Connecting the dots: the role of connexins in the pulmonary vascular response to hypoxia

To the Editor:

We read with great interest the recent manuscript by Bouvard *et al.* [1], which suggested the gap junctional protein connexin-43 (Cx43) to be a promising target for the treatment of chronic hypoxic pulmonary hypertension (CHPH). Therein, the authors demonstrated increased Cx43 expression in human pulmonary arteries of CHPH patients, while heterozygous Cx43 deficient mice were partially protected from CHPH.

These findings align with a series of previous studies highlighting the role of connexins in the pulmonary vascular response to hypoxia: I. McMurtry and co-workers first reported that the non-specific gap junction blocker 18α-glycyrrhetinic acid blunts the acute vasoconstrictive response to hypoxia in isolated perfused rat lungs [2]. Using a combination of small peptide inhibitors and gene deficient mice, subsequent work by our own groups demonstrated that both connexin-40 (Cx40) and Cx43 contribute additively to hypoxic pulmonary vasoconstriction, and that Cx40 deficient mice are largely protected from the development of CHPH [3]. Attenuation of the vasoconstrictive response of pulmonary arteries to hypoxia *in vivo* and *ex vivo* by 18α-glycyrrhetinic acid was further confirmed by the laboratory of J.P.T. Ward and P.I. Aaronson [4]. Consistent with these reports, the findings by Bouvard *et al.* [1] consolidate the requirement of connexins for the pulmonary vascular response to hypoxia. Notably, however, this concept has recently been challenged by a report that Cx40 deficient mice develop more severe CHPH, which can be attenuated by adenoviral Cx40 overexpression [5]. The reason for the obvious discrepancy between these [5] and earlier [3] findings remains unclear.

Connexins are the building blocks of gap junctions, *i.e.* they form intercellular communication "channels" composed of two opposing homo- or heterotypic (*i.e.* identical or not) connexons, each of which is again a hexamer consisting of six identical (homomeric) or different (heteromeric) connexins [6]. In the vasculature, gap junctions are typically composed of one of three connexins, namely connexins 37, 40 and 43 [6]. In the systemic circulation, vascular gap junctions have been long recognised as important "highways" for retrograde signal conduction from capillaries to upstream arterioles in order to match resistance vessel tone and, thus, blood flow to local demands in the "downstream" tissue. In the lung, an analogous signal propagation from capillaries to upstream arterioles mediates the pulmonary vascular response to hypoxia. This signal conduction is required due to the spatial separation between the site of gas exchange, *i.e.* the alveolo-capillary compartment, and the site of vasoconstriction and vascular remodelling in response to acute and chronic hypoxia, respectively, which are localised in upstream arterioles [3, 7]. Although direct visualisation of gas exchange in intact lungs by multispectral oximetry has revealed relevant precapillary oxygenation, indicating that alveolar hypoxia can directly impact oxygen tension (PO_2) in the vascular wall of precapillary pulmonary vessels, this phenomenon is restricted to arterioles of <30 µm in diameter [8]. PO_2 in the vascular wall of larger vessels is, conversely, determined by central venous PO_2, which is, however, not an adequate trigger for hypoxic vasoconstriction and remodelling in the lung [9]. Hence, vasoconstriction and remodelling of pulmonary arteries >30 mm in response to acute or chronic hypoxia requires retrograde signal propagation along the vascular wall. Gap junctions facilitate such intercellular communication by propagating changes in cell membrane potential along either the endothelial or the smooth muscle layer, or between the two cell types *via* so-called

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Gap junctions, composed of connexins 37, 40 or 43, mediate the pulmonary vascular response to acute and chronic hypoxia as they propagate the hypoxic signal from the site of gas exchange retrogradely to the feeding arteri(ol)es https://bit.ly/3orJKXM

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myo-endothelial gap junctions. In line with the concept of such a conducted response, direct imaging of endothelial membrane potential in intact lungs revealed endothelial depolarisation in alveolar capillaries in response to hypoxia that propagated upstream to feeding arterioles in wild type, but not in Cx40-deficient mice [3].

While the expression of Cx40 in pulmonary vessels is restricted to the arteriolar and capillary endothelium, Cx43 is expressed in both endothelial and smooth muscle cells and may as such propagate changes in membrane potential along the intimal or medial layer, or between both. Notably, combined application of inhibitory mimetic peptides for both Cx40 and Cx43 causes an additive inhibitory effect on hypoxic pulmonary vasoconstriction as compared to each inhibitory peptide alone, indicating that different connexins serve distinct roles in the propagation of hypoxia. Consistent with this concept, even the vasoconstrictive response to hypoxia in isolated pulmonary arterioles, which are exposed *in toto* to hypoxia and, hence, do not require retrograde signal propagation, has been found to require gap junctional communication, suggesting an important role for myo-endothelial gap junctions in the pulmonary vascular response to hypoxia [4]. Yet, whether Cx43-composed gap junctions form the structural correlate for this functional observation remains to be determined.

In addition to the need for a better understanding of the exact cell type(s) connected by Cx43-mediated signalling in the pulmonary vascular response to hypoxia, future studies may also aim to elucidate hypoxia-dependent post-translational modifications in Cx43, as Cx43-mediated gap junctional intercellular communication is critically regulated *via* phosphorylation of several serine residues in its carboxyl-terminus [10].

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