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Minireview

Apelin-APJ Signaling: a Potential Therapeutic Target for Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a progressive disease characterized by the vascular remodeling of the pulmonary arterioles, including formation of plexiform and concentric lesions comprised of proliferative vascular cells. Clinically, PAH leads to increased pulmonary arterial pressure and subsequent right ventricular failure. Existing therapies have improved the outcome but mortality still remains exceedingly high. There is emerging evidence that the seven-transmembrane G-protein coupled receptor APJ and its cognate endogenous ligand apelin are important in the maintenance of pulmonary vascular homeostasis through the targeting of critical mediators, such as Krűppel-like factor 2 (KLF2), endothelial nitric oxide synthase (eNOS), and microRNAs (miRNAs). Disruption of this pathway plays a major part in the pathogenesis of PAH. Given its role in the maintenance of pulmonary vascular homeostasis, the apelin-APJ pathway is a potential target for PAH therapy. This review highlights the current state in the understanding of the apelin-APJ axis related to PAH and discusses the therapeutic potential of this signaling pathway as a novel paradigm of PAH therapy.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a devastating disease localized to the small pulmonary arterioles, which is characterized by increased pulmonary vascular resistance and right ventricular failure. The latter is the most common cause of death. PAH is clinically defined by an elevation in mean pulmonary artery pressure exceeding 25 mmHg at rest (Simonneau et al., 2009). PAH is histologically characterized by endothelial and smooth muscle cell proliferation, medial hypertrophy, muscularization of the arterioles, and thrombosis *in situ* (Rabinovitch et al., 2007). The etiology of PAH is complex and largely undetermined, and it remains a fatal disease if left untreated.

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Currently, there are few approved drugs for the treatment of PAH. Most such as endothelin-1 (ET-1) receptor antagonists, prostacyclin analogues, and phosphodiesterase-5 inhibitors, are primarily pulmonary vasodilators that maintain the balance of endothelium-derived vasoactive mediators (McLaughlin and McGoon, 2006). However, despite these advances, PAH remains a fatal disease without curative therapies. Mortality rates remain exceedingly high with 15%, 30%, and 45% mortality at 1, 2, and 3 years after diagnosis, respectively (Humbert et al., 2010). Identifying novel targets for PAH therapy is crucial.

The aim of most current therapies is to improve hemodynamic parameters and functional lung capacity through vasodilatation of the pulmonary vasculature. Emerging data suggests that targeting abnormal proliferation of vascular cells may, in fact, be a viable and efficacious therapeutic option. In line with this possibility, disruption of apelin-APJ signaling was recently proven to be involved in vascular remodeling *via* abnormal proliferation of vascular cells, as well as in the progression of PAH (Kim et al., 2013).

This review discusses the links between apelin-APJ signaling and pathogenesis of PAH, and its possibility as a potential therapeutic target for PAH.

APELIN-APJ SIGNALING IN VASCULAR BIOLOGY

Characterization of apelin and APJ

The APJ receptor was first cloned from a human gene based on homology to other known G protein coupled receptors (O'Dowd et al., 1993). The human APJ (approved gene symbol: *APLNR*) gene encodes a 377 amino acid G-protein coupled receptor with seven transmembrane domains and shares 31% sequence identity at the protein level with the Angiotensin II receptor type I (AT1R). However, the APJ receptor does not bind with angiotensin II (Ang II).

The ligand for APJ was identified in 1998 and was named apelin (Tatemoto et al., 1998). Initially characterized as a 36 amino acid peptide, cloning of the cDNA identified it as a pre-pro-protein of 77 amino acids that is cleaved to generate shorter biological active peptides of 13 and 17 amino acids that exist *in vivo* (Hosoya et al., 2000; Kawamata et al., 2001; Tatemoto et al., 1998). These shortened forms have more potent effects than the longer form (Tatemoto et al., 1998). A pyroglutamylated form of apelin-13 is produced endogenously improves resistance to enzymatic cleavage, and has been more recently used experimentally as a more suitable peptide for chronic infusion.

