symptoms of overt RVF (at least not until late in the disease) (43, 44). Similarly, pulmonary artery banding in rats (a model of pure mechanical pressure overload) generates significant RV hypertrophy (mean RV/LV+S 0.52) with preserved RV function (11). Despite the fact that some investigators do not report mouse RV/LV+S data at all, it is surprising that only two studies attempted to evaluate cardiac function by means of echocardiography (13, 16) and both studies reported systemic cardiac output rather than RV cardiac output or contractility parameters [i.e., tricuspid annular plane systolic excursion (TAPSE)]. Although informative, systemic cardiac output does not characterize RV function.

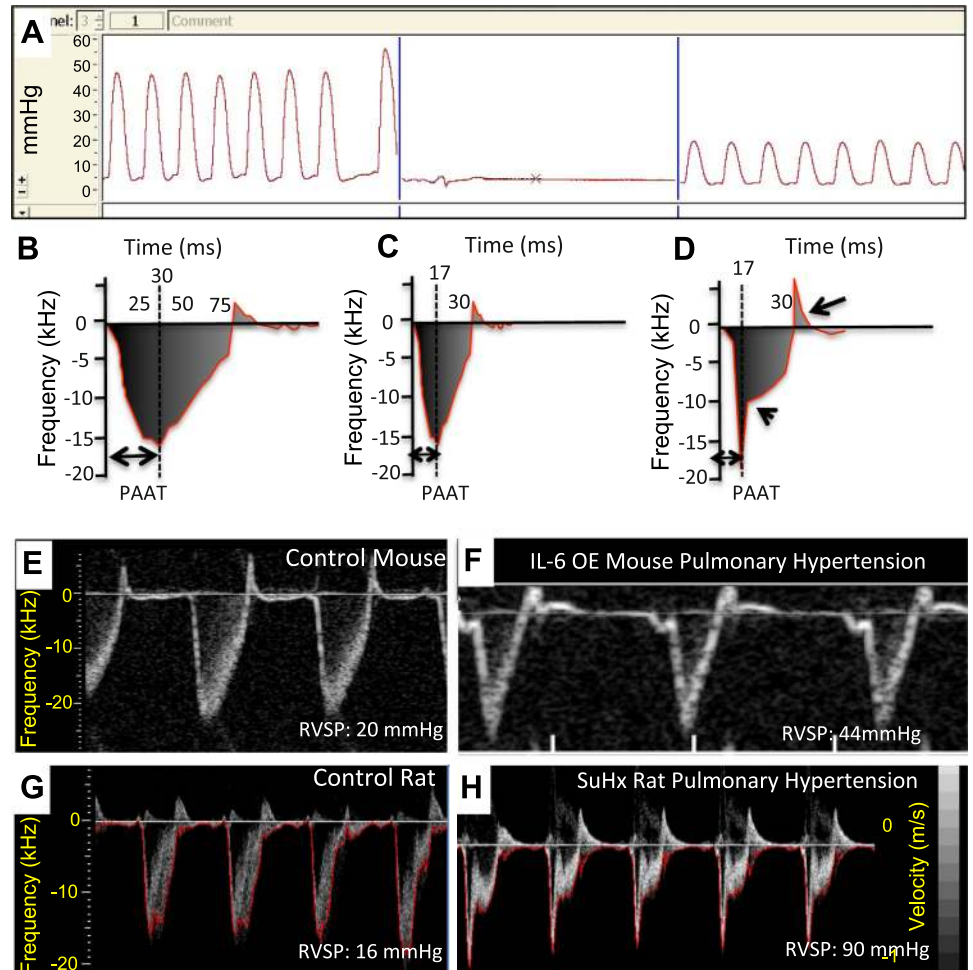
It is true that the presence of RV hypertrophy supports the diagnosis of PH; however, it does not imply that the RV of these animals is failing. As such, RV function should be

evaluated invasively by RV catheterization (cardiac output/index) or noninvasively by echocardiogram (TAPSE or RV fractional area change) or cardiac magnetic resonance (10, 12, 104, 107). Another useful echocardiographic finding to demonstrate RVF is the presence of paradoxical septal movement (Supplemental Video S1). When multiple echocardiographic signals such as RV dilatation, decreased TAPSE, and paradoxical septal movement are present, RVF is likely. Because severe chronic PAH is a cardiopulmonary disease, the study of PH in animal models should not only include assessment of RV hypertrophy but also evaluate whether the increase in PH pressure is affecting the function of the right ventricle.

