

## Preventable ATII Proliferation after Hyperoxia: The “Tempo” of Folate Metabolism in the Neonatal Lung

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that occurs at high rates in survivors of premature birth (1). BPD was originally described in 1967 by Northway and colleagues as an airway and parenchymal lung injury in preterm infants with a history of respiratory distress syndrome concurrent with exposures to oxygen and mechanical ventilation (2). More than 50 years later, the clinical features of BPD have evolved, together with abilities to care for extremely premature and low birthweight infants born in the late canalicular/early saccular phases of lung development. Thébaud and colleagues recently highlighted the complex intersection of lung development, plasticity, injury, and repair in the clinical disease BPD (1). Despite marked advances in our understanding of the disease, no significant progress has been made in reducing its prevalence (3), and therapeutic options remain limited.

Hyperoxia-induced neonatal lung injury (4) is used to model BPD, as supraphysiologic oxygen exposure is a common source of lung injury in premature infants (5, 6) and the pathophysiology induced by early oxygen exposure parallels that seen with BPD. Murine models also offer unique opportunities to evaluate early effects of oxygen exposure, as newborn mice exhibit immature morphometry resembling the saccular phase of lung development (7). Understanding how hyperoxia inflicts injury and compromises ongoing alveolar and vascular (8, 9) development is paramount to understanding BPD pathogenesis.

Distal airspace alveoli are lined by alveolar epithelial type I and II (ATI and ATII) cells. ATI cells are squamous epithelial cells that facilitate gas exchange and serve barrier functions. ATII cells, which normally cover 5% of the alveolar surface area, produce surfactants that decrease surface tension and thereby protect against atelectasis. ATII cells normally proliferate in the healthy mouse lung from Postnatal day 0 (PN0) to PN14, with a peak of proliferation at PN7. After lung injury, ATII cells become both proliferative and resistant to apoptosis, with increased telomerase activity (10–12). These characteristics, in addition to their ability to repopulate ATI cells lost after lung injury (13), identify ATII cells as a critical progenitor population and a key contributor to the developmental phenotype observed with BPD.

A significant knowledge gap remains in understanding the contribution of neonatal hyperoxia to the number and complement of alveolar epithelial cells in simplified alveoli that are pathognomonic for modern BPD (1). Multiple studies have demonstrated oxygen-induced alterations of alveolar epithelial proliferation, apoptosis, and/or cell-cycle regulation (14–16). Using *Sftpc*-EGFP transgenic mice, Yee and colleagues previously demonstrated that mice exposed to a fraction of inspired oxygen of 1.0 during PN0–4 exhibited increased proliferating (Bromodeoxyuridine<sup>+</sup>) ATII cells starting at PN1, with rapid expansion of the ATII cell population by PN4 (17, 18). This

expanded ATII population is not static, as they identified fewer ATII cells in lungs of adult mice 8 weeks after neonatal hyperoxia exposure (19).

In this issue of the *Journal*, Yee and colleagues (pp. 402–414) now identify a novel—and targetable—mechanism by which hyperoxia causes atypical proliferation of ATII cells in the newborn mouse lung (20). First, the authors demonstrate that an early and aberrant wave of ATII cell proliferation (PN1–4) after neonatal hyperoxia exposure is associated with genetic programs directing serine synthesis and one-carbon-coupled folate metabolism. These metabolic pathways affect cell proliferation through multiple mechanisms, nicely outlined by the authors to include redox defense, epigenetic maintenance, and biosynthesis. Second, they show that these gene expression pathways were also associated with the normal wave of postnatal ATII proliferation that peaks at PN7.

Narrowing in on mechanism, the authors focused on *Mthfd2* (methylene tetrahydrofolate dehydrogenase 2), an enzyme in mitochondrial folate metabolism expressed by proliferative cells (21), and the ATII mitogen *Fgf7/Kgf* (22–24). In this study, *Mthfd2* is found to be a central mediator of alveologenesis, induced in aberrant (hyperoxia-treated) and normal (room air) proliferating neonatal ATII cells and in *Fgf7/Kgf*-treated proliferating adult ATII cells. Third, the authors identify a mechanism of serine/one-carbon-coupled folate metabolism gene expression through mitochondrial stress-related expression of *Atf4* (activating transcription factor 4). *Mthfd2* is required for *Fgf7/Kgf*-induced ATII proliferation and *Atf4* induction.

An exciting finding is that the early and aberrant ATII proliferation during hyperoxia exposure was mitigated by concurrent administration of the SOD (superoxide dismutase) mimetic/antioxidant mitoTEMPO. MitoTEMPO did not affect normal ATII proliferation in control experiments. These results strengthen previous studies showing preservation of lung development during neonatal hyperoxia using transgenic expression of human extracellular SOD or treatment with mitoTEMPO (25, 26). Interestingly, a recent study also showed the therapeutic potential of mitoTEMPO to attenuate cardiac injury after nicotine exposure associated with mitochondrial oxidative stress in rats (27).

Finally, the authors identified increased ATII proliferation and increased expression of ATF4 and MTHFD2 in lungs of human patients with severe type 2 BPD. Though not specified, these patients, by definition, had histories of significant hyperoxia exposure associated with a diagnosis of severe disease (28). These translational findings mirror those in premature baboons, where oxygen and mechanical ventilation treatments were associated with ATII proliferation (Reference 29 and current manuscript).

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Correlation of aberrant neonatal ATII proliferation (20) with short- and long-term functional consequences would be of great interest. To this end, lung function testing of mice that have recovered from their brief hyperoxia exposure, with and without mitoTEMPO treatment, would provide insight. It will also be important to follow the cellular fates after the early wave of ATII proliferation, as the authors previously demonstrated reduced pro SP-C expression and decreased ATII cell numbers after exposure to hyperoxia from PN1 to PN4. Finally, the authors acknowledge that a limitation of their study is the unknown effects of chronic hyperoxia on these mechanisms, which remain interesting given the prolonged oxygen dependency in severe BPD.

Another goal would be to clarify the effect of FGF7/KGF on ATII proliferation in BPD, as *Fgf7* is associated with early ATII proliferation in rabbits but not mice. Increased pulmonary FGF7/KGF concentration is protective in human infants, as tracheal concentrations are increased in those who do not develop BPD compared with those who do (30). Finally, future directions may include elucidating a specific role of *Atf4* with loss-of-function experiments *in vivo* and/or *in vitro*. The authors are well poised to address these and other important questions in subsequent basic and translational studies.

In summary, this study elucidates a novel mechanism by which hyperoxia results in mitochondrial oxidative stress-dependent induction of folate metabolism programs as well as ATII proliferation in the developing lung. The combination of multiple preclinical models and samples from patients with BPD strengthen the translational significance of this work by identifying possible clinical implications for mitigation of alveolar maldevelopment in premature infants with BPD. ■

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