Hungry for More
Autophagy in the Pathogenesis of Pulmonary Arterial Hypertension

Dustin R. Fraidenburg, Jason X.-J. Yuan

Genetic alterations in pulmonary arterial hypertension (PAH) have become increasingly recognized in both known familial or heritable and sporadic or idiopathic PAH (IPAH). Unrecognized genetic alterations have now been found in up to 40% of the IPAH in which no familial predisposition is apparent. Bone morphogenetic protein (BMP) receptor II (BMPR-II) is the most common gene implicated in this hereditary form of PAH; furthermore, it is implicated in the pathogenesis of non-hereditary forms of PAH with a significant reduction in the expression of BMPR-II in both IPAH and experimental animal models of pulmonary hypertension (PH). BMPs represent the largest group of cytokines involved in the transforming growth factor-β superfamily and regulate growth, differentiation, and apoptosis in multiple cell types, whereas BMPR-II has been shown to have unique roles in differing cells.

BMPR-II is constitutively active at the cell membrane, and ligand stimulation initiates cross-linking with BMPR-I to form a receptor complex that is necessary to activate intracellular signaling. BMPR-II is most highly expressed in endothelial cells in the pulmonary vasculature, and BMPR-II activation leads to the increased proliferation and decreased apoptosis through Smad signaling. This is in contrast to pulmonary arterial smooth muscle cells, where BMP activation leads to the inhibition of proliferation and increased apoptosis through Smad signaling in large vessels, although in small pulmonary arteries, a proliferative effect is seen through activation of extracellular signal–regulated kinase and mitogen-activated protein kinase, which inhibits Smad signaling. It is these unique yet complementary functions that make BMPR-II mutations particularly damaging in the pulmonary circulation, leading to development of PAH. A dysfunctional mutation of BMPR-II, as in heritable PAH, or downregulation of protein expression, as in IPAH and animal models, can lead to endothelial dysfunction hallmarked by abnormal barrier function through increased apoptosis while also leading to vascular medial hypertrophy through increased proliferation and decreased apoptosis of distal arteriole pulmonary arterial smooth muscle cells.

Autophagy represents a homeostatic mechanism essential for cell survival. Cell stress, including hypoxia, nutrient deprivation, or reduction in growth factor stimulation can lead to autophagy responses, in which cytoplasmic contents are collected and recycled to produce amino acids and fatty acids necessary for cellular response and ATP production. Another function is to clear unnecessary or toxic components of the cell cytoplasm, either to salvage the cell or as a mechanism to trigger cell death in a nonapoptotic fashion. After collecting cellular debris, mature autophagosomes fuse with lysosomes, resulting in degradation and recycling of this collected material. Each step of this process is highly regulated, and dysfunction of this system has been implicated in multiple disease processes, including malignancy, neurodegeneration, liver, and heart disease. Recently, dysfunctional autophagy has been implicated in pulmonary diseases, with particularly strong evidence in chronic obstructive pulmonary disease, in which cigarette smoke–induced emphysema is associated with increased numbers of autophagosomes, which is thought to be the result of imbalance in autophagosome production versus clearance. Many of the disease-related autophagy studies have implicated this imbalance as the mechanism by which autophagy influences disease development and progression.

In IPAH, autophagy has been shown to be upregulated, with the marker for mature autophagosomes, light chain 3B (LC3B-II), having increased expression compared with healthy controls. The role of autophagy in PH remains inconclusive but seems to be important in vascular remodeling. Pulmonary artery endothelial cells exposed to hypoxia have increased autophagy, which is thought to be a protective mechanism, because LC3B-II knockout mice have exaggerated PH in response to chronic hypoxia. In contrast, persistent PH in fetal lambs, an experiment model of persistent PH of the newborn, is associated with increased autophagy thought to be detrimental to fetal angiogenesis, inhibiting autophagy can lead to restoration of adequate angiogenesis. Yet, as with any highly regulated cellular mechanism, proper cellular balance seems to be essential for normal function, and these early data have confirmed the complicated nature of autophagy in disease processes. This seems to be the case with regard to BMPR-II and autophagy. Previous work by Morrellet al has shown that the lower levels of BMPR-II seen in experimental models of PH seem to be at least in part attributable to BMPR-II being targeted for ubiquitination and degradation via the lysosome (Figure).