Assessing the presence of PH by echocardiogram should also be part of the protocol when studying animal models of PAH. This is particularly important because, although RV catheterization is relatively simple in rats, the small hearts of mice impose a bigger challenge (79). Figure 2A illustrates how two different positions of the catheter tip, in a C57BL6/J W mouse heart, only 5 mm apart from each other, can significantly modify the measurement of RVSP. Thus a precatheterization echocardiographic assessment of PH, as well as an assessment of the RV/LV+S, complement the hemodynamic findings.

Two echocardiographic parameters obtained by pulsed-wave Doppler of the main pulmonary artery, can be used to address the presence of PH: 1) pulmonary artery acceleration

Fig. 2. A: hemodynamic measurements in a normal C57BL6 mouse assessed in 2 different locations in the right ventricle. We can appreciate that a slight difference in the site of catheter insertion can cause dramatic changes in pressure recordings, even if the shape of the curve looks apparently normal. B–D: schematic representation of a pulsed-wave Doppler recording of the pulmonary artery. Animals with pulmonary hypertension show a pattern consistent with decreased pulmonary artery acceleration time (PAAT) with or without midsystolic notching (arrowhead). A bidirectional flow pattern appears when pulmonary valve insufficiency is present (D, arrow). E–H: pulsed-wave Doppler recordings from normal mouse (E), mouse overexpressing IL-6 with pulmonary hypertension (F, reproduced from Thibault HB, Kurtz B, Raheer MJ, Shaik RS, Waxman A, Derumeaux G, Halpern EF, Bloch KD, Scherrer-Crosbie M. Noninvasive assessment of murine pulmonary arterial pressure: validation and application to models of pulmonary hypertension. *Circ Cardiovasc Imaging* 3: 157–163, 2010), normal Sprague-Dawley rat (G), and a SU5416/hypoxia exposed Sprague-Dawley rat (H). Compared with mice, rats develop a pulsed-wave Doppler pattern consisting of decreased PAAT, midsystolic notching, and bidirectional flow.



time (PAAT) and 2) midsystolic notching. In humans, the pulmonary artery systolic pressure is usually estimated by measuring the tricuspid regurgitation peak flow velocity (Bernoulli's equation). However, tricuspid regurgitation is uncommon in rodents, and a proper apical/subcostal view, for adequate measurement of tricuspid regurgitation, is particularly challenging in mice (104). RVSP can be estimated by measuring the PAAT, which is the time from the onset of pulmonary flow to peak velocity by pulsed-wave Doppler recording (Fig. 2B). In response to an increase in pulmonary artery pressure, the pulmonary valve tends to close prematurely (118) and a peak flow velocity is reached earlier in systole (Fig. 2C). This is usually the pattern seen in mouse models of PH (Fig. 2, E and F). The PAAT inversely correlates with the mean pulmonary artery pressure in humans and RVSP in rats and mice (53, 104, 107).

In a similar way, when PH is present, the pulsed-wave Doppler demonstrate an alternative ejection pattern consisting of an abnormally rapid rise in flow velocity to a peak level, preceded by a rapid deceleration, followed in turn by a secondary slower rise to form a notch (Fig. 2D). The midsystolic notching is observed in patients with PAH (53) and is usually the pattern found in the SU5416/hypoxia (Fig. 2, G and H) and monocrotaline rat models of PH (107). Both flow patterns have been reported in PH (53); nonetheless, the second pattern (with midsystolic notching) also presents with a bidirectional broadening spectrum of the Doppler frequency during early diastole, indicating pulmonary regurgitation (Fig. 2D), an indirect sign of increased pulmonary pressure.

Three of the published mouse studies provided unpredicted and puzzling results which ought to be followed up to better understand the integrated response of the lung circulation-heart axis, in the setting of PAH and chronic hemodynamic stress. For instance, Daley et al. (19) reported very pronounced pulmonary artery muscularization, but no RV hypertrophy. In contrast, the studies of Shifren et al. (86) demonstrate that mice lacking elastin develop impressive elevation of RVSP without RV hypertrophy. Finally, in hemoxygenase-1 KO mice, Yen et al. demonstrated that, after chronic hypoxia exposure, mice did not develop RV hypertrophy, but developed RV dilatation. All together, these last three studies exemplify that, in mice, 1) severe muscularization does not necessarily indicate PH, 2) PH is not always translated into RV hypertrophy, and 3) RV remodeling could occur in the absence of PH. Whether increased pulmonary arterial pressure and RV remodeling are two mechanistically distinct processes that are usually coupled in PAH remains to be investigated.