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Tissue and cellular distribution of apelin and APJ

Multiple tissues express apelin and APJ, including the heart, lungs, and vasculature (Medhurst et al., 2003). In the cardiovascular system, animal studies using mice and rat tissues have documented the elevated expression of both apelin and APJ messenger RNA (mRNA) in the heart and lungs (Medhurst et al., 2003), and apelin has been localized to endothelial cells associated with small vessels in various tissues (Sheikh et al., 2008; Tatemoto et al., 2001).

Apelin-APJ signaling in cardiovascular physiology

The apelin-APJ pathway appears to modulate cardiac contractility and vascular tone. Apelin is an arterial and venous dilator *in vivo* (Cheng et al., 2003; Lee et al., 2000; Tatemoto et al., 1998), and induces a potent dose-dependent positive inotropic effect in isolated perfused rat hearts (Szokodi et al., 2002). *In vivo*, apelin infusions for two weeks improves cardiac contractility and reduces cardiac loading (Ashley et al., 2005). More recent studies have demonstrated a link between apelin and Ang II signaling pathways. The apelin-APJ pathway mediates opposing actions to the renin-angiotensin system (RAS) in a number of physiologic and pathophysiologic settings. Indeed, while Ang II increases vascular tone and raises blood pressure, apelin is a vasodilator and lowers blood pressure; in heart failure, Ang II levels rise, whereas apelin levels fall (Chen et al., 2003; Cheng et al., 2003; De Mota et al., 2004; Ishida et al., 2004; Lee et al., 2000; O'Carroll et al., 2006; Tatemoto et al., 1998). The apelin-APJ pathway also appears to physiologically antagonize RAS, suggesting that apelin-APJ signaling can suppress Ang II actions in vascular disease (Chun et al., 2011). In addition, there is evidence for direct counter-regulation of apelin-APJ signaling by RAS. Differences in blood pressure response in mice lacking both the AT1R and APJ, compared to mice lacking only AT1R were described (Ishida et al., 2004) and Ang II inhibits expression of apelin and APJ, perhaps by contributing to cardiac decompensation in a hypertensive model of heart failure (Iwanaga et al., 2006). Most recently, Kang et al. (2013) demonstrated that apelin-APJ signaling plays an important role in cardiovascular development, controlling the activation of myocyte enhancer factor-2 (MEF2) transcription factors by regulating G alpha 13, histone deacetylase (HDAC) 4, and HDAC5.

APELIN-APJ SIGNALING IN PAH

Vascular remodeling in PAH

The pathogenesis of PAH is a multifactorial process leading to functional and structural changes in the pulmonary vasculature. Common histopathologic patterns include: 1) intimal hyperplasia, 2) medial hypertrophy, 3) adventitial proliferation/fibrosis, 4) occlusion of small arteries, 5) thrombosis *in situ*, and 6) plexiform lesions comprised of hyperproliferative pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth-muscle cells (PASMCs) (Rai et al., 2008; Sakao et al., 2010). The majority of cases are sporadic, but genetic studies in cases of familial PAH have identified the bone morphogenetic protein receptor type II (*BMPR2*) gene as the gene for familial PAH (Machado et al., 2009).

Although the exact causes of PAH remain for the most part unknown, endothelial dysfunction is likely an important contributor to the progression of the disease (Budhiraja et al., 2004). The endothelial disruption that occurs in PAH is complex, and is thought to manifest in part by the imbalance of locally produced vasoconstrictors and vasodilators, including nitric oxide (NO), prostacyclin, endothelin-1 (ET-1), serotonin, and thromboxane. Clinical studies have demonstrated perturbation in these factors in the lungs of patients with PAH (Ozkan et al., 2001; Raja et al., 2010). The vascular remodeling results from dysregulation of endothelial and vascular smooth muscle cell growth. Abnormal proliferation of endothelial cells is seen in the plexogenic lesions expressing angiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor 2 (FGF2) (Benisty et al., 2004; Izikki et al., 2009). These factors from endothelial cells affect smooth muscle cells growth in a paracrine manner (Kim et al., 2013). In addition, the pulmonary vascular smooth muscle cells also undergo proliferation and hypertrophy. These changes seem to be mediated by loss of anti-mitogenic factors and increase in mitogenic factors. The significant evidence sup-porting the importance of endothelial disruption in PAH makes this cell type an ideal target for therapeutic development in PAH patients. This novel paradigm involving a link between dysfunctional endothelial cells and apelin-APJ axis in these cells may have important implications for understanding the pathogenesis of PAH and possibly lead to more effective treatments (Fig. 1).

