S
ince its first description,1,2 pulmonary arterial hypertension (PAH) has fascinated clinicians and scientists, and, despite recent progress, it remains an intriguing and mysterious disease. An impressive array of molecular abnormalities has been described over the past few years and is summarized in recent reviews.3–8 Generally, these abnormalities result in an obliterator remodeling of the pulmonary circulation, characterized by occlusion of the lumen in medium-sized and small pulmonary arteries (PAs) due to excessive cellular proliferation in the vascular wall and in situ thrombosis, as well as loss of microvessels and capillaries (Figure 1A). The afterload of the right ventricle increases, resulting in right heart failure and premature death.9 However, the relevance to human disease and the translational potential of several of those described abnormalities remain unclear. Only 3 abnormalities have been translated into clinical practice: the downregulation of the prostacyclin axis (reversed by exogenous prostacyclin analogues), the downregulation of the NO/cGMP axis (reversed by inhaled NO and sildenafil), and the upregulation of the endothelin axis (reversed by endothelin receptor antagonists).10 All of these therapies were developed on the basis of the “vasodilator” hypothesis of pulmonary hypertension. Despite the realization that PAH is not a disease of vascular tone (<10% of patients respond acutely to vasodilators), these therapies continue to dominate the field, although some of them were also, in retrospect, found to have some antiproliferative properties. Furthermore, these therapies were all designed and originally developed for nonpulmonary vascular diseases.

The parenteral use of prostacyclin analogues can significantly slow down the progression of the disease, but this is limited by their lack of specificity for pulmonary vessels, difficulty in administration, and many serious adverse effects.10 Oral therapies for PAH such as endothelin receptor antagonists (eg, bosentan) and phosphodiesterase inhibitors (eg, sildenafil) may improve symptoms and the quality of life of PAH patients, but it remains to be determined whether they improve survival. The overall improvement in hemodynamics is minimal, and the functional capacity improvement with these therapies is similar to improvements achieved by exercise training alone.10,11 The ease of delivery of these therapies contributes to their heavy use. The emerging idea that the next breakthrough in management of PAH may be a combination of existing therapies is of concern if this in any way impedes the development of new testable ideas. An urgent need exists for experimental therapies that address mechanisms intrinsic to PAH pathology. Such experimental therapies with high translational potential need urgent evaluation in phase I and II clinical trials.

This review is dedicated to discussing emerging concepts in the molecular biology of PAH that have a strong translational potential and near clinical applicability. We propose a framework on which current experimental therapies can be developed further and one that might facilitate the design, development, and application of new therapies (Figure 1B). Recent evidence suggests that the proliferative and antiapoptotic environment in the vascular wall of medium and small PAs shares common features with neoplasia. On the other hand, the loss of endothelial cells and microvessels has features of a degenerative disease. Circulating and vascular inflammatory cells and mediators also appear to play an important role in the pathology of PAH.

Furthermore, new therapies ideally have to be selective to the pulmonary circulation; for this, the unique features of the pulmonary circulation have to be better understood because they will likely provide targets for such PAH-specific therapies. For example, hypoxic pulmonary vasoconstriction might provide therapeutic targets that are unique to the pulmonary circulation.12 Although most systemic vessels dilate to hypoxia, the pulmonary circulation contracts in order to maintain ventilation-perfusion match. Mitochondria have emerged as important oxygen sensors in a variety of oxygen-sensing systems in the body,13 and important differences have been shown between the PAs and systemic arteries smooth muscle cell (SMC) mitochondria.14 Mitochondria can regulate vascular tone via the production of diffusible activated oxygen species and their effects on redox-sensitive membrane K+ channels,12 and they are also important regulators of apoptosis. Therefore, as discussed below, targeting PASMC mitochondria might offer selectivity, sparing systemic arter
ies, and effectiveness in reversing the PA remodeling in PAH. A PA-specific approach is important in the development of future therapeutic strategies in PAH.

Until the 1990s, the focus of PAH therapeutics was on vasodilators. More recently, interest has been growing in antiproliferative strategies. In this review, we propose to extend this shift in thinking to include proapoptotic, regenerative, and anti-inflammatory strategies.