The SU5416/Hypoxia Rat Model of Pulmonary Arterial Hypertension: A Brief Summary

The combined VEGF receptor 1 (Flt) and 2 (KDR) blocker, SU5416, was one of the first agents discovered by screening for growth inhibitory activity of cultured endothelial cells incubated with the potent angiogenic VEGF ligand (27). Kindly provided by Dr. Peter Hirth (Sugen, South San Francisco, CA), for preclinical studies, SU5416 was tested with the hypothesis that inhibition of VEGF signaling would result in pulmonary emphysema. Indeed, a single subcutaneous injection of 20 mg/kg of SU5416 caused air space enlargement and mild PH in adult rats (51). Unexpectedly, rats injected with

SU5416 (20 mg/kg) and exposed to chronic hypoxia in a hypobaric chamber (hereafter SUHx), as an attempt to worsen the air space enlargement, surprisingly developed severe PAH, which was not reversible when the animals were returned to Denver altitude (100) or in later experiments to sea level (11). The PH was associated with angioobliterative pulmonary lesions that were preventable by treating the animals concomitantly with a pan-caspase inhibitor, indicating that apoptosis was necessary for the development of the pulmonary vascular lesions (100). This rat model has served as a model for preclinical drug studies designed to examine whether the PH and pulmonary vascular disease in the SUHx rats could be reversed once established. Simvastatin (a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor) and a bradykinin agonist had a small effect on established PH and RVH, whereas a Ca²⁺ channel blocker (nifedipine), angiotensin converting enzyme inhibitor (lisinopril), angiotensin receptor blocker (ibersartan) and bosentan (a nonselective endothelin receptor blocker) were all not effective (102, 103). Interestingly, when treated with infused prostacyclin, the animals died acutely, presumably because prostacyclin caused overwhelming peripheral vasodilation, dropping RV perfusion pressure and RV preload (72). Because in this rat model the PH is severe, is accompanied by plexiform-like lesions, and is largely refractory to treatment, it was concluded that the SUHx rat model of angioobliterative severe PH had a number of features that resembled the human disease. Moreover, Abe et al. (1) have shown the histopathological similarities of the SUHx lung vascular lesions compared with the plexiform lesions in human disease. In addition to severe PH, rats treated with SU5416 and exposed to chronic hypoxia also develop severe RV failure characterized by a cardiac output reduction, decreased TAPSE, increased RV diastolic diameter, capillary rarefaction, RV fibrosis, and cardiomyocyte apoptosis (11, 12, 72).

The SUHx model has been modified to uncover immunological mechanisms in the pathobiology of severe PAH. We have shown that treatment of athymic rats with SU5416 is sufficient to cause severe angioobliterative PAH, indicating that hypoxia is not necessary (101) and that early immune reconstitution with rat regulatory T lymphocytes prevents the development of severe PAH (99, 101). For further information regarding the SUHx model the reader is referred to Refs. 11, 81, 82, 100.

Attempts To Generate a SU5416/Hypoxia Mouse Model of Pulmonary Hypertension

Given the wealth of mechanistic insights gained from genetically engineered mice (Table 1), it was only intuitive to combine the SUHx model with gene knockout technology. Subsequent to the first publication describing the SUHx rat model (100), multiple attempts to generate a SUHx mouse model of severe PH have been pursued but, until now, none have been fruitful. Here we present some of our data utilizing different mouse strains, genetic manipulations, and multiple SU5416 dosing as we tried to generate a SU5416-based mouse model. All attempts to generate a SUHx mouse model are summarized in Table 2.

The first attempt goes back 10 years ago, shortly after the first report of Taraseviciene-Stewart in 2001 (100). We injected 8–12 wk old adult C57BL6 mice with either 20 or 50 mg/kg of SU5416 at Denver (altitude 5,600 ft above sea level)

Table 2. Right ventricular systolic pressure measurements and pulmonary vasculature remodeling in mice treated with SU5416 with or without hypoxia

Mouse Strain	Genetic Modification	RVSP, mmHg		RV/LV+S		Histology	
		Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
C57BL6/J	Wild type/normal with single SU5416 dose	N/A	25	N/A	N/A	N/A	↑ musc.
C57BL6/J	Wild type/normal with single SU5416 + 2 wk Denver altitude	N/A	33	N/A	N/A	N/A	↑ musc.
C57BL6/J	ADAM 10TG Wild type	20	N/A	0.26	N/A	No changes	N/A
C57BL6/J	ADAM 10TG OE	41	N/A	0.28	N/A	No changes	N/A
C57BL6/J	ADAM 10TG Wild type with SU5416	22	18	0.25	0.23	No changes	↑ musc.
C57BL6/J	ADAM 10TG OE with SU5416	25	20	0.27	0.22	No changes	↑ musc.
R26R	PVEC BMPR ^{-/-} KO	21	N/A	0.28	N/A	No changes	N/A
R26R	PVEC BMPR ^{-/-} KO with SU5416	N/A	23	N/A	0.26	N/A	↑ musc.
NU/NU	Nude mice Foxn1 ^{mu}	21	N/A	0.21	N/A	No changes	N/A
NU/NU	Nude mice Foxn1 ^{mu} with SU5416	22	N/A	0.25	N/A	No changes	N/A
NU/NU	Nude mice Foxn1 ^{mu} 3 doses once a week with SU5416	22	N/A	0.23	N/A	No changes	N/A
NU/NU	Nude mice Foxn1 ^{mu} 4 doses once a week with SU5416						

