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Pulmonary epithelial cell differentiation in the nitrofen-induced congenital diaphragmatic hernia☆

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Abstract

Background/Aim: In congenital diaphragmatic hernia (CDH), there is pulmonary neuroendocrine cell (PNEC) hyperplasia and Clara (nonendocrine) cell hypoplasia, the meaning of which remains unknown. In embryonic/fetal lung, an intricate cross talk between Notch pathway and basic helix-loop-helix transcription factors Mash1 and Hes1 determines the balance between endocrine and nonendocrine epithelial cell fate. Differences at the molecular level in pulmonary epithelial cell differentiation between control and CDH hypoplastic lungs were investigated.

Material and Methods: The nitrofen-induced CDH rat model was used. At 15.5 days postconception (dpc), fetuses were assigned to 2 experimental groups: control and nitrofen (exposed to nitrofen, without CDH), whereas at 17.5, 19.5, and 21.5 dpc, fetuses were assigned to 3 experimental groups: control, nitrofen, and CDH (exposed to nitrofen, with CDH). The fetal lungs were processed for expression quantification of *CC10*, *Hes1*, *Mash1*, and *Dll1* by real-time polymerase chain reaction.

Results: In control fetuses, expression of all studied genes increased with gestational age. In nitrofen-exposed fetal lungs, endocrine cell marker *Mash1* was downregulated only at the earliest studied gestational age, whereas *Dll1* expression levels were significantly increased in the CDH group at 19.5 and 21.5 dpc. Regarding nonendocrine markers, *Hes1* presented increased expression at 15.5 and 19.5 dpc, whereas *CC10* was downregulated at 17.5 and 19.5 dpc but not at term.

Conclusions: This study suggests that PNEC hyperplasia in CDH fetal lung is likely because of Notch signaling deregulation, whereas Clara cell hypoplasia in CDH lungs could be a consequence of protein synthesis delay, reflecting a functional maturation hindrance and not a cell fate commitment problem.

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Notch comprises a family of transmembrane receptors (Notch 1-4) whose ligands (Delta-like1-3 and Jagged 1,2) are expressed on adjacent cells. Throughout vertebrate and invertebrate development, Notch signaling regulates spatial patterning, timing, and outcomes of cell fate decisions. In a common progenitor background, Notch signal-sending cell and -receiving cell adopt distinct fates via lateral inhibition event [1].

Lung development initiates with a primitive foregut endodermal bud surrounded by mesenchyme, evolving to a highly branched tracheobronchial tree with several specialized cell types. During the early pseudoglandular period of lung development, columnar immature cells line the forthcoming bronchial system where activation or repression of differentiation genes, by specific transcription factors, is essential for cell phenotype establishment. Relevant transcription factors involved in airway epithelial differentiation are hepatocyte nuclear factor 3α and 3β, hepatocyte nuclear factor/forkhead homolog 4, thyroid transcription factor 1, GATA transcription factor 6 [2], mammalian achaete-scute homolog 1 (Mash1), and hairy and enhancer of split 1 (Hes1) [3]. During fetal lung development, Notch pathway provides a regulation pattern that determines the balance between endocrine and nonendocrine epithelial cell fate [4,5] (Fig. 1). In addition, reports from knockouts showed fundamental relationships between Notch pathway and regulation of basic helix-loop-helix (bHLH) transcription factors Mash1 and Hes1 as activator and repressor of cell fate decisions in the developing lung, respectively [4,5]. Pulmonary neuroendocrine cells (PNECs) are the first cell type to differentiate in the airway epithelium [6].

Congenital diaphragmatic hernia (CDH) is a severe anomaly characterized by pulmonary hypoplasia and neonatal hypertension [7,8]. Pulmonary neuroendocrine cell hyperplasia, as well as increased expression of neuroendocrine-secreted substances (bombesin, calcitonin gene-related peptide [CGRP], and ghrelin), has been well documented to be associated to this pathology [9-12], whereas Clara cell hypoplasia has been reported [13]. These reports suggest alterations in normal epithelial cell differentiation during lung development related to CDH.

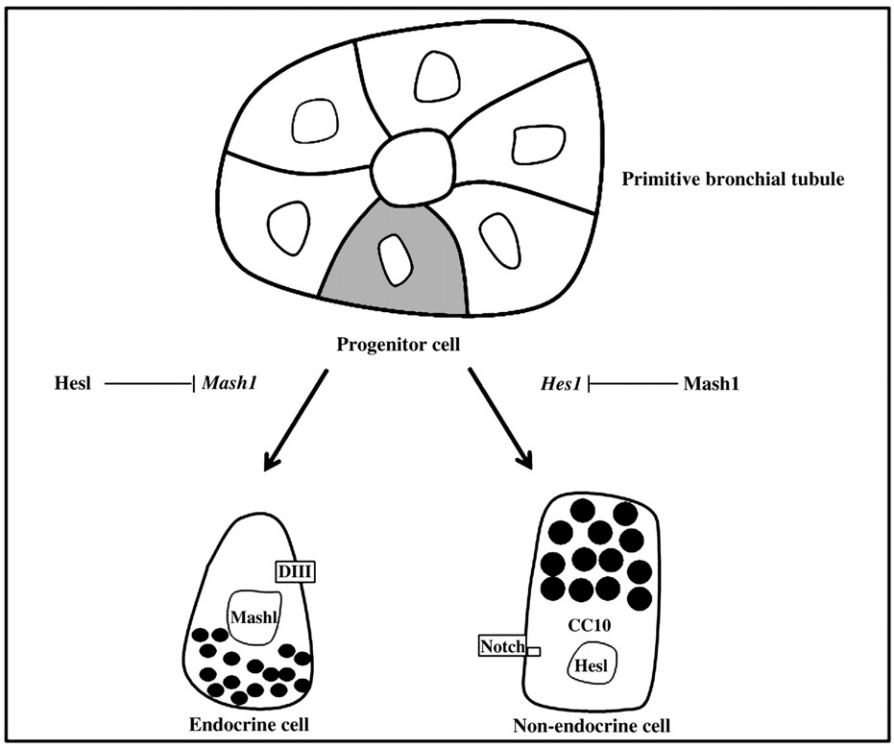

Fig. 1 Endocrine and nonendocrine epithelial cell fate during fetal lung development. In the pseudoglandular phase of lung development, the undifferentiated primitive airway expresses a variety of markers and comprises a population of multipotential epithelial progenitors. The highly conserved Notch signaling is upstream of bHLH regulation of epithelium differentiation and acts via lateral inhibition. Mash1 and Hes1, bHLH activator and repressor transcription factors, regulate endocrine differentiation by concurrently regulating their expression. Hes1 is present in Notch1- and Notch3-positive nonendocrine cells (Clara cell precursors). *Dll1* temporal and spatial expression pattern is similar to *Mash1* and restricted to endocrine cells. Thus, Dll1 positioned in Mash1-positive epithelial cells (endocrine precursors) can inhibit endocrine differentiation in Clara cell precursor (nonendocrine) adjoining cells by interaction with Notch1 or Notch3 inducing *Hes1* expression ($\perp$ inhibitory effect).