Spatio-Temporal Diversity of Apoptosis Within the Vascular Wall in Pulmonary Arterial Hypertension

Heterogeneous BMP Signaling May Have Therapeutic Implications

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“The temptation to form premature theories is the bane of our profession.”

Sherlock Holmes

Although pulmonary arterial hypertension (PAH) was originally thought to be a disease of increased pulmonary arterial (PA) tone, we now know that vasoconstriction is important only in a minority of patients. PAH is characterized by increased proliferation of PA endothelial cells (PAECs) and PA smooth muscle cells (PASMCs), leading to narrowing or even obliteration of the PA lumen, increased pulmonary vascular resistance (PVR), right ventricular failure and premature death.

Voelkel and Tuder suggested that the proliferative remodeling in the PAs resembles cancer. Several features, in addition to excessive proliferation, make this hypothesis attractive. For example, as in cancer, the development of PAH appears to result from a “multiple-hit” mechanism, where environmental factors (virus, inflammation, anorexiogens, shunt-induced shear stress, etc) interact with a genetic predisposition (loss-of-function mutations in the bone morphogenetic protein receptor II, BMPR-II) culminating in disease. That PAH is characterized by a cancer-like apoptosis resistance is supported by recent reports that proapoptotic chemotherapies are the mainstay of treatment. Several experimental PAH treatments (including dichloroacetate, sildenafil, imatinib, anti-survivin, and K+ channel replacement gene therapies) induce apoptosis of PASMCs, leading to reversal of vascular remodeling and PAH. In sharp contrast, strategies designed to promote survival and inhibit apoptosis of PAECs (cell-based gene transfer of angiopoietin-1 or eNOS, caspase inhibitors) also improve PAH, particularly at early stages. These apparently conflicting reports can be rationalized by the hypothesis that vascular apoptosis is regulated in a compartment-specific manner.

The role of apoptosis in the pathogenesis of PAH is also supported by the discovery that mutations in BMPR-II predispose patients to familial PAH. Like most mediators involved in embryological development, BMPs are important regulators of apoptosis. It was initially assumed that loss-of-function BMPR-II mutations would suppress apoptosis and increase proliferation in the PA wall, perhaps fully explaining the vascular remodeling in PAH. However it is now recognized that although most familial PAH patients carry mutations, they are found in only ≈10% of patients with sporadic idiopathic PAH (iPAH) and their presence confers only a 15% to 20% chance of PAH in a carrier’s lifetime. Nevertheless, this discovery offered new insights into the biology of PAH.

**BMP Signaling in the Pulmonary Circulation**

BMP4 inhibits growth of PASMC from normal but not iPAH patients and, similarly, BMP2 and -7 induce more apoptosis in normal than in iPAH PASMCs. Interestingly, these human data are not supported by transgenic models. Heterozygous knockout mice lacking exons 4 to 5 of the BMPR-II gene have only mild pulmonary hypertension at baseline and, surprisingly, after exposure to chronic hypoxia the muscularization of distal PAs is not different than in wild mice. A new transgenic model, where a dominant-negative BMPR-II gene from a familial PAH patient was conditionally overexpressed in SMCs, does have the predicted increase in PA pressure, but it too lacks PA muscularization. The dissociation of hemodynamics from vascular remodeling in these models and the species-specific effects of BMPs in PASMCs raise questions regarding the clinical relevance of some mouse models and casts doubt on the central/obligatory importance of PASMC as the primary targets of abnormal BMP signaling. Nevertheless, the BMP axis is highly active in PAs (more so in the PAECs than in PASMCs) and may be involved in the pathogenesis of PAH through means beyond those related to BMPR-II mutations. Surprisingly, studies on the direct role of the BMP axis on human PAEC are lacking, despite the recognition that endothelial dysfunction is an critical and early event in PAH. Therefore, the work presented in this issue of *Circulation Research* by Dr Stewart’s group on BMP signaling in human PAECs is welcome.

They show that BMP2 and -7 inhibit apoptosis (induced by serum-deprivation or TNF-exposure) in normal human
PAECs. Also, inhibition of BMPR-II expression by 50% using siRNA increases basal PAEC apoptosis 3-fold. More importantly, they studied circulating endothelial precursor cells (EPCs) from both normal volunteers and 15 patients with iPAH. The level of BMPR-II expression was similar in normal and iPAH EPCs, but their response to exogenous BMP2 was quite different. BMP2 inhibited apoptosis in normal but not iPAH EPCs ($P<0.05$). Interestingly, the severity of PAH (based on invasively measured mean PA pressure) showed a positive (although weak) correlation with the response of EPC to BMP2. They conclude that loss-of-function mutations in BMPR-II could increase PAEC apoptosis and initiate PAH.

