Pediatric Surgery International (2019) 35:41–61
https://doi.org/10.1007/s00383-018-4375-5

ORIGINAL ARTICLE

Assessment of the nitrofen model of congenital diaphragmatic hernia and of the dysregulated factors involved in pulmonary hypoplasia

Louise Montalva[1,2] · **Augusto Zani**[1,2]

Accepted: 18 October 2018 / Published online: 1 November 2018

Abstract

Purpose To study pulmonary hypoplasia (PH) associated with congenital diaphragmatic hernia (CDH), investigators have been employing a fetal rat model based on nitrofen administration to dams. Herein, we aimed to: (1) investigate the validity of the model, and (2) synthesize the main biological pathways implicated in the development of PH associated with CDH.

Methods Using a defined strategy, we conducted a systematic review of the literature searching for studies reporting the incidence of CDH or factors involved in PH development. We also searched for PH factor interactions, relevance to lung development and to human PH.

Results Of 335 full-text articles, 116 reported the incidence of CDH after nitrofen exposure or dysregulated factors in the lungs of nitrofen-exposed rat fetuses. *CDH incidence*: 54% (27–85%) fetuses developed a diaphragmatic defect, whereas the whole litter had PH in varying degrees. Downregulated signaling pathways included FGF/FGFR, BMP/BMPR, Sonic Hedgehog and retinoid acid signaling pathway, resulting in a delay in early epithelial differentiation, immature distal epithelium and dysfunctional mesenchyme.

Conclusions The nitrofen model effectively reproduces PH as it disrupts pathways that are critical for lung branching morphogenesis and alveolar differentiation. The low CDH rate confirms that PH is an associated phenomenon rather than the result of mechanical compression alone.

Keywords Retinoic acid · Fibroblast · Branching morphogenesis · Alveolar differentiation · Lung development

Introduction

Congenital diaphragmatic hernia (CDH) is a severe birth defect with an incidence of 1 in 3000 live-births [1, 2]. Despite advances in prenatal diagnosis and postnatal management, the morbidity and mortality rates of babies with CDH remain high [3]. Pulmonary hypoplasia (PH), which is characterized by the abnormal and delayed lung development, has been recognized to be the main determinant of CDH outcome [4]. As a result, most research efforts have focused on the understanding of PH pathogenesis and on the search for a PH treatment strategy that would improve lung maturation and growth [5]. To study PH associated with CDH, investigators have conducted experimental studies using a number of animal models of CDH [6]. Surgical models of CDH have been employed mainly to test promising antenatal therapies, but have not been used to study the earliest origins of CDH and PH, as the diaphragmatic defect is created late in gestation. Although several genetic models have been reported to reproduce a diaphragmatic defect as part of their phenotype, these cannot really be employed to investigate the etiology and pathophysiology of CDH and PH as these are multifactorial and not associated with a single gene mutation [7]. Moreover, genetic models do not reflect the true nature of human CDH, that only a minority of cases is associated with known genetic defects [8]. In 1981, a toxicology report established for the first time a link between the administration of a herbicide called nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether) to a pregnant rat and the development of CDH and PH in a relatively high proportion of the offspring [9]. Since then,

✉ Augusto Zani
augusto.zani@sickkids.ca

[1] Developmental and Stem Cell Biology Program, Peter Gilgan Centre for Research and Learning, The Hospital for Sick Children, Toronto, ON, Canada

[2] Division of General and Thoracic Surgery, Department of Surgery, The Hospital for Sick Children, 1524C-555 University Ave, Toronto, ON M5G 1X8, Canada

the nitrofen-induced rat model of CDH/PH has been extensively studied, modified and widely employed, because of the early development of CDH/PH and the similarities with the human condition [10, 11]. The exact mechanism underlying nitrofen-induced CDH/PH remains still unclear. It is now known that lung development requires regulation by many factors and constant epithelial–mesenchymal interactions [12]. In 1993, Suen et al. suggested the "biochemical immaturity" of lungs in the nitrofen model [13]. Since then, many pathways have been reported to be disrupted in nitrofen-induced CDH/PH and several hypotheses have been suggested over the years.

To the best of our knowledge, no study has synthetized the accumulated data on the factors contributing to the pathogenesis of CDH/PH and on the dysregulated signaling pathways leading to impaired lung maturation and growth in this model. The aim of the present study was to investigate the validity of the nitrofen-induced CDH/PH model and to integrate the current available knowledge on the main biological factors and pathways implicated in the development of CDH/PH. Lastly, we explored and reported the similarities between the nitrofen-induced CDH/PH model and the human condition.

Methods

To investigate the factors and pathways that are affected in the nitrofen-induced CDH/PH model, we conducted a systematic review of the literature that was drafted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement [14]. Eligible studies were identified by searching scientific databases (PubMed, Medline, Cochrane Collaboration, Embase and Web of Science) involving articles published in English from the first available date in each database to August 2018. The search strategy combined the keywords: "congenital diaphragmatic hernia" and "nitrofen". Reference lists were searched to identify relevant cross-references. Case reports, reviews and opinion articles were excluded from the review. All grey literature publications (i.e., reports, theses, conference proceedings, bibliographies, commercial documentations, and official documents not published commercially) were excluded. The full text of potentially eligible studies was retrieved and assessed for eligibility. We included all studies reporting the incidence of CDH after nitrofen exposure at embryonic day (E)9 and/or at least one dysregulated factor with a potential role in the development of PH. Dysregulation of the factor was defined as a significant downregulation or upregulation of mRNA and/or protein and/or enzyme activity quantification in the lungs of nitrofen-exposed pups with CDH compared to control pups (statistical significance defined as $p < 0.05$). Data were collected regarding the gene or protein expression in the lungs of nitrofen-exposed pups without CDH if available. Due to the big number of articles published on experimental CDH, we deliberately excluded studies reporting models of CDH/PH other than the rat nitrofen model and studies that reported dysregulated factors and/or pathways involved in diaphragm development, as well as those on vascular remodeling and pulmonary hypertension (Table 1). Finally, we searched for PH factor interactions, relevance to human PH and to lung development.

Results

Of the 401 records screened, 116 reported either the incidence of CDH after nitrofen exposure or dysregulated factors with a potential role in PH. These articles were published between 1990 and 2018 (Fig. 1).

Validity of the model

In 1990, Kluth et al. showed that most diaphragmatic hernias occurred after administration of 100 mg of nitrofen to pregnant rats on E9 (42%) and E11 (59%) [10]. Left-sided hernias only occurred upon administration at E9, whereas right-sided hernias developed after exposure on E10 or later [10]. For these reasons, it was commonly accepted that nitrofen should be administered at E9 when establishing the model of CDH to reproduce the human condition. Since this study, 28 articles have reported the incidence of CDH after nitrofen exposure at E9 (Table 2). Overall, administration of 100 mg of nitrofen to pregnant rats on E9 resulted in CDH in 54% (27–85%) fetuses [15–42]. Conversely, nitrofen reproduces PH in the totality of fetuses in varying degrees, though this is

Table 1 Inclusion and exclusion criteria for the systematic review

Publication	
Language	English
Date	Until August 2018
Subject	Rat
Study type	Experimental
Excluded study types	Reviews, opinion articles, letters Case reports Grey literature
Keywords	Congenital diaphragmatic hernia Nitrofen
Exclusion criteria	Studies reporting on: Dysregulated factors involved in diaphragm or other organ development Dysregulated factors involved in vascular remodeling/pulmonary hypertension Incidence of CDH after exposure at E10 or later

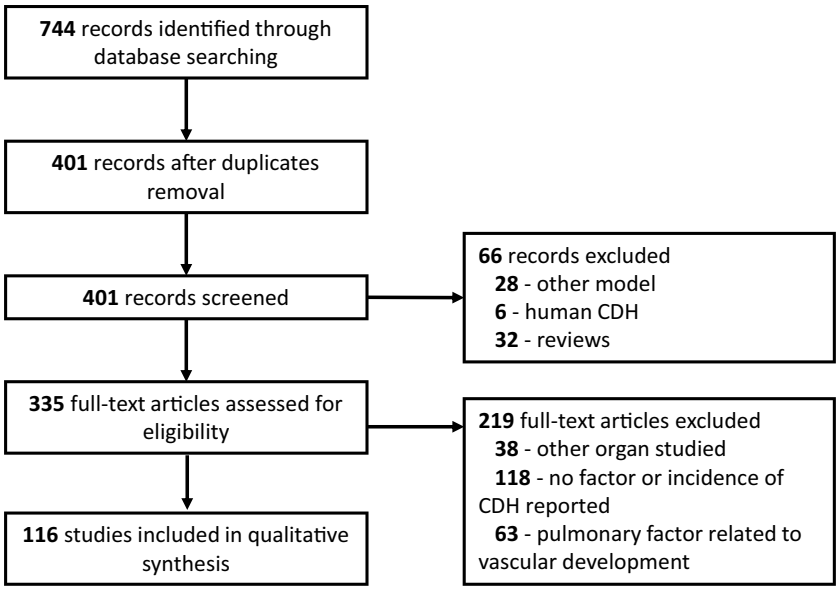

Fig. 1 Diagram of workflow in the systematic review according to PRISMA statement

difficult to quantify. Nitrofen-exposed pups have a decreased lung to body weight ratio macroscopically and fewer alveoli microscopically compared to controls [11]. At birth, PH is more severe in nitrofen-exposed pups with CDH which have fatal respiratory distress than in those without CDH which may behave as control pups [11].

