CME Section

Endothelial Cells Caught in the Crosshairs of Pulmonary Arterial Hypertension

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The purpose of this overview is to provide a framework for understanding the fundamental mechanisms underlying the initiation and progression of pulmonary arterial hypertension and suggest a unifying concept that may better guide the development of therapies based on the central role of endothelial cell injury and loss by apoptosis.

Despite the major advances in diagnosis and treatment of pulmonary arterial hypertension (PAH) over the last several decades, this disorder remains largely an enigma from the point of view of our understanding of its underlying mechanisms. Consequently, our current therapeutic approaches fall short of being truly effective. Indeed, there currently exist several different ideas concerning the initiation and progression of the vascular lesions of PAH, each of which suggest exciting new directions for the development of innovative therapies; but, these concepts appear on the surface to be contradictory and even mutually exclusive. Yet each is supported by credible evidence that has stood the test of peer review, so how are we to reconcile these differences? The purpose of this overview is to provide a framework for these diverse views and suggest a unifying concept that may better guide the development of therapies based on the central role of endothelial cell (EC) injury and loss by apoptosis.

THE ENDOTHELIUM AND PAH

Even in some of the earliest models, injury to the endothelium of pulmonary arteries has been recognized as an inciting mechanism of experimental pulmonary hypertension.[1] Since the early 1980s, it has been understood that the importance of this innermost lining of all blood vessels extends far beyond its barrier and physical properties, and that a derangement in the release of potent endothelial vasoactive factors, such as nitric oxide (NO), prostacyclin, and endothelin-1, was a major determinant of vascular disease.[15]

Indeed, most of the current therapies in the modern era of PAH treatment were designed to address the problem of endothelial dysfunction in PAH, either by supplementing the loss of an endothelium-derived vasodilator factor such as prostacyclin or NO, and blocking the effects of a vasoconstrictor factor, eg, endothelin receptor antagonists. While there is no doubt that the introduction of these agents has benefited countless PAH patients, it is also clear that their efficacy is limited. Even when a good response is obtained, it is often incomplete and the vast majority of patients will eventually progress. Thus, although the prognosis for this disease has improved, it remains a dire and ultimately fatal disease in absence of lung transplantation.

VASCULAR REMODELING AND "ANGIOPROLIFERATIVE" CHANGES

The current focus of much research is on the events that underlie the dramatic arterial remodeling and pruning that characterize this disease. In particular, the development of occlusive intimal and plexiform lesions is a typical pathological feature in group 1 patients, regardless of whether PAH is familial or associated with any of the disorders that are known to lead to this disease.[1,2] While there is still debate about the importance of these lesions in the pathogenesis of this disease, it is hard to ignore the possibility that unrestrained vascular cell proliferation could be a major contributor to the progression of the functional loss of pulmonary microvasculature that is a fundamental problem in PAH. Indeed, the "angioproliferative" hypothesis likens PAH to a neoplastic condition, and therefore suggests that cancer-like treatments to block cell growth may be the answer.[3] However, it is clearly not quite that simple, as was evidenced by a now seminal report describing the effects of blockade of the key receptor tyrosine kinase, VEGFR2, which supports EC growth.[4]

Rather than preventing PAH in the rat hypoxia model, VEGF blockade using SU5416 exacerbated both the hemodynamic and remodeling changes, resulting in proliferative intimal lesions that resembled the plexiform lesions of human PAH. Of great interest, all these effects of VEGF blockade could be completely prevented by inhibition of apoptosis, using nonspecific inhibitors of caspases. This landmark study has led to the concept that EC apoptosis may be a trigger for both degenerative and reactive proliferative events that may ultimately result in increased pulmonary vascular resistance and the typical vascular pathology of PAH, including the plexiform lesions (Figure 1). However, it has also created some ambiguity concerning what potential therapeutic strategies may be most promising. Should one focus on inhibiting cell growth, or attempt to address the underlying reasons for injury and loss of the endothelium? As with the SU compound, related tyrosine kinase inhibitors could have unexpected effects to the extent that they block EC survival signaling through VEGF receptors. It is perhaps a telling reflection of the lack of clarity in the field that VEGF blockade in the hypoxic rat is being widely adopted as a more relevant model of PAH, while at the same time clinical trials are underway using a different tyrosine kinase inhibitor, sorafenib, which also blocks VEGFR2 activity.

