DDR1 Deficiency in Mice: A Spontaneous Model of Bronchopulmonary Dysplasia-associated Pulmonary Hypertension?

Bronchopulmonary dysplasia (BPD) is the most common morbidity associated with preterm birth, affecting up to 50–60% of babies born with birth weights less than 1,000 grams when the lungs are at the saccular stage of development (1). The multifactorial pathogenesis of BPD is governed by a complex interplay between various antenatal and postnatal exposures and immature lung tissue. At the molecular degree, these interactions trigger an inflammatory response and ultimately result in the characteristic pathology of BPD: disrupted alveolarization and vascularization with variable degrees of fibrosis (2). Patients with severe BPD continue to require invasive or noninvasive ventilatory support when they reach term age and are at high risk of developing yet another potentially lethal complication: pulmonary arterial hypertension (3, 4). As the clinicians are pushing the envelope on the limits of viability, we are likely to see an increasing number of infants with severe BPD and pulmonary arterial hypertension. Hence, there is an urgent need for a better understanding of the molecular mechanisms underlying these disorders for the development of safe and efficient therapies, which are still lacking.

In this issue of the *Journal*, Bonafiglia and colleagues (pp. 562–573) from the Bendeck Laboratory report their findings on cardiovascular and pulmonary effects of DDR1 (discoidin domain receptor 1) deficiency in mice (5). DDRs are unique receptor tyrosine kinases that are activated by collagens (6, 7). *In vitro* experiments have demonstrated that phosphorylated DDR1 activates intracellular signal transduction pathways that regulate cell migration and differentiation and matrix metalloproteinase expression (8, 9). *Ddr1*-deficient ($Ddr1^{-/-}$) mice, which were generated more than 2 decades ago, have provided additional important insights into the biological role of DDR1 (10). These mice exhibit normal embryonic development, but homozygous females display defects in implantation and lactation. DDR1 is overexpressed in lung samples from patients with idiopathic pulmonary fibrosis, and $Ddr1^{-/-}$ mice are resistant to bleomycin-induced pulmonary fibrosis and inflammation (11).

As a result of carefully conducted, comprehensive studies by Bonafiglia and colleagues, we now have become aware of new phenotypic features associated with DDR1 deficiency, which include impaired alveolarization and PH. These findings, to a large extent, help explain the increased mortality observed in $Ddr1^{-/-}$ mice between 1 and 4 months of age. A combination of hemodynamic measurements obtained by right ventricle catheterization and echocardiograms and histologic analyses demonstrated elevated right ventricle systolic pressures, right ventricle hypertrophy and systolic dysfunction, right atrial enlargement, and increased number of muscularized distal arteries, all consistent with a diagnosis of pulmonary arterial hypertension, in 1-month-old $Ddr1^{-/-}$ mice. Although these cardiac anomalies were absent in younger mice at postnatal Day (P)7, $Ddr1^{-/-}$ pups displayed increased muscularization of distal pulmonary arteries compared with wild-type mice at this time point, thus revealing a plausible time course and pathogenesis for the development of pulmonary arterial hypertension. Furthermore, $Ddr1^{-/-}$ mice demonstrated fewer and larger alveoli, akin to the classic BPD pathology, and reduced body weight at P7. However, in contrast to the human and other murine models of BPD, these structural alterations were not associated with increased inflammation at P7 in $Ddr1^{-/-}$ mice.

How does DDR1 deficiency impair alveolarization and cause pulmonary arterial hypertension? Do these two pathologic processes occur through independent actions of DDR1 on the developing air saccules and distal pulmonary arteries, or are they linked to each other as that occurs in human infants with BPD? To begin to answer these mechanistic questions, knowledge of the cell types which express Ddr1 in murine lungs is a prerequisite. In the normal human lung, Moll and colleagues have reported that DDR1 is expressed in epithelial cells lining the airways and the alveoli by immunohistochemistry using a well-characterized anti-human DDR1 antibody (12). These immunolocalization studies are corroborated by recent single-cell RNA sequencing, which has detected *DDR1* transcripts in fibroblasts in addition to epithelial cells in the human lung (13). Unfortunately, the lack of a commercially available anti-mouse Ddr1 antibody has precluded Bonafiglia and colleagues from examining the expression of Ddr1 in murine tissues. However, based on single-cell RNA sequencing from the LungMap project, Ddr1 expression is detected in fibroblasts, endothelial cells, airway epithelial cells, and $CD163^{+}$ macrophages in murine lungs (13). These data combined with the studies conducted in A549 cells, albeit not primary alveolar epithelial cell cultures, and decreased number of proliferating nuclear cell antigen (PCNA)-positive alveolar epithelial cells strongly suggest impaired alveolar epithelial cell proliferation and migration as the underpinnings of alveolar simplification observed in $Ddr1^{-/-}$ mice. Furthermore, from a mechanistic standpoint, the authors propose an interesting concept of "partial" epithelial to mesenchymal transition (EMT) as a requirement for alveolarization to link their finding of decreased markers of EMT to impaired alveolarization in $Ddr1^{-/-}$ mice. Although the contribution of decreased EMT to DDR1-mediated antifibrotic effects is well recognized, the notion of decreased EMT during alveologenesis would require further experimental evidence.

In conclusion, while it is tempting to attribute the pulmonary vascular and cardiac pathology in $Ddr1^{-/-}$ mice to alveolar simplification as that occurs in BPD, the finding of *Ddr1*expression in other cell types besides alveolar epithelial cells raises the possibility that

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Ddr1 expression in these cell types also might be contributing to this phenotype. Ultimately, this question can only be unequivocally answered by studies performed in tissue-specific conditional $Ddr1^{-/-}$ mouse models. As for the potential of DDR1 as a target of antifibrotic therapy in the lung and other organs, preclinical studies will need to include a detailed assessment for pulmonary arterial hypertension, as Bonafiglia and colleagues have performed. For now, we congratulate the authors on their rigorous rephenotyping of the $Ddr1^{-/-}$ mouse model, which paves the way for further mechanistic studies toward potentially unraveling another double-edged sword in the lung. ■

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