Dysregulated factors and pathways involved in lung development (Fig. 2; Table 3)

Fibroblast growth factors (FGF)

Together with heparan-sulfate proteoglycans, FGF and FGF receptors (FGFRs) form a complex signaling network between the epithelium and mesenchyme that is essential for the initiation of the embryonic lung bud formation, branching morphogenesis and cell proliferation and differentiation. In the pseudo-glandular and canalicular stages, epithelial FGF-9 and mesenchymal FGF-18 have been reported as decreased in the lungs of nitrofen-exposed fetuses with and without CDH, compared to control, suggesting an early disruption of this pathway, before the occurrence of the hernia [47–49]. In the saccular stage, all five main FGFs that are important for lung development have been reported as decreased in the nitrofen model [43, 45, 47–49]. In the same way, several regulators of this signaling pathway have decreased expression in canalicular and saccular stages [52–54]. Heparan sulfate, an essential component of the extracellular matrix (ECM), required for FGFR activation, has a decreased expression and modified structure and function in the pseudoglandular stage [55]. Contradictory results have been reported regarding the receptors of this signaling pathway. Although unchanged at E18, FGFR-2 and FGFR-3 have been reported as increased in saccular stages in lungs of nitrofen-exposed pups, regardless of the presence of a hernia, compared to control pups [51]. This could be part of a regulatory loop, due to the deficit of FGF ligands, occurring as early as the pseudo-glandular stage. A decrease in FGFR-2 and 3 has also been found in micro-array analysis, but was not confirmed after RT-PCR [50]. Finally, FGF-FGFR signaling is transduced through mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt, which have both been shown to have a reduced activity in this CDH model [56, 57]. Decrease in FGF-18 signaling between mesenchymal cells can result in decreased production of lysyl oxidase (Lox); an extra-cellular enzyme that catalyzes the cross-linking of ECM proteins is necessary for the structural and functional integrity of the connective tissue and can influence the transcriptome of lung fibroblasts [87]. This enzyme is mainly produced by interstitial fibroblasts after stimulation by FGF-18, and its expression has been shown to be decreased in lungs and diaphragms of nitrofen-exposed CDH fetuses from E15 to E18 [58, 88]. In human CDH, FGF-18 is the only factor involved in this pathway that has been studied and was reported as decreased in lung tissue at autopsy [49]. FGF10 and FGF7 are decreased at amniocentesis of CDH pregnancies compared with normal [44].

Bone morphogenetic proteins (BMP)

These growth factors belong to the transforming growth factor (TGF)-β family and play a critical role in airway branching morphogenesis, alveolar and vascular formation, and epithelial differentiation. Ligands BMP-4, BMP-7 and the

Table 2 Incidence of CDH after maternal exposure to nitrofen

References	Incidence (%)	Number of pups with CDH/number of pups studied
Tovar et al. [19]	59	24/41
Alfonso et al. [16]	58	26/45
Alles et al. [20]	27	33/122
North et al. [21]	63	42/67
Alfonso et al. [22]	46	18/39
Allan and Greer [23]	52	95/181
Xia et al. [24]	63	57/90
Migliazza et al. [25]	62	80/130
Migliazza et al. [26]	63	57/90
Hoydu et al. [27]	48	76/159
Migliazza et al. [28]	53	76/143
Utsuki et al. [18]	43	44/103
Yu et al. [29]	69	22/32
Yu et al. [30]	41	16/39
Correia-Pinto et al. [31]	64	157/246
Rodriguez-Matas et al. [32]	68	53/78
Gonzalez-Reyes et al. [33]	74	70/94
Martinez et al. [34]	72	47/65
Chapin et al. [15]	31	85/274
Baptista et al. [35]	50	77/153
Oshiro et al. [36]	85	77/91
Folkesson et al. [37]	48	46/96
Montedonico et al. [38]	62	40/65
Lin et al. [17]	53	68/128
Baird et al. [39]	58	64/110
Sakai et al. [40]	50	13/26
Tsuda et al. [41]	48	44/92
Zhu et al. [42]	63	32/51
Total ($N=28$)	54	1539/2850

receptor BMPR-2 are downregulated in the CDH nitrofen model from pseudoglandular to saccular stages [59–61]. Downstream factors and markers of the BMPR signaling pathway activity are mostly reported as downregulated, whereas BMP antagonist, Gremlin-1, is upregulated [60, 62, 63]. Interestingly, T-box transcriptions factors 2, 4 and 6, mediators of BMP-Smad signaling, are essential for FGF-10 and Wnt2 expression in the mesenchyme and are decreased in the CDH nitrofen model from E15 to E21 [64].

Sonic Hedgehog (Shh)

Throughout lung organogenesis, Shh protein is strongly expressed in the epithelium at the tips of the primary and secondary lung buds and developing bronchi, with a peak in expression during the pseudoglandular stage, suggesting a polarizing role in branching morphogenesis [89]. Shh signaling also contributes to the mesenchymal thinning of late pseudoglandular and early canalicular stages, by inhibiting fibroblast proliferation through the activation of kinesin family-7 (Kif-7) and Forkhead box-1 (FoxF1) [89]. Shh is known to interact with many other pathways: it induces BMP4 in early lung development, restricts FGF-10 expression to the distal tips of lung buds, and induces FoxF1 expression in sub-epithelial lung mesenchyme [89, 90]. In return, Shh is upregulated by FGF-9 and downregulated by the non-canonical Wnt-5 [89]. In the nitrofen model, the expression of Shh in the epithelium and FoxF1 and Kif7 in the mesenchyme is decreased from the early pseudo-glandular stage until the alveolar stage, correlating with low BMP-4 expression levels [65–67]. Shh downregulation results in branching arrest, fibroblast proliferation, thickened mesenchyme, and alveolar immaturity. In human CDH, the normal pseudoglandular peak in Shh expression is delayed to late canalicular/saccular

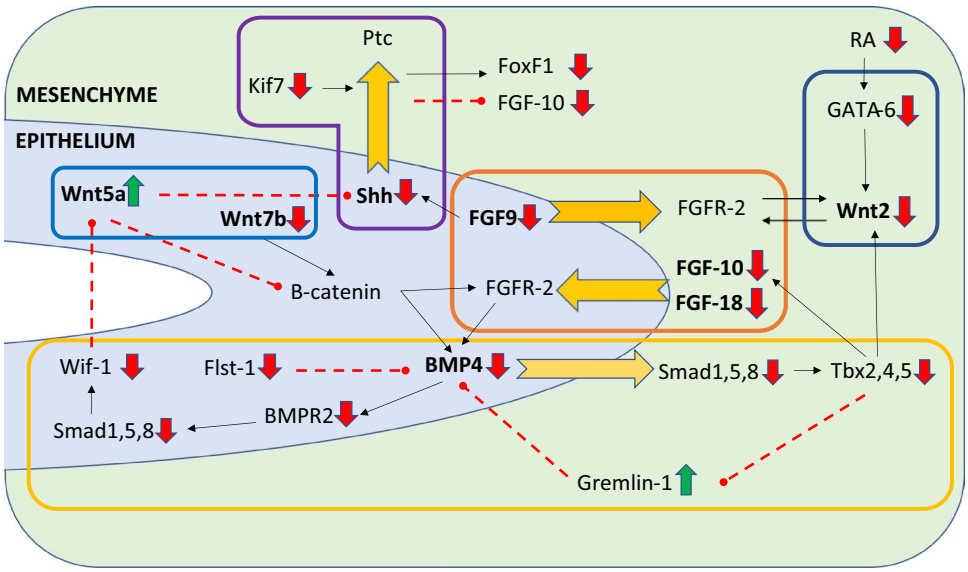

Fig. 2 Main dysregulated pathways in the nitrofen model involved in lung development (Lung bud)

Table 3 Main dysregulated factors and signaling pathways involved in lung development

Factor	Change in CDH model	Location	Role	Human CDH
FGF signaling				
FGF-10	Decreased E21 (CDH vs nitrofen without CDH and controls) [43]	Mesenchyme	Controls of branching morphogenesis Epithelial cell proliferation and differentiation Induces SP-C, downregulates BMP4	Decreased in amniotic fluid [44]
FGF-2 (=basic FGF)	Decreased E21 [45]	Mesenchyme and epithelium	Branching morphogenesis Epithelial differentiation Vasculogenesis	
FGF-7 (=keratocyte growth factor)	Decreased E21 [43]	Mesenchyme	Branching morphogenesis Pulmonary septation Epithelial proliferation and differentiation	Decreased in amniotic fluid [44] Unchanged in lung [46]
FGF-9	Decreased E15 → E18 [47]	Airway epithelium and mesothelium (E15)	Branching morphogenesis Subpleural mesenchymal cell proliferation	
FGF-18	Decreased E18 → E21 [48] Decreased E21 [49]	Mesenchymal cells surrounding branching airways	Pulmonary septation Alveolar maturation Lung fibroblast proliferation	Decreased in lung [49]
FGFR1	Decreased E21 [50] Unchanged E18 and E21 [51]		FGF receptor	
FGFR2	Increased E21 [51] No change E18 [51] Decreased E21 [50]	Distal airway epithelium and mesenchyme	FGF receptor	
FGFR3	Increased E21 [51] No change E18 [51] Decreased E21 [50]	Distal airway epithelium and mesenchyme	FGF receptor	
c-cBl	Decreased E18–E21 [52]	Alveolar epithelium	Regulator of FGFR	
Sprouty-2	Decreased E18–E21 [53, 54]	Distal airway epithelium	Regulator of FGFR	
Sprouty-4	Decreased E18 [54]	Distal airway epithelium	Regulator of FGFR	
SPRED-1 and 2	Decreased E18 [54]	Mesenchymal cells surrounding newly formed airways	Regulator of FGFR	
Heparan sulfate	Decreased E15.5–17.5 + modified structure [55]	Extracellular matrix	Required for FGFR signaling	
ERK-1 and ERK-2 (=MAPK 1 and 2)	Decreased E19.5 [56]		Transduces mitogen and differentiation signals	
PI3K	Decreased E21 [57]	Epithelium and mesenchyme	Downstream of FGF regulates AKT activity	
AKT	Decreased E18 [57]	Mesenchyme	Mediates epithelial cell proliferation, differentiation and survival signal	
Lox	Decreased E15–18 [58]	ECM, produced by mesenchymal cells	Controls cross-linking of ECM proteins Downstream of FGF-18	
BMP signaling				
BMP-4	Decreased E15–21 [59, 60]	Epithelium	Downstream of Wnt Branching, epithelial differentiation	
BMP-7	Decreased E17, no change E21 [60]			

