Targeting Vessel Formation in Pulmonary Arterial Hypertension: Is the Endostatin–*Id1*–Thrombospondin 1 Pathway a New Hope?

Pulmonary endothelial dysfunction is a characteristic of pulmonary arterial hypertension (PAH) and is believed to be an early event that determines many of the other key pathological processes in the development of this devastating condition. Pathways involved in the regulation of blood vessel formation and repair are relevant to the formation of pulmonary vascular lesions and restoration of distal pulmonary perfusion in PAH. Thus, established angiopoietic and angiostatic factors have been a focus of targeted research studies in recent years.

A decade ago, Kümpers and colleagues reported finding elevated circulating concentrations of angiopoietin 1 and its inhibitor angiopoietin 2 in a study of 104 patients with idiopathic PAH (1). Angiopoietin 2 alone was prognostic of, and expression in patient lung tissues was associated with, the formation of plexogenic lesions, where it might function to destabilize established vessels at initiation of remodeling. A more recent study confirmed the lack of association between circulating angiopoietin 1 and outcomes in patients with pulmonary hypertension, with no differences in etiologies, including connective tissue disease, left heart dysfunction, and chronic thromboembolic disease–associated forms of pulmonary hypertension (2). Following from an *in vivo* observation of aggravated pulmonary hypertension in hypoxic mice overexpressing another regulator of endothelial function, angiostatin (3), Jurasz and colleagues observed elevated angiostatin concentrations in platelets of six patients with idiopathic PAH compared with matched healthy control subjects (4). Angiostatin was able to trigger endothelial microfragment formation, consistent with its role as a stimulator of endothelial apoptosis.

More recently, Damico and colleagues documented elevated circulating concentrations of the peptide derived from the carboxy terminus of collagen XVII, α1, endostatin (ES), in a cohort of 132 patients with PAH compared with healthy control subjects (5). ES was associated with multiple measures of disease severity, and concentrations above 95 ng/ml correlated with worse survival in two independent cohorts of patients ($n = 82$ with PAH and $n = 50$ with idiopathic PAH, respectively). The authors also screened 12 genomic variants in the *COL18A1* gene (encoding collagen XVII, α1, and hence ES) and identified one, rs12483377, at higher minor allele frequency (21.6%) than available controls (7.5%). This variant encodes an asparagine (N) residue at position 104 of ES in place of the ancestral aspartic acid (D). Heterozygotes for this variant had approximately half the circulating ES concentration of reference patients and significantly better outcomes in both cohorts studied. This led to the suggestion that the enrichment of this variant may be driven by survival bias in recruitment, which may potentially explain why the locus was not one of those found to be associated with development of PAH in the recent international genome-wide association study of PAH (6). In over 120,000 individuals registered in the Genome Aggregation Database (gnomad.broadinstitute.org), the minor allele frequency is 0.066, with the highest listed frequency at 0.10 in the Ashkenazi Jewish population, making the 0.21 observed in PAH notable (5). However, in the largest substudy of the PAH genome-wide association study, the National Institute for Health Research BioResource PAH population had an allele frequency of 0.084 ($P = 0.69$), indicating no enrichment (6). Further analysis in large, genotyped PAH populations with sufficient follow-up should establish the relevance of rs12483377 to PAH progression.

In this issue of the *Journal* (pp. 524–534), Goyanes and colleagues (7) show that ES inhibits pulmonary endothelial cell (PEC) proliferation and migration and promotes apoptosis in *in vitro* analyses. Published data in human umbilical vein endothelial cells suggested that ES reduces expression of *ID1* (inhibitor of differentiation/DNA binding 1), well known in PAH as a downstream target of *BMPR2* (bone morphogenetic protein receptor type 2) signaling. Surprisingly, the authors found little effect of ES on *Id1* mRNA in PECs but substantially reduced protein expression, which they were able to rescue with inhibition of the proteasome using MG132, suggesting a difference in regulation of *Id1* by ES in PECs versus systemic endothelial cells. Rescue of ES effects was also possible through overexpression of *Id1*. MG132 was also able to prevent the induction of release of the *Id1* target, TSP-1 (thrombospondin 1), from PECs by ES, again emphasizing the importance of the proteasome in the effects of ES stimulation. TSP-1 itself was of particular interest because it is also a known endostatic regulator that was elevated in plasma samples from 93 patients with PAH, also relating to poor outcomes (8). Furthermore, knockdown of TSP-1 was able to prevent the PEC functional effects of ES in this study. TSP-1 can bind multiple receptors, including CD36 and CD47, and the authors demonstrated that CD36 was required for inhibition of migration by ES or TSP-1, whereas both receptors were required for their effects on proliferation and apoptosis. Finally, short ES-derived peptides containing either reference or variant residue 104 (ES99–111D104 and ES99–111N104, respectively) were shown to have differential effects on migration but no effect on proliferation. This again contrasts with studies on human umbilical vein endothelial cells that indicated equal function of the variant peptides on tube formation (which requires migratory function) (9). The differential effect on migration and

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proliferation suggests that distinct mechanisms are relevant to these functions of ES. For example, as well as circulating concentrations, residue 104 may affect activation of or binding to CD36 but not CD47, but this requires further experimental evidence to prove.

The inability of circulating ES to inform prognosis independent of established clinical severity markers, including 6-minute-walk distance, World Health Organization functional class, and N-terminal pro-brain natriuretic peptide (5), precludes its use as a prognostic biomarker in PAH, but it could still be of value, alongside genotyping of the key variant, to select patients for therapy if strategies targeting the ES–ID1–TSP-1 axis were successful. It would also be interesting to investigate whether TSP-1 concentrations are altered by the genetic variant that determines ES expression. Some of the rescue strategies used in *in vitro* studies (MG132, CD36 knockdown) are not sufficiently selective, so more precise strategies, potentially targeting the interaction between TSP-1 and the CD receptors or enhancing ID1 activation, may prove worthwhile to pursue and could perhaps be tested *in vivo* in models overexpressing ES or its derived, functional peptides. ■

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