PAH and cancer paradigm

PAH involves abnormal proliferation of multiple cell types, such as PAECs and PASMCs, in the pulmonary vasculature. Dysregulated cellular growth leads to formation of vascular plexiform lesions, which is the hallmark of severe PAH. These plexiform lesions are composed of hyperproliferative PAECs and PASMCs. The concept of the endothelial cell as "quasi-malignant" was initially suggested in 1998 (Rai et al., 2008; Tuder et al., 2001) and has been the focus of more recent attention in the context of clinical trials demonstrating efficacy of anti-cancer therapies, such as tyrosine kinase inhibitors in the treatment of PAH (Barst, 2005; Ghofrani et al., 2010). The complex vascular lesions of PAH appear to be governed by the same traits that control cancer growth, including angiogenesis, absence of apoptotic cells in the lesions, and the presence of antiapoptotic proteins in the lesion cells (Hanahan and Weinberg, 2000). These studies have provided support for targeting aberrant cellular proliferation in treatment of PAH.

Given the similarities between hyperproliferative phenotype in cancer and PAH, the most recent study is germane. The study showed that miR-424 and miR-503 regulated by apelin-APJ signaling in PAECs exert anti-proliferative effects and inhibit the proliferation of PASMC in a paracrine manner, which may bridge the current gap in PAH specific mechanism concerning how these PAECs undergo a switch from a quiescent homeostatic state to a hyperproliferative state and affect PASMC growth (Kim et al., 2013). These data provide support for targeting aberrant cellular proliferation in treatment of PAH (Figs. 1 and 2).

Regulation of apelin signaling by BMPR2 and hypoxia

BMPR2 is a member of the transforming growth factor beta (TGF-β) superfamily of growth factor receptors. Mutations in this gene are causally linked to PAH, where loss-of-function mutations in the *BMPR2* gene have been found in approximately 70% of PAH patients with a family history of PAH, in 10-40% of PAH patients without a family history of the disease (Deng et al., 2000; Machado et al., 2009; Simonneau et al., 2009), and in 6-9% of patients with secondary forms of PAH associated with a number of other conditions, including connective tissue disease and congenital heart disease. However, the penetrance of heritable PAH is low; on average, only 15% of

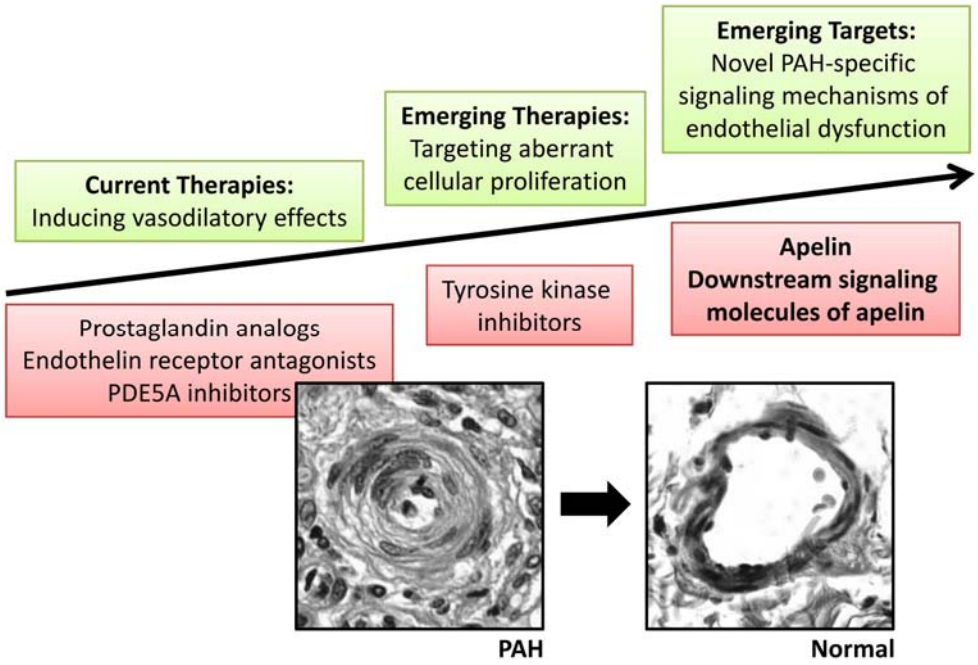

Fig. 1. Proposed novel paradigms for PAH therapy. Augmentation of apelin-APJ signaling in endothelial cells might be an attractive therapeutic venue for PAH treatment.