Table 3 (continued)

Factor	Change in CDH model	Location	Role	Human CDH
BMPR-2	Decreased E17–21 [60, 61]	Epithelial and vascular cells	BMP receptor	
Gremlin-1	Increased E17, no change E21 [60]	Epithelial and vascular cells	BMP antagonist	
Fstl1	Decreased E18–21 [62]	Distal alveolar epithelium	Regulator of alveolar formation and maturation	
Smad1/5/8	Decreased E17–E21 [60, 63]	Epithelium, surrounding mesenchyme	Downstream factor of BMPR-2	
Id1	Decreased E17–21 [60]		Marker of BMP activity	
Wif1	Decrease E18–21 [63]	Epithelium	Downstream of Smad1	
T-box 2, 4, 5	Decreased E15–21 [64]	Mesenchyme around branching airways	Downstream of Smad Mediates production of branching signals Essential for FGF-10 and Wnt2 expression in mesenchyme	
Shh signaling				
Sonic HedgeHog	Decreased E12–22 [65]	Epithelium	Branching morphogenesis Inhibits lung fibroblast proliferation	Delayed peak [65]
Kif7	Decreased E15–E18 [66]	Mesenchyme	Controls proliferation of mesenchymal cells. Necessary for Shh signaling to mesenchyme	
FoxF1	Decreased E21 [67]	Mesenchyme	Controls fibroblast proliferation Promotes VEGF	
Wnt signaling				
Wnt7b	Decreased E15, unchanged E17–E21 [59]	Epithelium		
Wnt2	Decreased E15, unchanged from E17 to E21 [59]	Mesenchyme		
Wnt5a	Increased E18–21 [68]	Epithelium and mesenchyme		
GATA-6	Decreased E13–18 [59, 69]	Mesenchyme	Branching morphogenesis Activates transcription of Wnt	
RSP				
Retinol	Increased E21 in plasma, decreased E21 in lung [70]		Converted to retinal then to retinoic acid	Decreased [71, 72]
RBP	Decreased E21 [73]	Whole lung	Plasmatic retinol transport	Decreased in plasma [71]
TTR	Decreased E21 [73]	Whole lung	Retinol transport	
CRBP-1	Increased E21 [70]	Whole lung	Intracellular retinol transport	CRBP2 extinct in human CDH [74]
RARα	Increased E17–21 [70, 75]	Epithelial and mesenchymal cells		
RARβ, RXRα	Increased E21 [70]	Epithelial and mesenchymal cells		
RARg	Increased E17 [75]	Epithelial and mesenchymal cells		
RALDH3	Increased E21 [70]		Oxidizes retinal to RA	
RALDH2	Unchanged [70, 74, 76]		Oxidizes retinal to RA	Increased in CDH lungs [74]

Table 3 (continued)

Factor	Change in CDH model	Location	Role	Human CDH
LRAT	Decreased E17–21 [74, 76]		Esterifies retinol to RE for storage	Absent from all newborn lungs [74]
CYP26b1	Decreased E17–21 [74, 76]		Degrades excess RA, stimulated by RA	Absent from CDH lungs [74]
LPL	Increased E21 [77]		Hydrolyzes maternal RE to retinol	
COUP-TFII	Increased E15 [78]	Mesenchyme	Represses RSP by sequestering RXR Modulates GATA 4–6	
Midkine	Decreased E15, unchanged E18–21 [79]	Mesenchyme ++ Alveolar epithelium (AT2)	RA-responsive Upregulated by TTF1 Mesenchymal thinning	
LGL-1 (crispld2)	Decreased E21 [80]	Fibroblasts	Role in branching morphogenesis and alveolarization Downstream of RSP	
LRP-1	Increased E21 [77]		Receptor allowing lungs to take in RE	
IGF signaling				
IGF-1	Decreased E21 [81]	Bronchiolar epithelium		Increased in lungs of newborns and stillborns [82]
IGF-2	Decreased E21 [73, 81]	Epithelium		
IGF-1R	Decreased E21 [83]	Proximal alveolar epithelium++, mesenchyme	Epithelial proliferation and differentiation	
IGF-2R	Decreased E21 [83]	Proximal alveolar epithelium++, mesenchyme	Alveolar maturation internalizes and transports IGF-2	
Insulin-R	Decreased E21 [84]	Proximal alveolar epithelium, mesenchyme	Regulates uptake of glucose by AT2 cells for surfactant	
IGFBP-3	Decreased E21 [85]	Proximal airway epithelium, mesenchyme	Stimulates RSP by activating RXR-α	
IGFBP-4	Increased E18–21 [86]	Mesenchyme	Inhibits IGF	
IGFBP-5	Decreased E18 [85]	Proximal airway epithelium, mesenchyme	Stimulates RSP by activating RXR-α	

stage and seems responsible of the thickened mesenchyme and sub-pleural alveolar immaturity [65].

Wnt signaling

FGF, BMP and Shh signaling can all three be regulated by a key player in lung development, Wnt signaling. Based on their distinct signal transductions, Wnt signaling can be categorized into two groups: canonical and non-canonical Wnt. Canonical Wnt, with ligands such as Wnt-7b and Wnt-2, regulates early branching morphogenesis and proximal–distal patterning. Epithelial Wnt-7b and mesenchymal Wnt-2 have decreased expression in the lung during the pseudoglandular stage in the CDH nitrofen model [59]. Decreased Wnt signaling in the epithelium results in the downregulation of its two target genes, BMP4 and FGFR-2. The decrease in Wnt ligands could be due to the downregulation of GATA-6 reported as early as E13 [59, 69]. GATA-6 is a transcription factor known to activate the Wnt-7b promoter in the lung epithelium [91]. Non-canonical Wnt factors, such as Wnt-5a, control lung distal morphogenesis, by promoting epithelial cell differentiation and alveolar septation, and inhibiting Shh signaling. In the CDH model, Wnt5a is increased in the alveolar epithelium from E18 to E21 [68]. This increase could be due to downregulation of Wnt inhibitor factor 1 (Wif1), caused by decreased BMP-4/Smad1 signaling [63].

Retinoic acid signaling pathway (RSP)

GATA-6 is a retinoic acid (RA) responsive gene product and its decrease from E13 to E18 results in a delay in branching morphogenesis and epithelial differentiation, possibly due to an RA deficit [92]. RSP disruption is considered to have a central role in the pathogenesis of CDH/PH nitrofen

model. Retinoic acid, the active form of vitamin A, is the ligand for retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which act as transcription factors for many genes involved in lung development. In 1953, it was shown that CDH was present in two-thirds of the offspring of a vitamin A deficient female rat and, later, incidence in CDH after nitrofen exposure was decreased with administration of large doses of vitamin A or RA [93–95]. Since then, multiple studies have shown RSP disruption in the nitrofen model. The embryo may be particularly susceptible to perturbations of retinoid levels, as these drop markedly between E10 and E14 due to a strong increase in retinol utilization [96]. The developing lungs and diaphragm, which express retinaldehyde dehydrogenase 2 (RALDH-2) and rely on RA for proper development, would then be compromised by a deficit in RA [97]. In vitro, nitrofen inhibits RALDH2 [98]. In vivo, RALDH2 is reported as unchanged and RALDH3 as increased from E15 onwards after nitrofen exposure [76]. However, after administration to a pregnant dam, embryonic nitrofen levels peak at 48 h post-administration and then starts decreasing slowly [99]. It has been speculated that temporary RALDH2 inhibition after nitrofen administration at E9 impairs left diaphragmatic and lung development before E15, followed by an increase in RALDH2 and RAR as part of a regulatory feedback loop due to the lack of RA. Indeed, RAR and RXR are overexpressed in epithelial and mesenchymal cells of nitrofen-exposed lungs from E17 to E21, [70, 75]. Indirect arguments for RA deficit include the downregulation of enzymes that store or degrade RA in case of RA excess, such as lecithin retinol acyltransferase (LRAT) and CYP26b1, and the activation of an alternate pathway to increase the uptake of retinol esther (RE) from maternal dietary RE through the placenta [76, 77]. In nitrofen-exposed fetuses, pulmonary and plasmatic retinol levels are decreased, whereas increased levels of RE are reported [70]. RE is transported from maternal dietary RE through the placenta, suggesting the increase in enzymes and proteins that process RE, such as LRP1 and LPL, in the fetal CDH lungs [77]. In human, newborns with CDH, cord blood retinol and retinol binding protein (RBP) have been reported as decreased compared to control [71, 72]. This possibly results in the reported intracellular RA depletion, reflected by RBP-2 and CYP26b1 downregulation in CDH newborn lungs compared to control [74]. RALDH2 is also strongly increased in the lungs of human newborns with CDH [74]. It could be speculated that RALDH2 increase is promoted by a positive feedback, secondary to low RA intracellular levels, which could be caused by either initial low RA plasma levels, or by RA excessive utilization for compensatory growth in reaction to CDH/PH. Pulmonary RALDH2 expression is unchanged in the nitrofen model, but is increased in the surgical rabbit model and in human CDH [74]. This suggests that pulmonary RALDH2 increase in human is at least in part due to the mechanical compression of the lung by the contents of the hernia [74]. Midkine, a growth factor involved in mesenchymal–epithelial interactions, is a retinoic acid responsive gene product that is involved in mesenchymal thinning during the pseudoglandular stage and later in the alveologenesis [100, 101]. In the CDH nitrofen model, Midkine expression is downregulated in the pseudoglandular stage, mainly in the mesenchyme [79].

