Review Article

New models of pulmonary hypertension based on VEGF receptor blockade-induced endothelial cell apoptosis

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ABSTRACT

In spite of treatment, severe angioproliferative pulmonary arterial hypertension (PAH) remains a disease characterized by great morbidity and shortened survival. New treatment strategies for patients with PAH are needed, and after drug development, preclinical studies are best conducted in animal models which present with pulmonary angio-obliterative disease and right heart failure. A rat model of severe pulmonary hypertension and right heart failure, described a decade ago, continues to be investigated and provide insight into the nature of the lung vascular lesions and mechanisms of cardiac adaptation to an altered lung circulation. This rat model is based on the combination of VEGF receptor blockade with Su5416 and chronic hypoxia; use of this pulmonary hypertension induction strategy led to developing the concept of apoptosis-dependent compensatory vascular cell growth. Although, often employed in experimental designs, chronic hypoxia is not necessary for the development of angio-obliterative pulmonary hypertension. Left pneumonectomy combined with Su5416 also results in severe pulmonary hypertension in normoxic conditions. Similarly, the immune insufficiency component of severe PAH can be modeled in athymic rats (lacking T-lymphocytes). In these rats housed under normoxic conditions, treatment with the VEGFR receptor blocker results in angioproliferative pulmonary hypertension; cardiopulmonary disease in these animals can be prevented by immune reconstitution of regulatory T-cells (Tregs). Finally, chronic hypoxia can be replaced with another stimulator of HIF-1α: Ovalbumin (Ova). Immunization of rats with Ova increases lung tissue HIF-1α protein expression, and in Su5416-treated rats causes lethal pulmonary hypertension. Finally, we postulate that these models may also be useful for "reverse translation"; that is, the mechanisms of lung vascular cell death and growth and the modifying influences of immune and bone marrow cells that have been identified in the Su5416 VEGFR inhibitor models can be informative about heretofore undescribed processes in human PAH.

Key Words: apoptosis, chronic hypoxia, pulmonary hypertension, regulatory/T-cells, Su5416

The designation "pulmonary hypertension" is an umbrella descriptor for all conditions characterized by an elevated pressure in the pulmonary circulation, regardless of a primary arterial, venous, or capillary involvement. PAH refers to a group of progressive and incurable pulmonary vascular diseases with a predominant involvement of small pulmonary arteries which exists in hereditary, idiopathic, and associated forms. While over the last decade dramatic progress has been made in the understanding of the pathogenesis of pulmonary hypertension and PAH in particular,[1-5] a recent meta-analysis has characterized the field of clinical pulmonary hypertension research as "a field in need of new drugs and new study designs."[6,7] Review papers have critically assessed the pros and cons of rodent models of pulmonary hypertension;[8,9] additionally a recent summary statement resulting from an NIH-NHLBI-sponsored

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workshop on pulmonary hypertension identified the need for the new development of new animal models.[10]§ The greater context for such a recommendation is the recognition that the presently-used drugs do not have a sufficiently strong impact on the overall outcome of PAH patients (and remain unexplored in other forms of pulmonary hypertension); furthermore "the major factor responsible for the high pulmonary vascular resistance in severe, established PAH is the formation of occlusive neointimal and plexiform lesions in small peripheral pulmonary arteries."[10]

Informative animal models of pulmonary vascular disease should both utilize and reflect the pathobiology of severe PAH. Ten years ago, in an attempt to better recreate the neointimal lesions observed in plexiform lesions, the Voelkel laboratory along with Dr. Rubin Tuder at the University of Colorado sought to replicate the human lesions by directly antagonizing vascular endothelial growth factor (VEGF). The notion of targeting VEGF arose out of evidence pointing to deregulated angiogenesis as the cause of abnormal endothelial proliferation in the vascular lumen of affected arterioles in the PAH lung. Given that VEGF is required for normal endothelial cell maintenance, function, and signaling, it was originally reasoned that the blockade of VEGF function (originally performed in association with chronic hypoxia) would induce endothelial cell dysfunction and death, promote apoptosis-resistant endothelial cell proliferation, and cause pulmonary hypertension. The study which emerged resulted in a new model of pulmonary hypertension in the rat.[11] Treatment with the VEGF receptor/tyrosine kinase antagonist, Su5416, generated severe angio-obliterative pulmonary vascular remodeling and pulmonary hypertension.[12] Unfortunately, as with other experimental triggers of pulmonary hypertension, the mouse model has generally proven less useful with this approach.[13] We believe that the introduction of VEGFR antagonism into rodent studies represents an important step toward recreating the complex vascular anomalies found in the plexiform lesions of PAH patients.[14,15]

In recent years, the concepts used to discuss the pathobiology of severe forms of PAH have expanded to include stem and progenitor cells,[5,16] Tregs,[17] and microRNAs.[18] Accordingly, PAH pathogenesis concepts now include a cancer paradigm,[19] as well as a prominent role for chronic inflammation and autoimmunity.[3,17] The following review highlights these studies, and reviews concepts that have emerged from using this animal model (Fig. 1).

