# Mesenchymal Cells and Bronchopulmonary Dysplasia: New Insights about the Dark Side of Oxygen

Bronchopulmonary dysplasia (BPD) is the most common chronic pulmonary complication of preterm birth (1). In recent years, the incidence of BPD has risen along with increased survival of very premature infants, reaching 10,000 new cases annually in the United States (1). Infants with BPD develop long-term respiratory complications, including wheezing, asthma, and airflow obstruction, during childhood and even adulthood (2, 3). As a result, BPD is now a leading cause of pediatric lung disease. However, there are few methods to prevent it, and treatment is limited to supportive care rather than targeted therapies.

Our ability to develop methods for BPD prevention or treatment depends on understanding the causal relationship between the risk factors for BPD and its pathology. The histopathologic changes observed in BPD include large, simplified alveolar structures; a dysmorphic microvasculature; and variable interstitial thickening due to myofibroblast accumulation (4, 5). Multiple factors have been implicated in the pathogenesis of BPD, including oxygen- and mechanical ventilation–mediated lung injury, infection/inflammation, and genetic risk factors. It is not yet understood how these factors interact to cause the pathology of BPD. In this issue of the *Journal*, Möbius and colleagues (pp. 592–600) present a study of the effects of supraphysiologic concentrations of oxygen on mesenchymal stromal cells (MSCs) isolated from human fetal lung tissue (6). This reflects oxygen exposure after preterm birth. The cells used in this study were obtained at 16–18 weeks gestation and thus are the youngest human fetal lung mesenchymal cells yet isolated. This period corresponds to the canalicular stage of lung development, when many premature infants who subsequently develop BPD are born (5).

The authors found that compared with hypoxic exposure (5% $O_2$, reminiscent of the normal prenatal environment), *in vitro* exposure to supraphysiologic oxygen concentrations (21% and 60% $O_2$) decreased the production of factors that favor normal lung development. These include proangiogenic (angiogenin, IL-8, and VEGF) and epithelial cell–protecting (FGF-10, stanniocalcin-1, and FGF-7) factors, as well as extracellular matrix components such as elastin and sulfated glycosaminoglycans. This work unveils a potential mechanism by which high concentrations of oxygen, presumably when it is diffusing through the pulmonary interstitium, can directly alter the function and secretome of the resident mesenchymal cells and impair normal lung growth. Mesenchyme-derived signals are critical for epithelial cell differentiation and vasculogenesis during the canalicular and later stages of lung development (reviewed in Reference 7). The authors also found increased MSC gene expression of profibrotic genes (collagen type 1–encoding *COL1A1*, *ACTA2*, *TAGLN*, *TGFB3*, and *POSTN*), decreased MSC colony-forming capacity, and increased MSC proliferative capacity. This indicates that in a hyperoxic environment, fetal lung MSCs may contribute to the interstitial thickening and myofibroblast accumulation seen in BPD.

The implication of hyperoxia-induced impaired lung-resident MSC function in aberrant lung development associated with BPD is intriguing but not unexpected. All premature infant lungs are exposed to supraphysiologic oxygen levels, and many infants are treated with supplemental inspired oxygen. Oxygen therapy is a risk factor for BPD development and correlates with long-term respiratory symptoms (8). Oxygen has also been shown to modulate the growth and differentiation of various mesenchymal cells. For example, when cultured in a physiologic hypoxic environment, bone marrow MSCs show greater survival, colony-forming capacity, and differentiation potential than they do under normoxia (21% $O_2$) (9, 10).

