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References

1. Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK. Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. *Am J Respir Crit Care Med* 2007;176:277–284.
2. Garcia CK. Idiopathic pulmonary fibrosis: update on genetic discoveries. *Proc Am Thorac Soc* 2011;8:158–162.
3. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease? *Proc Am Thorac Soc* 2006;3:293–298.
4. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949–1961.
5. Kass DJ, Kaminski N. Evolving genomic approaches to idiopathic pulmonary fibrosis: moving beyond genes. *Clin Transl Sci* 2011;4:372–379.
6. Yang IV, Schwartz DA. Epigenetic control of gene expression in the lung. *Am J Respir Crit Care Med* 2011;183:1295–1301.
7. Yang IV. Epigenomics of idiopathic pulmonary fibrosis. *Epigenomics* 2012;4:195–203.
8. Pandit KV, Milosevic J, Kaminski N. MicroRNAs in idiopathic pulmonary fibrosis. *Transl Res* 2011;157:191–199.
9. Rabinovich EI, Selman M, Kaminski N. Epigenomics of idiopathic pulmonary fibrosis: evaluating the first steps. *Am J Respir Crit Care Med* 2012;186:473–475.
10. Rabinovich E, Yakhini Z, Benos P, Pandit K, Milosevic J, Kapetanak M, Richards T, Chensny L, Kaminski N. Human CpG islands arrays reveal changes in global methylation patterns in idiopathic pulmonary fibrosis. *PLoS ONE* 2012;7:e33770.
11. Sanders YY, Ambalavanan N, Halloran B, Zhang X, Liu H, Crossman DK, Bray M, Zhang K, Thannickal VJ, Hagood JS. Altered DNA methylation profile in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;186:525–535.
12. Dakhlallah D, Batte K, Wang Y, Cantemir-Stone CZ, Yan P, Nuovo G, Mikhail A, Hitchcock CL, Wright VP, Nana-Sinkam SP, *et al*. Epigenetic regulation of *miR-17∼92* contributes to the pathogenesis of pulmonary fibrosis. *Am J Respir Crit Care Med* 2013;187:397–405.
13. Concepcion CP, Bonetti C, Ventura A. The microRNA-17–92 family of microRNA clusters in development and disease. *Cancer J* 2012;18:262–267.
14. Butz H, Racz K, Hunyady L, Patocs A. Crosstalk between TGF-beta signaling and the microRNA machinery. *Trends Pharmacol Sci* 2012;33:382–393.
15. Issa JP, Kantarjian HM. Targeting DNA methylation. *Clin Cancer Res* 2009;15:3938–3946.
16. Kaminskas E, Farrell AT, Wang YC, Sridhara R, Pazdur R. FDA drug approval summary: azacitidine (5-azacytidine, Vidaza) for injectable suspension. *Oncologist* 2005;10:176–182.
17. Saba HI. Decitabine in the treatment of myelodysplastic syndromes. *Ther Clin Risk Manag* 2007;3:807–817.
18. Amatori S, Bagaloni I, Donati B, Fanelli M. DNA demethylating antineoplastic strategies: a comparative point of view. *Genes Cancer* 2010; 1:197–209.
19. Brock MV, Hooker CM, Ota-Machida E, Han Y, Guo M, Ames S, Glockner S, Piantadosi S, Gabrielson E, Pridham G, *et al*. DNA methylation markers and early recurrence in stage I lung cancer. *N Engl J Med* 2008;358:1118–1128.
20. Juergens RA, Wrangle J, Vendetti FP, Murphy SC, Zhao M, Coleman B, Sebree R, Rodgers K, Hooker CM, Franco N, *et al*. Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. *Cancer Discov* 2011;1:598–607.
21. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R. Induction of tumors in mice by genomic hypomethylation. *Science* 2003;300:489–492.
22. You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* 2012;22:9–20.
23. Heard E, Tishkoff S, Todd JA, Vidal M, Wagner GP, Wang J, Weigel D, Young R. Ten years of genetics and genomics: what have we achieved and where are we heading? *Nat Rev Genet* 2010;11:723–733.

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Mitochondria in Hypoxic Pulmonary Vasoconstriction

Potential Importance of Compartmentalized Reactive Oxygen Species Signaling

About 15 years ago, Paul Schumacker and his then student, Nav Chandel, reported that in cultured Hep3b cells hypoxia caused mitochondrial production of reactive oxygen species (ROS) that were necessary for accumulation of the master transcriptional regulator in hypoxia, Hif1, and attendant gene expression (1). Since then—and including an important contribution in this issue of the *Journal* (pp. 424–432) (2)—Greg Waypa and the Schumacker research team have shown that specific disruption of mitochondrial complex III function by genetic deletion of the nuclear gene encoding the Riske iron-sulfur protein (RISP) inhibits hypoxia-induced ROS generation, calcium mobilization, and acute pulmonary vasoconstriction. The current work is significant for at least two reasons. First, it translates previous observations in cultured pulmonary arterial smooth muscle cells (PASMCs) to intact RISP-deficient mice, thus confirming in an integrated system that complex III is indeed a source of hypoxia-induced ROS generation. And second, the current article continues an evolving theme that ROS-mediated signaling is compartmentalized.