The Neoplasia Model: Increased Proliferation and Suppressed Apoptosis in the Vascular Wall

Endothelial dysfunction and damage are well recognized in PAH and result in an imbalance of vasoconstrictors (eg, endothelin, thromboxane, serotonin) versus vasodilators (eg, prostacyclin, NO), favoring the former. All of these well-characterized abnormalities eventually result in an increase in PASMC \([Ca^{2+}]\). In addition to vasoconstriction, the increase in \([Ca^{2+}]\) also results in increased PASMC proliferation. The endothelial damage and the increased proliferation of cells within the vascular wall are central features of the pathology of PAH in both clinical and animal models, and in that sense this model also resembles the “vascular injury” model of disease (Figure 1).

However, increased proliferation alone cannot explain vascular remodeling. Cells have the ability to block excessive proliferation in order to avoid mismatch of fuel supply and demand, resulting in oxidative stress and genetic damage. One of the ways this can be achieved is by activation of apoptosis, when cells inappropriately enter phases in the cell cycle associated with division or growth. The same is true in cancer, in which most modern anticancer therapies aim to activate apoptosis, in addition to or instead of inhibiting proliferation. Over the past few years, a number of experimental therapies have been shown to reverse established PAH in animal models by inducing apoptosis in the vascular wall of remodeled PAs (Figure 2). These include diverse therapies such as elastase inhibitors, epidermal growth factor (EGF) receptor inhibitors, simvastatin, dichloroacetate, sildenafil, cyclosporine, and imatinib. Despite the very different mechanisms of these therapies, the fact that all increase PASMC apoptosis suggests that the apoptosis regulation that occurs distally in diverse signaling pathways plays an important role in the pathogenesis of PAH. Evidence
exists that both receptor-mediated and mitochondria-induced apoptosis are suppressed in PAH.

**Receptor-Mediated Apoptosis in PAH**

The recently discovered loss-of-function mutations in the bone morphogenetic protein receptor II (BMPRII) gene and abnormalities in the Smad signaling downstream of the receptor in familial PAH and in ~10% of patients with idiopathic PAH (iPAH) lead to suppressed apoptosis in PASMCs.30–33 Interestingly, similar mutations in BMP receptors and their transforming growth factor receptor superfamily are also linked with familial colon cancer syndromes.34 Intriguingly, the same germline mutations were recently shown to cause increased apoptosis in PA endothelial cells (PAECs).35,36 The translational potential of this discovery remains unclear because of the complexity and the multiple feedback loops in the BMPR downstream signaling. For example, augmentation therapy, in which exogenous normal BMPRII is delivered via gene therapy in rat PAH, does not improve PAH.37

**Mitochondria-Induced Apoptosis in PAH**

Recent evidence suggests that a mitochondrial remodeling also contributes to apoptosis resistance in PASMCs from several animal models of PAH as well as clinical specimens. This remodeling is evident by the significant mitochondrial hyperpolarization that characterizes PAH PASMC mitochondria compared with those from healthy PASMCs.26,38,39 The mitochondrial membrane potential is a surrogate for mitochondrial function because its regulation is directly linked to respiration, oxidative phosphorylation, and energy production.40 The efflux of proapoptotic mediators from mitochondria (like cytochrome c or apoptosis-inducing factor) is associated with mitochondrial depolarization; thus, the hyperpolarization of mitochondria in PAH indicates resistance to apoptosis. Interestingly, mitochondria are also hyperpolarized in many cancers compared with noncancerous cells.42,43 The cause of this hyperpolarization remains unknown. In cancer, evidence exists that this is associated with an active suppression of mitochondrial function, with a shift of energy production from mitochondrial-based oxidative phosphorylation to cytoplasm-based glycolysis.42 A glycolytic phenotype (the basis of the Warburg effect in cancer) is associated with resistance to apoptosis, and many key enzymes of glycolysis are also involved in inhibition of apoptosis.44,45 Therefore, the cancer cells (or the PAH PASMCs) might suppress mitochondrial function because this also suppresses apoptosis and gives them a proliferative advantage. This is turn makes them dependent on the energetically less efficient glycolysis for energy production (2 ATP production per molecule of glucose versus 36 ATP with oxidative phosphorylation). To compensate, cancer cells upregulate glucose uptake, which explains why cancer can be detected so easily with 2-[18F] fluoro-2-deoxy-d-glucose/positron emission tomography scan imaging. This has not yet been studied directly in PAH, but it is intriguing that the PAs of PAH patients in vivo also have a very high glucose uptake when studied by positron emission tomography imaging of the lung, mimicking cancer.46 Further support of the emerging idea that metabolism is involved in the pathogenesis of PAH comes from the recent suggestion that insulin resistance and low plasma adiponectin levels might be risk factors in PAH; male apolipoprotein E knockout mice fed a high-fat diet developed PAH, which was reversed by the clinically used rosiglitazone, an activator of peroxisome proliferator-activated receptor γ.47