THE SU5416/CHRONIC HYPOXIA MODEL OF PULMONARY HYPERTENSION

The first batch of the orange, granular material, known as Su5416 or semaxinib, was provided to the Voelkel laboratory by Dr. Peter Hirth, then Chief Scientific Officer of Sugen, South San Francisco. The compound was originally

§Current convention generally favors referring to animal models of pulmonary vascular disease as models of pulmonary hypertension rather than models of PAH. The Su5416 animal model likely best replicates the significant occlusions of small-to-mid-sized pulmonary arterioles characteristic of PAH.

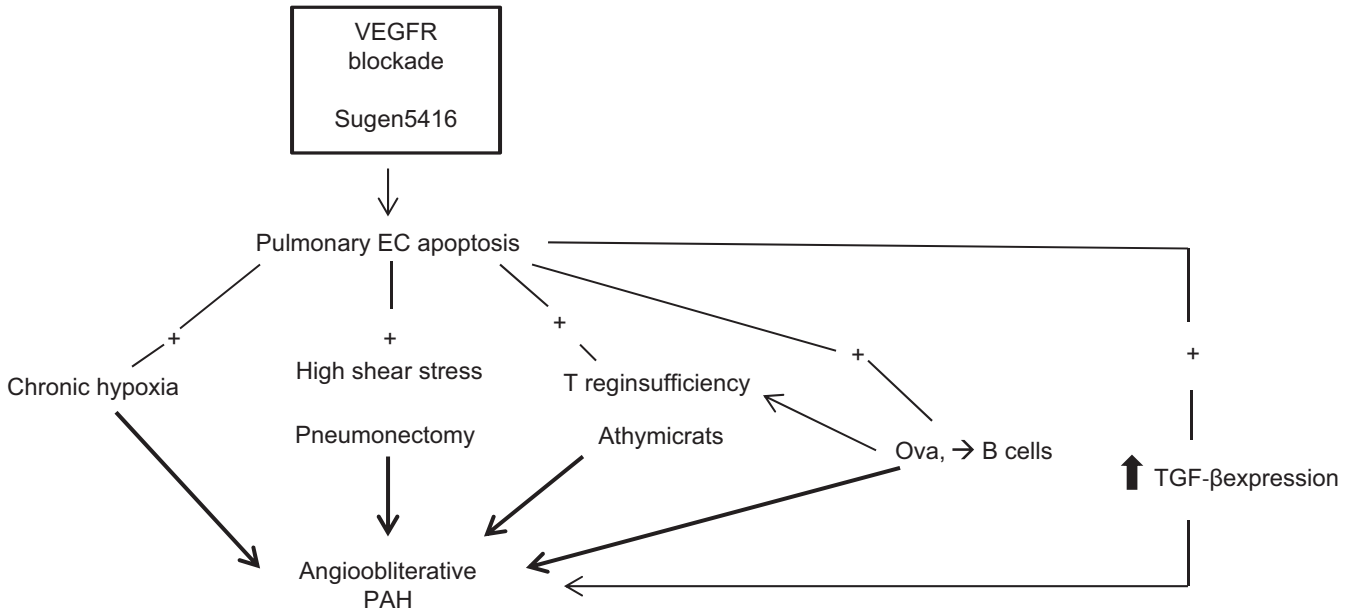


Figure 1: VEGF receptor blockade (subcutaneous injection of Su5416) is the "first hit" common to all models which cause lung vascular endothelial cell (EC) apoptosis. This initiating event in combination with chronic hypoxia, elevated lung vascular shear stress, regulatory T lymphocyte dysfunction, ovalbumin (OVA) immunization, or increased lung tissue expression of TGF-β causes severe angioobliterative pulmonary hypertension. Chronic hypoxia is not necessary as the "second hit."

identified in a high-throughput screening process as a powerful inhibitor of VEGF-dependent endothelial cell growth.[12] Su5416 is a small molecule inhibitor of the cytoplasmic tyrosine kinase segment of the VEGF receptors flt and KDR (VEGFR1 and VEGFR2)[12] and was developed as a drug for cancer treatment. When injected subcutaneously into adult rats, a single dose treatment caused lung endothelial cell apoptosis, emphysema, and a mild increase in the pulmonary artery pressure; pulmonary angiography documented a significant loss of lung vessels.[20] Concurrently, it was shown that Su5416 inhibited neovascularization of the cornea and angiogenesis stimulated by adipose tissue-derived stem cells. Su5416 affects the proliferation of hematopoietic progenitor cells,[21-23] blocks the VEGF- dependent growth of liver cysts,[24] and limits post-ischemic endothelial cell proliferation.[25] Jakkula and coworkers[26] showed that Su5416 inhibited angiogenesis and alveolarization in neonatal rats. Thus, several groups of investigators established that Su5416 caused endothelial cell apoptosis and inhibited endothelial cell proliferation. These findings prompted further investigation into use of this agent as a means to induce experimental pulmonary hypertension.