Regarding the first issue, the murine reagent described by Waypa and colleagues should enable more detailed studies of the links between complex III–derived ROS and pathophysiologic processes that can be examined only in an intact model. Among many possible uses of the model, several seem especially pertinent. For example, it may now be possible to determine whether the long-appreciated suppression of localized hypoxic vasoconstriction and dysregulation of ventilation–perfusion matching in sepsis (3) are associated with defective signaling at the level of mitochondrial complex III. In addition,

and as the authors point out, although the available data support the view that complex III–derived ROS are important for the calcium mobilization underlying acute hypoxic vasoconstriction, the question of whether a similar ROS-dependent pathway drives sustained pulmonary vasoconstriction and vascular remodeling in chronic hypoxic pulmonary hypertension should now be amenable to resolution. Finally, the article by Waypa and colleagues raises intriguing questions about the cellular basis of hypoxic pulmonary vasoconstriction. Whereas the current findings support the concept that the PASMC is both a sensor and an effector of hypoxic pulmonary vasoconstriction (3), Wang and coworkers, using multiple strategies to inhibit connexin 40 (Cx40)-mediated gap junctional signaling in intact mice, recently presented evidence that the vasoconstrictor response to alveolar hypoxia is initiated by depolarization of pulmonary capillary endothelial cells (4). In their paradigm, identified as "out of the box" in an accompanying editorial (5), the pulmonary microvascular endothelium, not the pulmonary arterial smooth muscle, functions as the cellular oxygen sensor that initiates a signal conducted retrogradely via endothelial Cx40-containing gap junctions to activate smooth muscle contraction in upstream muscular arteries. Some of the observations reported by Waypa and colleagues may bear on this divergence of evidence. They noted in cultured PASMCs that hypoxia caused only a transient increase in cytosolic calcium that was inhibited by RISP depletion, whereas in small arteries observed in precision-cut lung slices, the RISP-sensitive calcium response was sustained over the course of hypoxic exposure. Perhaps these temporal differences in cytosolic calcium accumulation reflect the contribution of endothelial cells to regulation of hypoxic vasoconstriction in the intact lung tissue that does not occur in cultured PASMCs. Taking this notion one step further, it is reasonable to consider whether the molecular mechanism of the hypoxia-induced depolarization of the pulmonary capillary endothelial cell is similar to that identified in the PASMC; that is, is the endothelial cell depolarization triggered by increased mitochondrial ROS production? Hopefully, the sophisticated strategies and reagents used by the Schumacker (2) and Kuebler (4) labs can be combined to address these questions.

The article by Waypa and colleagues also contributes to the evolving concept that ROS-dependent signaling is highly compartmentalized. Using reduction-oxidation–sensitive green fluorescent protein (roGFP) redox probes targeted to the mitochondrial matrix, intermembrane space, and cytoplasm, they found that hypoxia exerted divergent effects on the redox status of these cellular compartments; whereas the mitochondrial matrix was progressively reduced, the intermembrane space and cytosol became more oxidized. This compartmentalized pattern of oxidant stress makes sense; the hypoxia-induced oxidant stress originating at complex III is "vectored" away from the mitochondrial matrix where oxidative damage to the sensitive mitochondrial genome could be expected to disrupt mitochondrial transcription, possibly triggering a bioenergetic crisis and/or cell death (6). And as shown by Waypa and coworkers and discussed below, the oxidant stress in the cytosol triggers calcium accumulation necessary for hypoxic pulmonary vasoconstriction.

