A Step toward Treating a Lethal Neonatal Lung Disease
STAT3 and Alveolar Capillary Dysplasia

Alveolar capillary dysplasia with misalignment of the pulmonary veins (ACDMPV) is a rare lethal lung developmental disorder in which the majority of affected infants present with neonatal respiratory failure and severe pulmonary hypertension that is refractory to treatment (1, 2). Pathologically, the disease is characterized by a paucity of distal capillaries and the presence of "misaligned" veins—pulmonary veins located within the same bronchovascular sheath as the pulmonary artery and airway (2). Recently, it has been shown that these misaligned veins are actually anastomotic shunt vessels (3). Most affected infants also have abnormalities in other organ systems, including the cardiac, gastrointestinal, and genitourinary systems (2, 4). Over the past several years, children with milder forms of ACDMPV who present later and survive longer with anti–pulmonary hypertensive therapies have been increasingly recognized, although the prognosis is still poor, with lung transplantation being the only available long-term therapy (5, 6).

A breakthrough in understanding the cause of ACDMPV came with the discovery that genic deletions of and mutations in the FOXF1 (forkhead box F1) gene account for the majority of ACDMPV cases (7). FOXF1 is a transcription factor essential for vascular development. Homozygous *foxf1*-null mice are embryonic lethal because of abnormal vascular development of the allantois and yolk sac (8). Haploinsufficient *foxf1*$^{+/-}$ mice recapitulate some of the features of ACDMPV, with affected animals having lung hypoplasia and reduced angiogenesis, abnormal gall bladder morphogenesis, and increased (but not universal) perinatal mortality. Interestingly the pathology of *foxf1*$^{+/-}$ mice does not include findings of misaligned pulmonary veins, as seen in the human disorder (8). Haploinsufficiency is the presumed mechanism for *FOXF1* mutations causing human lung disease, as disease results from monoallelic gene deletions and null (nonsense and frameshift) mutations (4, 7). Regulation of *FOXF1* is complex, as disease-associated mutations are clustered within the DNA-binding domain of FOXF1, and deletions in the 5′ untranslated region involving two long noncoding RNAs also result in the phenotype of ACDMPV (9).

In this issue of the *Journal*, Pradhan and colleagues (pp. 1045–1056) expand our knowledge of the molecular mechanisms by which *FOXF1* mutations cause disease and offer a glimmer of hope for treatment for this universally fatal disorder (10). They selected for study a mutation identified in an infant with ACDMPV that resulted in the substitution of phenylalanine for S52F (serine in codon 52). The S52F mutation is located within an evolutionary conserved, frequently mutated, computationally predicted SH2-binding domain important for interactions with the protein STAT3 (signal transducer and activator of transcription 3). The authors demonstrated that the S52F-FOXF1 protein did not bind STAT3 *in vitro*, indicating the importance of the serine at position 52 in the interaction of FOXF1 and STAT3, although several other *FOXF1* mutations within another computationally predicted SH2 binding domain (Y284A, I285Q, S291*) did not disrupt FOXF1's interaction with STAT3. They then used CrispR/Cas9 to generate a mouse model with one allele expressing the S52F mutation. Perinatal mortality was increased in the wild type (WT)/S52F mice, although, similar to *foxf1*$^{+/-}$ mice, it was not uniformly lethal, and the reasons some WT/S52F pups survive remains unclear. However, this murine model largely recapitulates the histopathology of the human phenotype, including pulmonary hypoplasia, misaligned pulmonary veins, pulmonary arterial hypertrophy, and alveolar simplification. Furthermore, decreased transcription of both the FOXF1 and STAT3 genes, as well as decreased transcription of additional downstream target genes important in endothelial cell proliferation and angiogenesis, was observed in the lungs of WT/S52F mice. Finally, they used nanoparticles to deliver STAT3 complementary DNA intravascularly into newborn WT/S52F mice and demonstrated efficient targeting of lung endothelial cells with increased STAT3 protein and phosphorylation, increased expression of endothelial cell markers indicating improved angiogenesis, improved alveogenesis, and decreased inflammation. Whether there was increased survival or improved lung function in treated mice was unaddressed.

Although ACDMPV is a rare disease, with the recognition of the causative role of *FOXF1* mutations and deletions, clinical genetic testing is now routinely available, allowing for noninvasive diagnosis. As a result, the number of identified cases has increased dramatically in recent years, as exemplified by the additional 28 cases included in the report (10). Could delivery of STAT3 complementary DNA using nanoparticles, which are being used in clinical trials for human malignancies, be used to treat human infants with ACDMPV? There are several important potential limitations and barriers to this approach. First, it is not clear how many other *FOXF1* mutations disrupt interactions with STAT3 and are associated with decreased STAT3 signaling, as the authors' data with respect to several other mutations indicated that they did not interfere with FOXF1–STAT3 interactions. Interestingly, decreased phosphorylated STAT3 was observed in human lung tissue from an infant with an unrelated *FOXF1* frameshift mutation downstream of the first STAT3 consensus binding sequence. Augmenting STAT3 signaling might thus be an effective approach for some *FOXF1* mutations, as well as an approach that could be applied to augment other downstream signaling critical for angiogenesis. A more practical barrier is that ACDMPV usually arises as a sporadic disorder due to *de novo* mutations (4, 9). Although familial cases are recognized and prenatal diagnosis has been performed (11), these cases are the exceptions. Most infants present after birth with respiratory failure and persistent pulmonary hypertension, which may result from other disease mechanisms. Even if the diagnosis is suspected initially, confirmatory genetic studies may take several weeks, and affected infants may die before a diagnosis is confirmed. By the time the diagnosis is established for surviving infants, secondary lung damage from oxygen toxicity and ventilator-induced

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injury will have complicated the infant's course. Lung biopsy may allow for more rapid diagnosis, but some infants with histologic ACDMPV do not have *FOXF1* mutations or deletions (9). Finally, gain-of-function mutations in STAT3 cause an autoimmune disease that can affect the lungs (12), so dosing considerations will be critical so as not to replace one disease with another. Despite these practical limitations, Pradhan and colleagues have generated an important animal model and an important advance in understanding the molecular pathogenesis of ACDMPV, and they suggest a path forward for the treatment of this devastating disorder. ■

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