Seminars in Pediatric Surgery 26 (2017) 123–128

Contents lists available at ScienceDirect

Seminars in Pediatric Surgery

journal homepage: www.elsevier.com/locate/sempedsurg

Abnormal lung development in congenital diaphragmatic hernia

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ARTICLE INFO

Keywords:
Lung development
Congenital diaphragmatic hernia
Pulmonary hypoplasia
Diaphragm development

ABSTRACT

The outcomes of patients diagnosed with congenital diaphragmatic hernia (CDH) have recently improved. However, mortality and morbidity remain high, and this is primarily caused by the abnormal lung development resulting in pulmonary hypoplasia and persistent pulmonary hypertension. The pathogenesis of CDH is poorly understood, despite the identification of certain candidate genes disrupting normal diaphragm and lung morphogenesis in animal models of CDH. Defects within the lung mesenchyme and interstitium contribute to disturbed distal lung development. Frequently, a disturbance in the development of the pleuroperitoneal folds (PPFs) leads to the incomplete formation of the diaphragm and subsequent herniation. Most candidate genes identified in animal models have so far revealed relatively few strong associations in human CDH cases. CDH is likely a highly polygenic disease, and future studies will need to reconcile how disturbances in the expression of multiple genes cause the disease. Herein, we summarize the available literature on abnormal lung development associated with CDH.

Introduction

Patients born with congenital diaphragmatic hernia (CDH) have poorly developed lungs with two main pathologic features, namely, pulmonary hypoplasia and persistent pulmonary hypertension. The defining characteristic of CDH is a defect in the diaphragm and displacement of abdominal viscera into the thoracic cavity. CDH occurs with an incidence of 1 in 2000–3000 births, thus on average, every 10 min a baby with CDH is born worldwide.[1–3] The pulmonary hypoplasia is particularly pronounced on the ipsilateral side, and pulmonary hypertension develops from abnormal pulmonary vasculature development. A posterolateral (Bochadalek) hernia occurs most frequently, as opposed to anterior (Morgagni) defects (~6%) or defects within the central tendon.[4] The diaphragmatic defect occurs on the left 80% of the time and is rarely bilateral (~2%). However, bilateral defects, when compared to unilateral defects, are more often associated with other major anomalies.[4,5]

The emergence of new therapeutic approaches, such as delayed surgery, gentle ventilation strategies including permissive hypercapnia, high-frequency oscillation, inhaled nitric oxide, and extracorporeal membrane oxygenation (ECMO), have improved overall survival rates that are now reported to be as high as 85% in isolated CDH.[6] Unfortunately, survival rates remain lower in cases associated with other congenital anomalies.[7] Improved survival rates likely reflect recent advances in treating high-risk CDH patients.[6] However, there may be a significant selection bias in mortality studies as there is evidence of a "hidden mortality," when only a subset of CDH patients makes it to referral centers and are reported.[6,8,9] Thus, mortality rates are probably higher than what is reported from institutional data.[9] Poor outcomes are observed in cases with pulmonary hypertension that persists despite intervention, with survival rates as low as 20%.[10] Here, we review the development of the diaphragm and lungs, and how this development is abnormal in CDH.

Normal development of the lungs and diaphragm

Human lungs start to develop around 4 weeks of gestation, with the separation of the anterior foregut into a trachea and two lung buds, and a dorsal esophagus (reviewed extensively elsewhere[11–13]). Specification of the respiratory system occurs with the appearance of thyroid transcription factor 1 (TTF-1 or NKX2-1) in the endoderm at the ventral wall of the anterior foregut. NKX2-1 expression is directed by supporting signals within the

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http://dx.doi.org/10.1053/j.sempedsurg.2017.04.011
1055-8586/

surrounding ventral mesenchyme.[14] WNT2 and WNT2b are expressed in the lung mesenchyme and participate in canonical WNT/β-catenin signaling on the endoderm progenitors to specify a lung fate.[15] Fibroblast growth factors (FGF), bone morphogenetic protein (BMP), retinoic acid (RA), and transforming growth factor β (TGF-β) are other crucial signals secreted by the ventral mesenchyme that mediate the formation of the trachea and primary lung buds.[14,16,17]

During the pseudoglandular stage of lung development (5–16 weeks of gestation) the two lung buds undergo repeated branching to generate a tree-like structure of airways. Critical for this airway branching morphogenesis is the expression of the transcription factor Sox2 in the non-branching, proximal airways and Sox9 in the distal, branching airways. Initially, Sox2 is expressed in the dorsal foregut and excluded from the ventral foregut through BMP inhibition.[16] During branching morphogenesis, Sox2 is repressed at the branching sites, but highly expressed in non-branching regions.[18] Sox9 is expressed at the distal tips of the branching airway epithelium and regulates the balance between proliferation and differentiation as well as the establishment of the extracellular matrix.[19] Marked also by the expression of Id2, the Sox9-positive distal endodermal airway progenitors will eventually give rise to type 1 and type 2 alveolar epithelial cells (AEC-1 and AEC-2).[12] Sox2-positive cells differentiate into the cells of the conducting airways, which are also capable of partially reprograming AECs-2 into those with ciliated or non-ciliated airway characteristics.[20] FGF10 signaling is essential for branching morphogenesis. Secretion of FGF10 from the mesoderm promotes the directional outgrowth of endodermal lung buds, and FGF10 transgenic knockout mice form tracheas, but branching morphogenesis of the airways is disrupted.[21,22]

