Hyperoxia and EGFL7: saving cells from too much of a good thing

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HIGH OXYGEN CONCENTRATIONS can damage cells. Whereas the detrimental effects of oxygen have been well-reported (1), respiratory diseases such as pneumonia and neonatal surfactant deficiency often cause hypoxemia and necessitate the use of supplemental oxygen as part of their management. Administering high oxygen concentrations for prolonged periods may contribute to adverse outcomes, including acute respiratory distress syndrome (ARDS) in adults (11) and bronchopulmonary dysplasia (BPD) in preterm infants (10). But what pathological mechanisms explain how hyperoxia and oxygen-induced free radicals lead to lung injury in ARDS and disrupted lung development in BPD? Damage to the pulmonary vasculature may contribute to the pathogenesis of lung disease in both adults and children, as hyperoxia can produce endothelial injury and eventually loss of vascular integrity (12, 14). Identifying the molecular targets of hyperoxia within the lung endothelium may improve our understanding of acute lung injury.

In this issue of *AJP-Lung*, Xu et al. (20) propose that the endothelial-specific growth factor EGFL7 may play a role in hyperoxia-induced vascular injury. EGFL7 is a member of the epidermal growth factor-like domain (EGFL) family of growth factors, is expressed by endothelial cells, binds the extracellular matrix surrounding blood vessels, and appears to act in an autocrine manner to promote angiogenesis (7, 13, 17). Present in *Xenopus*, zebrafish, and mammals, EGFL7 expression is highest during early vascular development and is required for tubular extension of newly formed vessels (13). Unlike the vascular growth factors VEGF (6), FGF-2 (3), and angiopoietin-1 (9), EGFL7 does not appear to stimulate cell proliferation. Rather, EGFL7 promotes endothelial cell migration and structural morphogenesis of the vascular bed (5, 17). Based on the data presented by Xu et al. (20), we may need to add preventing cell death in response to injury to the EGFL7 repertoire.

The authors tested the hypothesis that hyperoxia could inhibit EGFL7 expression, leading to endothelial injury and abnormal vascular development in the lung. Placing neonatal rats in 95% oxygen reduced EGFL7 expression in the lung. The effect was reversed when the hyperoxia-treated pups were put back in room air. Hyperoxia also inhibited EGFL7 expression in cultured human umbilical vein endothelial cells (HUCEC). These data established that EGFL7 was indeed a target of hyperoxia, and additional experiments revealed an important role for EGFL7 in protecting endothelial cells from hyperoxic damage.

In the next set of experiments, the authors proposed that reduced EGFL7 expression might contribute to the injury and cell death seen in endothelial cells following hyperoxia exposure. Using a line of cultured endothelial cells stably transfected with EGFL7, Xu et al. (20) showed that EGFL7 overexpression protected endothelial cells from hyperoxia-induced apoptosis. This effect appeared to involve the intrinsic apoptotic pathway, as EGFL7 expression prevented release of cytochrome c into the cytoplasm. In addition, EGFL7 increased expression of the antiapoptotic protein Bcl-xL and prevented the hyperoxia-induced expression of the proapoptotic protein Bax. Therefore, increased EGFL7 expression in endothelia was protective against hyperoxia.

Endothelial cell adhesion and migration is critical for normal vascular development (16), and EGFL7 may regulate endothelial adhesion and directed migration (13, 17). Once secreted by endothelial cells, EGFL7 colocalizes with fibronectin on the basal surface of newly formed vessels. Endothelial cells can bind EGFL7-coated surfaces but not as robustly as surfaces coated with the matrix proteins fibronectin or collagen (13, 17). Of note, Xu et al. (20) used a cell line overexpressing EGFL7 in testing if increasing EGFL7 levels could prevent hyperoxia-induced cell death. This approach made use of the endogenous protein synthesis, packaging, and release mechanisms, possibly resulting in a more physiological incorporation of EGFL7 into the extracellular matrix. One intriguing possibility is that EGFL7 is a modulator of endothelial adhesion, promoting cell stability and integrity while still allowing newly formed vessels to migrate toward target sites of angiogenesis. Factors that inhibit or decrease EGFL7 expression may therefore decrease vessel migration, leading to defects in vascular development. Similarly, decreased endothelial cell adhesion in the absence of EGFL7 may lead to apoptosis, just as decreased adhesion in many cells tissues can lead to programmed cell death (15).

The fetal lung undergoes extensive vascular growth and remodeling from the canalicular stage through the saccular and alveolar stages of development (4, 8). During canalicular development (16–25 wk in humans; embryonic days 20–22 in rats), formation of a primitive capillary bed surrounding terminal airways will allow enough gas exchange to permit fetal viability. Formation of alveoli begins months later in human fetuses, and vascular remodeling with formation of new alveoli may continue for months to years after birth. In extremely preterm infants born in the late canalicular stage, subsequent vascular development is complicated by exposure to hyperoxia, mechanical trauma, and inflammation (18). Findings like those presented here by Xu et al. (20) may provide us with new insight into how these environmental factors may interfere with normal postnatal development in extremely preterm infants. The idea that abnormal vascular development in the lung leads to BPD has been proposed (19), although experimental data supporting this idea are still lacking.

Hyperoxia also contributes to lung disease in adults. Although increased oxygen exposure is seldom the sole elucidating agent in lung disease pathogenesis, it may well exacerbate acute lung injury. During the recovery phase of ARDS, the lung must repopulate areas of damaged endothelium to regenerate an intact microvasculature (2, 11). By establishing that EGFL7 is important for cell survival and is suppressed by hyperoxia, the authors identify a potential therapeutic target for

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prevention or treatment of lung injury. Hyperoxia has been reported to increase expression of angiopoietin-2, which destabilizes the pulmonary vascular bed and increases endothelial permeability (2). Future studies may be able to determine if the same processes that induce angiopoietin-2 also suppress EGFL7 expression and if these two molecules antagonize each other or work via distinct mechanisms.

Many interesting questions now arise about how EGFL7 might regulate vascular development. Do changes in oxygen delivery to peripheral tissues in congenital and acquired disease states regulate EGFL7 expression and therefore vascular development and remodeling? Is there genetic heterogeneity in the EGFL7 gene that could predispose patients to endothelial damage? Why does EGFL7 expression persist in the adult lung but not other tissues? Answering these questions will require using both disease models and further investigation of disease mechanisms in human patients. As investigators continue to identify factors such as EGFL7 that regulate vascular development and injury, we may better understand the contribution of endothelial biology to the pathogenesis of lung disease.

REFERENCES
