Pulmonary Circulation

The changes in endothelial cytoskeleton and calcium in vascular barrier breakdown: a response of ever-growing complexity

Pulmonary Circulation 2018; 8(1) 1–3
DOI: 10.1177/2045893218754854

The endothelium forms a tightly regulated semi-permeable barrier that dictates the rate of passage of fluid and macromolecules from the vessel lumen towards the surrounding extravascular space. This endothelial barrier is a critical aspect of fluid homeostasis. Many inflammatory stimuli promote the disruption of this barrier, leading to the formation of tissue edema. While this is a necessary aspect of the inflammatory response, when dysregulated, it can lead to tissue damage and organ injury, such as during acute respiratory distress syndrome.[1] Given its importance, it is not surprising that multiple mediators regulate the strength of this barrier, including a large number of inflammatory and edemagenic factors that include cytokines, growth factors, proteases, and small molecules acting systemically or in a paracrine fashion to activate a plethora of intracellular signaling mechanisms.[2,3] As Hamilton et al. now show in their article in this issue of *Pulmonary Circulation*, a novel immunophilin is a new player in this complex system of endothelial regulators.

The best understood mechanisms of endothelial barrier disruption involve acute responses that lead to short-term opening of the barrier, mediated in large part by the activation of the Src family of kinases and the Rho family of small GTPases.[2,3] However, many aspects of this response are still a matter of intense research. Some the questions that are still unanswered are: How do these pathways become activated? When is the activation of these pathways required? For which edemagenic mediators and under which pathological conditions? Thrombin, a protease originally identified as the enzyme that mediates the cleavage of fibrin to regulate clotting, promotes in cultured endothelial cells a dramatic increase in permeability that lasts for approximately 2 h via the activation of the protease-activated receptor (PAR) 1, a member of the G protein-coupled receptors (GPCR) superfamily. PAR1 activation promotes RhoA-mediated actin stress fibers formation and the disassembly of the endothelial cell–cell junctions, thus leading to monolayer gap formation barrier breakdown.[2] Multiple GPCRs induce calcium entry via the store-operated calcium entry (SOCE) pathway, causing RhoA and myosin light chain kinase (MLCK) activation to form new actin stress fibers. This way, SOCE promotes an acute endothelial response that includes a sharp decrease in barrier function that is very similar to thrombin. Whether calcium mediates thrombin/PAR1-induced endothelial permeability increases is still a matter of active debate.[2,4]

SOCE is a physiological cellular response to the depletion of inositol 1,4,5-trisphosphate (IP_3)-sensitive endoplasmic reticulum calcium contents (Fig. 1). This Ca^{2+} depletion promotes extracellular calcium entry via the activation of several plasma membrane channels, including the highly selective Ca^{2+} release-activated Ca^{2+} (CRAC) channel (mediated by STIM-1/Orai-1) and the less Ca^{2+}-selective store-operated channel (SOC) mediated by the transient receptor potential (TRP) proteins.[5] Normally, endoplasmic reticulum calcium stores are maintained through the activity of the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), a Ca^{2+} pump. Thapsigargin, a SERCA inhibitor, prevents Ca^{2+} pumping into the endoplasmic reticulum, thus effectively inducing the depletion of the calcium stores[5] (Fig. 1). It has been widely used to induce SOCE independently of IP_3. Thapsigargin can also induce other responses that are initiated by endoplasmic reticulum Ca^{2+} depletion, such as the unfolded protein response.

In the paper published in this issue, Hamilton et al. used thapsigargin to study a new regulator of SOCE-induced barrier function loss. Using rat pulmonary artery endothelial cells (PAECs) as a model, the authors show that the immunophilin FK506 binding protein (FKBP) 51, a protein that was previously known to bind SOC channel components, negatively regulates an inward Ca^{2+} current in cells treated with thapsigargin that is compatible with the SOC current. Cells overexpressing FKBP51 not only show reduced SOC current, but also a stabilization of microtubules, reduced formation of actin stress fibers, and a drastically diminished barrier function loss after thapsigargin treatment, mechanistically linking SOCE and FKBP51 to cytoskeletal changes and endothelial barrier disruption. Because of the observed