ADAM10, A Disintegrin And Metalloproteinase 10.

and then exposed the mice to a simulated altitude of 17,000 ft. Littermates injected with saline instead of SU5416 served as controls. No significant differences in the RV/LV+S or the lung vascular morphology were detected between control and SU5416 groups. In the aggregate, the data indicated that, at least C57BL6, mice subjected to the same experimental protocol that had been used to generate severe PAH in WT rats did not develop PAH, nor did they develop pulmonary vascular remodeling or RVH (Table 2). Because the protocol used to generate severe PAH in rats did not generate in mice PH beyond the degree observed with chronic hypoxia exposure alone, we postulated that mouse-specific genetic background factors played an important role and did not publish these results.

On the basis of the findings that athymic nude rats lacking T cells exposed to SU5416 generated PH and vascular remodeling without hypoxia (101), we next attempted to induce PH in nude (immune-insufficient) mice (SCID). Given that a single dose of SU5416 did not induce the expected changes in WT mice, we decided to expose the SCID mice to one injection per week for 3 wk (3 doses total) and for 4 wk (4 doses total). Table 2 shows that there was no significant change in the mean RVSP when compared with nude mice without SU5416. SU5416 did not induce PH in nude mice.

As a further attempt to explore the role of abnormal immune regulation in the pathobiology of PAH, we investigated transgenic mice overexpressing the A Disintegrin And Metalloproteinase 10 (ADAM10). Saleem, Conrad, and colleagues (34) had reported that overexpression of ADAM10 abrogated B cell development, delayed T cell development, and strikingly induced systemic expansion of CD11b+Gr-1+ myeloid-derived suppressor cells. MDSCs were first characterized in tumor-bearing mice and in patients with cancer; however, there are other numerous pathological conditions that markedly induce MDSC expansion (29). MDSCs are characterized by a remark-

able ability to suppress T cell responses and regulate innate immune responses by modulating cytokine production by macrophages (29, 89). Furthermore, circulating MDSCs can migrate to tumor sites and rapidly differentiate into tumor-associated macrophages (TAMs) (54). TAMs acquire the ability to produce several cytokines, such as IL-1 β , IL-6, and TGF- β (60). Because 1) circulating MDSCs have been described in patients with PAH (127), 2) MDSCs/TAMs can produce large amounts of IL-6, 3) IL-6 is increased in patients with PAH (47) and overexpression of IL-6 generates PH (94), and finally, 4) abnormal T-cell function is associated with the development of plexiform-like lesions in rats exposed to SU5416 (99, 101), we postulated that MDSCs in ADAM10 transgenic mice (ADAM10Tg) would facilitate the development of pulmonary arterial hypertension and vascular remodeling in mice. Figure 3 shows the data obtained from the first ADAM10Tg mouse studied. The baseline echocardiogram showed RV dilatation (Fig. 3A) and hemodynamic measurements demonstrated a RVSP of 57 mmHg (Fig. 3B). Histological analysis revealed the presence of multiple occluded vessels (Fig. 3, C and D); however, immunohistochemistry for Von Willebrand factor revealed that none of the cells occluding the vessels were endothelial cells (Fig. 3, E and F). When additional animals were evaluated, we observed that, whereas some of the mice exhibited a high RVSP (range 40–60 mmHg) (Fig. 3H), the majority of the ADAM10Tg mice did not spontaneously develop PH. Given that few ADAM 10 mice (on the C57BL6 background) exhibited high RVSP values, we treated WT and ADAM10Tg animals with SU5416, on the basis of the double-hit hypothesis of PAH. Mice were injected with 20 mg/kg of SU5416 subcutaneously, once a week for 4 consecutive wk; however, we did not find an increase in the RVSP compared with WT mice (Table 2).

