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Discovery of a murine model of clinical PAH: Mission impossible?

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ABSTRACT

Pulmonary arterial hypertension (PAH) is a lung vascular disease characterized with a progressive increase of pulmonary vascular resistance and obliterative pulmonary vascular remodeling resulting in right heart failure and premature death. In this brief review, we document the recent advances in identifying genetically modified murine models of PH, with a focus on the recent discovery of the mouse model of *Tie2* Cre-mediated deletion of prolyl hydroxylase 2, which exhibits progressive obliterative vascular remodeling, severe PAH, and right heart failure, thus recapitulating many of the features of clinical PAH. We will also discuss the translational potential of recent findings arising from experimental studies of murine PH models.

Key words: Animal model, Endothelium, Hypoxia inducible factor, Prolyl hydroxylases, Vascular remodeling.

Introduction

Pulmonary arterial hypertension (PAH) is characterized by progressive increase in pulmonary vascular resistance and arterial obliteration leading to right heart failure and premature death [1–3]. Intimal, medial and adventitial thickening, vascular fibrosis, augmented oxidative/nitrative stress, vascular occlusion, and formation of complex plexiform lesions are histopathological features of clinical PAH including idiopathic PAH (IPAH) [4–6]. Current therapies targeting abnormalities in the prostacyclin, nitric oxide, and endothelin signaling pathways result in only modest improvements in morbidity and mortality [1,3]. None of these therapeutic agents target the underlying mechanisms of obliterative vascular remodeling. Although two rat PH models induced by either monocrotaline (MCT) challenge or chronic hypoxia plus Sugen 5416 treatment (best known as an inhibitor of vascular endothelial growth factor receptors 1 and 2) exhibit severe vascular remodeling and are widely used for preclinical studies of PH, these treatments fail to induce severe PH with stable obliterative vascular remodeling in mice. Thus, the identification of mouse model(s) with severe PH and obliterative vascular remodeling (i.e. recapitulating the pathological features of clinical PAH) is critical in order to delineate the molecular mechanisms that are responsible for obliterative vascular remodeling, and thereby provide valuable druggable targets and novel therapeutic approaches for PAH patients.

Here we review recent advances in murine models of PH since 2012 (based on ref [7]) and highlight a novel mouse model established by our group with pathology resembling clinical PAH. The mouse model of *Tie2* Cre-mediated

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disruption of *Egln1* [encoding prolyl-4 hydroxylase 2 (PHD2)], designated *Egln1*^{Tie2}, is the first mouse model of PAH exhibiting spontaneous progressive PAH with extensive pulmonary vascular remodeling including stable vascular occlusion and complex plexiform-like lesions [8]. As seen in PAH patients, these mice also die of right heart failure. We also discuss the obligatory role of HIF-2α signaling in the pathogenesis of PH and potential novel therapeutic strategies for the treatment of PAH.

Recent advances in murine models of PH

A number of genetically engineered mouse models have been generated to study the pathogenesis of PH (Table 1) since the publication of a preceding review in 2012 [7]. Hemodynamic measurement shows that 14 of the 23 mouse models have basal right ventricular systolic pressure (RVSP) of more than 30 mm Hg, while 4 of these 14 have an RVSP of more than 50 mm Hg. These 4 recently identified mouse models are *Erg*$^{-/-}$ mice (WT: KO, 20:50 mm Hg) [9], *Hif2a*$^{G536W/G536W}$ knockin mice (28:66 mm Hg) [10], *Cdh5Cre*-mediated *Egln1* knockout mice (*Egln1*^{Cdh5}) (25:54 mm Hg) [11], and *Tie2Cre*-mediated *Egln1* knockout mice (*Egln1*^{Tie2}) (22:72 mm Hg) [8]. The latter 3 of these models target the same pathway. Intriguingly, both Erg and its downstream target, Apelin receptor, are also downregulated in the lungs of *Egln1*^{Tie2} mice [8]. Although all of these mouse models show increased muscularization of the distal pulmonary arterioles, only a few of them show evidence of occlusive pulmonary vascular remodeling. Horita et al. reported that smooth muscle cell (SMC)-specific deletion of *Pten* in mice resulted in marked pulmonary vascular remodeling including occlusion of small pulmonary arteries when exposed to chronic hypoxia but not under normoxic condition [12]. The RVSP of these mice after 4-week exposure of hypoxia is less than 40 mm Hg with a right ventricular hypertrophy index (RV/LV+S ratio) of 0.42. No evidence of right heart failure was observed in these mice. Lathen et al. showed that mice with deficiency of either *Erg or Aplnr* developed occluded pulmonary venues but not arterioles [9]. *Erg*$^{-/-}$ mice develop an RVSP as great as 50 mm Hg and all die by the age of 3 months. These mice also develop RV hypertrophy (RV/LV+S ratio ~0.5), but it is unknown whether they die of right heart failure, given that no echocardiography or hemodynamic measurement of RV function have been carried out. In an experimental rat model of PAH, it has been shown that endothelial cell apoptosis is a trigger for the development of severe PAH induced by chronic hypoxia combined with Sugen5416 treatment. A recent study employing a mouse model with Fas-induced apoptosis of ECs showed evidence of occlusive vascular remodeling [13]. However, only a small portion (~20%) of these mice developed mild PH with scarce occlusive lesions. Previously, Steiner et al. have shown occlusive vascular remodeling in transgenic mice overexpressing IL-6 following 3-weeks exposure to hypoxia but not under normoxic conditions [7,14]. Although these models are helpful for us to understand the mechanisms that regulate pulmonary vascular remodeling, none of them fully resembles the pathology of clinical PAH. We recently demonstrated for the first time that *Tie2* Cre-mediated deletion of *Egln1* in ECs and hematopoietic cells spontaneously induces severe PAH with progressive vascular remodeling including vascular occlusion and the formation of complex plexiform-like lesions, recapitulating clinical PAH.