Insulin-like growth factors (IGFs)

The IGF family, which closely interacts with RSP, is essential for proximal and distal airway epithelial differentiation [102, 103]. IGF-binding protein 4 (IGFBP-4), known to inhibit IGF, is reported as increased from E18 to E21 in the mesenchyme of lungs of nitrofen-exposed pups with or without CDH compared to controls [86]. The expression of the ligands IGF-1 and IGF-2, as well as the different receptors and regulators of this pathway, is reported as decreased in nitrofen-exposed lungs during the saccular stage [73, 81, 83, 84]. Epidermal growth factor (EGF) increases IGFBP-4 production by fetal lung fibroblast [104]. Interestingly, the lungs of human babies with CDH have higher levels of IGF-1 [82].

Dysregulated factors involved in early differentiation of airway epithelium (Fig. 3; Table 4)

Pulmonary neuro-endocrine cells (PNECs) are the earliest differentiated airway epithelial cells, arising from multipotent epithelial progenitors, expressing Sox2, at E14–15. Mash1 is a transcription factor that directs epithelial progenitors towards neuroendocrine differentiation, whereas Dll1 is a Notch ligand secreted by $Mash1^{+}$ cells that inhibits neuroendocrine differentiation, thus promoting differentiation towards club cells [105]. Mash1 and Dll1 downregulation between E15.5 and 17.5 suggests a delay in the normal epithelial neuro-endocrine cell commitment [105]. After E17.5, the lungs of fetuses with CDH have increased levels of both Mash1 and Dll1 that at E21 reach normal levels for Mash1 and levels higher than control for Dll1 [105]. This suggests that CDH stimulates neuro-endocrine differentiation and compensatory growth.

Moreover, the expression of Hes1, a factor that influences undifferentiated epithelium into a non-endocrine fate, is increased at E15.5 in nitrofen-exposed lungs [105]. In parallel, there seems to be a delay in airway epithelial non-neuroendocrine differentiation, as suggested by the decreased expression of CC10, a club cell marker, at 17.5 and 19.5 compared to controls [105]. CC10 levels increase back to normal at E21 in lungs of fetuses that develop CDH, whereas they remain lower in the lungs of those who did not develop CDH [105].

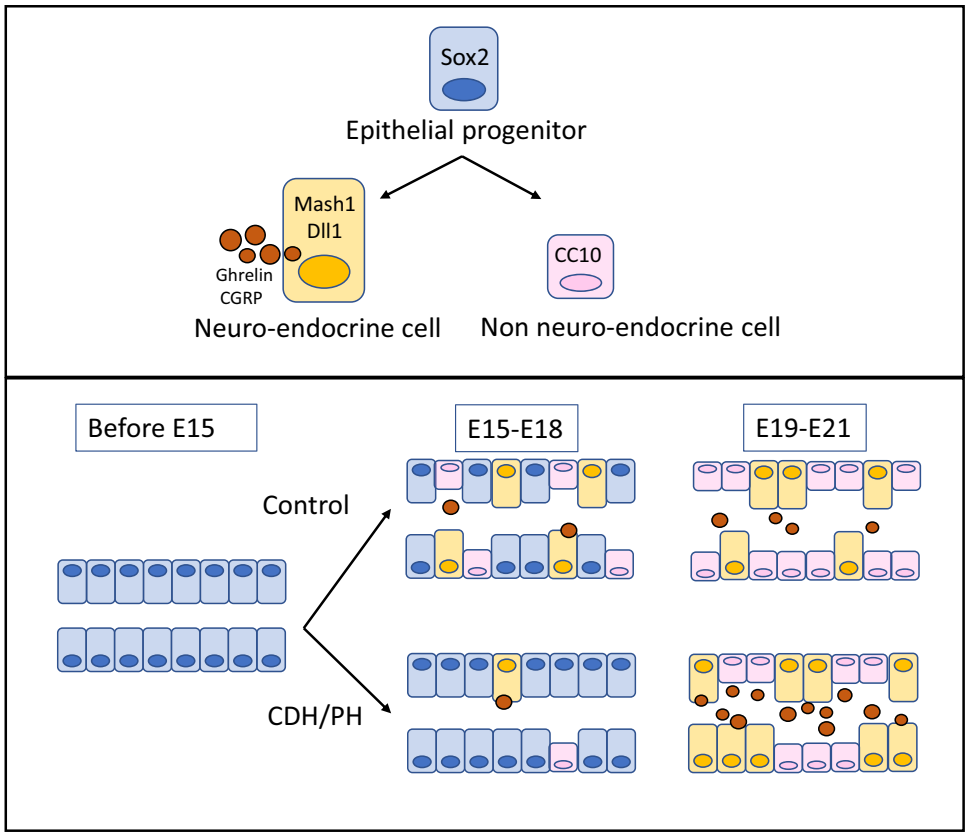

Fig. 3 Dysregulated factors involved in early differentiation of airway epithelium

Table 4 Dysregulated factors involved in early differentiation of airway epithelium

Factor	Change in CDH	Location	Role, properties	Human
Mash1 (=Ascl1)	Decreased 15.5–17.5, then increased E21 [105]	Airway epithelium	Neuro-endocrine cell (PNEC) differentiation	
Dll1	Decreased E15.5, then increase E19.5 [105]	Airway epithelium	NE cell Notch ligand	
Hes1	Increased 15.5 [105]	Airway epithelium	Neurogenic gene repressor-non NE cell	
CC10	Decreased E17.5–19.5, back to normal levels E21 [105]	Airway epithelium	Club cell marker	
Calcitonin growth-related peptide $(GGRP)^+$ cells	Increased E22 [106–108]	Bronchiolar epithelium	Peptide secreted by PNEC Epithelial and endothelial cell proliferation vasodilation	
Protein Gene Product $(PGP)^+$ cells	Increased E21 [109]	Airway epithelium	Marker of PNEC	
Ghrelin	Increased E17.5–E21.5 [75, 110]	Epithelium	Increases expression of RARα/γ	Increased in lung [110]

PNECs secrete peptides, such as calcitonin gene-related protein (CGRP), ghrelin, and bombesin. Between E18 and P7, CGRP stimulates proliferation of endothelial and epithelial cells and plays a role in controlling bronchoconstriction and vasodilation. In nitrofen CDH fetuses, whereas decreased CGRP levels are noticed at E18, there is evidence for an increased number of CGRP secreting PNECs in the airway epithelium at E22 compared to lungs of control and nitrofen-exposed without CDH fetuses [106, 107, 109]. Interestingly, the number of $CGRP^+$ cells/mm^3 is significantly higher in ipsilateral CDH lungs vs contralateral lungs and control lungs at E22 [108]. This suggests that nitrofen-exposed hypoplastic lungs experience an initial delay followed by a rapid increase in the differentiation of CGRP-secreting PNECs, possibly stimulated by the mechanical compression of the lung by the herniated organs.

Ghrelin plays a role in stimulating branching morphogenesis by increasing the expression of retinoic acid receptors (RAR) RARα and RARy ex vivo [75, 110]. Higher levels of ghrelin are reported in the lungs of all nitrofen-exposed pups (with or without CDH) from E17 to E19, compared to controls, whereas at E21 only lungs of CDH pups have higher expression of ghrelin compared to controls [110]. In lung explants from nitrofen-exposed fetuses (E13.5), administering a ghrelin antagonist decreases the expression of RAR-α/y, but administering ghrelin does not change the expression of these receptors, suggesting that receptors are already overexpressed, correlating with other studies reporting on the overexpression of RARs in CDH lungs [70, 75]. This suggests that the RA deficit stimulates RAR overexpression through ghrelin, whereas CDH further stimulates PNEC differentiation, with an increased ghrelin and CGRP production. Higher ghrelin expression has been reported in lungs of fetuses with CDH, especially during pseudoglandular and canalicular stages [110].

Lungs of human neonates with CDH showed a significant increase in bombesin expression compared with the lungs from controls or from neonates with PH without CDH [111, 112]. These findings suggest a hyperplasia of PNEC in human CDH lungs, possibly as a compensatory mechanism due to the compression of the lung by the hernia contents.

Dysregulated factors involved in late differentiation of airway epithelium (Fig. 4; Table 5)

Alveolar type 1 (AT1) cells

During the canalicular stage, alveolar type 2 (AT2) cells differentiate from distal epithelial cells and start secreting surfactant or differentiate into flat, non-secreting AT1 cells. Several markers of AT1 cells, such as Aquaporin-5, podoplanin and ICAM-1, have been reported as decreased during the saccular stages in the lungs of nitrofen-exposed CDH pups compared to nitrofen-exposed pups without CDH and control pups, suggesting that compression of the lungs by the hernia contents impairs differentiation from AT2 to AT1 cells [113–115]. In the nitrofen model, the levels of thyroid hormone and its receptors TRα1 and TRβ1, that are essential to AT1 maturation, are decreased in pups with CDH compared to control and nitrofen-exposed pups without CDH [116, 117]. This suggests that the mechanical compression of the lung by the hernia contents impairs AT1 maturation, in part through TR downregulation. Moreover, nitrofen-exposed lungs have lower levels of other factors essential to AT1 maturation and regulated by thyroid hormone receptors, such as the transcription factor Krüppel Like Factor 2 (Klf2) and connexin 43, a gap junction protein located between alveolar epithelial cells, which are both essential to differentiation from AT2 to AT1 [118, 119]. The decreased expression of ion and water channels in nitrofen-exposed