Because Su5416 had caused a mild elevation in pulmonary artery pressure in wild-type rats, the Voelkel/Tuder group tested whether Su5416 treatment combined with another known stimulus of pulmonary hypertension, chronic hypoxia, would cause more severe hemodynamic and vascular changes. The expectation at the time was that Su5416 and chronic hypoxic pulmonary vasoconstriction would be synergistic. Even still, the results of the Su5416/hypoxia combination experiments were surprising: The degree of right ventricular pressure overload was very severe, and the lungs were characterized by PAH-like vascular lesions not before observed in rats.[11,27] Subsequently, it was shown that the severe, angio-obliterative pulmonary hypertension was associated with right ventricular failure and that the degree of pulmonary hypertension linearly related to the number of fully or partially occluded pulmonary arterioles (Fig. 2).[28] This finding suggested that the reopening of obliterated lung vessels would be expected to reduce the pulmonary artery pressure and afterload of the right ventricle. Concomitant administration of the pan-caspase inhibitor Z-asp was subsequently shown to prevent lesion development and pulmonary hypertension, indicating that endothelial cell apoptosis was required for Su5416 to induce this disease.[11] Whereas neither Su5416-induced endothelial cell apoptosis nor hypoxic pulmonary hypertension were individually sufficient to cause angio-obliterative pulmonary hypertension, their combination was notably effective. In time, it became evident that chronic hypoxia was not required as the "second hit" for pulmonary hypertension induction. For example, chronic hypoxia is not necessary when left pneumonectomy is combined with Su5416 treatment, nor as described in detail below, when athymic rats receive Su5416.

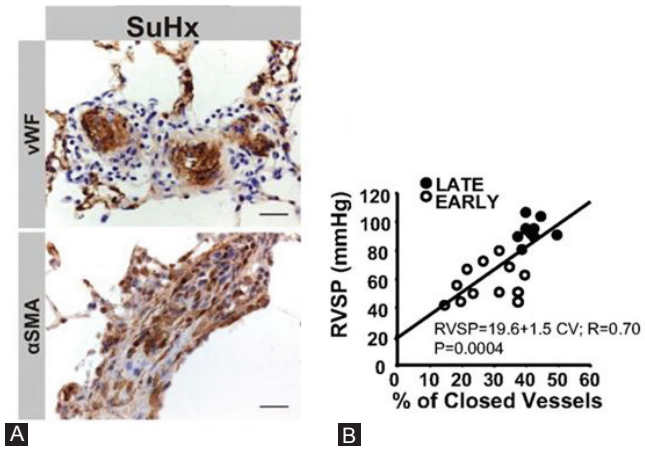


Figure 2: Panel **A** shows characteristic complete occlusions of arterioles in the lungs from rats with established severe Su5416/hypoxia induced pulmonary hypertension. Von Willebrand factor stains endothelial cells, α smooth muscle actin the smooth muscle cells. The model is characterized by a tight correlation between percentage of closed vessels (CV) and right ventricular systolic pressure (RVSP; Panel **B**). Adapted from references 28 and 47.

The Su5416/chronic hypoxia model proved to be a particularly robust model of pulmonary hypertension. Drugs, with demonstrated efficacy in other rodent models of pulmonary hypertension, such as calcium-entry blockers, ACE inhibitors, bosentan, cyclosporine, taxol, and methotrexate, had little effect in this model.[29] Thus, the Su5416/hypoxia model appeared, especially relevant for human PAH because the disease in the rat was severe, progressive and refractory to treatment as it is in most PAH patients. More recently, this model was also utilized to investigate mechanisms of right heart failure.[30]

PULMONARY HYPERTENSION IN ATHYMIC RATS

It has been known for decades that severe PAH is associated with autoimmune diseases, such as scleroderma, systemic lupus erythematosus, and mixed connective tissue disease, and that it also occurs in a relatively high incidence in virally-infected individuals such as in patients with HIV/AIDS.[31] In these clinical PAH conditions and in the Su5416/hypoxia model of pulmonary hypertension, pulmonary inflammation is particularly prominent (Fig. 3). Subsequently, we investigated, using the Su5416 model, whether this inflammation was directly contributing to pulmonary hypertension pathogenesis. An opposing but tractable

alternate hypothesis was that the inflammation was merely a byproduct of the high cardiovascular pressures and not important in pulmonary hypertension development. We originally hypothesized that animals lacking T-cells (athymic nude rnu/rnu rats) would actually exhibit less inflammation and therefore have only a mild elevation in pulmonary artery pressures. To our surprise, these animals developed significantly worse pulmonary vascular disease to the extent that exposing them to hypoxic conditions was not required for them to develop severe pulmonary hypertension. Paradoxically, inflammation was worse in these athymic animals; the inflammation consisting mainly of macrophages, B cells, and evidence of anti-endothelial antibodies[32] (Fig. 4).