affected family members develop PAH (Hamid et al., 2009; Machado et al., 2009; Roberts et al., 2004), suggesting the importance of abnormalities in downstream signaling of BMPR2 or environmental influences that could disrupt BMPR2 function through the inhibition of its expression, thereby contributing to PAH development (Hansmann et al., 2007; Zamanian et al., 2009). Recently, apelin signaling was identified as a key downstream target in pulmonary vascular homeostasis of the BMPR2 signaling (Alastalo et al., 2011). Given that dysfunctional BMPR2 signaling is involved in the pathogenesis of PAH, the authors showed that BMPR2 signaling mediated by a complex between β-catenin and peroxisome proliferator activated receptor gamma (PPARγ) is able to regulate apelin expression. The authors also found that disruption of a complex between β-catenin and PPARγ through abnormal BMPR2 signaling leads to decreased apelin expression. In turn autocrine effects result in increased PAECs apoptosis, as well as paracrine effects in suppression of abnormal proliferation of PASMCs. The authors further reported reversal of PAH by exogenous apelin peptide administration in PPARγ knockout mice, suggesting that apelin could be a beneficial target in treating PAH through the restoration of BMPR2 signaling.

Hypoxia-induced pulmonary vasoconstriction is a well-established animal model of pulmonary arterial hypertension (Zaidi et al., 2002). Apelin expression is significantly increased by hypoxia in cultured cell lines, including human coronary artery endothelial cells, human microvascular endothelial cells, and human PAECs (Sheikh et al., 2008). A recent study also demonstrated that mRNA expression of heart and lung resulted in the marked upregulation of apelin and APJ in mice after 1 week of hypoxia. However, after 3 weeks of hypoxia, there was a significant reduction in the expression of both genes (Chandra et al., 2011). These findings suggest that the initial up-regulation of apelin and APJ during hypoxia may reflect a compensatory mechanism that antagonizes the vasoconstrictive mediators of hypoxia. However, with prolonged hypoxia, this pathway is down-regulated, leading to a failure to compensate for the progressive vasoconstriction and pulmonary vasculature remodeling.

Disruption of apelin-APJ signaling in PAH

Recent studies have focused on the endothelial signaling pathways that are disrupted in PAH. Disruption of apelin-APJ signaling pathway in PAECs was chronicled in patients with PAH and in experimental pulmonary hypertension rodent models subjected to monocrotaline (MCT), chronic hypoxia, and SU-5416/hypoxia (Chandra et al., 2011; Kim et al., 2013). In addition, apelin knockout mice developed more severe PAH compared with wild-type mice, and displayed significant loss of pulmonary microvasculature when subjected to chronic hypoxia (Chandra et al., 2011). More importantly, other studies have reported significantly reductions of apelin levels in the serum and pulmonary endothelium of patients with PAH, as well as in the lungs of rats with MCT-induced pulmonary hypertension (Alastalo et al., 2011; Chandra et al., 2011; Falcão-Pires et al., 2009; Goetze et al., 2006; Kim et al., 2013). Furthermore, PAH in both the MCT model and endothelial-specific PPARγ knockout mice, in which apelin is reduced, is reversed by exogenous apelin peptide administration (Alastalo et al., 2011; Chandra et al., 2011; Falcão-Pires et al., 2009; Kim et al., 2013). The findings of hyperproliferative and antiapoptotic activity of PAH PAECs (Kim et al., 2013; Masri et al., 2007; Tuder et al., 1994), in conjunction with other studies showing decreased apelin expression in PAH (Alastalo et al., 2011; Chandra et al., 2011; Goetze et al., 2006; Kim et al., 2013) supports the view that disruption of apelin-APJ signaling in PAH contributes to the aberrant activation of a downstream secondary signaling cascade that induces the abnormal proliferation of pulmonary vascular cells, leading to progressive vascular remodeling.