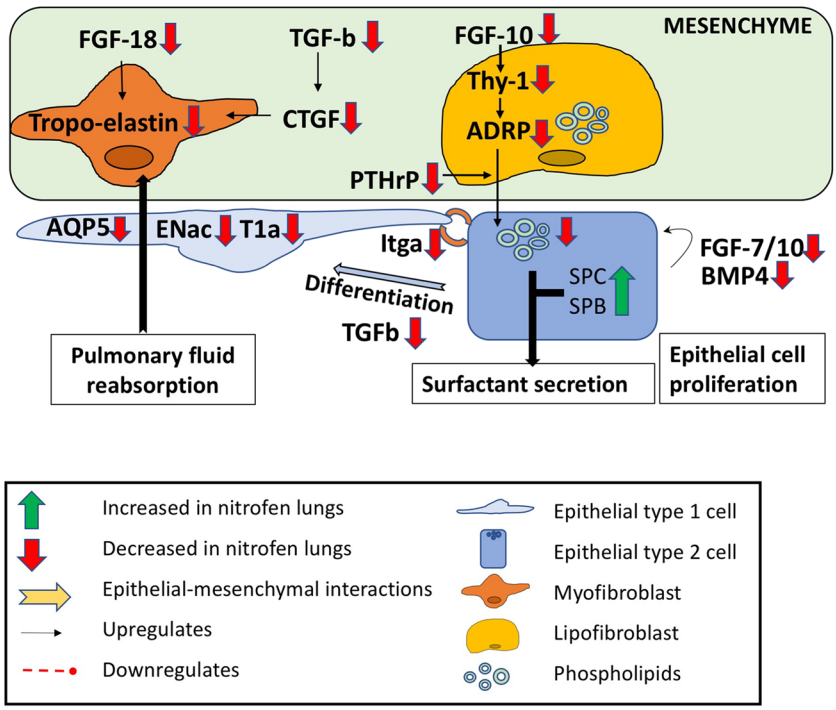

Fig. 4 Dysregulated factors involved in alveolar maturation

Table 5 Dysregulated factors involved in late differentiation of airway epithelium

Factor	Change in CDH model	Location	Role, properties	Human CDH
AQP-5	Decreased E21, no difference E15–E19 [113]	AT-1	Marker of AT-1 Reabsorption of water	
T1α (podoplanin/RTI(40))	Decreased E21 [114]	AT-1	Marker of AT-1	
ICAM-1	Decreased E21 [114, 115]	AT-1	Marker of AT-1	
T3 and T4	Decreased E21 in fetal plasma, unchanged in the lung [116]	Fetal plasma	Thyroid hormones	
TRα1	Decreased E21 [117]	Epithelium	AT-1 maturation Lung fluid absorption	
TRβ1	Decreased E21 [117]	Epithelium		
Klf2 (=Lung Klf)	Decreased E18–21 [118]	AT1	AT1 maturation Inhibits TNFα	
Connexin 43	Decreased E18–E21 [119]	Between alveolar epithelial cells	Major gap junction protein Differentiation from AT2 to AT1	
Ion and water channels	Decreased E21 [37, 50, 113, 120]	AT1	Secretion and reabsorption of lung fluid	
TTF-1	Increased E21 [40, 114] No difference [115] Decreased E21 [121–123]	AT-2 + non-ciliated bronchiolar epithelial cells	Lung morphogenesis Surfactant protein synthesis	No difference [46, 124]
Hepatocyte nuclear factor − 3β	Decreased E21 [123]	AT2	Role in surfactant synthesis	
SP-A	Decreased E21 [18, 125, 126] No change E15–22 [127, 128]			No difference [46]
SP-B	Decreased E15 [129] Increased E21 [114] Higher proportion of cells expressing SP-B, no difference < E20 [127] Decreased E21 [121–123, 126, 128, 130]	AT-2 + bronchiolar epithelial cells		Decreased mature SP-B in tracheal aspirates [131]
SP-C	Decreased E15 [129] Increased E21–22, no difference < E20 [114, 127] Decreased E21 [128] No changes [126]	AT-2		No difference [46]
SP-D	Decreased E21 [126] No change [128]			No difference [46]
Disaturated phosphatidyl-choline (PC)	Decreased E21 [18, 132]	AT2	Lipid component of surfactant	Decreased PC synthesis in infants with CDH and ECMO [133] No difference between overall CDH and controls [131, 134]
TNFα	Increased E21 [135]	Bronchiolar and alveolar epithelium	Inhibits surfactant synthesis Stimulates fibroblast proliferation and collagen	Increased in epithelial cells [136] and in serum [137]

Table 5 (continued)

Factor	Change in CDH model	Location	Role, properties	Human CDH
EGF	Increased E21 [17]	Bronchiolar epithelium Pulmonary arteries	Epithelial cell differentiation Stimulated by TNFα	Increased in epithelium [138]

lungs impairs the reabsorption of pulmonary fluid at birth [37, 50, 113, 120].

Alveolar type 2 (AT2) cells

During the pseudoglandular stage, there is a decreased expression of surfactant protein (SP)-B and SP-C, suggesting an initial delay in differentiation of distal epithelial cells [129]. During the saccular stage, nitrofen-exposed lungs have a distal epithelium mainly populated by AT2 cuboidal cells, compared to control lungs which have many AT1 flat cells, as evidenced by the increased expression of AT2 cell markers (Surfactant protein (SP)-B, SP-C and Thyroid transcription factor (TTF)-1) in the peripheral lung tissue [40, 114, 127]. One of the causes for increased markers for AT2 cells could be an increased stimulation by tumor necrosis factor (TNF)-α. Following mechanical strain, TNFα converting enzyme (TACE) can induce SP-C expression in AT2 by signaling through EGF and transforming growth factor (TGF)-α [139, 140]. TNF-α inhibits AQP-5 expression in lung epithelial cells [141]. In nitrofen-exposed lungs, higher levels of TNFα and EGF are reported in the bronchiolar epithelium and pulmonary arteries in the saccular stage [17, 135]. In CDH human neonates, a strong expression of TNF-α has been reported in pulmonary AT2 and macrophages as well as elevated serum levels of TNFα at birth [136, 137]. EGF and TGFα expression levels are upregulated in the bronchial and bronchiolar epithelium of infants with CDH compared to controls [138]. Higher rates of EGF are also found in cord blood samples from neonates with CDH at birth and in amniotic fluid just before delivery from pregnancies with CDH compared to control [142, 143]. The cause for this increase in TNFα in epithelial cells is unclear, but this might occur as a compensation to lung immaturity.

Surfactant

Several articles have reported that nitrofen-exposed lungs have decreased or unchanged levels of surfactant protein [18, 121–123, 125, 126, 128, 130]. These could seem contradictory to the reported increase in AT2 cells above mentioned. However, all these studies measured the surfactant expression in the whole lung, which does not reflect the status of the distal airways. Nitrofen-exposed lungs have lower levels and altered secretion of phosphatidylcholine (PC), the lipid component of surfactant [18, 132]. Moreover, factors involved in stimulating the maturation of surfactant lipids, such as PTHrP, ADRP, Thy-1 and retinoic acid, are decreased, whereas factors that inhibit surfactant phospholipid synthesis, such as TNFα, are increased [70, 135, 144–148].

Dysregulated factors involved in lung mesenchymal development (Fig. 4; Table 6)

Lipofibroblasts (LIFs)

In the developing lung, interstitial fibroblasts can differentiate into lipid-containing interstitial fibroblasts (LIFs) and myofibroblasts. LIFs are critical for normal alveolar development and for stimulating the de novo synthesis of surfactant phospholipids in AT2. LIFs expression is reduced in the mesenchyme of nitrofen-exposed lungs, as evidenced by the decrease of LIF-specific markers thymocyte antigen 1 (Thy-1) and adipocyte differentiation-related protein, as well LIF receptors parathyroid hormone-related receptor (PTHrP-R) and PPARg [144, 146, 147, 169]. PTHrP levels are decreased also in the epithelium of nitrofen-exposed lungs at E21 [147]. In these lungs, LIFs have a decreased expression of micro-RNA 200b, which is both downstream and regulates TGFβ/Smad signaling [150]. Several studies reported an altered TGFβ signaling in the lungs of nitrofen-exposed pups [45, 50, 151–153].

Myofibroblasts

Alveolar myofibroblasts (AMF) lacking Thy-1 produce ECM components, such as elastin, α-smooth muscle actin (α-SMA), and collagen, and are essential to secondary septation [170]. The platelet-derived growth factor A (PDGF)/PDGF-receptor-α pathway is crucial for the formation of secondary alveolar septa, by stimulating myofibroblast proliferation and migration [171]. In the CDH nitrofen model, PDGFA/PDGFRα signaling is increased in the pseudoglandular stage, inducing an excessive proliferation of myofibroblast progenitors in the mesenchyme [155]. PDGFA/PDGFRα pathway upregulation activates NADPH oxidase and results in an increase of oxidative stress, which has been reported in several studies [155–159]. An associated decrease in anti-oxidant enzymes has been found in these lungs [157, 160]. Excessive stimulation of AMF is also suggested by the increased expression of collagen, α-SMA and elastin [129, 161]. Procollagen, a soluble protein precursor of collagen, is significantly increased in nitrofen-exposed lungs at E22 [161]. Shh signaling drives AMF migration towards the sites of secondary septation, whereas FGF-10 is essential for AMF maturation [170]. However, as Shh and FGF-10 signaling are defective in nitrofen-exposed lungs, AMF migration is impaired, resulting in defective secondary septation, as shown by the immature and disorganized distribution of elastin in nitrofen-exposed lungs [49, 161, 162].

In human CDH, elastin deposition is decreased at the tips of growing septa, reflecting deficient secondary septation [49]. High levels of PDGF have been reported at amniocentesis and in cord blood of infants with CDH [142, 143].

Other mesenchymal factors

Integrin receptors link collagen to fibroblasts in the fetal lung and are essential for cell–matrix interaction and branching morphogenesis. Integrin sub-units α 3, 6 and 8 are reported as decreased in CDH nitrofen mesenchyme from pseudoglandular to saccular stage [163].

Connective tissue growth factor (CTGF) is expressed in the fetal rat lung as early as E14 and increases throughout gestation, in response to TGF-β [164]. In CDH nitrofen lungs, CTGF expression is decreased compared to controls at E21 [164, 165].