In a follow up study utilizing inbred euthymic (T-cell replete) and athymic rats on the same genetic background, we were able to test the hypothesis that the lack of anti-inflammatory Treg activity in athymic rats is the reason why these T-cell deficient animals develop such significant inflammation in response to Su5416 and why pulmonary hypertension occurs in T-cell-deficient rats and not T-cell replete rats.[17] This study subsequently demonstrated that missing Treg activity does explain the predisposition

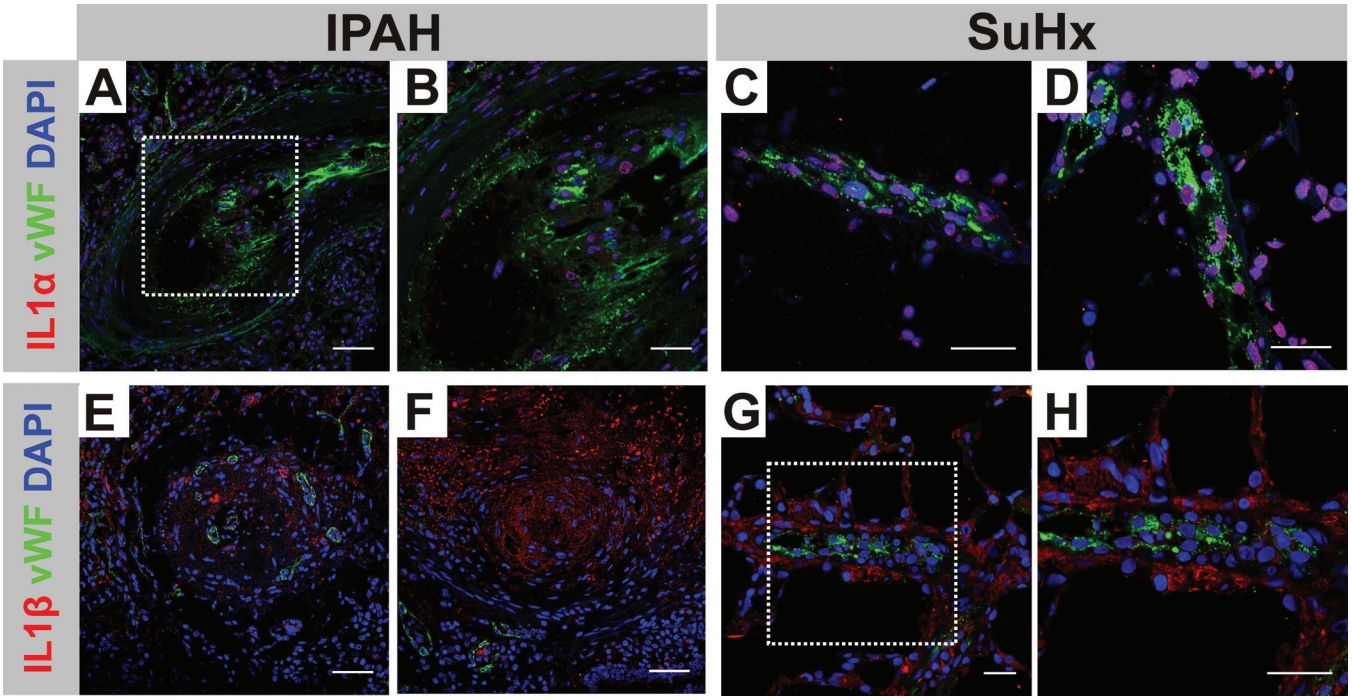
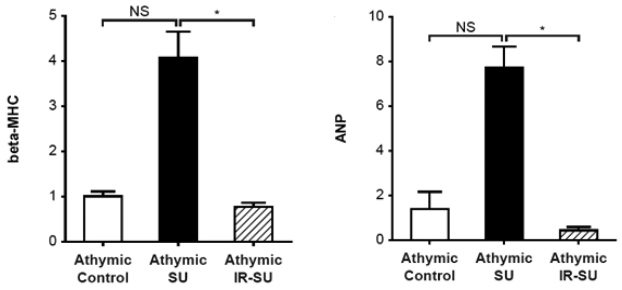


Figure 3: Juxtaposition of human lung vascular lesions from a patient with IPAH and lung vascular lesions from rats exposed to Su5416 and three weeks of chronic hypoxia. Interleukin 1α (IL-1α) is expressed in endothelial cells (green) and non-endothelial cells, predominantly in the nucleus (purple and red) **(A-D)**. Interleukin 1-β (IL-1β) shows mostly a cytoplasmic expression pattern of endothelial cells and non-endothelial cells **(E-H)**.IL-1β is abundantly expressed in the IPAH patient lesions. Of note, phenotypically switched endothelial cells may lose von Willebrand factor (vWF) staining. DAPI = nuclear stain.