Dr Stewart and his team are to be congratulated for their efforts to present data from PAH patients and correlate in vitro data with clinical parameters. Translational investigator-driven research of this kind is very much needed but is often challenging because of ethical and logistical complexities of studying seriously ill patients. Nonetheless, this study does have some limitations. BMPR-II genotyping was not performed, and the worthy attempt to correlate a hemodynamic clinical parameter with EPC apoptosis was compromised by lack of control for the type of therapy and patient demographics. Moreover, the use of PA pressure rather than PVR is suboptimal, as cardiac output is not accounted for. Despite these limitations, the data significantly advance our knowledge on the primary role of PAECs in the pathogenesis of PAH and, as discussed below, inspire for the proposal of an apoptosis-based theory of PAH.

More work is required to expand this new field that the authors open with their work. First, confirmation of their findings in larger cohorts of patients is needed. Second, work is also required to reveal the role of EPCs in the biology of PAH. In the meantime, the in vitro response of circulating EPCs to therapies targeting the BMP axis or other apoptotic pathways might evolve as a predictor of the clinical response to such therapies in individual patients. Measuring the function and/or numbers of circulating EPCs might also prove to be a biomarker that could detect early PAH or be used in risk-stratifying patients with advanced disease.

Does the current article explain the restriction of the vascular pathology in PAH to the pulmonary circulation? The authors suggest that the increased shear stress in the pulmonary microvessels (the lung is the only organ that experiences the entire cardiac output) makes the PAECs more vulnerable than systemic ECs to apoptosis. A basally elevated shear stress in the pulmonary circulation might also allow for a selection pressure and emergence of apoptosis-resistant PAECs, which localizes proliferative vascular remodeling. This concept is supported by elegant experiments describing the emergence of apoptosis-resistant PAECs (expressing survivin, an inhibitor of apoptosis and tumor marker), after shear-stress–induced apoptosis in vitro.

Furthermore, a significant difference between the pulmonary and systemic microvessels is their exposure to very different redox environments, because the lung microvessels are uniquely exposed to much higher PO$_2$ and thus oxidative stress than systemic vessels. Weir and Archer have shown that such redox differences provide the basis for the fact that hypoxic pulmonary vasoconstriction is unique to the pulmonary circulation. The observation that the BMP axis can be enhanced by redox mechanisms in EC (H$_2$O$_2$ directly increases BMP2 expression$^{21}$) suggests that it can be enhanced locally in the pulmonary circulation despite the expression of BMP receptors (normal or mutated) throughout the vasculature.

**An Apoptosis-Based Theory for the Development of PAH: Being at the Right Place the Right Time**

The Toronto group’s report does allow development of a testable hypothesis for the pathogenesis of PAH in humans. Central to this hypothesis is the fact that apoptosis shows a spatio-temporal diversity within the vascular wall as PAH develops. An abnormality in the BMP axis, inherited or acquired, will promote the apoptosis of PAECs, particularly in response to injury (viral infection, increased shear stress, etc). Initial PAEC death will cause loss of small capillaries (which are essentially PAEC tubes), increasing the flow and shear stress in the remaining vessels, amplifying the effect. The emergence of apoptosis-resistant PAECs, expressing survivin, will lead to the proliferation in the intima and in plexogenic lesions. At the same time, loss of PAECs would allow for exposure of PASMCs to circulating growth factors that are normally excluded, except in vascular injury. Such a factor, PDGF, has been shown to induce the expression of survivin in vascular SMCs.$^{22}$ Survivin itself also induces the production of PDGF in human vascular SMCs.$^{23}$ This positive feedback allows for amplification of the survivin pathway and thus the resistance to apoptosis. Indeed, patients and animals with PAH, but not normal controls, show high levels of survivin expression in PAs.$^7$ Selective delivery of a dominant-negative construct to the small PAs (using an inhaled adenovirus) induces apoptosis, decreases proliferation in the media, and reverses PAH.$^7$ Also, inhibition of the PDGF pathway either by fish oil$^{24}$ or imatinib$^6$ reverses PAH in animals.

In summary, early PAH is characterized by increased apoptosis in the endothelial layer. In contrast, late PAH is characterized by suppressed apoptosis and increased proliferation in both the intima and the media. This view has potential therapeutic implications (Figure). Patients in early stages of PAH may benefit more from antiapoptotic approaches, whereas patients presenting in late stages (which unfortunately represent the majority) will benefit from proapoptotic strategies. Patients with intermediate stages of PAH might require cell-specific or vascular compartment–specific therapies.

PAH in individual patients might need to be properly “staged” to select the appropriate pro- or antiapoptotic therapies, much like in cancer. Unfortunately, open lung biopsies carry a significant risk and are rarely used in PAH, although catheter-based approaches have shown promise in large animal models.$^{25}$ The direct assessment of apoptosis within the pulmonary circulation in vivo will be very important in this approach, and this is already used in cancer. Emerging molecular imaging techniques show promise for the in vivo assessment of vascular apoptosis in humans.$^{26}$
An apoptosis-based theory for the development of pulmonary arterial hypertension and its therapeutic implications.

The proposal of an apoptosis theory for PAH, inspired by recent publications including the one under discussion, might seem premature but perhaps can be more kindly received in light of Einstein’s view that “it is theory that decides what can be observed.”

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References


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