The cause of the metabolic/mitochondrial remodeling in cancer is multifactorial, but several key molecules and transcription factors involved in metabolism and carcinogenesis are directly involved, including AKT, myc, HIF, PTEN, and p53 (reviewed in Pan and Mak44). Many of these pathways should also be studied in PAH and might open several novel therapeutic windows.

**Caspases and K+ Channels in PAH**

Evidence also exists that caspases, the executors of apoptosis, are directly inhibited in PAH. Intracellular K+ is a direct inhibitor of caspases in many cell types, including neurons, cancer, and PASMCs.48 The voltage-gated K+ channels (Kv) are downregulated in every available model of animal and human PAH26,39,42,49–51; this limits the normal outward flux of K+ down its concentration gradient,48 increasing the intracellular K+ and thus suppressing caspases.

It remains unknown whether the changes in mitochondria and K+ channels are coordinated, but recent findings suggest that they might be. For example, NFAT (nuclear factor of activated T cells52), a transcription factor that regulates T-cell activation, cardiac development/hypertrophy, and vascular remodeling, was recently shown to be activated in PAH in vitro and in vivo.28 NFAT is known to regulate the expression of several mitochondrial enzymes, Kv1.5 and bcl-2 (which is upregulated in human PAH53 and inhibits apoptosis by interacting with the mitochondrial membrane). Inhibition of NFAT reverses the PAH phenotype (upregulating Kv1.5, downregulating bcl-2, and depolarizing mitochondria), induces PA wall apoptosis, and improves established rat PAH. NFAT is also activated in cancer, and its inhibition in cancer cells has exactly the same effects as its inhibition in PAH.42 Direct and strong evidence now exists, with immediate clinical implications, of similar mechanisms in neoplasia and PAH. The following anticancer therapeutic strategies have been shown to reverse established PAH.

**Epidermal Growth Factor Receptor Inhibitors**

The prosurvival signaling in PAH PASMCs can be driven by many growth factors. In addition to the direct exposure of PASMCs to growth factors from which they are normally protected (for example, PASMCs are exposed to circulating platelet-derived growth factor [PDGF] when the protective layer of endothelial cells is damaged), growth factor signaling is also activated within the interstitial matrix. The activated serine elastases23 within the PA wall can directly activate EGF receptors.24 Therefore, inhibition of EGF signaling might mimic inhibition of serine elastases; this is important because, although elastase inhibitors are not clinically available, EGF inhibitors and anti–EGF receptor antibodies are clinically used in oncology.34 Indeed, PICIN166, which inhibits phosphorylation and activation of the EGF receptor, reverses vascular remodeling, activates PASMC apoptosis,
and decreases PA pressure and survival in rats with established PAH. PCI166 has been shown to decrease tumor growth in vivo and has significant translational potential in PAH because it is an orally available small molecule. Much of the proapoptotic effects of EGF receptor inhibition are mediated by negative downstream effects on AKT, an oncoprotein that induces a glycolytic phenotype and directly inhibits mitochondrial-dependent apoptosis.

Metabolic Modulators (Dichloroacetate)

In that sense, intriguing similarities are present with dichloroacetate, another orally available small molecule that has been shown to inhibit both cancer growth and established PAH. Through a different pathway, dichloroacetate also activates mitochondria-dependent apoptosis, reversing vascular remodeling, normalizing hemodynamics, and improving survival in several models of rat PAH. Dichloroacetate inhibits mitochondrial pyruvate dehydrogenase kinase and thus increases the influx of pyruvate into the mitochondria, promoting glucose oxidation over glycolysis. Dichloroacetate reverses mitochondrial hyperpolarization in PASMCs from patients and rats with iPAH, reverses Kv channel downregulation by a mechanism that involves an increase in complex I–produced activated oxygen species and NFAT, and opens the mitochondrial transition pore, allowing the efflux of proapoptotic mediators. Intriguingly, dichloroacetate, by exactly the same mechanism, inhibits cancer growth in vitro and in vivo in several human cancer cell lines and xenotransplant models (Figure 3). Dichloroacetate has been used for >30 years in the treatment of the lactic acidosis that complicates congenital mitochondrial diseases, and phase I to II clinical trials in cancer are in progress. Dichloroacetate, a generic drug, should also be tried in human PAH.