Final remarks

The nitrofen model is the most commonly studied model of CDH and has provided valuable insight into the development of PH over almost three decades. Our review shows that nitrofen induces a diaphragmatic defect in half of the litter. Nonetheless, we demonstrated that this is a good model to study PH as all fetuses in the litter develop perturbations in signaling pathways essential to lung development. Our review confirms the "dual hit hypothesis" formulated in 2000 by Keijzer et al. which suggested that PH associated with CDH results from two insults, one affecting both lungs before diaphragm closure and one affecting the ipsilateral lung after defective diaphragm development [172]. Whereas perturbations in several signaling pathways are present early in the pseudoglandular stage, before diaphragmatic closure, dysregulation of some other factors and pathways appear later and are present only in lungs of fetuses that develop CDH. Accordingly, an increase in AT2 cells is observed in all nitrofen-exposed lungs, but only lungs of fetuses with CDH experience also a decrease in AT1.

We acknowledge that the results of this review are limited by the quality of the studies reported in the literature. We have noticed that many studies excluded lungs of nitrofen-exposed pups which did not develop CDH. Moreover, some dysregulated factors have been described but their role in known lung development pathways remain unexplained. Lastly, we noticed some discrepancies between the model and the human condition for factors that have discordant levels of expression. Nonetheless, the majority of factors and pathways described in the fetal rat model are relevant to human lung development, as they are conserved across species.

Table 6 Dysregulated factors involved in lung mesenchymal development

Factor	Change in CDH model	Location	Role, properties	Human CDH
Thy-1	Decreased E21 [144]	Lipofibroblasts (LIFs)	Marker of LIFs Upregulates ADRP	
ADRP (=perilipin 2)	Decreased E18–21 [144]	LIFs	Uptake of lipids by LIFs and transport to AT2	
PPARg	Decreased E21 [146]	LIFs	Increases ADRP, AT2 maturation inhibits MCP-1	
MCP-1	Increased E21 [146]	Mesenchyme, epithelium	Monocyte chemo-attractant Increased by TNFα	Increased in plasma [149]
PTHrP	Decreased E21 [147]	Epithelium (AT2)	Increases AQP5 expression by AT1	
PTHrP-R	Decreased E18–E21 [147]	Lipofibroblasts (LIFs)	Increases the transfer of TG by LIFs to AT2 for production of surfactant	
miR-200b	Decreased E21 [150]	Lipofibroblasts	Decreases TGFβ/Smad signaling	
TGF-β	TGFβ1 decreased E17–E21 [45, 50, 151] TGFβ1 increased E16.5–E21 (peripheral lung or bronchiolar epithelium) [152, 153] TGFβ3 decreased E21 [50]		Alveolar maturation Signals through smad2/3 Target of miR-200b Enhances miR-200 expression (negative feedback loop)	TGF-β2 in umbilical cord blood increased in neonates requiring ECMO [154]
ACTA-2 (smooth muscle actin)	Increased E21 [129]	Myofibroblast	Marker of myofibroblasts	
PDGF-α	Increased E18 [155]	Epithelium and mesenchyme	Secondary septation Myofibroblast proliferation and migration	Increased in amniotic fluid [142, 143]
PDGFR-α	Increased E15 [155]	Mesenchyme	Myofibroblast progenitors	
NADPH oxidase	Increased E21 [156, 157]		Generates reactive oxygen species (ROS)	
Hydrogen peroxide	Increased E15–E18 [155]		ROS	
Oxidative damaged proteins	Increased E21 [158]			
8-Hydroxy-2′-deoxyguanosine	Increased E21 [159]		Marker of oxidative stress	
Antioxidant enzymes	Decreased E21 [157, 160]		Eliminates excess ROS	
α1-Procollagen	Increased E21 [161]	ECM	Collagen precursor	
Tropo-elastin	Increased E21 [161] Decreased E21 [49, 162]	ECM	Elastin precursor	Decreased in lungs [49]
Integrin sub-unit α 3, 6, 8	Decreased E15–E21 [163]	Mesenchyme	Branching morphogenesis, cell-matrix interaction	
CTGF (=CCN2)	Decreased E21 [164, 165]	Mesenchyme, epithelium	Alveolar maturation, Stimulates fibroblasts and ECM Production Increased by TGFβ1	
NPAS3 (neuronal PAS domain 3)	Decreased E21.5 [166]	Mesenchyme	Branching morphogenesis, alveolarization Regulates Shh signaling	
Slit2 and 3	Increased E15 and E21 [167]	Mesenchyme, airway epithelium	Organization of mesenchyme	
Epimorphin	Increased E16, decreased E20 [168]	Mesenchyme	Epithelial–mesenchyme interactions	
miR-33	Decreased E21 [42]		Predicted target gene: PDGFR-α	

Funding This work was supported by Sickkids start-up funds and by the Canadian Institute of Health Research (CIHR)—SickKids Foundation New Investigator Research Grant (NI18-1270R).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Not applicable.

References

1. Burgos CM, Frenckner B (2017) Addressing the hidden mortality in CDH: a population-based study. J Pediatr Surg 52:522–525. https://doi.org/10.1016/j.jpedsurg.2016.09.061
2. McGivern MR, Best KE, Rankin J, Wellesley D, Greenlees R, Addor M-C, Arriola L, de Walle H, Barisic I, Beres J, Bianchi F, Calzolari E, Doray B, Draper ES, Garne E, Gatt M, Haeusler M, Khoshnood B, Klungsoyr K, Latos-Bielenska A, O'Mahony M, Braz P, McDonnell B, Mullaney C, Nelen V, Queisser-Luft A, Randrianaivo H, Rissmann A, Rounding C, Sipek A, Thompson R, Tucker D, Wertelecki W, Martos C (2015) Epidemiology of congenital diaphragmatic hernia in Europe: a register-based study. Arch Dis Child Fetal Neonatal Ed 100:F137–F144. https://doi.org/10.1136/archdischild-2014-306174
3. Balayla J, Abenhaim HA (2014) Incidence, predictors and outcomes of congenital diaphragmatic hernia: a population-based study of 32 million births in the United States. J Matern Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet 27:1438–1444. https://doi.org/10.3109/14767058.2013.858691
4. Cauley RP, Potanos K, Fullington N, Bairdain S, Sheils CA, Finkelstein JA, Graham DA, Wilson JM (2015) Pulmonary support on day of life 30 is a strong predictor of increased 1 and 5-year morbidity in survivors of congenital diaphragmatic hernia. J Pediatr Surg 50:849–855. https://doi.org/10.1016/j.jpedsurg.2014.12.007
5. Russo FM, De Coppi P, Allegaert K, Toelen J, van der Veeken L, Attilakos G, Eastwood MP, David AL, Deprest J (2017) Current and future antenatal management of isolated congenital diaphragmatic hernia. Semin Fetal Neonatal Med 22:383–390. https://doi.org/10.1016/j.siny.2017.11.002
6. Chiu PPL (2014) New insights into congenital diaphragmatic hernia—a surgeon's introduction to CDH animal models. Front Pediatr 2:36. https://doi.org/10.3389/fped.2014.00036
7. Kardon G, Ackerman KG, McCulley DJ, Shen Y, Wynn J, Shang L, Bogenschutz E, Sun X, Chung WK (2017) Congenital diaphragmatic hernias: from genes to mechanisms to therapies. Dis Model Mech 10:955–970. https://doi.org/10.1242/dmm.028365
8. Wynn J, Yu L, Chung WK (2014) Genetic causes of congenital diaphragmatic hernia. Semin Fetal Neonatal Med 19:324–330. https://doi.org/10.1016/j.siny.2014.09.003
9. Costlow RD, Manson JM (1981) The heart and diaphragm: target organs in the neonatal death induced by nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether). Toxicology 20:209–227
10. Kluth D, Kangah R, Reich P, Tenbrinck R, Tibboel D, Lambrecht W (1990) Nitrofen-induced diaphragmatic hernias in rats: an animal model. J Pediatr Surg 25:850–854
11. Tenbrinck R, Tibboel D, Gaillard JL, Kluth D, Bos AP, Lachmann B, Molenaar JC (1990) Experimentally induced congenital diaphragmatic hernia in rats. J Pediatr Surg 25:426–429
12. Herriges M, Morrisey EE (2014) Lung development: orchestrating the generation and regeneration of a complex organ. Dev Camb Engl 141:502–513. https://doi.org/10.1242/dev.098186
13. Suen HC, Catlin EA, Ryan DP, Wain JC, Donahoe PK (1993) Biochemical immaturity of lungs in congenital diaphragmatic hernia. J Pediatr Surg 28:471–475 **(discussion 476–477)**
14. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 62:1006–1012. https://doi.org/10.1016/j.jclinepi.2009.06.005
15. Chapin CJ, Ertsey R, Yoshizawa J, Hara A, Sbragia L, Greer JJ, Kitterman JA (2005) Congenital diaphragmatic hernia, tracheal occlusion, thyroid transcription factor-1, and fetal pulmonary epithelial maturation. Am J Physiol Lung Cell Mol Physiol 289:L44–L52. https://doi.org/10.1152/ajplung.00342.2004
16. Alfonso LF, Vilanova J, Aldazabal P, Lopez de Torre B, Tovar JA (1993) Lung growth and maturation in the rat model of experimentally induced congenital diaphragmatic hernia. Eur J Pediatr Surg Off J Austrian Assoc Pediatr Surg Al Z Kinderchir 3:6–11. https://doi.org/10.1055/s-2008-1063498
17. Lin H, Wang Y, Xiong Z, Tang Y, Liu W (2007) Effect of antenatal tetrandrine administration on endothelin-1 and epidermal growth factor levels in the lungs of rats with experimental diaphragmatic hernia. J Pediatr Surg 42:1644–1651. https://doi.org/10.1016/j.jpedsurg.2007.05.017
18. Utsuki T, Hashizume K, Iwamori M (2001) Impaired spreading of surfactant phospholipids in the lungs of newborn rats with pulmonary hypoplasia as a model of congenital diaphragmatic hernia induced by nitrofen. Biochim Biophys Acta 1531:90–98
19. Tovar JA, Alfonso LF, Aldazabal P, Lopez de Torre B, Uriarte S, Vilanova J (1992) The kidney in the fetal rat model of congenital diaphragmatic hernia induced by nitrofen. J Pediatr Surg 27:1356–1360
20. Alles AJ, Losty PD, Donahoe PK, Manganaro TF, Schnitzer JJ (1995) Embryonic cell death patterns associated with nitrofen-induced congenital diaphragmatic hernia. J Pediatr Surg 30:353–358 **(discussion 359–360)**
21. North AJ, Moya FR, Mysore MR, Thomas VL, Wells LB, Wu LC, Shaul PW (1995) Pulmonary endothelial nitric oxide synthase gene expression is decreased in a rat model of congenital diaphragmatic hernia. Am J Respir Cell Mol Biol 13:676–682. https://doi.org/10.1165/ajrcmb.13.6.7576705
22. Alfonso LF, Arnaiz A, Alvarez FJ, Qi B, Diez-Pardo JA, Vallisi-Soler A, Tovar JA (1996) Lung hypoplasia and surfactant system immaturity induced in the fetal rat by prenatal exposure to nitrofen. Neonatology 69:94–100. https://doi.org/10.1159/000244283
23. Allan DW, Greer JJ (1997) Pathogenesis of nitrofen-induced congenital diaphragmatic hernia in fetal rats. J Appl Physiol Bethesda Md 83:338–347. https://doi.org/10.1152/jappl.1997.83.2.338
24. Xia H, Migliazza L, Diez-Pardo JA, Tovar JA (1999) The tracheobronchial tree is abnormal in experimental congenital diaphragmatic hernia. Pediatr Surg Int 15:184–187
25. Migliazza L, Xia H, Alvarez JI, Arnaiz A, Diez-Pardo JA, Alfonso LF, Tovar JA (1999) Heart hypoplasia in experimental congenital diaphragmatic hernia. J Pediatr Surg 34:706–710 **(discussion 710–711)**
26. Migliazza L, Xia H, Diez-Pardo JA, Tovar JA (1999) Skeletal malformations associated with congenital diaphragmatic hernia: experimental and human studies. J Pediatr Surg 34:1624–1629
27. Hoydu AK, Kitano Y, Kriss A, Hensley H, Bergey P, Flake A, Hubbard A, Leigh JS (2000) In vivo, in utero microscopic magnetic resonance imaging: application in a rat model of diaphragmatic hernia. Magn Reson Med 44:331–335