Downstream signal transduction of apelin-APJ signaling in PAH

Given that the apelin-APJ signaling is disrupted in PAH and

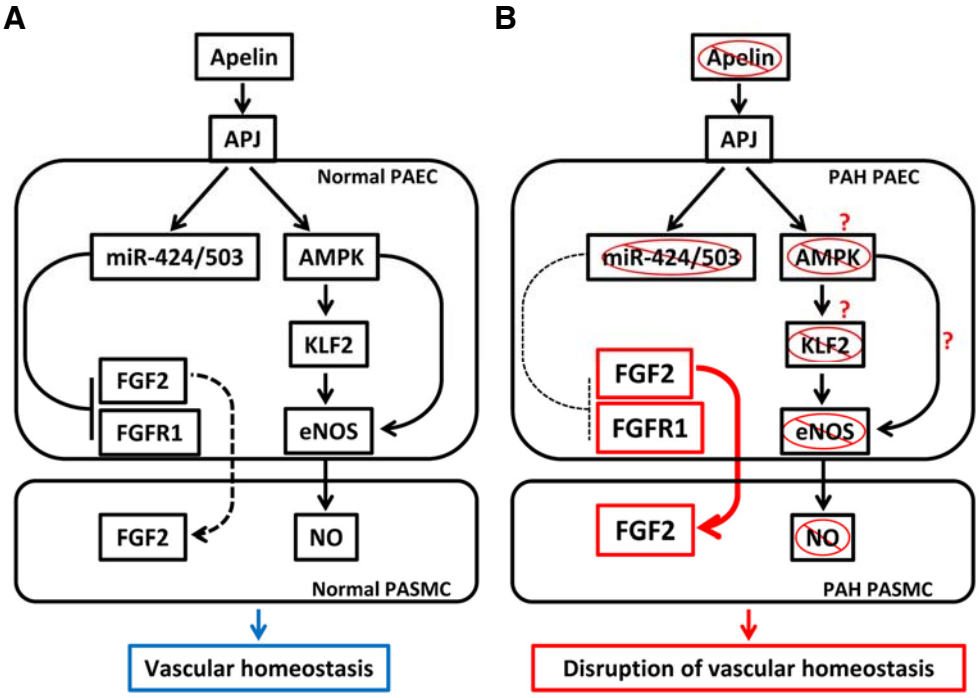

Fig. 2. Putative model of the mechanism of vascular homeostasis mediated by apelin-APJ signaling. (A) Basal apelin-APJ signaling maintains expression of miR-424/503, KLF2, and eNOS, as well as activity of AMPK. miR-424/503 in turn inhibits FGF2/FGFR1 expression, and eNOS induces the production of NO. (B) In PAH, there is decreased apelin, miR-424/503 and eNOS expression, leading to abnormal activation of FGF signaling pathway and reducing NO level that target both the PAECs and PASMCs.

represents a novel, potentially fruitful therapeutic target, pursuing various downstream signaling mechanisms that mediate the vascular protective effects of apelin-APJ signaling could yield important mechanistic data. The apelin-APJ pathway targets critical mediators of endothelial homeostasis, including KLF2, (Chandra et al., 2011; McLean et al., 2012) and endothelial nitric oxide synthase (eNOS) (Chandra et al., 2011). A potential role of eNOS in PAH is supported by the finding of decreased expression of eNOS in the lungs of PAH patients (Chandra et al., 2011; Giaid and Saleh, 1995). Apelin deficient mice are more susceptible to hypoxia-induced PAH, at least in part secondary to decreased eNOS expression in the lung of apelin-null mice as well as in the PAECs with apelin knockdown, leading to decreased NO production (Chandra et al., 2011). Although KLF2 regulation of eNOS expression has been well established (Lin et al., 2005; Sen-Banerjee et al., 2005), AMPK and KLF2 appear to be critical intermediaries for apelin/APJ regulation of eNOS expression *in vitro* knockdown studies.