Discovery of the first murine model of progressive PAH recapitulating clinical PAH

As pointed out by Gomez-Arroyo et al., an ideal animal model of clinical PAH should display some or all of the key features found in humans, including markedly increased RVSP (>60 mm Hg), pulmonary artery lumen obliteration, formation of plexiform-like lesions, severe RV hypertrophy (RV/LV+S ratio >0.45), RV chamber dilation, and RV failure [7]. The *Egln1*^{Tie2} mouse model is the only mouse model that closely resembles many clinical features of severe PAH in patients [8]. Under basal conditions, *Egln1*^{Tie2} mice develop progressive PAH with RVSP levels ranging from 60 to 90 mm Hg at the age of 3.5 months. As seen in patients, these mice die progressively (i.e. starting at the age of 2 months and with 80% mortality by the age of 6 months). Remarkably, these mice also develop unprecedented RV hypertrophy (RV/LV+S ratio ranging from 0.75 to 1.1). Echocardiography reveals a 3-fold increase in RV wall thickness, a marked dilatation of the RV chambers, and a decrease in RV fraction area change indicating RV dysfunction. Pulmonary artery dysfunction is evidenced by a marked decrease in the ratio of pulmonary artery acceleration time: ejection time (Fig. 1 and ref [8]). Molecular analysis also shows induced expression of atrial natriuretic factor and skeletal α-actin in the RV, indicating heart failure [15]. These data demonstrate that *Egln1*^{Tie2} mice develop spontaneous progressive PAH that results in RV failure and premature death as seen in patients with severe PAH.

Histological examination of lung sections from *Egln1*^{Tie2} mice demonstrates various forms of vascular remodeling, including thickening of intima, media and adventitia, and neointima occlusion as well as the formation of plexiform-like lesions (Fig. 1). Pulmonary vascular obliterative remodeling is prominent in both large and small vessels, and this remodeling is progressive and irreversible. Proliferation of vascular cells including both ECs and SMCs is evident in the lesions. Besides CD11b^{+} monocyte infiltration in the lesions, we also observed increased expression of IL-6 (indicating inflammation), vascular fibrosis, and increased oxidative/nitrative stress in *Egln1*^{Tie2} lungs (unpublished observations). These are also the characteristic features of the pathology of clinical PAH [4,6,16–18]. In human, it was shown that female gender is a risk factor for PAH, but that men with PAH have high mortality [19,20]. In *Egln1*^{Tie2} mice, however, we did not observe significant differences in RVSP or RV hypertrophy between genders.

In support of the concept that *Egln1*^{Tie2} mice may be the long-sought-after murine model of clinical PAH, expression of many of the PH-causing genes is altered in *Egln1*^{Tie2} lungs. Expression of *Arg1, Lcn2, Slc39α12, Il13, Retnla, Ngf, Serpine 1, Il6, Cxcl12, Csf2, Ptger3, Arg2, Eln, Edn1, Trpv4*, and *Sphk1* is upregulated while *Aplnr, Ccr7, Ccr2, Bmpr2, Cav1, EphA1, Erg, Apln, Prkg1, Acvr2b, Acvr11*, and *Eng* expression is downregulated. It remains unclear whether altered expression of these genes collectively causes severe PAH in *Egln1*^{Tie2} mice. Nevertheless, we have