28. Migliazza L, Xia HM, Arnaiz A, Alvarez JI, Alfonso LF, Diez-Pardo JA, Valls i Soler A, Tovar JA (2000) Prenatal dexamethasone rescues heart hypoplasia in fetal rats with congenital diaphragmatic hernia. J Pediatr Surg 35:1757–1761
29. Yu J, Gonzalez S, Rodriguez JI, Diez-Pardo JA, Tovar JA (2001) Neural crest-derived defects in experimental congenital diaphragmatic hernia. Pediatr Surg Int 17:294–298. https://doi.org/10.1007/s003830100597
30. Yu J, Gonzalez S, Diez-Pardo JA, Tovar JA (2002) Effects of vitamin A on malformations of neural-crest-controlled organs induced by nitrofen in rats. Pediatr Surg Int 18:600–605. https://doi.org/10.1007/s00383-002-0865-5
31. Correia-Pinto J, Baptista MJ, Pedrosa C, Estevão-Costa J, Flake AW, Leite-Moreira AF (2003) Fetal heart development in the nitrofen-induced CDH rat model: the role of mechanical and nonmechanical factors. J Pediatr Surg 38:1444–1451. https://doi.org/10.1016/S0022-3468(03)00494-9
32. Rodriguez-Matas MJ, Gonzalez-Reyes S, Martínez L, Martínez I, Rodriguez JI, Diez-Pardo JA, Tovar JA (2003) The adrenal cortex in experimental congenital diaphragmatic hernia. J Pediatr Surg 38:682–684. https://doi.org/10.1016/jpsu.2003.50182
33. Gonzalez-Reyes S, Alvarez L, Diez-Pardo JA, Tovar JA (2003) Prenatal vitamin E improves lung and heart hypoplasia in experimental diaphargamatic hernia. Pediatr Surg Int 19:331–334. https://doi.org/10.1007/s00383-003-1005-6
34. Martínez L, González-Reyes S, Burgos E, Tovar JA (2004) The vagus and recurrent laryngeal nerves in experimental congenital diaphragmatic hernia. Pediatr Surg Int 20:253–257. https://doi.org/10.1007/s00383-003-1121-3
35. Baptista MJ, Melo-Rocha G, Pedrosa C, Gonzaga S, Teles A, Estevão-Costa J, Areias JC, Flake AW, Leite-Moreira AF, Correia-Pinto J (2005) Antenatal vitamin A administration attenuates lung hypoplasia by interfering with early instead of late determinants of lung underdevelopment in congenital diaphragmatic hernia. J Pediatr Surg 40:658–665. https://doi.org/10.1016/j.jpedsurg.2005.01.034
36. Oshiro T, Asato Y, Sakanashi M, Ohta T, Sugahara K (2005) Differential effects of vitamin A on fetal lung growth and diaphragmatic formation in nitrofen-induced rat model. Pulm Pharmacol Ther 18:155–164. https://doi.org/10.1016/j.pupt.2004.11.004
37. Folkesson HG, Chapin CJ, Beard LL, Ertsey R, Matthay MA, Kitterman JA (2006) Congenital diaphragmatic hernia prevents absorption of distal air space fluid in late-gestation rat fetuses. Am J Physiol Lung Cell Mol Physiol 290:L478–L484. https://doi.org/10.1152/ajplung.00124.2005
38. Montedonico S, Nakazawa N, Shinkai T, Bannigan J, Puri P (2007) Kidney development in the nitrofen-induced pulmonary hypoplasia and congenital diaphragmatic hernia in rats. J Pediatr Surg 42:239–243. https://doi.org/10.1016/j.jpedsurg.2006.09.062
39. Baird R, Khan N, Flageole H, Anselmo M, Puligandla P, Laberge J-M (2008) The effect of tracheal occlusion on lung branching in the rat nitrofen CDH model. J Surg Res 148:224–229. https://doi.org/10.1016/j.jss.2007.07.019
40. Sakai K, Kimura O, Furukawa T, Fumino S, Higuchi K, Wakao J, Kimura K, Aoi S, Masumoto K, Tajiri T (2014) Prenatal administration of neuropeptide bombesin promotes lung development in a rat model of nitrofen-induced congenital diaphragmatic hernia. J Pediatr Surg 49:1749–1752. https://doi.org/10.1016/j.jpedsurg.2014.09.015
41. Tsuda H, Kotani T, Nakano T, Imai K, Hirako S, Li H, Kikkawa F (2016) Lipocalin 2 as a new biomarker for fetal lung hypoplasia in congenital diaphragmatic hernia. Clin Chim Acta Int J Clin Chem 462:71–76. https://doi.org/10.1016/j.cca.2016.08.023
42. Zhu S, He Q, Zhang R, Wang Y, Zhong W, Xia H, Yu J (2016) Decreased expression of miR-33 in fetal lungs of nitrofen-induced congenital diaphragmatic hernia rat model. J Pediatr Surg 51:1096–1100. https://doi.org/10.1016/j.jpedsurg.2016.02.083
43. Teramoto H, Yoneda A, Puri P (2003) Gene expression of fibroblast growth factors 10 and 7 is downregulated in the lung of nitrofen-induced diaphragmatic hernia in rats. J Pediatr Surg 38:1021–1024
44. Candilera V, Bouchè C, Schleef J, Pederiva F (2016) Lung growth factors in the amniotic fluid of normal pregnancies and with congenital diaphragmatic hernia. J Matern Fetal Neonatal Med 29(13):2104–2108. https://doi.org/10.3109/14767058.2015.1076387
45. Oue T, Shima H, Taira Y, Puri P (2000) Administration of antenatal glucocorticoids upregulates peptide growth factor gene expression in nitrofen-induced congenital diaphragmatic hernia in rats. J Pediatr Surg 35:109–112
46. Boucherat O, Benachi A, Chailley-Heu B, Franco-Montoya M-L, Elie C, Martinovic J, Bourbon JR (2007) Surfactant maturation is not delayed in human fetuses with diaphragmatic hernia. PLoS Med 4:e237. https://doi.org/10.1371/journal.pmed.0040237
47. Takahashi H, Friedmacher F, Fujiwara N, Hofmann A, Puri P (2014) Pulmonary FGF9 gene expression is downregulated during the pseudoglandular stage in nitrofen-induced hypoplastic lungs. Eur J Pediatr Surg Off J Austrian Assoc Pediatr Surg Al Z Kinderchir 24:75–78. https://doi.org/10.1055/s-0033-1351392
48. Takahashi H, Friedmacher F, Fujiwara N, Hofmann A, Kutasy B, Gosemann J-H, Puri P (2013) Pulmonary FGF-18 gene expression is downregulated during the canalicular-saccular stages in nitrofen-induced hypoplastic lungs. Pediatr Surg Int 29:1199–1203. https://doi.org/10.1007/s00383-013-3387-4
49. Boucherat O, Benachi A, Barlier-Mur A-M, Franco-Montoya M-L, Martinovic J, Thébaud B, Chailley-Heu B, Bourbon JR (2007) Decreased lung fibroblast growth factor 18 and elastin in human congenital diaphragmatic hernia and animal models. Am J Respir Crit Care Med 175:1066–1077. https://doi.org/10.1164/rccm.200601-050OC
50. Burgos CM, Uggla AR, Fagerström-Billai F, Eklöf A-C, Frenckner B, Nord M (2010) Gene expression analysis in hypoplastic lungs in the nitrofen model of congenital diaphragmatic hernia. J Pediatr Surg 45:1445–1454. https://doi.org/10.1016/j.jpedsurg.2009.09.023
51. Friedmacher F, Doi T, Gosemann J-H, Fujiwara N, Kutasy B, Puri P (2012) Upregulation of fibroblast growth factor receptor 2 and 3 in the late stages of fetal lung development in the nitrofen rat model. Pediatr Surg Int 28:195–199. https://doi.org/10.1007/s00383-011-2985-2
52. Friedmacher F, Gosemann J-H, Takahashi H, Corcionivoschi N, Puri P (2013) Decreased pulmonary c-Cbl expression and tyrosine phosphorylation in the nitrofen-induced rat model of congenital diaphragmatic hernia. Pediatr Surg Int 29:19–24. https://doi.org/10.1007/s00383-012-3191-6
53. Friedmacher F, Gosemann J-H, Fujiwara N, Alvarez LAJ, Corcionivoschi N, Puri P (2013) Spatiotemporal alterations in Sprouty-2 expression and tyrosine phosphorylation in nitrofen-induced pulmonary hypoplasia. J Pediatr Surg 48:2219–2225. https://doi.org/10.1016/j.jpedsurg.2013.07.003
54. Friedmacher F, Gosemann J-H, Fujiwara N, Takahashi H, Hofmann A, Puri P (2013) Expression of Sproutys and SPREDs is decreased during lung branching morphogenesis in nitrofen-induced pulmonary hypoplasia. Pediatr Surg Int 29:1193–1198. https://doi.org/10.1007/s00383-013-3385-6
55. Thompson SM, Connell MG, van Kuppevelt TH, Xu R, Turnbull JE, Losty PD, Fernig DG, Jesudason EC (2011) Structure and epitope distribution of heparan sulfate is disrupted in experimental lung hypoplasia: a glycobiological epigenetic cause for malformation? BMC Dev Biol 11:38. https://doi.org/10.1186/1471-213X-11-38