Because the roGFP used in the study by Waypa and colleagues was diffusely distributed throughout the cytoplasm, it was not possible to determine if the hypoxia-induced, mitochondria-dependent oxidant stress was more or less prominent in specific cytoplasmic domains. This becomes an interesting issue because it is widely appreciated that mitochondria are motile organelles whose distribution has the potential to determine their functional activities (7). For example, kinesin-dependent movement of mitochondria to a submembrane region in close proximity to the "immunologic synapse" in activated immune cells increases local calcium buffering capacity to sustain transmembrane calcium influx through calcium release–activated membrane calcium channels (8). More germane to the focus of the study by Waypa and coworkers, Al-Mehdi and colleagues recently showed in hypoxic pulmonary artery endothelial cells that dynein-driven perinuclear clustering of ROS-producing mitochondria creates a nuclear oxidant stress (9). This nuclear oxidant stress leads to oxidative base modifications in promoter sequences of hypoxia-inducible genes that seem to be important for transcriptional activation. Noted but not pursued in this latter report was the observation that perinuclear mitochondrial clustering was accompanied by a diminution in the peripheral cytosolic density of mitochondria. Putting these findings in the context of Waypa and colleagues' article, it is tempting to speculate that acute hypoxia-induced mitochondrial ROS production interacts with time-dependent changes in mitochondrial distribution to create signaling microdomains (Figure 1). One critical domain could be at the mitochondrial–sarcoplasmic reticulum (SR) interface, where local ROS might function to regulate SR calcium release and cytosolic calcium accumulation. By contrast, the relative diminution of mitochondria in the vicinity of the cell membrane could serve to create a local environment relatively deficient in mitochondria-derived signaling molecules or functions, thereby impacting the operation of plasma membrane ion channels and other processes.

The mechanism underlying the reported link between mitochondrial-derived oxidant stress and the hypoxia-induced cytosolic calcium accumulation required for pulmonary vasoconstriction is not understood. It is possible, however, that a compartmentalization of the oxidant stress may contribute to these regulatory processes. In this regard, experiments in cultured PASMCs suggest that hypoxia may induce SR calcium release by causing ROS-mediated dissociation of FK506 binding protein 12.6 from ryanodine receptor 2 (10, 11). Another contributory mechanism might be that perimitochondrial ROS impair mitochondrial calcium sequestration, as reported for carotid glomus cells (12), thereby exaggerating cytosolic calcium accumulation triggered by SR calcium release. In this scenario, SR calcium release and mitochondrial calcium uptake would be reciprocally regulated by complex III–generated ROS.

The concept of compartmentalized regulation of ROS signaling in hypoxia as advanced above and inferred from the data of Waypa and colleagues is far from proven. However, we think that it makes intuitive sense. After all, ROS are intrinsically dangerous and can react with macromolecules necessary for cell survival as well as adaptation. Mechanisms restricting access of otherwise cytotoxic ROS to targets important for their signaling function have been described and include their generally short half-lives, the various ROS-scavenging enzymes, the proximity

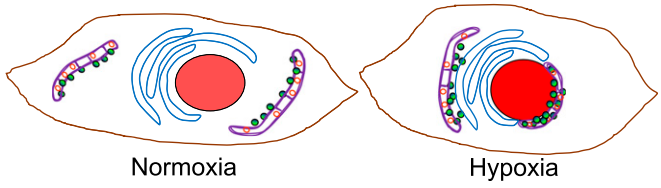

Figure 1. Signal compartmentalization by source translocation. Mitochondria (*purple*), being motile organelles, can create localized signaling environments by clustering to specific cellular compartments. In normoxia, mitochondria in pulmonary arterial smooth muscle cells are distributed throughout the cytoplasm. With hypoxia, they cluster in the central areas of the cell, creating a reactive oxygen species–, ATP-, and calcium-enriched signaling environment (*green*) in the vicinity of the nucleus (*red*) and sarcoplasmic reticulum (*blue*) and a mitochondrial signal–deficient environment near the plasma membrane (*brown*). Creation of a signaling gradient by movement of the source is a novel paradigm in cellular signaling and may be a mechanism of regulation at the cellular systems biology level.

of reactive target molecules, etc. But the Schumacker group's findings that ROS produced from complex III in the mitochondria—a motile organelle—are directed away from the mitochondrial matrix and into the cytosol point to new concepts by which ROS compartmentalization could be engendered.