Survivin Inhibitors

Survivin is an “inhibitor of apoptosis protein,” previously thought to be expressed only in cancer cells in adult animals. Survivin was also recently found to be expressed in the PAs of patients with PAH and rats with monocrotaline-induced PAH but not in normal PAs from rats or humans. Gene therapy with inhalation of an adenovirus carrying a dominant-negative construct reverses established rat PAH. Endogenous survivin inhibition improved vascular remodeling, hemodynamics (without affecting systemic arterial pressure), and survival. Both in vitro and in vivo, inhibition of survivin induced PASMC apoptosis, decreased proliferation, depolarized mitochondria, caused efflux of cytochrome c and apoptosis-inducing factor in the cytoplasm, and increased Kv current; the opposite effects were observed with gene transfer of wild-type survivin, both in vivo and in vitro. Early phase clinical trials of survivin inhibitors in oncology are currently taking place, and application of these therapeutic strategies to PAH is indicated.

PDGF Inhibitors

Survivin induces the expression of PDGF receptors, and PDGF itself has also been shown to induce survivin expression, suggesting a strong positive feedback loop. As in cancer, increased expression of PDGF receptors has been shown in human and rat PAH. The PDGF inhibitor imatinib (Gleevec), in clinically relevant doses, reverses established monocrotaline- and hypoxia-induced rat PAH. As with all the aforementioned therapies, imatinib induced apoptosis and decreased proliferation in the PA media, reversed vascular remodeling, and improved hemodynamics and survival. Remarkably, imatinib was also shown to dramatically improve a case of severe PAH, in which the patient was failing with maximal medical therapy. Clinical trials with imatinib in PAH are now under way.

The Degenerative Disease Model: Increased Apoptosis and Loss of Microvascular Endothelial Cells

Vascular remodeling in PAH eventually decreases perfusion of lung tissue. This is a direct consequence of lumen obliteration of PAs due to excessive growth of cells in the...
media and neointima, distal muscularization, and, to a lesser degree, vasoconstriction. It has been suggested that, in addition to these changes, loss of distal arteries and capillaries occurs in PAH. There has been controversy surrounding this vessel loss, fueled, at least in part, by weaknesses in the methodology used. For example, classic methods like barium-gelatin perfusion of vessels or contrast-based microcomputed tomographic imaging are limited by the fact that intrinsic vascular tone and proximal lumen obliteration will limit the perfusion of contrast to distal vessels, giving a false impression of vessel loss.

However, compelling evidence has recently appeared that loss of distal vessels does take place in PAH, and this has been documented in the monocrotaline model along with direct evidence that this is likely due to increased apoptosis of endothelial cells or pericytes (as opposed to the suppressed PASMC apoptosis) further supports this idea. It is generally agreed that endothelial dysfunction and injury is 1 of the earlier (if not the earliest) abnormalities in PAH. This dysfunction (eg, genetic predisposition, direct endothelial injury by environmental toxins, viral infection, increased shear stress in left-to-right shunts) might in fact result in endothelial cell loss and loss of microvessels. The diversity of distal PA versus proximal PAECs provides some biological basis for the localization of this response within the distal vessels. The increased angiogenesis that has been shown in clinical specimens and animal models might represent an attempt for repair. It appears, however, that often this repair is suboptimal, resulting in dysregulated vascular networks (plexiform lesions), resembling the abnormal angiogenesis that takes place in tumors.