NO regulated by the apelin-APJ-AMPK-KLF2-eNOS axis plays an important role in normal pulmonary vascular homeostasis and is a mediator of pulmonary vascular remodeling. It is a potent pulmonary vasodilator produced locally in the lungs and mediates smooth muscle relaxation and proliferation. eNOS catalyzes the conversion of L-arginine to citrulline, producing NO in a number of tissues, including the lungs. NO diffuses into PASMCs, leading to production of guanosine 3′,5′-cyclic monophosphate (cGMP) (Moncada and Higgs, 2006) *via* activation of guanylate cyclase. In turn, increased cGMP leads to hyperpolarization and PASMC relaxation. Patients with PAH have low levels of NO in their exhaled breath (Kaneko et al., 1998). Although NO administration is effective therapy for PAH, technical limitations of gas delivery have limited widespread use (Chan-nick et al., 1996; Rubin, 1997). Rather, multiple therapies may use NO as a mediator of their efficacy. For example, infusion of epoprostenol, a prostacyclin analogue, increases the NO levels in the exhaled breath of patients (Ozkan et al., 2001) (Fig. 2).

MiRNAs are a class of small, noncoding RNAs that have critical post-transcriptional regulatory roles targeting mRNA with their main function being down-regulation of gene expression (Kim, 2005). MiRNAs are emerging as key, powerful trans-acting factors that regulate gene expression and fundamental cellular processes (Kim and Kim, 2012), and which may play an important role in the pathogenesis of PAH, including miR-17, miR-20a, and miR-21 (Brock et al., 2012; Parikh et al., 2012; Pullamsetti et al., 2012). In addition, a recent study identified two key endothelial miRNAs, miR-424 and miR-503, which are regulated by apelin-APJ signaling and which target two molecules of the FGF signaling pathway (FGF2 and fibroblast growth factor receptor 1, FGFR1), associated with the cellular hyperproliferation and vascular remodeling found in PAH. These miRNAs are highly expressed in normal PAECs, but are also markedly decreased in PAECs isolated from patients with clinically diagnosed PAH (Kim et al., 2013). FGF2 expression is increased in PAECs from PAH patients (Izikki et al., 2009). Kim et al. (2013) also demonstrated that restoration of miR-424/503 expression in the experimental PAH models by intranasal lentiviral delivery can significantly ameliorate the severity of the disease through reversal of a progressive vascular remodeling by inhibition of abnormal proliferation of PAECs and PASMCs, suggesting that they may serve as potential therapeutic agents in clinical PAH (Fig. 2). The existing approved therapies primarily target achieving vasodilatation, whereas emerging data suggest that targeting abnormal proliferation of the vascular cells may be a viable therapeutic venue.

In the context of the endothelium, a number of miRNAs have emerged as critical regulators of cellular development and function (Urbich et al., 2004). However, the elucidation of their role in the PAECs and PAH is in its infancy. Further characterization of their involvement in PAH will provide new insight and poten-

tially novel therapeutic targets for this deadly disease through reversal of the hyperproliferative, antiapoptotic phenotype of the PAECs associated with PAH. Given that apelin-APJ signaling could regulate various downstream molecules, such as KLF2, eNOS and miR-424/503, that are able to modulate the vascular remodeling in PAH, apelin-APJ signaling is a very attractive therapeutic target for PAH therapy (Fig. 1).

CONCLUSIONS

In summary, PAH is characterized by vascular remodeling resulting from hyperproliferation of PAECs and PASMC in the pulmonary vasculature. Disruption of apelin-APJ signaling appears to play a major pathogenic role in these events. This view is also supported by restoration effects of PAH animal model caused by rescue of disrupted apelin signaling. Emerging evidence indicates that apelin-APJ signaling targets various key genes, such as eNOS, KLF2, miR-424, and miR-503, involved in the pathogenesis of PAH. Although apelin-APJ signaling-related targets in PAH need further elucidation, apelin may affect both reversal pulmonary vascular remodeling through the antiproliferatory effect of PAECs and PASMCs and its properties as a vasodilator, implicating it as a potentially useful therapeutic target in the treatment of PAH. Ongoing studies will yield substantial increase in our understanding of the molecular actions of various targets of apelin-APJ signaling in PAH.

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