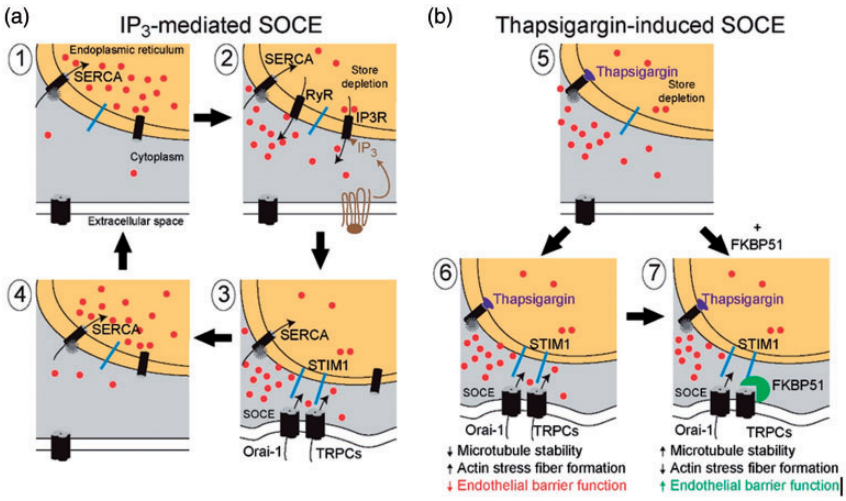

Figure 1. IP3- and thapsigargin-induced SOCE. (a) In non-activated endothelial cells (1), the endoplasmic reticulum is a major store of Ca^{2+}. Upon IP_3 binding to the IP_3 receptor (IP3R) and/or Ca^{2+} binding to ryanodine receptor (RyR), Ca^{2+} is released from the endoplasmic reticulum, leading to a depletion of the endoplasmic Ca^{2+} store (2). This, in turn, induces STIM1-mediated SOCE via Orai-1 and TRPC channels (3). SERCA Ca^{2+} pumping restores the calcium stores to return to resting conditions (4). (b) By using a SERCA inhibitor such as thapsigargin, Ca^{2+} leak without replenishment leads to receptor-independent calcium store depletion (5), activating all the molecules involved in SOCE and leading to cytoskeletal disruption and endothelial barrier loss (6). Hamilton et al. now showed that cells overexpressing FKBP51 have reduced SOCE by blocking TRPC-mediated Ca^{2+} entry, reducing the cytoskeletal changes and restoring barrier function (7).

effects of thapsigargin and FKBP51 on microtubule stability, the authors then tested whether preventing microtubule polymerization (using nocodazole) altered Ca^{2+} influx. While nocodazole treatment in control cells reduced SOC currents, the same treatment in FKBP51-overexpressing cells rescued the FKBP51-mediated SOC current inhibition, suggesting that this current depends on a delicate balance of microtubule polymerization.

In addition, Hamilton et al. showed that lungs from mice lacking FKBP51 had a marked increase in the response to thapsigargin-induced barrier breakdown assays, providing strong evidence that FKBP51 limits SOCE-induced barrier function loss in vivo. These assays were performed using global knockouts. As reviewed by Dr. Townsley recently in *Pulmonary Circulation*, the Ca^{2+} response in the pulmonary vasculature is very heterogeneous.[6] It remains to be determined which endothelial cells express FKBP51 and whether expression of FKBP51 outside the endothelium (e.g. smooth muscle cells [SMCs]) plays any role in the regulation of SOCE-induced edema.

A number of FKBPs have been identified in a variety of cell types and been shown to mediate many cellular processes. Several reports indicate that FKBP12 is expressed in pulmonary vascular endothelial cells and may play an important role in pulmonary artery hypertension (PAH); as such, FK506 (also called tacrolimus) has been shown to dissociate FKBP12 from all three type 1 bone morphogenetic protein receptors (ALK1, ALK2, and ALK3), activate BMPR2-mediated signaling, and regulate endothelial-specific genes (e.g. apelin), thereby inhibiting the development of PAH.[7,8] The signaling molecules described herein are also expressed in PASMCs; thus, it would be interesting to determine their potential contribution in the effect of FK506 on PAH.

