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INVITED EDITORIAL

Cyclophilin D and p66Shc contribute to KCl-induced Ca^{2+} increase in pulmonary artery smooth muscle cells: a potentially relevant phenomenon awaiting a definite mechanism

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This editorial refers to 'Genetic deletion of p66Shc and/or cyclophilin D results in decreased pulmonary vascular tone' by M. Gierhardt *et al.*, pp. 305–315.

A recent report in this journal[1] demonstrated that deletion of cyclophilin D (CypD) and/or p66Shc decrease contraction and intracellular [Ca^{2+}] in response to hypoxia or KCl stimulation in pulmonary arterial smooth muscle cells (PASMCs). One merit of this study is to shed light upon the role of those two mitochondrial proteins in lung pathophysiology, especially with regard to pulmonary circulation.

CypD is a matrix prolyl isomerase that is noted for its involvement in the opening of the mitochondrial permeability transition pore (PTP). CypD binding to the inner mitochondrial membrane favours PTP opening in a process stimulated by a matrix increase in Ca^{2+} and reactive oxygen species (ROS). A prolonged PTP opening causes mitochondrial depolarization, ATP and NAD depletion, as well as a wide array of cellular alterations incompatible with cell survival.[2] Consistent with the notion that mitochondrial Ca^{2+} overload and oxidative stress are causally related to cell death, inhibition or deletion of CypD elicits cytoprotective effects, especially in the context of myocardial ischaemia. However, a transient PTP opening has been suggested to act as a fast and efficient mitochondrial pathway for releasing Ca^{2+} accumulated within the matrix space.[2] Accordingly, PTP inhibition is associated with an increase in intramitochondrial Ca^{2+}.

The adaptor protein p66Shc is involved in mitochondrial ROS formation. Stress conditions result in p66Shc phosphorylation followed by its translocation into the mitochondrial intermembrane space, where it catalyzes the electron transfer from cytochrome c to oxygen causing H_2O_2 formation. p66Shc deletion antagonizes ageing, decreases oxidative stress, and protects against cardiac injury induced by ischaemia and reperfusion.[3] Based upon its role in facilitating mitochondrial ROS formation, p66Shc has been associated with an increased propensity to PTP opening and cell death. In addition, p66Shc-induced oxidative stress is likely to impair intracellular Ca^{2+} homeostasis by oxidizing transporters involved in the maintenance of optimal Ca^{2+} levels within the various cellular compartments.[4]

PTP opening has been shown to increase ROS formation that might prolong the duration of the initial event and/or cause opening in adjacent mitochondria exacerbating the detrimental consequences of ensuing mitochondrial dysfunction. Notably, while ROS are likely to stimulate PTP opening by oxidizing its components and modulators, such as CypD, a convincing mechanism is not yet available to explain how PTP opening increases ROS levels.[4]

Due to their role in oxygen sensing, ROS formation and intracellular Ca^{2+} homeostasis, mitochondria are likely to contribute to mechanical activity in PASMCs, including contraction of pulmonary arteries in response to alveolar hypoxia.[5] In this respect, the study of Gierhardt *et al.* adds novel information on the role of p66Shc and CypD in acute and chronic responses of lungs and PASMCs to hypoxia. The study was carried out by investigating the effects of CypD and/or p66Shc deletion. The absence of either of these two proteins did not affect pulmonary vascular remodelling caused by chronic hypoxia. On the other hand, deletion of CypD, p66Shc, or both decreased pulmonary vascular resistance. In addition, systemic arterial pressure was decreased only in $CypD^{-/-}$ mice. Notably, this effect has not been described before, despite the numerous studies carried out in animals lacking CypD or in humans treated with PTP inhibitors. Additional experiments in isolated cells demonstrated that changes in vascular resistance were associated with modifications of intracellular Ca^{2+} homeostasis. Indeed, in isolated PASMCs all deletions attenuated both KCl-induced pulmonary vasoconstriction and KCl-induced increase in cytosolic [Ca^{2+}] in the absence of changes in ROS levels. In addition, KCl-induced mitochondrial [Ca^{2+}] and the ability to accumulate Ca^{2+} in the matrix (i.e. the so-called calcium retention capacity) were increased only in mitochondria isolated from lungs of $CypD^{-/-}$ mice.

Overall, the report of Gierhardt *et al.* provides novel and interesting evidence that CypD and p66Shc shape changes in Ca^{2+} levels in the

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cytosol in a redox-independent manner. An explanation for the findings of Gierhardt is not immediately available. Actually, based upon our current understanding of PTP and p66Shc relationships with intracellular Ca^{2+} homeostasis, those results appear somewhat counterintuitive.

An increased CRC is expected in mitochondria devoid of CypD. However, this does not necessarily imply that PTP opening occurred and/or was affected in isolated PASMCs subjected to hypoxia and/or KCl stimulation. It should be demonstrated that the observed increase in $[Ca^{2+}]_i$ is sufficient to cause a transient or prolonged PTP opening in situ. On the other hand, CypD deletion causes an increase in intramitochondrial Ca^{2+} under basal conditions, but this could hardly cause a decrease in cytosolic Ca^{2+} levels, especially in cells such as PASMCs with a relatively low abundance of mitochondria. Notably, PTP inhibition increases matrix Ca^{2+} in mitochondria-rich cardiomyocytes without affecting cytosolic Ca^{2+} transients. Furthermore, CypD has been shown to interact with the VDAC/Grp75/IP3R1 complex in the heart facilitating the Ca^{2+} transfer from endoplasmic/sarcoplasmic reticulum (ER/SR) to mitochondria. Accordingly, genetic or pharmacologic inhibition of CypD in cardiomyocytes decreased SR Ca^{2+} release and the consequent mitochondrial uptake.[6] Therefore, *in situ* CypD might also affect mitochondrial Ca^{2+} accumulation along with SR Ca^{2+} release that could contribute to the results of Gierhardt *et al*. An additional possibility is that CypD deletion might cause adaptive changes, such as profound metabolic remodelling previously described in hearts devoid of CypD[7] that might interfere with intracellular Ca^{2+} movements.

Finally, it is even more difficult to explain the effect of p66Shc deletion on cytosolic Ca^{2+} movements. A previous report showed that in primary kidney smooth muscle cells stimulated with endothelin-1, p66Shc deletion led to the activation of transient receptor potential cation (TRPC) channels and increased cytosolic Ca^{2+} influx.[8] While cytosolic p66Shc functions as an inhibitor of TRPC channel activity and Ca^{2+} influx, the mitochondrial effects of p66Shc on cytosolic Ca^{2+} levels are less clear. For instance, although no change in ROS levels was observed in Gierhardt study, the notion of the antioxidant property of p66Shc deletion would suggest an increase, rather than a decrease, in cytosolic Ca^{2+} leading to vasoconstriction. Indeed, in hypoxic PASMCs a decrease in mitochondrial H_2O_2 formation has been causally related to inhibition of the plasma membrane K^+ channel Kv1.5. The consequent depolarization promotes Ca^{2+} entry and vasoconstriction.[5] Whether p66Shc plays a role in this process remains to be established. Although no change in cytosolic ROS levels was observed in the Gierhardt study, mitochondrial ROS levels were not assessed and it cannot be excluded that ROS hotspots modulate Ca^{2+} levels in WT PASMCs.

In conclusion, the interesting findings in Gierhardt's report should prompt future studies aimed at elucidating how CypD and p66Shc, and, more generally mitochondria, modulate Ca^{2+} movements in PASMCs.

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