	Athymic control (n=12)	Athymic SU (n=15)
mPAP (mmHg)	20.52 ± 11.82	65.31 ± 7.7*
LVEDP (mmHg)	7.15 ± 4.71	7.37 ± 6.43
RVEDP (mmHg)	4.8 ± 2.81	9.10 ± 4.39*
RV ESPVR (mmHg/RVU)	2.63 ± 1.77	5.23 ± 3.38
RV EDPVR (mmHg/RVU)	0.51 ± 0.51	0.98 ± 1.39

mPAP, mean pulmonary artery pressure; L(R)VEDP, left (right) ventricular end-diastolic pressure, RV ES(D)PVR, right ventricular end-systolic (diastolic) pressure volume relations. Values are means ± SEM. *p<0.05 vs athymic controls.

(A)



(B)

Figure 4: Right and left ventricular hemodynamic parameters in athymic control and athymic Su5416 rats at Day 21. **(A)** IR of athymic rats prevents activation in the right ventricle of the pathological fetal gene program. **(B)** Real-time PCR of right ventricular myocardial tissue for detection of pathologic β-MHC and ANP at Day 21.

backgrounds). Regulatory (anti-inflammatory) activity was most strongly exhibited in $CD4^+$ T-cells. Immune reconstitution of athymic rats with $CD4^+CD25^{hi}$ T-cells (obtained from major histocompatibility complex (MHC)-identical euthymic rats) that were also strongly forkhead box P3 (FOXP3) positive (i.e.,"classic" Tregs), effectively attenuated the development of pulmonary hypertension; of some interest, but with precedence in rat tolerance studies, $CD4^+CD25^-$ cells injected into athymic rats also exhibited regulatory activity in preventing disease. This study also presented evidence that this latter T-cell population may have been converted to classic Tregs in vivo. 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 a mechanistic explanation of why patients with autoimmune diseases or viral infections, who also have abnormal Treg activity and immune dysregulation, are possibly predisposed to developing PAH following vascular injury (reviewed in Voelkel et al.[34,35])

SEVERE PULMONARY HYPERTENSION IN THE SU5416/OVALBUMIN MODEL

A series of carefully-conducted experiments in mice by Daley et al.[36] demonstrated that repeated immunization of mice with OVA 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. Because like hypoxia, OVA immunization has been shown to increase the lung levels of HIF-1α protein,[37] we hypothesized that a combination of OVA immunization with VEGFR blockade-induced lung cell apoptosis would produce severe angio-obliterative pulmonary hypertension. Mice treated with Su5416 and immunized with OVA did not develop pulmonary hypertension (unpublished data). As noted above, the issues and problems of mouse models of pulmonary hypertension have been recently reviewed[13]). However, when wild type Sprague-Dawley rats were subjected to combined Su5416/OVA treatment, severe pulmonary hypertension developed, and 20% of the animals died after eight weeks from right ventricular failure.[38] The lung tissue expressed high levels of IL-6, especially in the endothelial cells of the pulmonary arteriolar lesions (Fig. 5).Unexpectedly, depletion of B cells using an anti-rat CD20 antibody (Genentech) prevented both pulmonary vascular remodeling and an increase in pulmonary artery pressure, indicating that B cells are necessary for the development of pulmonary hypertension in this Su5416/OVA model.[38]

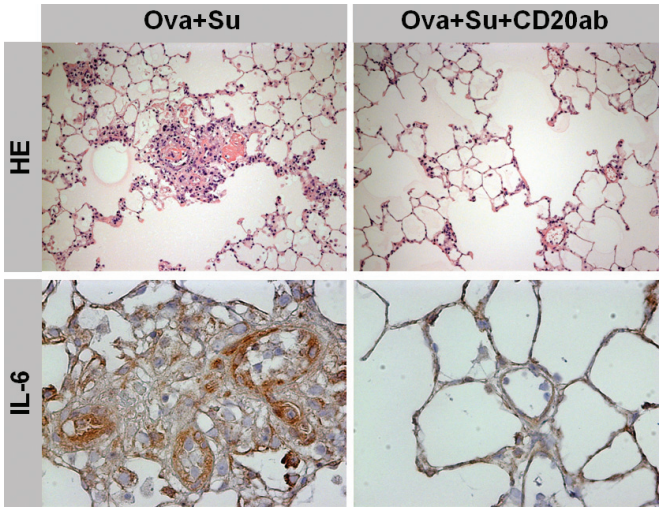


Figure 5: Histology and immunohistochemistry of lung tissue samples harvested from rats immunized with ovalbumin (Ova) and treated with Su5416 (left) or additionally with an antibody directed against rat B-lymphocytes (right). HE = hematoxylin eosin, IL-6 = interleukin 6.