It is possible that these changes in distal vessels follow, or are at least exaggerated by, the proximal vessel remodeling, resulting in decreased flow, although increased flow and shear stress can also directly induce vascular structural changes. For example, in right-to-left shunts, the proximal vessel remodeling precedes the development of plexogenic lesions; also, when a lung with monocrotaline PAH is transplanted to a normal rat in order to completely normalize perfusion of the distal vessels, this distal remodeling normalizes, and apoptosis can no longer be detected. Whether primary or secondary, the loss of distal vessels and the dysregulated attempt to repair it increase the pulmonary vascular resistance in PAH. In the monocrotaline model, microvasculature regeneration is possible with the use of endothelial precursor cells (injected directly to the pulmonary vasculature in PAH. The fact that these cells can be selectively and directly infused in the pulmonary circulation through the jugular vein and “trapped” in the lung microvessels, where they are expected to regenerate the vasculature, makes this approach in humans feasible and relatively selective. In fact, Wang et al have reported encouraging preliminary results in 25 PAH patients, in whom injection of endothelial precursor cells (taken from the peripheral blood) resulted in both hemodynamic and functional improvement in PAH. This was the first report of gene- or cell-based therapy ever attempted in human PAH. Dr Stewart’s group is also conducting a human trial, translating the protocol followed in their rat model, reporting encouraging preliminary results (personal communication, Duncan J. Stewart, MD, 2008).

### The Inflammatory Disease Model: Generalized and Vascular Inflammation

Despite strong evidence that inflammation is heavily involved in PAH, this is the least studied aspect of the disease. Several forms of PAH (collagen vascular disease related or HIV related) are characterized by generalized activation of inflammation. It is interesting that these are also the subgroups of PAH with the worst prognosis.

Several types of inflammatory cells including activated T cells, B cells, and macrophages have been documented to infiltrate the PA wall in iPAH (Figure 5). Whether the infiltrating inflammatory cells play a primary role in PAH, represent a reaction to viral factors, or represent a nonspecific reaction to vascular injury remains unknown. A number of interleukins and cytokines that can serve as chemoattractants
for these inflammatory cells are also present in the PAH vessel wall and plasma.67,72–74

One of the most fascinating developments in the field is the proof of direct involvement of viruses in the pathology of PAH. Viral proteins can affect vascular remodeling through direct effects on vascular wall cells and extracellular matrix75 or indirectly through the dysregulation of inflammation and immunity. The HIV Nef (negative factor) was recently found in endothelial cells from patients with HIV PAH.76 Nef can affect several signaling pathways: prosurvival, proapoptotic, and proangiogenic.77,78 Human herpesvirus 8 (the Kaposi-associated herpesvirus, which carries several inflammatory, proangiogenic, and proproliferative genes79) has also been found in human plexogenic lesions.80

Similar findings have been reported in circulating inflammatory cells, which show activation of NFAT, a key transcription factor that regulates the activation of T cells and the expression of many inflammatory mediator and cytokine genes.28,52 In addition, the levels of NFAT mRNA in the buffy coat of a small cohort of patients with PAH were higher than those in patients with secondary pulmonary hypertension or normal controls; in fact, they were the highest in a subgroup of PAH patients with scleroderma.28 This suggests that, if confirmed in larger cohorts, this master regulator of T cells might even be a potential PAH biomarker. CD3-positive cells with activated NFAT are also found in the PA wall in patients with PAH but not in normal patients. It is intriguing, however, that NFAT is also activated in PASMCs in PAH in vivo and in vitro but not in normal PAs and PASMCs (Figure 5). In addition, inhibition of NFAT by VIVIT (a competing peptide) or cyclosporine A normalized several features of the PAH phenotype that persist in vitro, ie, the downregulation of Kv1.5 (which has been described in all human and animal models of PAH26,38,39,49,51,81), the upregulation of the antiapoptotic bcl-2,53 and the hyperpolarization of PAH PASMC mitochondria. More important, this inhibition of NFAT and normalization of phenotype in vitro were associated with improvement of established monocrotaline PAH in vivo.28

It is possible that many patients diagnosed with iPAH are “masked” cases of unrecognized mild inflammatory diseases or viral infections. In addition to diagnosis, the inflammatory disease platform can also be useful in the consideration of anti-inflammatory strategies for the treatment of PAH. More specific inhibitors of NFAT, for example, are being developed for myocardial diseases that might lack the nonspecific toxic effects of cyclosporine.82 Such therapies will need to be considered for the treatment of PAH.