Recent studies have drawn attention to the role of mesenchymal cells in lung development and neonatal lung disease. Experimental animal studies have demonstrated that lung mesenchymal cells expressing ACTA2, elastin, and platelet-derived growth factor receptor α (PDGFRα) are required for alveogenesis (7). By contrast, hyperoxia-exposed lungs with arrested alveolar development show thickened interstitium with ACTA2-, periostin-, and collagen type I–positive myofibroblasts (11). Lung-resident MSCs from hyperoxia-exposed lungs show decreased pathways that promote lung and vascular growth (12). In premature infants who develop BPD, myofibroblasts accumulate in the interstitium within the first week after birth (4). Furthermore, tracheal aspirate–derived neonatal lung MSCs from infants developing BPD show decreased PDGFRα expression (13), suggesting these cells' phenotype is deficient in promoting alveolarization. The fetal lung MSCs used in the present study were found to secrete many growth factors that favor normal lung development. The findings indicating that hyperoxic exposure can disrupt the growth-promoting paracrine function of lung MSCs represent a novel concept that is clinically relevant to lung development in premature infants.

Möbius and colleagues also found that compared with normal fetal lung MSCs, umbilical cord–derived MSCs cultured in hypoxic conditions (5% $O_2$) produced similar and in some cases higher levels of lung-protective proteins and growth factors. They suggest that umbilical cord–derived MSCs may be developed therapeutically to counteract the effects of hyperoxic exposure on resident lung cells. However, because the umbilical cord–derived MSCs were not exposed to hyperoxia, the question of whether these cells would undergo the same fate as resident lung MSCs if they were delivered to a lung receiving hyperoxic ventilation remains unanswered. Thus, this and other studies (reviewed in Reference 14)

highlight the potential of exogenous MSCs or their secretome as therapeutics for prematurity-related lung disease. However, it is important to acknowledge the need for exhaustive functional validation of the described observations.

The results presented here have to be interpreted with caution. In addition to producing factors known to participate in normal lung development when cultured under normal (hypoxic) conditions, the fetal lung MSCs also produced significant amounts of proinflammatory cytokines, such as IL-6 and IL-8, as well as the monocyte chemoattractant MCP-1/CCL2. Similar to the effect on factors required for normal lung development, the expression of proinflammatory cytokines was downregulated after hyperoxic exposure. Furthermore, the suggestion of administering umbilical cord MSCs as a potential treatment for BPD requires further investigation. As with normal fetal lung MSCs, when cultured in hypoxic conditions the umbilical cord–derived MSCs also produced significant quantities of proinflammatory IL-6 and IL-8, as well as activin-A, a member of the TGF superfamily that is involved in BPD pathogenesis. Because inflammation has long been associated with BPD development, the ultimate effect on lung development of hypoxia- or hyperoxia-conditioned fetal lung MSCs or umbilical cord MSCs is unclear. The functional effects of these MSCs require further investigation using *in vivo* animal models of hyperoxic lung injury or *in vitro* models of lung development.

It is also unclear how well the *in vitro* hyperoxic exposure of fetal lung MSCs represents the *in vivo* microenvironment of a lung being exposed to a high concentration of inspired oxygen. Although MSCs residing in the pulmonary interstitium could be exposed directly to higher oxygen concentrations as oxygen diffuses to reach the microvasculature, prolonged hyperoxic exposure likely has an effect on other cells, which release mediators that can also affect the MSCs. For example, prolonged hyperoxic exposure is associated with necrotic airway epithelial cell death (15). Necrotic cells release damage-associated molecular patterns, inducing MSC proliferation (16). Moreover, it will be of interest to investigate the effect of reactive oxygen species on the function and secretome of lung-resident MSCs. To develop strategies to counteract the inhibitory effect of oxygen on the secretion of growth-promoting factors by MSCs, we will need to gain insight into the potential mechanism by which hyperoxia affects lung-resident MSCs.

Overall, this study highlights a pathogenic mechanism by which oxygen disrupts the function and secretome of lung-resident MSCs, and may impair normal lung development. The development of methods to counteract this effect of oxygen may provide new tools to prevent or treat BPD. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

Antonia P. Popova, M.D.
*Division of Pediatric Pulmonology*
*University of Michigan Medical*
*Ann Arbor, Michigan*

ORCID ID: 0000-0002-0404-6124 (A.P.P.).

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