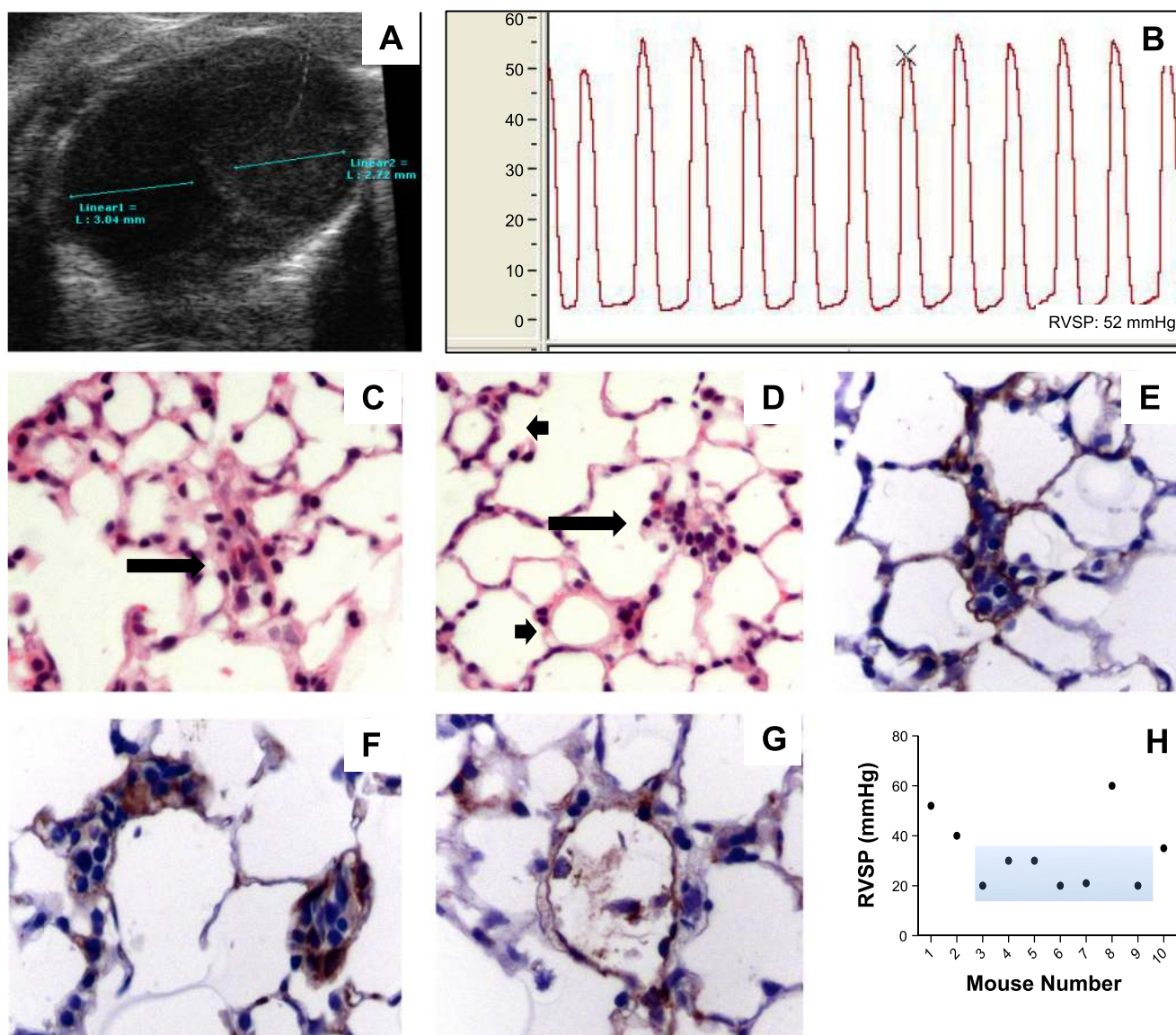


Fig. 3. *A*: short-axis view obtained from a mouse overexpressing ADAM10 illustrates significant right ventricular dilatation compared with the left ventricle. *B*: hemodynamic measurement illustrating an increase in the right ventricular systolic pressure. *C* and *D*: HE-stained lung section illustrating occluded vessel (arrows) and normal vessels (arrowheads). *E*–*F*: immunohistochemistry of lung sections illustrating that the occluded vessels are not occluded by von Willebrand-positive endothelial cells. *G*: normal vessel. *H*: RVSP measurements in ADAM10 transgenic mouse. Light blue area marks mice that had a normal pulmonary artery pressure. Although some mice demonstrated high RVSP, the measurements were highly variable.