The conducting airways of the lungs have formed after 16 weeks of development and what follows is the canalicular stage of lung development and formation of the respiratory zone (16–26 weeks of gestation). Terminal bronchioles divide into respiratory bronchioles, which branch into alveolar ducts. The blood vessels form *de novo* through vasculogenesis with the airways acting as a template for the formation of the pulmonary vessels.[23] The blood–air interface is established and can provide enough gas exchange to sustain life.[23] AECs-1 and AECs-2 differentiate from a bipotent progenitor.[24] The saccular stage of lung development (26–38 weeks of gestation) is denoted by the formation of epithelial sacs, which become more closely connected with the maturing pulmonary capillary bed. Near birth, these sacs will form alveoli through secondary septation, at which point the capillary bed will be exposed to the surfaces of two alveoli simultaneously. These later processes occur during the final, alveolar stage of lung development, lasting from term to 7 years of age.

The development of the diaphragm begins around the same time as the lungs, 4 weeks of gestation, and is completed by 10 weeks of gestation. The fully formed diaphragm is composed of the dorsomedial crural muscle, the ventrolateral costal muscle, and centrally the amuscular central tendon, which connects to the muscular components of the diaphragm. Multiple embryonic structures contribute to the development of the diaphragm. The septum transversum is the first structure to form and serves as a barrier between the abdominal and thoracic cavities.[25] It is unlikely that the septum transversum contributes to the musculature of the diaphragm,[26] and it also does not contribute to the central tendon.[27] However, the septum may provide a scaffold for diaphragm morphogenesis.[28] The somites are the source of the diaphragm muscle cells, which must migrate as precursors to transient pyramid-shaped mesodermal structures called the pleuroperitoneal folds (PPFs).[27] Migration of these precursors relies on the chemoattractant hepatocyte growth/scatter factor (HGF/SF) signaling and its c-Met tyrosine kinase receptor.[29,30] In the dermomyotome derivatives of the somites, particularly in the ventral-lateral lip, c-Met is strongly expressed.[30] The lateral lip of the dermomyotome generates the hypaxial musculature, giving rise to the muscles of the diaphragm.[29] HGF/SF is expressed along the migratory path, including the PPFs, of migratory muscle precursors.[27,29] The muscle precursors will then spread to all regions of the diaphragm that will be muscularized.[26]

Abnormal development of the lungs in CDH

Two unique animal models have been developed to study the mechanical and developmental aspects of CDH.[31] The herbicide 2,4-dichloro-phenyl-p-nitrophenylether (nitrofen) is a teratogen widely used to model CDH. When administered to a pregnant rat dam on day 9 of gestation, it produces diaphragmatic hernias and abnormal lung development like human patients with CDH. Despite the immense value in uncovering a multiplicity of pathogenetic factors, the nitrofen model has been criticized for being a toxicological model and because nitrofen has not been linked to human CDH.[31] The surgical model is used in rabbits and sheep. In this model, the diaphragmatic defect is created surgically following a period of normal development. This model has been particularly useful for studying the mechanical aspects of the herniated abdominal organs on the lungs, and for testing interventional therapeutic strategies. However, the hernia is created late in gestation, making the surgical model less useful to study earlier pathogenetic events.[31]

The degree of pulmonary hypoplasia and hypertension most directly determine the outcome of an infant diagnosed with CDH. The lungs ultimately have a reduced surface area for gas exchange due to hypoplasia, and particularly the lung parenchyma suffers from reduced distal branching and alveoli.[32] The alveoli that do exist have thicker walls, impairing the close association of the airspaces to the capillaries, and abnormal composition of the pulmonary interstitium and reduced compliance. Patients with a poorer outcome tend to have anatomic anomalies of the tracheobronchial tree and bronchial hypoplasia,[33] and many patients have impaired lung perfusion.[34]

The characteristics of the pulmonary interstitium critically influence the ability of the lungs to perform gas exchange (Figure 1). Elastin, collagen, and proteoglycans significantly determine the reversible dispensability during respiration.[35] Procollagen and tropoelastin levels are elevated in the nitrofen model, likely contributing to decreased lung compliance.[36] However, it was more recently determined that deposition of elastin in human CDH lungs is rare at the tips of secondary septa of the developing alveoli, in the few places where secondary septa are forming.[37] These findings also extend to the surgical sheep model, and tropoelastin mRNA is reduced in nitrofen.[37] Elastin itself is likely causing a portion of the alveolar defects in human CDH and animal models. Elastin-null mouse lungs show not only dilated alveolar airspaces but also disturbed distal airway branching.[38]

Lung tissue from human CDH patients, sheep with surgically induced diaphragmatic hernias, and nitrofen rats, also showed reduced FGF18 expression.[37] FGF18 is normally expressed in the interstitial cells of septa and increases later in development and coincides with the onset of alveolarization.[37] Likely, decreased FGF18 plays a role in impaired septation in CDH.[37] FGF18 plays a role in stimulating the proliferation of lung cells during the terminal saccular stage, remodeling the distal lung and expanding the alveolar airspace.[39] Transgenic FGF18 knockout mice also have thicker interstitial mesenchymal compartments.[39] Expression of FGF18 and elastin can be rescued in the nitrofen pups and sheep with surgically induced diaphragmatic hernias by prenatal treatment with vitamin A and tracheal occlusion, respectively.[37] The