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

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 (adenovirus) AdTGF-β1 related experimental lung fibrosis.[39] When the AdTGF-β1 model of lung fibrosis and pulmonary hypertension was combined with a single infection of the VEGFR inhibitor Su5416, the results were (a) obliteration of small pulmonary arteries with von Willebrand $Factor^+$ cells, (b) a further reduction of lung capillarization, and (c) a much more severe increase in pulmonary artery pressure.[40,41] Although anti-angiogenic tyrosine kinase inhibitors are currently investigated as a potential therapy for idiopathic pulmonary fibrosis, the combination of lung tissue overexpression of active TGF-β1 and Su5416 did not prevent the development of lung fibrosis, but instead increased the fibrotic activity and pulmonary angioproliferation found in this AdTGF-β1/Su5416 model. The degree of pressure overload is usually mild when pulmonary hypertension develops in patients with idiopathic pulmonary fibrosis, and angioproliferative lesions are rarely seen in these patients. However, there is a subset of these patients who develop severe pulmonary hypertension. The AdTGF-β1/Su5416 may be used to model the disease of this subset of patients with idiopathic pulmonary fibrosis and severe pulmonary hypertension.

TRANSLATION OF SU5416 BASED RAT MODELS OF PULMONARY HYPERTENSION TO HUMAN PAH

When the Su5416/hypoxia model of severe angioproliferative pulmonary hypertension was originally published in 2001[11] a question was raised about how a drug used as an antiangiogenic therapy in cancer[12] could paradoxically cause angiogenesis (albeit deregulated angiogenesis) in the lungs of laboratory rats. In this process, endothelial apoptosis, followed by compensatory but uncontrolled endothelial hyperproliferation, may lead to pulmonary arteriolar occlusion. In the development of human PAH, lung endothelial cell apoptosis may be triggered by (viral) infections, anti-endothelial cell antibodies, cytotoxic lymphocytes, or toxins.[42] This is modeled in the rat models by VEGF inhibition which also induces endothelial cell apoptosis.[43] In the 1990s, it was believed that VEGF was primarily produced and released from epithelial cells and macrophages to act on endothelial cells. Subsequently, an autocrine role for VEGF synthesized by endothelial cells and acting on endothelial cells was described.[44,45] Blockade of this autocrine VEGF signaling causes endothelial cell apoptosis.[43] While the initial trigger for endothelial cell apoptosis in the Su5416/hypoxia model is clearly VEGFR inhibition, there is to date no evidence to suggest that some forms of human PAH, at any stage of disease development, are also dependent on the inhibition of VEGF signaling. The exception to this rule is possibly the occurrence of pulmonary hypertension in patients treated with the tyrosine kinase inhibitor dasatinib.[46]

Peter Carmeliete has used the term 'escape angiogenesis' for angiogenesis that occurs and is perhaps triggered after anti-angiogenic drug therapy (Personal communication at Keystone Conference of Pulmonary Hypertension, Sept. 2012). This term may not only describe the lung vascular obliterative cell growth we observe in the SuHx model, but also the unexpected occurrence of pulmonary hypertension after tyrosine kinase inhibitors."

Assuming that lung microvessel endothelial cell apoptosis is a key initiating event in human and experimental pulmonary hypertension, other mechanisms are required to explain subsequent endothelial proliferation, phenotypic switching, and progressive angio-obliteration. Using the combination of VEGFR blockade and increased flow (shear stress) to challenge endothelial cells in an artificial capillary system, Sakao et al. showed that the initial endothelial cell apoptosis was followed by cell proliferation and, as in the rat model described above, could be blocked by a pan-caspase inhibitor.[38,47] One possible explanation for this experimental result is that endothelial cell growth in the context of VEGFR inhibition depends on signals which are not routed through the VEGF receptors VEGFR1 and VEGFR2. Subsequent to the clinical introduction of VEGF receptor blocking drugs for the treatment of colon and breast cancer,[48,49] it had become clear that in spite of VEGFR blockade, or because of it, other growth factors could conceivably dominate the control of endothelial cell growth (e.g., Fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and others[50,51]) and that a compensatory apoptosis-dependent cell proliferation ensues.[52,53] Indeed, some of these growth factors are highly expressed in the Su5416/chronic hypoxia rat lung (Fig. 6). As we have recently shown, their expression, as well as the associated angio-obliteration, require the presence of thyroid hormones[54] and sufficient amounts of dietary copper.[55] The role of thyroid hormones is intriguing when considering the copresentation of PAH and thyroid diseases. Whereas up to 30% of all patients with PAH have a hypothyroid disorder,[56] postulated to be the consequence of an autoimmune thyroiditis, echocardiography now detects a large number of patients with hyperthyroidism and concomitant pulmonary hypertension.[57] Because thyroid hormones are angiogenic,[58] we subjected rats with a subtotal thyroidectomy to the Su5416/hypoxia protocol. While thyroidectomized rats did not develop angio-obliterative pulmonary vascular lesions,[54] implanting a slow-release pellet of thyroxin (T4) in thyroidectomized animals did allow angio-obliterative pulmonary hypertension to develop.