Mizuno et al. (64) had demonstrated that mice lacking the p53 gene developed severe pulmonary vascular muscularization and mild PH upon exposure to chronic hypoxia (Table 2). Thus we examined the impact of SU5416 in combination with hypoxia in p53 mice (FVB/B6-Trp53^{tm1tyj/J}, Jackson Laboratory, Sacramento, CA), hypothesizing that the lack of “the guardian of the genome” p53 would perhaps be permissive and lead to a more severe form of PH. Mice were injected with a single subcutaneous dose of SU5416 and exposed for 4 wk to 10% hypoxia, in keeping with the rat SUHx protocol. We did not find differences between SUHx p53^{-/-} mice and p53^{-/-} mice exposed to hypoxia alone (data not published).

In a recent experiment, we injected C57Bl6 WT mice with 20 mg/kg of SU5416 subcutaneously, once a week for 3 consecutive wk, and exposed the animals to nitrogen dilution hypoxia (10% oxygen). We then compared the data to those

obtained with a group of C57BL6 mice exposed to hypoxia alone. The animals were killed following echocardiography and measurement of RVSP, and the lungs were inflated according to our standard protocol. The data derived from these studies are displayed in Fig. 4.

Mice Are Not Small Rats

There are several factors that could explain the differences between the data obtained and published in mice following the implementation of SU/Hx protocols and the data obtained with rats subjected to the SU/Hx protocols. In the study by Ciucan et al. (16), more than one dose of SU5416 was apparently required to generate PAH, strongly suggesting that mice are less responsive to SU5416 and that the compound is differently or more rapidly metabolized and inactivated in mice compared

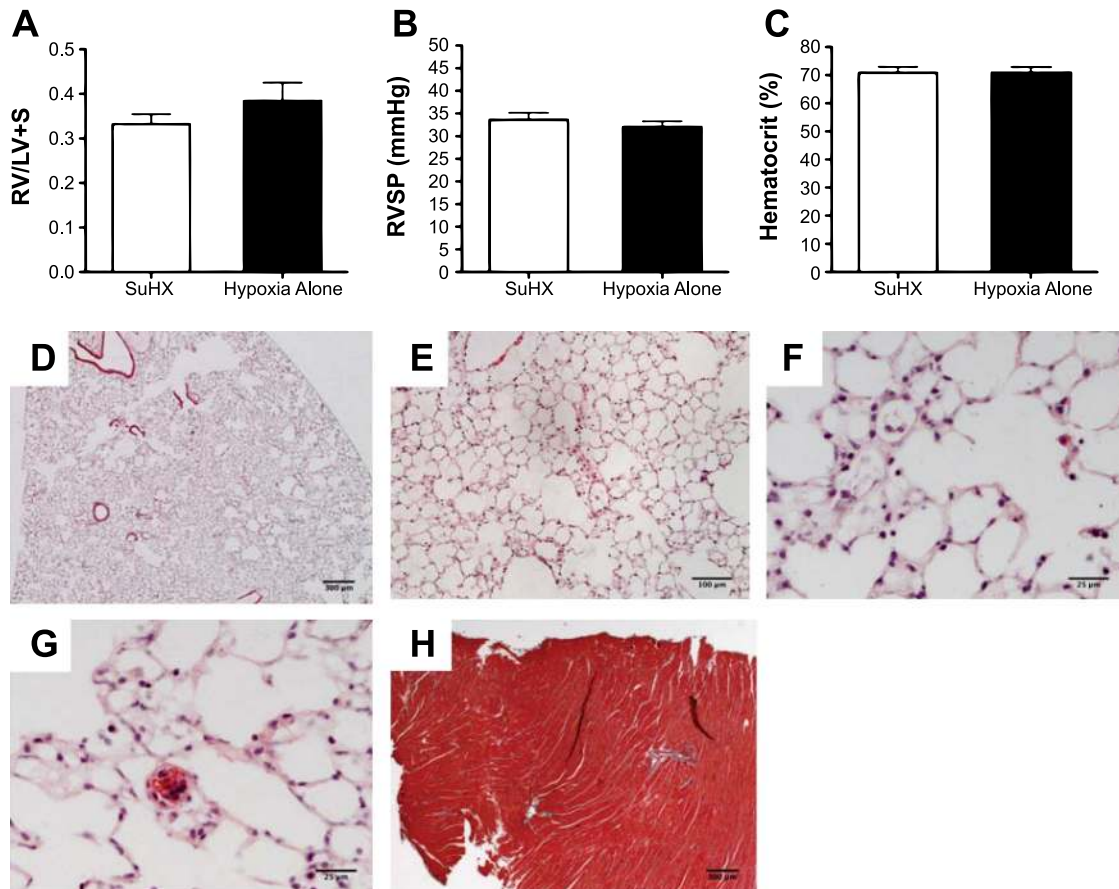


Fig. 4. Mice administered SU5416 (20 mg/kg sc) once a week for 3 consecutive wk and exposed to nitrogen dilution hypoxia for 3 wk ($n = 5$) are compared with mice exposed only to chronic hypoxia ($n = 5$). Neither right ventricular hypertrophy nor RVSP were different when the 2 groups were compared (A and B); there was no difference in the hematocrit (C). Lung histology (HE staining) shows some degree of air space enlargement (D–F). Rare small vessel thrombi were observed (G). As evidenced by trichrome staining, RV fibrosis was absent (H).