An FKBP12 analogue, FKBP12.6, is expressed in both PAECs and PASMCs.[7,9,10] The role of FKBP12.6 has not been yet investigated in PAECs. On the other hand, FKBP12.6 may be dissociated from ryanodine receptors (RyRs, particularly RyR2) and subsequently induce Ca^{2+} release, contributing to endothelial dysfunction and hypertension. Evidently, FKBP12.6 is important for pulmonary vasoconstriction and remodeling, thus playing a significant role in hypoxia-induced and related pulmonary hypertension.

Finding new therapeutic avenues to block vascular leakage and edema will undoubtedly improve the outcomes of acute and chronic inflammatory pulmonary diseases by reducing pulmonary edema and thus improving gas exchange. The challenge is now to establish whether FKBP51 acts to limit the vascular response to edemagenic factors in inflammatory conditions. Given that loss of TRPC channels can lead to an increased response to endotoxins,[2] it is tempting to speculate a role for FKBP51 in limiting the inflammatory response through the modulation of SOC Ca^{2+} currents. Because the expression of this protein can be regulated by steroids, it will be important to determine when and how the pulmonary vasculature expresses FKBP51 and whether anti-inflammatory agents can be used to increase its expression. The activity of many other cell types, including neutrophils and other circulating cells, may depend on SOC currents during the inflammatory response.[6] Understanding how Ca^{2+} signaling in these other cells may affect the extent of vascular barrier

breakdown remains an important challenge. Lastly, further studies are necessary to ascertain the role of each individual FKBPs and their cooperative functions in the pulmonary vasculature in health and disease.

References

1. Millar FR, Summers C, Griffiths MJ, et al. The pulmonary endothelium in acute respiratory distress syndrome: insights and therapeutic opportunities. *Thorax* 2016; 71: 462–473.
2. Komarova YA, Kruse K, Mehta D, et al. Protein interactions at endothelial junctions and signaling mechanisms regulating endothelial permeability. *Circ Res* 2017; 120: 179–206.
3. Adam AP. Regulation of endothelial adherens junctions by tyrosine phosphorylation. *Mediators Inflamm* 2015; 2015: 272858.
4. Shinde AV, Motiani RK, Zhang X, et al. STIM1 controls endothelial barrier function independently of Orai1 and Ca^{2+} entry. *Sci Signal* 2013; 6: ra18.
5. Earley S and Brayden JE. Transient receptor potential channels in the vasculature. *Physiol Rev* 2015; 95: 645–690.
6. Townsley MI. Permeability and calcium signaling in lung endothelium: unpack the box. *Pulm Circ* 2018; 8: 2045893217738218.
7. Bonner JM and Boulianne GL. Diverse structures, functions and uses of FK506 binding proteins. *Cell Signal* 2017; 38: 97–105.
8. Spiekerkoetter E, Tian X, Cai J, et al. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest* 2013; 123: 3600–3613.
9. Zheng YM, Mei QB, Wang QS, et al. Role of FKBP12.6 in hypoxia- and norepinephrine-induced Ca^{2+} release and contraction in pulmonary artery myocytes. *Cell Calcium* 2004; 35: 345–355.
10. Liao B, Zheng YM, Yadav VR, et al. Hypoxia induces intracellular Ca^{2+} release by causing reactive oxygen species-mediated dissociation of FK506-binding protein 12.6 from ryanodine receptor 2 in pulmonary artery myocytes. *Antioxid Redox Signal* 2011; 14: 37–47.

Alejandro Pablo Adam[1,2], Yun-Min Zheng[1] and Yong-Xiao Wang[1]

[1]Department of Molecular and Cellular Physiology, Albany Medical College, Albany, NY, USA; [2]Department of Ophthalmology, Albany Medical College, Albany, NY, USA

Corresponding author: Yong-Xiao Wang, Department of Molecular and Cellular Physiology, Albany Medical College, 47 New Scotland Avenue, Albany, NY 12208, USA.
Email: WangY@mail.amc.edu