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Endothelial Oxygen Sensing in Alveolar Maintenance

In this issue of the *Journal*, Pasupneti and colleagues (pp. 983–995) explore the role of HIF (hypoxia-inducible factor) in the pathogenesis and treatment of emphysema (1).

HIF is a transcription factor that orchestrates oxygen homeostasis. HIF stability is regulated by the oxygen-sensitive PHD (prolyl hydroxylase domain) enzymes, with the hydroxylated HIF-α subunit ultimately targeted for proteasomal degradation. In hypoxia, HIF-α escapes degradation and activates the transcription of genes that enable adaptation to reduced oxygen availability, including aspects of systems physiology that optimize oxygen delivery (2).

Invertebrates express a single HIF-α homolog, and the appearance of the HIF-2α paralog coincides with the evolution of complex oxygen delivery systems incorporating the lungs and vasculature. HIF-2 is abundantly expressed in these tissues and appears to have a particular role in the regulation of the systemic and pulmonary circulation (3). Chronic hypoxia induces pulmonary vasoconstriction and vascular remodeling, resulting in pulmonary hypertension, but mice with genetic inactivation of HIF-2 are protected from these effects (4). Although less well explored, hypoxia also stimulates proliferation of airway epithelial cells (5), which is similarly HIF-2 dependent (6).

Although originally defined as a hypoxia-inducible system, there is evidence that HIF also contributes to the maintenance of oxygen delivery systems in steady-state conditions. Genetic inactivation of HIF-α isoforms in mice has phenotypic consequences evident without hypoxia exposure or ischemic injury. Mice lacking *Hif-2α* have impaired iron absorption and erythropoietin production (7, 8), resulting in significant anemia following induced postnatal deletion of *Hif-2α*, which excludes confounding developmental effects (9). This might reflect incomplete HIF-α degradation, even in normoxia, or physiological

hypoxic niches that result in HIF-α stabilization, such as the intestinal epithelium and renal interstitium, where imbalances in blood flow and $\dot{V}O_2$ result in marked oxygen gradients.

This study by Pasupneti and colleagues provides evidence that HIF-2 contributes to the steady-state maintenance of alveolar architecture. They used an inducible form of genetic recombination to specifically knock out *Hif-2α* in the endothelial cells of adult mice, which then developed features of emphysema over the subsequent 14–28 days. This included evidence of pneumocyte apoptosis, airspace enlargement, and obstructive ventilatory failure, which were not observed in mice with conditional *Hif-1α* deletion, demonstrating a HIF-2–specific function in alveolar maintenance (1).

It is perhaps surprising that this function is intrinsic to endothelial HIF-2, although the pulmonary vascular endothelium may be well placed to sense inadequate pulmonary function and oxygen delivery. Most of the airway epithelium is exposed to high oxygen tensions, close to ambient levels. In contrast, the pulmonary arterioles conduct deoxygenated blood to gas exchange sites, exposing the endothelium to a relatively hypoxic environment. This is illustrated by the effects of carbon monoxide, which impairs the oxygen transport capacity of Hb and strongly induces HIF-2 in the pulmonary endothelium (10). The physiological function of HIF-2–dependent survival signals may be localized, coupling the growth and survival of the alveolar epithelium and endothelium to optimize the gas exchange surface. Alternatively, it may have a general role in matching lung capacity to systemic $\dot{V}O_2$. The compensatory lung growth observed in many species after pneumonectomy is modulated by oxygen availability, with hypoxia stimulating growth (11). In this context, it is significant that the current study does not restrict HIF-2 inactivation to the lungs and the alveolar maintenance signals may be generated in the systemic circulation.

The mechanism of this process remains unclear, although the authors propose impaired paracrine signaling. Reduced expression of the mitogenic hormone HGF (hepatocyte growth factor) was noted in the lungs of *Hif-2α* deficient mice, suggesting this might act as an endothelial-derived growth factor supporting pneumocyte survival (1). Interestingly, HGF is also implicated in

postpneumonectomy lung growth (12). However, endothelial-specific HIF-2 loss may not capture all HIF-2–dependent effects on lung epithelium. Previous studies using ubiquitous HIF-2 inactivation demonstrated impaired hypoxia-induced proliferation of club cells (6), which did not change in number following the endothelial-restricted intervention (1). HIF-2 is highly expressed in the bronchiolar epithelium and may also have mitogenic functions intrinsic to these cells.

Small-molecule prolyl hydroxylase domain inhibitors (PHIs) that enhance HIF signaling are in advanced stages of clinical development for the treatment of renal anemia (13). There has been considerable interest in also applying these agents to the treatment of vascular insufficiency, but could this be extended to the treatment of airway degeneration?

Pasupneti and colleagues assessed this using a model of emphysema induced by the kinase inhibitor SU5416, which results in combined alveolar and microvascular degeneration as a consequence of impaired growth factor signaling. They found that these phenotypes are rescued by enhanced HIF-2 function through gain-of-function mutations specific to the endothelium, or by generalized HIF stabilization using iron chelation, which nonspecifically inhibits activity of the PHDs and other iron-dependent enzymes.

SU5416-induced injury is similar to the observed phenotype of endothelial HIF-2 inactivation, and many of its pharmacological targets and associated signaling pathways are known to be HIF regulated, so this model might be specifically sensitive to rescue by HIF-2 stabilization. It will be important to determine whether the protective effects of HIF-2 extend to more standard models of chronic obstructive pulmonary disease (COPD), such as cigarette smoke, which may have a distinct mechanistic basis. Future work should also test whether airway degeneration can be reversed as well as prevented, and if this can be achieved with a clinically tested PHI.