In addition to the influence of other growth signals on endothelial cells, it is possible that in the Su5416-based

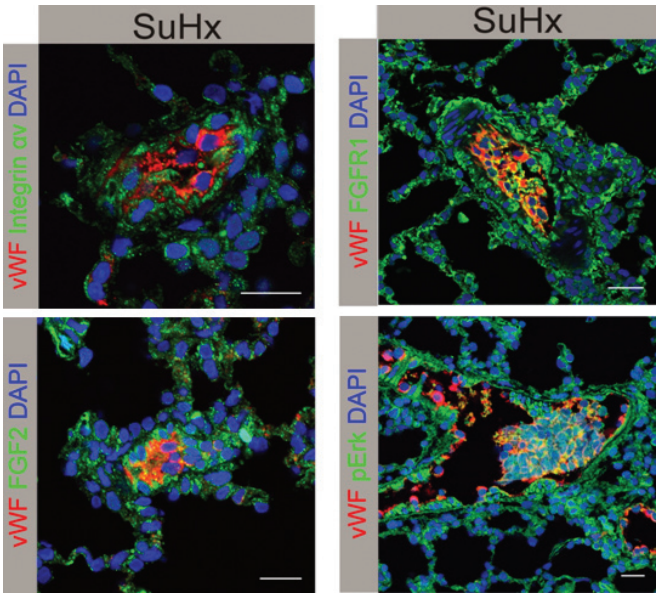


Figure 6: Immune histochemistry (triple labeling) of lung vascular lesions. Obliterated arterioles express the integrin αvβ3, FGF receptor 1 and phosphorylated Erk (pErk). Adapted from reference 46.

pulmonary hypertension models there are still ways for VEGF to stimulate endothelial cell growth, despite the presence of VEGFR blockade. First, illustrated in Figure 7, VEGFA protein can bind directly to the integrin $\alpha v\beta_3$(expressed on the cell surface of activated endothelial cells) and initiate signaling.[59] Second, the VEGF C isoform, commonly associated with lymph vessel growth, may bind and activate VEGFR3 (flt4) to stimulate endothelial cell growth.[60,61] Third, VEGF can activate transcription via nuclear VEGF receptors.[62,63] VEGF receptor blockade also affects the bone marrow,[64] and this inhibition of tumor-derived endothelial cell progenitors' maturation into endothelial cells could conceivably contribute to disease progression.[65] It is possible that, in the Su5416-based rat models of severe pulmonary hypertension, interactions between the injured lung and the bone marrow are impaired-again including bone marrow-derived stem cell maturation, and that immune responses are becoming unbalanced.

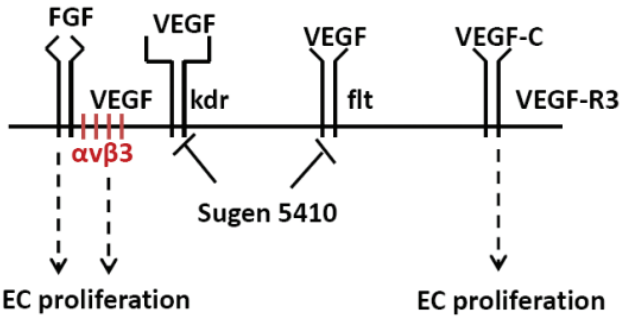


Figure 7: VEGF blockade may together with hypoxia induce a pro-tumorigenic, inflammatory state. Hypoxia renders, via HIF-1α, EC responsive to angiogenic signals. Hypoxia releases SDF-1, recruits bone marrow-derived-CXCR4+ cells and precursor cells, and increases VEGF protein expression. When the intracellular tyrosine kinases of the VEGF receptors VEGFR1 (flt) and VEGF receptor 2 (kdr) are blocked, the VEGF ligand CEGFC can bind to VEGFR3 (flt4). Both FGF and VEGF can bind to the cell membrane integrin αvβ3.Lastly, there may be VEGF-R intracellular signaling - independent of tyrosine kinase activation via nuclear receptors.