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ENHANCING VASCULAR REPAIR: THE ROLE OF PROGENITOR CELLS

An alternative approach is to focus on ways of limiting endothelial injury and promoting vascular repair as a strategy to reduce the downstream consequences of EC apoptosis in the distal lung arteriolar bed. One such strategy takes advantage of the recent discovery that even in the adult, there exist stem-like cells capable of differentiating into ECs and repairing or regenerating damaged blood vessels.[5] These so called endothelial progenitor cells (EPCs) reside in the bone marrow and circulate in the blood. However, at present there is considerable confusion about what represents an EPC and how best these cells can be identified from the rest of the mononuclear cell (MNC) fraction. To date, there is no agreement about what surface markers (ie, CD34, CD133, VEGFR2, Tie2) can be used to best select a "true" EPC population,[6] and as more stringent combinations of positive and negative selection markers are used, the efficiency of the isolation becomes vanishingly small yielding fewer and fewer cells, such that scaling up for clinical therapy becomes increasingly problematic.

An alternative approach is to place MNCs, isolated for example by Ficoll gradient centrifugation, into culture conditions that favor their differentiation into an endothelial phenotype.[7] This method initially produces "early growth" EPCs appearing within 3-5 days that are typically rod-shaped and highly motile cells and, while maintaining MNC (CD14) and panleukocyte markers (CD45), begin to express a variety of EC determinants. These cells are essentially nonproliferative but have consistent and potent angiogenic activity in a variety of in vitro and in vivo models. Interestingly, over a period of 1 to 2 weeks, the early growth cells begin to wane and clusters of highly proliferative cells appear that have a very strong EC phenotype and rapidly overgrow the cultures producing lawns of a typical cobblestone appearance. These "late outgrowth" EPCs have lost all leukocyte markers, and show a typical EC gene expression profile. Less is known about their therapeutic efficacy in neovascularization models; however, it has been suggested that they can act synergistically with the early growth cells, complementing their largely paracrine actions on vascular regeneration by engrafting and differentiating into new ECs in situ.[8]

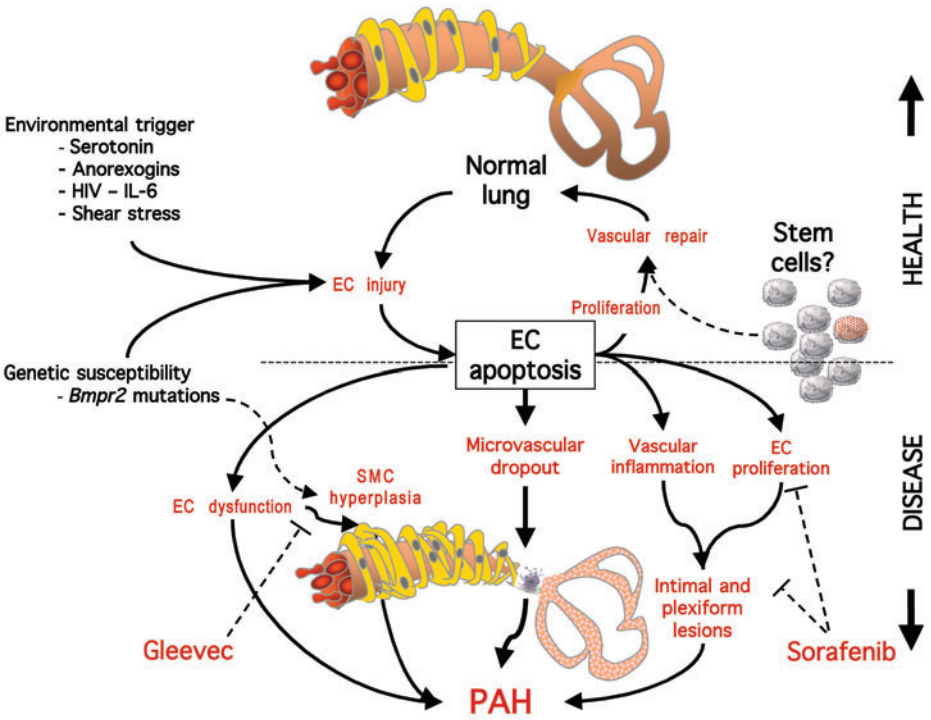

Figure 1: Modified from Yelle et al. In: Dauphinee S, Karsan A, eds. *Endothelial Dysfunction and Inflammation, Progress in Inflammation Research*. New York, NY: Springer: 2010.