In this issue of Circulation Research, Long et al discuss the role of autophagy and lysosomal BMPR-II degradation in the pathogenesis of experimental PH. They provide new evidence...
linking increased autophagy with downregulation of BMPR-II/Smad pathway in the development of PAH. In a disease in which new therapeutic targets are desperately needed, this may well be a novel target. The authors were able to show both prevention and partial reversal of monocrotaline-induced PH in rats using chloroquine, an inhibitor of autophagy. They further were able to show that the effect of chloroquine was through inhibition of autophagy both in vivo and in vitro, with downstream effects resulting in reduced proliferation and increased apoptosis of pulmonary arterial smooth muscle cells. The diminished protein expression of BMPR-II and Smad signaling in this experimental rat model of PH was restored with the use of chloroquine. This inhibition of autophagy and lysosomal degradation is established in vitro by using knockdown of autophagy-specific ATG5 and concanamycin A treatment, a specific inhibitor of lysosomal degradation, which were both associated with increased expression of BMPR-II and decreased LC3B-II. The authors have concluded that chloroquine ameliorates monocrotaline-induced PH in the rat and that this effect is mediated through downregulated autophagy inhibiting lysosomal degradation of BMPR-II.

The results from this study represent very interesting insight into the potential mechanism of attenuated BMPR-II expression in human PAH. Enhanced autophagy and lysosomal degradation may be a novel pathogenic mechanism and potential therapeutic target for this disease. A compound that has made a resurgence as of late, chloroquine and its less toxic cousin, hydroxychloroquine, have remained mainstays in malaria treatment and chemoprophylaxis, and the steroid-sparing anti-inflammatory effects of these medications have proved useful in systemic lupus erythematousus, rheumatoid arthritis, and sarcoidosis. Repurposing of a medication that is both well tolerated and inexpensive makes it an attractive agent to clinicians, especially to physicians in the developing countries where the commonly used drugs for PAH are not available. The present results provide a plausible mechanism by which experimental PAH was both prevented and reversed, yet both chloroquine and hydroxychloroquine have multiple known and unknown cellular and molecular targets in the human body, and it is unlikely that inhibition of autophagy is the sole effect on the pulmonary vasculature.

There are growing data on the role of inflammation in PAH. Cytokines, particularly interleukin-6, have largely been implicated in the disease process, affecting both vascular remodeling and vasoconstriction, which should not be unexpected, given the similarities between IPAH and associated PAH as a result of chronic inflammatory disorders such as systemic lupus erythematousus, rheumatoid arthritis, and systemic sclerosis. The fact that hydroxychloroquine has in more recent years been found to be a significant immunomodulator with steroid-sparing effects that are beneficial in many of these diseases would potentially implicate its anti-inflammatory properties in the treatment of PAH. Indeed, because hydroxychloroquine treatment is associated with reduced levels of interleukin-6 in patients with systemic lupus erythmatousus, it would be reasonable to assume similar effects in the pulmonary vasculature, which could have potential benefits in a disease characterized by inflammation and elevated interleukin-6 levels, although studies are lacking at this time.15 This immunomodulatory effect may also be contributing to PAH prevention and reversal.
in this animal model, although this effect would still support potential clinical efficacy and safety trials in human patients.

Animal models have always presented problems in research. The complexity of PAH makes this particularly challenging, and experimental models are invariably flawed. Monocrotaline, a plant-derived pyrrolizidine alkaloid, leads to endothelial cell dysfunction and vascular remodeling through unclear mechanisms, although many believe that inflammatory mechanisms play an important role in the pathogenesis of monocrotaline-induced experimental PH. Given the propensity for an exaggerated inflammatory response, it may not represent an ideal model of PAH and would presumably give preference to therapeutic agents with immune-related effects, although other experimental models such as hypoxia-induced PH or Sugen/hypoxia-mediated PH are not without their limitations. It is more likely that these models each emphasize a small part of a large and complicated disease process, and using them in a complementary manner may likely provide better insight into a disease process than simply using a single model alone.

The complexity of human disease is becoming ever apparent. New and exciting molecular pathways and pathogenic mechanisms have met with both success and failure in translation to real-world disease. The complexity that surrounds PAH and BMPR-II, in particular, makes it reasonable to assume that the answer will not come in a single pill. Still, with the undeniable knowledge that this single receptor plays such an important role in the pathogenesis of PAH, each new discovery is a piece of the puzzle leading to a potential cure. This article provides a new and important piece to that puzzle and a better understanding of the abnormal degradation of BMPR-II that will ultimately lead to a better understanding of the mechanisms behind PAH. The present article by Long et al provides encouraging data on chloroquine and hydroxychloroquine, and further studies will help enhance our understanding of the effects on the pulmonary circulation and the potential role in the treatment of patients with PAH.

Disclosures

None.

References


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