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References

1. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci USA* 1998;95:11715–11720.
2. Waypa GB, Marks JD, Guzy RD, Mungai PT, Schriewer JM, Dokic D, Ball MK, Schumacker PT. Superoxide generated at mitochondrial complex III triggers acute responses to hypoxia in the pulmonary circulation. *Am J Respir Crit Care Med* 2013;187:424–432.
3. Sylvester JT, Shimoda LA, Aaronson PI, Ward JP. Hypoxic pulmonary vasoconstriction. *Physiol Rev* 2012;92:367–520.
4. Wang L, Yin J, Nickles HT, Ranke H, Tabuchi A, Hoffmann J, Tabeling C, Barbosa-Sicard E, Chanson M, Kwak BR, *et al*. Hypoxic pulmonary vasoconstriction requires connexin 40-mediated endothelial signal conduction. *J Clin Invest* 2012;122:4218–4230.
5. Bhattacharya J. Lung capillaries raise the hypoxia alarm. *J Clin Invest* 2012;122:3845–3847.
6. Grishko V, Solomon M, Wilson GL, LeDoux SP, Gillespie MN. Oxygen radical-induced mitochondrial DNA damage and repair in pulmonary vascular endothelial cell phenotypes. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L1300–L1308.
7. Frederick RL, Shaw JM. Moving mitochondria: establishing distribution of an essential organelle. *Traffic* 2007;8:1668–1675.
8. Quintana A, Schwarz EC, Schwindling C, Lipp P, Kaestner L, Hoth M. Sustained activity of calcium release-activated calcium channels requires translocation of mitochondria to the plasma membrane. *J Biol Chem* 2006;281:40302–40309.
9. Al-Mehdi AB, Pastukh VM, Swiger BM, Reed DJ, Patel MR, Bardwell GC, Pastukh VV, Alexeyev MF, Gillespie MN. Perinuclear mitochondrial clustering creates an oxidant-rich nuclear domain required for hypoxia-induced transcription. *Sci Signal* 2012;5:ra47.
10. Liao B, Zheng YM, Yadav VR, Korde AS, Wang YX. 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.
11. Csordas G, Hajnoczky G. SR/ER-mitochondrial local communication: calcium and ROS. *Biochim Biophys Acta* 2009;1787:1352–1362.
12. Yan L, Lee AK, Tse FW, Tse A. Ca^{2+} homeostasis and exocytosis in carotid glomus cells: role of mitochondria. *Cell Calcium* 2012;51:155–163.

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Central Sleep Apnea: Effects on Stroke Volume in Heart Failure

Sleep-disordered breathing (SDB) is highly prevalent in patients with heart failure (1–4). Central sleep apnea with Cheyne-Stokes respiration (CSA-CSR), in particular, is found in approximately 30 to 40% of treated patients with heart failure and is associated with increased morbidity and mortality (1, 4, 5).

In this issue of the *Journal*, Yumino and colleagues (pp. 433–438) compared changes in stroke volume (SV) in obstructive versus central apnea events during sleep in patients with heart failure (6). Forty patients with heart failure (New York Heart Association I–III, ejection fraction $\leq 45\%$) with mixed obstructive and central sleep apnea underwent beat-to-beat measurement of SV by noninvasive digital photoplethysmography (DPP) during overnight polysomnography. The authors found that SV decreased by $6.8 \pm 8.7\%$ during obstructive events but increased by $2.6 \pm 5.4\%$ during central events ($P < 0.001$). The results of this study are interesting but with limitations.

The authors (6) aimed at determining the effects of obstructive and central sleep apnea in patients with heart failure by comparing changes in SV extrapolated from digital photoplethysmography measurement, a novel noninvasive method (DPP: Portapres; Finapres Medical Systems BV, Amsterdam, the Netherlands). The extraction of SV is based on a complex model developed by the commercial company selling a blood pressure device using the "Modelflow" method. This method uses noninvasively acquired blood pressure in combination with a nonlinear time-varying three-element model of the vascular system to derive stroke volume and cardiac output (CO). The "Modelflow" was developed from a study in open-heart surgery patients (7). Yumino and colleagues (6) attempted to calibrate SV and CO by calculating the percentage of change and comparing data to the event-free baseline value, and validating this method with Doppler echocardiogram in five healthy male subjects undergoing breathing maneuvers (simulating obstructive and central apneas). However, the three-element model is only supported by data recorded from healthy subjects.

Follow-up research showed that the model could be used to quantify SV and CO in healthy subjects with noninvasively acquired blood pressure (8). However, characteristics of the vascular system may be much different in heart failure versus healthy subjects, and so it is questionable whether the model should be used in this setting (7). Even if the model can be applied to patients with heart failure, characteristics of the vascular system (e.g., compliance) change during sleep stages, and this should be taken into account, specifically during stage 2 non-REM sleep for the study by Yumino and colleagues (6). The "Modelflow" method has a tendency to report large errors as CO increases (8), and thus it is critical to calibrate the derived CO with the CO estimated from standard approaches and to use patients with heart failure rather than healthy subjects to validate the DPP.

CSA-CSR is more prevalent than obstructive sleep apnea (OSA) in patients with heart failure (9). CSA-CSR describes a distinct respiratory pattern characterized by crescendo–decrescendo changes in tidal volume alternating with central apneas or central hypopneas. Recurrent episodes of central apnea or central hypopnea followed by hyperpnea are associated with relatively large negative deflections in intrathoracic pressure, recurrent episodes of hypoxemia, reoxygenation, sympathetic activation, and cortical arousals. These changes result in an imbalance in myocardial oxygen delivery and consumption ratio, activation of sympathetic and neurohormonal systems, and increased right and left ventricular afterload (1, 10).