Further challenges to clinical translation may arise from other HIF-2–dependent effects on the pulmonary vasculature, chiefly the risk of pulmonary arterial hypertension (PAH), a common complication of COPD. Although a cause for concern in the clinical development of PHIs, phase III trials completed on several compounds do not demonstrate increased adverse events due to PAH, despite this also being common in patients with chronic kidney disease. However, the etiology of PAH in these patient groups may be different, with a major contribution from chronic hypoxemia in COPD, and the effects of superimposed pharmacological pseudohypoxia difficult to predict.

There are also potential implications for the use of HIF-2 antagonists, which are in clinical trials for treatment of renal carcinoma and have demonstrated efficacy in animal models of PAH. Hypoxemia was reported as an adverse event in early clinical studies (14). This could partly reflect effects on ventilatory control, as these compounds reduce hypoxic ventilatory drive in mice (15). However, from the current study, the risk of degenerative lung disease and the need for pulmonary function monitoring may need consideration.

Going forward, it will be important to further characterize pulmonary HIF-2 expression: how this varies across the normal pulmonary vasculature, and with pharmacological or hypoxic HIF stabilization. Genetic mouse studies, such as this, indicate a role for HIF-2 in regulating the airways and pulmonary vasculature. Establishing the threshold and precise location of each function may be the key to clinically exploiting these responses. ∎

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∂ Machine Learning Classifier Models: The Future for Acute Respiratory Distress Syndrome Phenotyping?

Acute respiratory distress syndrome (ARDS) is a heterogeneous clinical syndrome characterized by severe respiratory failure requiring mechanical ventilatory support for which there is no therapy and in which mortality remains approximately 40% (1, 2). A recent important advance has been the subclassification of ARDS into subphenotypes that have potential prognostic and/or therapeutic significance. Calfee and colleagues reported that an approach called latent class analysis (LCA) could identify one-third of patients with ARDS with a "hyperinflammatory" phenotype, with the remainder having a "hypoinflammatory" pattern (3). The hyperinflammatory phenotype had higher levels of proinflammatory biomarkers and poorer outcomes, including fewer ventilator-free and organ failure–free days and higher mortality. Biological plausibility to these subphenotypes has been suggested by differential responses to treatment (3, 4). A recent LCA of a large negative randomized controlled trial of simvastatin in ARDS demonstrated potential benefit in the hyperinflammatory group (5). Other approaches to ARDS phenotyping exist, including a "physiologic phenotyping" approach (based on the focal versus diffuse distribution of lung infiltrates) (6) and transcriptomics-based approaches (7).

Classifying patients into hyperinflammatory and hypoinflammatory subgroups in real time would enable rapid implementation of a precision medicine approach. Key impediments to the real-time use of these approaches are that nonstandard assays for Il-6 and sTNFR1 are required and that LCAs are computationally complex.

Computational modeling approaches are increasingly being applied in medicine. Machine learning (ML) is when a computer algorithm is trained with known input and output values to predict outcomes from novel data with similar input characteristics. In this issue of the *Journal*, Sinha and colleagues (pp. 996–1004) present data demonstrating the potential of an ML approach using readily available clinical data to identify ARDS subphenotypes (8).

ML Classification and ARDS

Sinha and colleagues examined whether an ML approach, specifically a variant of the gradient-boosting machine (GBM) algorithm (9), could accurately identify inflammatory subtypes of ARDS (8). They used standard clinical and laboratory parameters to categorize patients from three prior clinical trials into inflammatory subtypes. This data were divided into subsets using 10-fold cross-validation to train and optimize the settings (hyperparameters) of the ML algorithm with the objective of building a model for the task of categorizing entries into the hypoinflammatory and hyperinflammatory subphenotypes. The performance of the optimized GBM classifier model was evaluated on a fourth separate dataset by comparing the classes it assigned to the "gold-standard" LCA classes. For the hypoinflammatory class, the GBM model (with a probability threshold of 0.5) gave the correct answer (assuming LCA is correct) in 98% of cases (460 of 468), but for the hyperinflammatory class, it was only correct in 63% of cases (175 of 277). The combined accuracy for both classes was 85%.

The probability threshold at which cases are assigned to each subphenotype can be varied, which, in essence, moves borderline cases into the hypoinflammatory or the hyperinflammatory class. For example, moving to a threshold of 0.3, the GBM model was correct in 93% of cases for the hypoinflammatory class and 78% of cases for the hyperinflammatory class, with an overall accuracy of 87%. This changing threshold depended on the etiology of ARDS, particularly sepsis-related ARDS, indicating that clinical judgment may still be required in the implementation and interpretation of these algorithms. Although the authors highlight the performance of their method as quantified using the area under the curve (AUC), which provides a valid and useful measurement of the performance of a probabilistic classifier, classification accuracy is more amenable to direct clinical interpretation.

The authors showed that these subphenotypes could be predicted using in-hospital data generated up to admission to an ICU with ARDS. They also demonstrated that inflammatory biomarkers and 90-day mortality were higher in the hyperinflammatory phenotype as classified by the model. Findings for treatment interactions for statins, fluid management, and positive end-expiratory pressure strategy in ARDS for the ML-derived classes were consistent with those seen previously for LCA-derived classes. An alternative GBM model built only with readily available laboratory and vital sign variables achieved a comparable performance (AUC = 0.94) with one using all variables (AUC = 0.95) and was able to identify similar treatment interactions. We note that Calfee and colleagues took care to keep training data separate from their evaluation data by holding out a cohort as the evaluation group.