with rats. Possibly the accumulation of SU5416 in the lung tissues is less in mice when compared with rats. Ye et al. (125) compared the distribution, metabolism, and excretion of SU5416 (semaxinib) between rats and mice and found that the systemic and renal clearance of SU5416 was much greater in mice and that SU5416 was hepatically metabolized by CYP1A, a cytochrome differentially expressed between animal species (61, 69). Of interest, repeated dosing with SU5416 induced an increase in the activity of several cytochrome *P*-450 enzymes. It is likely that pharmacogenomic differences between rats and mice can explain the difference in pulmonary vascular responses observed after administration of the alkaloid monochromaline (35). This hypothesis of a significantly different performance and action pattern of SU5416 between the mouse and rat species is also supported by the observation that, at least in the SUHx mouse model, PH is not sustained after the hypoxic exposure had been discontinued (16). This is in clear contrast to the response of SUHx rats in which PH is maintained for many weeks after the hypoxic exposure had been terminated, and in which lung lesion maturation continues for at least 13 wk after the initial and single dose of SU5416 had been administered (1).

The spontaneous reversal of PH is the hallmark of most (or all) “single-hit” chronic hypoxia models and is unfortunately not a feature of human forms of idiopathic PAH (113). Thus it

appears that the recently reported mouse model of SUHx exposure (16) is most likely a variant of chronic hypoxia-related PH models in that the reported pulmonary vascular changes are predominantly arteriolar muscularization (16). Importantly, as documented by the studies by Daley et al. (19), very impressive degrees of muscularization of the pulmonary arterioles can occur in mice without the development of PH and RV hypertrophy.

Because PH researchers are increasingly investigating mice (Table 1), consideration should be given to the known important differences between the rat and the mouse species (5, 6, 17, 25, 55). Such differences, which may categorically determine the mechanisms leading to the development of pulmonary vascular diseases, can be found on anatomical, cellular, and gene expression levels and also in pharmacogenomics. For instance, rat lungs have less alveolar and more blood vessel walls and higher mean alveolar diameters than mouse lungs (25), which translate into distinct tissue mechanical properties. In mice, the systemic blood vessels that supply the trachea and mainstem bronchi do not penetrate into the intraparenchymal airways, as they do in other species (63). Although we do not know whether the alveolar space design influences the development of PAH, the presence or absence of a fully developed bronchial circulation is likely important for the pathobiology of PAH. Precursor cells, derived from the bone marrow, can be

transported into the pulmonary vascular adventitia via vasa vasorum (via bronchial circulation) (126) and affect vascular remodeling.

In the heart the proportion of fibroblasts and myocardiocytes differs between the rat and mouse (5) and the cytochrome *P*-450 gene expression pattern, critically important for drug and steroid hormone metabolism both in the liver and the lung, differs between the two species (17, 61) (see above). As mentioned before, Hoshikawa et al. (45) demonstrated differences in lung tissue gene expression between WT mice and Sprague-Dawley rats. Some relevant differences included a sixfold upregulation of the expression of the gene encoding the integrin- α chain, a sixfold increase in the expression of the gene encoding MHC class II antigen and a sevenfold upregulation of the osteopontin gene in chronically hypoxic rat lungs compared with mice. In contrast, the gene encoding the CYP1A1 isozyme was more than fivefold downregulated in the lungs from hypoxic mice. Whether any of these gene expression differences can explain differences in the degree of lung vascular remodeling remains unclear but can be investigated.

