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ACE2 and pACE2: A Pair of Aces for Pulmonary Arterial Hypertension Treatment?

Pulmonary arterial hypertension (PAH) is primarily a consequence of pulmonary arterial remodeling and vasoconstriction. Limited therapeutic successes coupled with complex disease etiology have been major impediments to the successful control and treatment of PAH. Therefore, this field needs innovative concepts and approaches for PAH therapy. It is in this regard that the paper in this issue of the *Journal* by Zhang and colleagues (pp. 509–520) on ACE2 phosphorylation and stability is extremely relevant (1).

ACE2 (angiotensin-converting enzyme 2), a carboxymonopeptidase, is a member of the vasoprotective axis of the renin–angiotensin system (RAS). Although both ACE2 and its homolog, ACE, are endothelial-bound enzymes and share 42% identity, ACE2 is insensitive to classical ACE inhibitors, is localized on the X chromosome, and acts essentially as an antagonist of vasoactive angiotensin II by catalyzing its conversion to Ang-(1–7) (angiotensin-[1–7]) (2). Therefore, ACE2/Ang-(1–7), along with its widely accepted receptor, Mas, plays an important role in maintaining normal cardiopulmonary homeostasis by balancing the deleterious ACE/Ang II/Ang II type I receptor axis. Identification of ACE2 as a functional receptor for coronavirus, implicating it in severe acute respiratory syndrome (SARS) during the SARS epidemic, was the first indication that ACE2 plays a role in lung diseases (3). Since then, an abundance of evidence has supported the fundamental concept that lung ACE2 is protective against a variety of pulmonary diseases, including pulmonary fibrosis, acute lung injury, acute respiratory distress syndrome, pulmonary hypertension (PH), asthma, and chronic obstructive pulmonary disease (4, 5).

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Because ACE2 has been shown to be decreased in the plasma of patients with PAH, several attempts have been made in animal models to increase ACE2 to provide proof of concept and evidence of translational value. Recombinant ACE2, pulmonary overexpression of ACE2, and the use of small-molecule ACE2 activators all attenuated PH pathophysiology (6). Furthermore, an innovative approach of orally feeding ACE2 in transplastomic preparations provided encouraging outcomes (7). However, all of these approaches have the inherent problem of a lack of ACE2 stability *in vivo*.

Zhang and colleagues (1) have discovered a novel mechanism-based concept to circumvent this problem. Their study showed that phosphorylation of Ser-680, located in the extracellular domain, is crucial to enhance ACE2 stability and hence expression of this enzyme. They demonstrate that AMPK (AMP-activated protein kinase) is the responsible enzyme in ACE2/Ser-680 phosphorylation leading to increased production of Ang-(1–7) and eNOS (endothelial nitric oxide synthase)-derived NO bioavailability. With the use of genetically modified mice and a combination of *in vitro* and *in vivo* experiments, they concluded that AMPK phosphorylation of ACE2/Ser-680–mediated stability could be due to inhibition of ubiquitin-related proteasomal degradation. The downstream signaling mechanism for enhanced NO production is primarily proposed to involve Ang-(1–7), Mas, and a cascade of traditional genes relevant to endothelial function. Thus, they concluded that activation of the AMPK/pACE2 axis counteracted the vasodeleterious axis of the RAS to attenuate PH pathophysiology. Finally, validation of the relevance of AMPK/pACE2 axis was provided by their data demonstrating decreased levels of pAMPK, pACE2, and ACE2 in lung biopsies from patients with idiopathic PAH. Therefore, this study by Zhang and colleagues not only reinforces the importance of AMPK and the ACE2/Ang-(1–7) axis for PH therapy but also provides an innovative way to address it.

This study also invites consideration of multiple issues related to the mechanism by which ACE2 produces its beneficial effects. PAH has traditionally been considered a disease of the pulmonary vasculature, which may have impeded progress toward identifying novel targets for drug development and therapy for this devastating disease. Targeting ACE2 and fully understanding its actions on the cardiopulmonary system are particularly relevant in this regard. Addressing the following issues could be crucial for translating the observations of Zhang and colleagues and other investigators regarding ACE2 to therapies for PAH:

1. ACE2 is a multifunctional enzyme. Thus, are all actions of ACE2 a result of its enzymatic activity to convert Ang II to Ang-(1–7)? There is strong evidence in support of this; however, other vasoactive targets for ACE2, such as des-Arg-bradykinin, which is involved in lung inflammation, should be considered (8). Furthermore, nonenzymatic actions of ACE2 directly or via remote organs should be kept in mind in view of its significant C-terminal homology with collectrin, which is involved in the transport of neutral amino acids (9) (e.g., arginine, the precursor of NO).
2. ACE2 is cleaved by ADAM17 and other sheddases in the lungs and other tissues, and circulates as soluble ACE2 (sACE2), which is enzymatically active (10). It remains to be seen whether sACE2 is phosphorylated or whether phosphorylation occurs in the circulation after its cleavage from the membrane. This would have important implications for its stability, half-life, and bioavailability.
3. Extrapulmonary expression of ACE2 (particularly in the gut) and its indirect impact on the lungs should be considered. The small intestine is the most abundant source of ACE2 and has been linked with regulation of antimicrobial peptides and microbiota (4, 11). Our studies have shown profound differences in gut microbiota in ACE2 knockin mice (12). Because the gut is the biggest immune organ and is highly innervated, it is reasonable to infer that gut ACE2-mediated effects on immune and neural systems might impact pulmonary functions. Oral delivery of ACE2 bioencapsulated in plant cells further suggests the role of a potential gut–lung communication (7).
4. Females have a three to four times higher risk of PAH, even though ACE2 is an X-linked enzyme. This is particularly intriguing in view of some evidence that estrogen positively influences ACE2 (13). This paradox needs to be resolved. Plasma levels of ACE2, pACE2, and sACE2 in pre- and postmenopausal females may shed some light on this.
5. Lung-selective ACE2 delivery to increase local activity of the vasoprotective axis of the RAS would be ideal to circumvent unpredictable immunogenicity and functional modifications in circulation. A chemically modified ACE2 mRNA delivered via lipid nanoparticles offers such an alternative for future consideration (14).

This study by Zhang and colleagues adds important new data to support the overall concept that an increase in the vasoprotective axis of the RAS is beneficial in PAH and indeed ACE2 stability. The AMPK-pACE2 pathway could be the basis for moving forward. Gaining a better understanding of the mechanism of ACE2, the role of sACE2, and the gut–lung axis would certainly be an important next step in developing novel therapeutic strategies involving this molecule. ■

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