A Unifying Theory for PAH
Attempts to propose unifying theories for the pathogenesis of PAH might help in the design of experimental therapies; for example, targeting distal signaling pathways might be more effective or selective than proximal or more fundamental pathways (Figure 6).
An abnormality in the BMP axis, inherited or acquired, will promote the apoptosis of PAECs, particularly in response to injury (e.g., viral infection, increased shear stress). Initial PAEC loss will cause loss of small capillaries (which are essentially PAEC tubes), increasing flow and shear stress in the remaining vessels, thereby amplifying the effect. The emergence of apoptosis-resistant clones of PAECs, expressing survivin, has been found to take place and contribute to the proliferative remodeling in the intima and plexogenic lesions. At the same time, the initial loss of PAECs would allow for exposure of PASMCs to circulating growth factors. Such a factor, PDGF, has been shown to induce the expression of survivin in vascular SMCs. Survivin itself also induces the production of PDGF receptor in human vascular SMCs. This positive feedback allows for amplification of the survivin and PDGF pathways and thus the resistance to apoptosis. The associated disruption of the interstitial matrix will promote the activation of elastase and metalloproteinases and the release of matrix-bound mitogenic growth factors, like EGF, further promoting an antiapoptotic and proproliferative environment in the media. Further changes in the matrix, such as the upregulation of the glycoprotein tenascin-c, also activate the EGF receptor. The initial endothelial dysfunction promoting high PASMC [Ca\textsuperscript{2+}] leads to NFAT activation and regulation of multiple genes that might positively reinforce NFAT activation (Figure 6). For example, the downregulation of Kv1.5 will lead to PASMC depolarization, opening of L-type Ca\textsuperscript{2+} channels, and sustaining of the increase in [Ca\textsuperscript{2+}]. The increase in PASMC [Ca\textsuperscript{2+}], might also be promoted by other possibly primary PASMC abnormalities, such as the dysregulated serotonin transporter or upregulation of transient receptor potential channels. The presence of such feedback loops might explain why the phenotype is preserved in the PASMCs in culture, in the absence of endothelium-derived or circulating factors.

It is possible that unifying features are present relative to the mechanisms for initial apoptosis of small-vessel PAECs early in the disease or relative to the predominance of apoptosis-resistant PAECs and PASMCs in late disease. This concept is supported by the observation that induction of apoptosis in SMCs does not cause apoptosis of PAECs but rather is often associated with distal vessel regeneration. This spatiotemporal diversity in apoptosis needs further study, and it might prove to be important to the timing and choice of therapy for different stages of PAH.

Clinical translation of therapies that address all 3 features of PAH (inflammation, apoptosis/proliferation, regeneration) is attractive. NFAT can integrate multiple signaling pathways in PAH, as discussed. Similarly, inhibition of elastase promotes both regeneration of distal vessels and apoptosis of PASMCs. Peroxisome proliferator-activated receptor agonists promote both survival of PAECs (through HO-1 and endothelial NO synthase) and apoptosis of SMCs (through GADD-45) and of course are antiinflammatory.

Translational Priorities in Preclinical Research

The complexity of the biology of PAH exposes many signaling pathways as targets for therapy. However, inhibition of 1 pathway or improvement of 1 aspect of the disease (for example, a decrease in systolic right ventricular pressure) is far from forming the basis of a promising therapy in humans. To create a list of really promising therapies that could be efficiently tested in phase I and II clinical trials, comprehensive studies in animals need to be performed, including clinically relevant end points. Below, we discuss a list of priorities that might help in bridging the gap from the bench to the bedside; before that, we also briefly discuss the critical issue of PAH animal models.

Models

The PAH models are a constant challenge because no single model currently exists that completely recapitulates the human disease. On the other hand, all of the currently approved therapies for PAH have shown some degree of efficacy in the existing models of PAH; these include the rat monocrotaline model and the rat and mouse chronic hypoxia model. The monocrotaline model has been criticized because it lacks accepted features of human disease, including the plexogenic lesion or neointimal hypertrophy; however, in a variant of the model, in which monocrotaline injection is combined with pneumonectomy, intimal hypertrophy is present. On the other hand, the primary effect of the major monocrotaline metabolite is PAEC toxicity, not unlike the earliest event in the pathogenesis of PAH, and the inflammatory changes that are described in the monocrotaline-treated rats might in fact play a more important role in human PAH than currently realized. In addition, the plexogenic lesions might only be a secondary change in human PAH.