Decoding the complete genome of the C57BL/6J mouse (Mouse Genome Sequencing Consortium) improved our ability to relate sequence to function and allowed, in an unprecedented way, the task of creating null alleles for all genes (68). However, genetic catalogs remain incomplete, and we are still largely ignorant of the molecular basis of the majority of genetically influenced phenotypes (52). For instance, Keane and collaborators (52) examined tissues in a single cross (C57BL/6J \times DBA/2J) and were able to detect high levels of allelic bias. Interestingly, they showed divergent allele-specific patterns between tissues. For example, an allele that was relatively highly expressed in one tissue was underexpressed in a different one. Furthermore, they demonstrated the nature of sequence variants and how their relative position to other genes, influences function. Thus it is necessary to be circumspect when extrapolating data from a certain knockout mouse strain to other strains, as the phenotype could also be the result of a combination of sequence variants and not only the result of a specific genetic manipulation. For example, Rabieyousefi et al. (74) reported that mice overexpressing OX40L, a tumor necrosis factor family molecule, develop vascular remodeling of large pulmonary arteries in C57BL6 mice but not in BALB/c mice, supporting the importance of genetic background (strain differences).

Mouse Models of Pulmonary Hypertension: Translational Potential

Having reviewed many of the published data on mouse models of PAH and having failed to establish a mouse model of severe angioproliferative PAH in our own laboratory, we feel obligated to ask 1) To what extent can the information derived from the mouse PAH models be related to human forms of severe PAH? 2) Are mouse models really productive when it comes to preclinical drug testing and the exploration of new treatment strategies? 3) What are the criteria we should apply to recommend a particular animal model of PAH? These questions certainly depend on the disease aspect and pathobiological mechanisms of interest. For instance, if the investigational focus is on vasoconstriction or mechanisms of pulmonary arteriolar muscularization, many of the PAH mouse mod-

els will likely be very informative, especially if the model produces highly reproducible data without large variability. In contrast, if the investigational focus is a model of severe angioobliterative PAH and RVF, the animal model ought to display at least some of the critical features of human forms of PAH (Table 3). One critical feature of any new animal models of pulmonary arterial hypertension should be the development of RVF, not just RV hypertrophy.

Highly specific molecular targets or the opportunity to block certain signaling pathways are pleasing ideas to many researchers. However, without the context of the human phenotype, gene deletion may just produce another descriptive association. Killing one bee is unlikely to modify the complex interactions occurring in the colony (unless, of course, it is the queen!). This example may be simplistic, but the message is clear: Ablating one gene, in one particular cell type, is likely insufficient to explain a complex disease such as PAH. Taking our own data as an example (Table 2), knocking out the p53 gene in mice certainly caused enhanced pulmonary arteriolar muscularization after chronic hypoxic exposure (64) but did not cause a severe angioobliterative pathology, even after SU5416/hypoxia exposure. Does this result mean that p53 is uninvolved in the mechanisms of pulmonary angioobliteration? Or do we have to consider that mice are, perhaps, not suitable to explore this question, because the combination of SU5416 and hypoxia causes highly reproducible, progressive, and irreversible severe angioobliteration in the rat, but not in mice?

Highly complex, multicellular vascular lesions characterize the lungs of humans with severe PAH, and many layers of redundant control mechanisms prevent the formation of such lesions in healthy, not genetically susceptible people. Thus how likely it is that one single gene will explain the entire pathobiology of PAH? We have already learned that BMPR2 mutations are not sufficient to trigger PAH, but to interpret that the presence of BMPR2 gene mutations in humans with familial and idiopathic PAH is just an epiphenomenon, not relevant to the disease, would be a mistake.

Many questions remain, but one question should be highlighted: What from the PAH mouse models can be translated and applied to the human disease?

Table 3. *Salient features of human pulmonary arterial hypertension that ought to be considered in animal models*

Hemodynamics
Mean pulmonary artery pressure >25 mmHg or RV systolic pressure >60 mmHg
Decreased cardiac output
Histology
Small pulmonary artery lumen obliteration
Muscularization of the media
Plexiform-like lesions
RV hypertrophy (RV/LV/S >0.45)
RV fibrosis and capillary rarefaction
Echocardiography
Dilatation of the right heart chambers
Hypertrophy of the RV
Paradoxical septal movement (or at least rectification of the interventricular septum)
Decreased tricuspid annulus plane systolic excursion (\sim <1.5 mm)

Conclusions

Here we have reviewed and compared a large number of representative mouse model studies of PAH and contrasted, in passing, the salient features of these models with those that are present in rat models. We addressed some of the potential problems that can arise when evaluating PH in mice and underlined the importance of evaluating right ventricular function. Stating the obvious: There is not a perfect or ideal animal model of PAH yet. However, we invite researchers interested in designing new models of PAH to consider, among others (Table 3), three critical features of the human disease: 1) obliteration of the lung arterioles, 2) nonreversibility of PAH, and 3) development of RVF.

Although there have been recent general reviews of animal models of PH (79, 95), here we have attempted to fill a gap by putting current mouse models of PAH into perspective, and tried to highlight some important innate murine characteristics, which partially explain why many of the mouse models could have been lost in translation.

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