Table 1: Gene and protein expression levels in lung and heart tissue lysates in experimental angioobliterative pulmonary hypertension

Name	Lung	Reference	Right ventricle	Reference
a-MHC			mRNA: Down	Bogaard, et al.[30] Tamosiuniene, et al.[17]
Ang1			mRNA: Down/Protein unaffected	Drake, et al.[67]
ANP			mRNA: Up	Drake, et al.[67] Tamosiuniene, et al.[17]
Apelin			mRNA: Down/Protein down	Drake, et al.[67]
a_v-integrin	Protein: Up	Bogaard, et al.[55]		
b_3-integrin	Protein: Up	Bogaard, et al.[55]		
b-MHC			mRNA: Up	Bogaard, et al.[30] Tamosiuniene, et al.[17]
BNP			mRNA: Up	Drake, et al.[67] Tamosiuniene, et al.[17]
Caspase 3 (cleaved)	Protein: Up	Bogaard, et al.[55]		
Col1a1			Protein: Up	Bogaard, et al.[30]
CTGF			mRNA: Up	Drake, et al.[67]
FGF2	Protein: Up	Al Husseini et al.[54]		
HIF-1a	Protein: Up	Unpublished	Protein: Up	Bogaard, et al.[30]
HO1	Protein: Up	Al Husseini et al.[54]	Protein: Down	Bogaard, et al.[30]
IGF1			Protein: Down	Drake, et al.[67]
IL-1a	IHC: Up	Unpublished		
IL-1b	IHC: Up	Unpublished		
MCIP-1			mRNA: Up	Bogaard, et al.[55]
OPN-1			mRNA: Up	Bogaard, et al.[30]
p-AKT	Protein: Down	Taraseviciene-Stewart et al.[11]	Protein: Down	Drake, et al.[67]
	Protien: Up	Al Husseini et al.[54]		
PCNA	Protein: Up	Bogaard, et al.[55]		
p-ERK	Protein: Up	Al Husseini et al.[54]		
PPAR-g	IHC: Down	Ameshima et al.[15]	mRNA: Unchanged	Unpublished
Rcan1			mRNA: Up	Drake, et al.[67]
Serca 2a			mRNA: Down	Tamosiuniene, et al.[17]
Src	Protein: Down	Taraseviciene-Stewart et al.[11]		
Survivin	Protein: Up	Bogaard, et al.[55]		
VEGF-A	Protein: Up	Unpublished	mRNA: Down/protein down	Bogaard, et al.[30]
VEGFR1	Protein: Down	Taraseviciene-Stewart et al.[11]	mRNA: Down	Bogaard, et al.[30]
VEGFR2	IHC: Up	Abe et al.[24]	mRNA: Down	Bogaard, et al.[30]
miR-133a			Down	Drake, et al.[67]
miR-139_3p			Down	Drake, et al.[67]
miR-208			Down	Bogaard, et al.[30]
miR-21			Down	Drake, et al.[67]
miR-34c			Down	Drake, et al.[67]

SUMMARY AND CONCLUSION

The Su5416/hypoxia model is the first new rat model of severe angio-obliterative pulmonary hypertension since Botney et al. reported that the combination of monocrotaline and pneumonectomy had caused obliterative pulmonary vascular remodeling.[66] The Su5416/hypoxia rat model is likewise a "second hit" model in that there are many hallmarks of the severe human PAH which are shared by this rat model. Perhaps the most salient features of the Su5416/hypoxia model are the plexiform-like lung vascular lesions, the development of right ventricular failure,[27,28] and the linear relationship between the degree of pressure overload and the number of occluded lung vessels. Table 1 provides an overview of the genes and proteins which are differentially expressed in both the lungs and the right ventricle from rats with established angio-obliterative PAH, when compared to the corresponding tissues from normal control rats. Like PAH patients, Su5416/hypoxia rats with established severe pulmonary hypertension are exercise-intolerant and respond to acute prostacyclin infusion with peripheral vasodilation and sudden death (unpublished observations). Su5416 causes severe pulmonary hypertension and right ventricular failure in T-cell deficient athymic rats housed in normoxic conditions; furthermore, cardiopulmonary disease is attenuated by immune reconstitution with Tregs prior to Su5416 administration.[17] Su5416 treatment causes extensive lung vascular endothelial cell apoptosis and likely impairs immune responses.[48,68] Immunization of rats with Ova potentiates Su5416-induced vascular injury with a unique inflammatory profile and causes lethal pulmonary hypertension. Further, this model of PH is attenuated by B cell depletion. Finally, thyroidectomy ablates Su5416 induction of angio-obliterative disease, which suggests a potentiating role for thyroid hormones in the experimental model. In summary, chronic hypoxia, immune dysregulation, inflammatory skewing, and endocrine contributors all synergize with VEGF signaling antagonists to cause severe pulmonary hypertension. Hopefully, ongoing investigations with these evolving models can be used to better discriminate unique mechanisms of disease uniquely applicable to distinct forms of clinical PAH.

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