The combination of hypoxia and a vascular endothelial growth factor inhibitor in the rat produces plexogeniclike lesions and is used increasingly. Mice overexpressing S100A4/Mts1 (a protein that confers a metastatic phenotype in tumor cells and that is increased in iPAH) also show evidence of spontaneous but rare (as in human disease) development of PAH with pulmonary vascular plexogenic lesions. The fawn hooded rat, which was thought to develop spontaneous PAH only at high altitude (e.g., Denver), has been found to develop PAH at lower altitudes (e.g., Alberta). Although the fawn hooded rat is perhaps the only rat model of spontaneous PAH, it might suffer from many other pathologies involving the left side of the circulation. Although no perfect animal model exists, several are now available, and it is important that potential therapies be tested in >1 model.

Use of clinically relevant end points in animal studies is critical. These include the following:

1. Reversal of established disease, rather than prevention. At this point, it is clinically impossible to predict the development of human PAH, and therefore “prevention” protocols represent only a starting point for animal studies.

2. Complete hemodynamic assessment with the calculation of pulmonary vascular resistance. This requires the measurement of mean PA pressure (jugular approach in anesthetized animals) and cardiac output simultaneously with systemic and left ventricular end-diastolic pressures. A decrease in systolic PA or right ventricular pressure does not mean a decrease in pulmonary vascular resistance and,
used in isolation, has little significance. Complete hemodynamic studies are now possible even in mice.91

3. Assessment of functional capacity of the treated animals (for example, using treadmill tests) to determine whether improvement in right ventricular output is “clinically significant.”

4. Survival studies that extend to at least 6 months, along with toxicity studies with commonly used blood tests including complete blood count, kidney function, and liver function tests. The use of noninvasive tests that can longitudinally follow the rats in these long-term studies (for example, measuring right ventricular mass or PA acceleration time with echocardiography or micro–computed tomography) is important.

5. Dose-dependent effects in hemodynamic, anatomic, survival, and toxicity end points.

Translational Priorities in Clinical Research

It is important for the medical community to realize that the disease remains essentially untreatable and that although the recently approved therapies are relatively easy to take, their effect on functional capacity is modest, they have minimal effects on hemodynamics, and, although they might slow down the progression of the disease, no evidence has been found that they prolong survival. Priority must be given to promising therapies that emerge from appropriately performed preclinical trials. If the industry will not invest, translation in phase I to II trials must be investigator led. Important issues in this direction include the following:

1. Networks of large clinical programs need to be established to enable group funding for new phase I to II trials, particularly for drugs that do not have industry support; the example of the Thrombolysis in Myocardial Infarction network in myocardial infarct trials could perhaps be followed in PAH.

2. Industry-independent clinical databases and tissue banks need to be formed; it is unfortunate that there have not been any industry-independent database initiatives following the National Institutes of Health registry in the 1980s. The need for such initiatives is much greater now than it was in the 1980s because the current classification of PAH is much more complex, and essential information on incidence, prevalence, natural history, and prognosis of PAH is essentially lacking. This is also critical for the development of biomarker discovery programs.

3. New end points need to be identified that are more appropriate for the new molecular therapies. Emphasis needs to be given to molecular imaging techniques, including magnetic resonance imaging and positron emission tomography, that have the ability to study cell proliferation, apoptosis,92,93 and tissue metabolism in vivo (see Champion et al in the present series). Clinician scientists with molecular biology and physiology expertise need to be directly involved in the design of these trials to include and interpret these mechanistic and molecular end points. As genomic, proteomic, and metabolomic projects identify molecular signatures in patients that would match them with the best molecular therapies, trials will become smaller in terms of sample size but more complicated in terms of end -points. Lessons from cancer will be important; for example, patients with high levels of EGF receptor or survivin will be treated with EGF receptor inhibitors or anti-survivin strategies. Catheters that can “biopsy” the pulmonary arteries endovascularly are being developed,94 and although this is too early, it is not hard to envision another cancer model in which molecular imaging techniques and minimally invasive techniques will “stage” patients with PAH for prognosis and therapy.

4. The function of the right ventricle is a critical determinant of the functional capacity and survival of patients with PAH,95 and efforts to explore the molecular mechanism of the quick failure of the right ventricle (at least compared with the left ventricle) are needed. Therapies that can target the right ventricle need to be considered in parallel with therapies that target the pulmonary vasculature96; right ventricular size and function need to be recognized and established as important end points in clinical trials.96

Disclosures

None.

References


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