ROLE OF EPCs IN PAH

While there has been considerable interest in the role of EPCs in the systemic vascular repair and regeneration, it is only recently that their importance in the pulmonary vasculature has begun to be addressed. As well, the field is complicated by the lack of consistency in nomenclature and methodology, such that it is not always clear what population of cells is being studied. Observational reports have shown that numbers of circulating EPCs can be increased or decreased depending on the methods used, and that stem-like cells of bone marrow origin can be found associated with vascular lesions of patients with PAH.[9] However, these studies are open to different interpretations and cannot firmly establish a beneficial or detrimental role in the development and progression of these lesions. There is more consistency in studies that have examined the effect of EPCs, usually isolated by culture selection (ie, early growth cells), in experimental models of PAH. In these studies there is consistent benefit in the administration of EPCs within days of inducing vascular injury, often by the administration of the plant alkaloid, monocrotaline (MCT).[10,11]

However, the mechanisms by which EPCs can prevent PAH are not yet fully understood. The early expectation was that these cells would act by transdifferentiation to replace damaged ECs and regenerate lost pulmonary arterioles; however, while evidence for this mechanism has been reported (Figure 2), the frequency of these events appears to be rather low. Thus, it is likely that other mechanisms must contribute to the near complete efficacy of this cell therapy in prevention models, including the paracrine release of growth factors and cytokines that could affect local vascular repair in the lung,[12,13] or their interaction with immune regulatory cells to reduce inflammation and promote healing.[14]

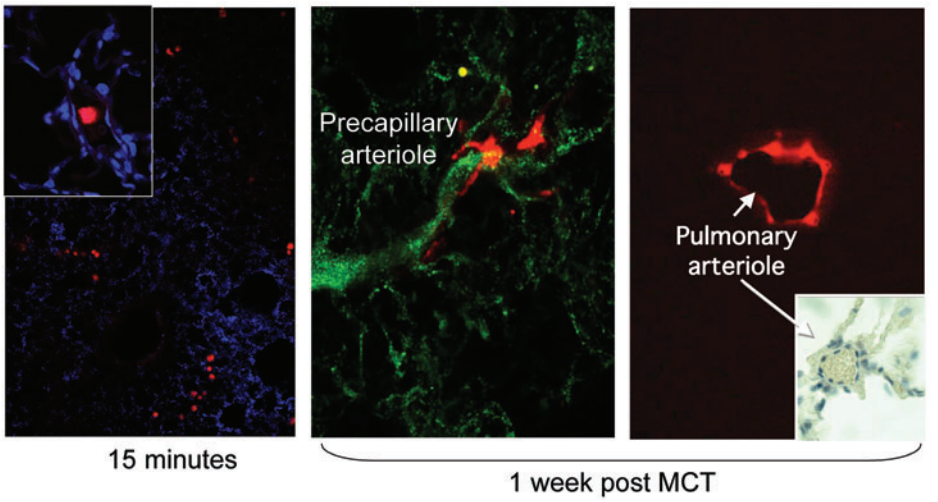

Figure 2: Modified from Zhao YD et al. *Circ Res*. 2005;96(4):442-450.

TRANSLATION OF EPC THERAPY INTO THE CLINIC

Although the preclinical results of EPC administration in a prevention model are compelling, this is not relevant to the clinical problem of treating established PAH in patients. For this reason, we assessed EPCs in a treatment model in which cell administration was delayed until 3 weeks after MCT, at which time severe PAH was already established. Again we saw efficacy with delayed EPC treatment, which prevented further progression in pulmonary pressures and improved survival over the following 2 weeks, but this did not produce a significant reduction in right ventricular systolic pressure (RVSP) at 5 weeks compared to 3 weeks measured in the same animals. However, the transfection of EPCs with endothelial NO-synthase (eNOS), a gene known to play a key role in vascular protection and repair, resulted in near complete rescue of established PAH.[11] Based on these preclinical data, we have embarked on the translation of this novel cell therapy into the first clinical trial using gene enhanced cell therapy for patients with severe PAH refractory to all available treatments.

The Pulmonary Hypertension And Cell Therapy (PHACeT) trial is a phase I, dose-escalation study that is now over halfway through enrollment, and the preliminary results support the safety of the administration of eNOS-transfected EPCs in patients with severe, refractory PAH—even suggesting potential efficacy largely attributable to the eNOS transgene based on a remarkable interaction with concomitant therapy with phosphodiesterase type 5 (PDE5) inhibitors. PDE5 inhibitors enhance the effects of NO by blocking the degradation of its second messenger, cGMP, and nearly half of the 7 PHACeT patients enrolled so far that were receiving sildenafil as part of their therapeutic regimen demonstrated by far the greatest hemodynamic and functional benefits.

CONCLUSIONS

Understanding the fundamental mechanisms underlying the initiation and progression of PAH is of critical importance to inform the development of novel therapies for this devastating disease. Based on the recent elucidation of the central role of EC apoptosis in this disease, leading to both degeneration of distal lung arteriolar continuity, as well as reactive vascular cell proliferation and possibly inflammation, we have proposed a novel strategy to enhance endothelial repair and regeneration using EPCs. This approach has been validated and optimized in preclinical studies, demonstrating the superiority of eNOS gene enhanced cells in reversing established PAH, and is now in the midst of early phase clinical studies.

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