56. Kling DE, Narra V, Islam S, Kinane TB, Alessandrini A, Ercolani L, Donahoe PK, Schnitzer JJ (2001) Decreased mitogen activated protein kinase activities in congenital diaphragmatic hernia-associated pulmonary hypoplasia. J Pediatr Surg 36:1490–1496
57. Doi T, Sugimoto K, Ruttenstock E, Dingemann J, Puri P (2010) Prenatal retinoic acid upregulates pulmonary gene expression of PI3K and AKT in nitrofen-induced pulmonary hypoplasia. Pediatr Surg Int 26:1011–1015. https://doi.org/10.1007/s00383-010-2654-x
58. Takahashi T, Friedmacher F, Takahashi H, Daniel Hofmann A, Puri P (2015) Lysyl oxidase expression is decreased in the developing diaphragm and lungs of nitrofen-induced congenital diaphragmatic hernia. Eur J Pediatr Surg Off J Austrian Assoc Pediatr Surg Al Z Kinderchir 25:15–19. https://doi.org/10.1055/s-0034-1386644
59. Takayasu H, Nakazawa N, Montedonico S, Puri P (2007) Downregulation of Wnt signal pathway in nitrofen-induced hypoplastic lung. J Pediatr Surg 42:426–430. https://doi.org/10.1016/j.jpedsurg.2006.10.018
60. Makanga M, Dewachter C, Maruyama H, Vuckovic A, Rondelet B, Naeije R, Dewachter L (2013) Downregulated bone morphogenetic protein signaling in nitrofen-induced congenital diaphragmatic hernia. Pediatr Surg Int 29:823–834. https://doi.org/10.1007/s00383-013-3340-6
61. Gosemann J-H, Friedmacher F, Fujiwara N, Alvarez LAJ, Corcionivoschi N, Puri P (2013) Disruption of the bone morphogenetic protein receptor 2 pathway in nitrofen-induced congenital diaphragmatic hernia. Birth Defects Res B Dev Reprod Toxicol 98:304–309. https://doi.org/10.1002/bdrb.21065
62. Takahashi T, Zimmer J, Friedmacher F, Puri P (2017) Follistatin-like 1 expression is decreased in the alveolar epithelium of hypoplastic rat lungs with nitrofen-induced congenital diaphragmatic hernia. J Pediatr Surg 52:706–709. https://doi.org/10.1016/j.jpedsurg.2017.01.020
63. Fujiwara N, Doi T, Gosemann J-H, Kutasy B, Friedmacher F, Puri P (2012) Smad1 and WIF1 genes are downregulated during saccular stage of lung development in the nitrofen rat model. Pediatr Surg Int 28:189–193. https://doi.org/10.1007/s00383-011-2987-0
64. Takahashi T, Friedmacher F, Zimmer J, Puri P (2017) Expression of T-box transcription factors 2, 4 and 5 is decreased in the branching airway mesenchyme of nitrofen-induced hypoplastic lungs. Pediatr Surg Int 33:139–143. https://doi.org/10.1007/s00383-016-4005-z
65. Unger S, Copland I, Tibboel D, Post M (2003) Down-regulation of sonic hedgehog expression in pulmonary hypoplasia is associated with congenital diaphragmatic hernia. Am J Pathol 162:547–555. https://doi.org/10.1016/S0002-9440(10)63848-5
66. Takahashi T, Friedmacher F, Takahashi H, Hofmann AD, Puri P (2015) Kif7 expression is decreased in the diaphragmatic and pulmonary mesenchyme of nitrofen-induced congenital diaphragmatic hernia. J Pediatr Surg 50:904–907. https://doi.org/10.1016/j.jpedsurg.2015.03.058
67. Zimmer J, Takahashi T, Hofmann AD, Puri P (2016) Down-regulation of Forkhead box F1 gene expression in the pulmonary vasculature of nitrofen-induced congenital diaphragmatic hernia. Pediatr Surg Int 32:1121–1126. https://doi.org/10.1007/s00383-016-3967-1
68. Doi T, Puri P (2009) Up-regulation of Wnt5a gene expression in the nitrofen-induced hypoplastic lung. J Pediatr Surg 44:2302–2306. https://doi.org/10.1016/j.jpedsurg.2009.07.069
69. Takahashi T, Friedmacher F, Zimmer J, Puri P (2018) Gata-6 expression is decreased in diaphragmatic and pulmonary mesenchyme of fetal rats with nitrofen-induced congenital diaphragmatic hernia. Pediatr Surg Int 34:315–321. https://doi.org/10.1007/s00383-017-4219-8
70. Nakazawa N, Montedonico S, Takayasu H, Paradisi F, Puri P (2007) Disturbance of retinol transportation causes nitrofen-induced hypoplastic lung. J Pediatr Surg 42:345–349. https://doi.org/10.1016/j.jpedsurg.2006.10.028
71. Major D, Cadenas M, Fournier L, Leclerc S, Lefebvre M, Cloutier R (1998) Retinol status of newborn infants with congenital diaphragmatic hernia. Pediatr Surg Int 13:547–549. https://doi.org/10.1007/s003830050399
72. Beurskens LWJE, Tibboel D, Lindemans J, Duvekot JJ, Cohen-Overbeek TE, Veenma DCM, de Klein A, Greer JJ, Steegers-Theunissen RPM (2010) Retinol status of newborn infants is associated with congenital diaphragmatic hernia. Pediatrics 126:712–720. https://doi.org/10.1542/peds.2010-0521
73. Kutasy B, Friedmacher F, Duess JW, Puri P (2014) Prenatal administration of retinoic acid increases the trophoblastic insulin-like growth factor 2 protein expression in the nitrofen model of congenital diaphragmatic hernia. Pediatr Surg Int 30:137–142. https://doi.org/10.1007/s00383-013-3449-7
74. Coste K, Beurskens LWJE, Blanc P, Gallot D, Delabaere A, Blanchon L, Tibboel D, Labbé A, Rottier RJ, Sapin V (2015) Metabolic disturbances of the vitamin A pathway in human diaphragmatic hernia. Am J Physiol Lung Cell Mol Physiol 308:L147–L157. https://doi.org/10.1152/ajplung.00108.2014
75. Pereira-Terra P, Moura RS, Nogueira-Silva C, Correia-Pinto J (2015) Neuroendocrine factors regulate retinoic acid receptors in normal and hypoplastic lung development. J Physiol 593:3301–3311. https://doi.org/10.1113/JP270477
76. Nakazawa N, Takayasu H, Montedonico S, Puri P (2007) Altered regulation of retinoic acid synthesis in nitrofen-induced hypoplastic lung. Pediatr Surg Int 23:391–396. https://doi.org/10.1007/s00383-006-1848-8
77. Kutasy B, Friedmacher F, Pes L, Paradisi F, Puri P (2014) Increased uptake of dietary retinoids at the maternal-fetal barrier in the nitrofen model of congenital diaphragmatic hernia. J Pediatr Surg 49:866–870. https://doi.org/10.1016/j.jpedsurg.2014.01.014
78. Doi T, Sugimoto K, Puri P (2009) Up-regulation of COUP-TFII gene expression in the nitrofen-induced hypoplastic lung. J Pediatr Surg 44:321–324. https://doi.org/10.1016/j.jpedsurg.2008.10.079
79. Doi T, Shintaku M, Dingemann J, Ruttenstock E, Puri P (2011) Downregulation of Midkine gene expression and its response to retinoic acid treatment in the nitrofen-induced hypoplastic lung. Pediatr Surg Int 27:199–204. https://doi.org/10.1007/s00383-010-2773-4
80. Ruttenstock EM, Doi T, Dingemann J, Puri P (2011) Prenatal retinoic acid treatment upregulates late gestation lung protein 1 in the nitrofen-induced hypoplastic lung in late gestation. Pediatr Surg Int 27:125–129. https://doi.org/10.1007/s00383-010-2783-2
81. Oue T, Taira Y, Shima H, Miyazaki E, Puri P (1999) Effect of antenatal glucocorticoid administration on insulin-like growth factor I and II levels in hypoplastic lung in nitrofen-induced congenital diaphragmatic hernia in rats. Pediatr Surg Int 15:175–179
82. Miyazaki E, Ohshiro K, Taira Y, Puri P (1998) Altered insulin-like growth factor I mRNA expression in human hypoplastic lung in congenital diaphragmatic hernia. J Pediatr Surg 33:1476–1479
83. Ruttenstock E, Doi T, Dingemann J, Puri P (2010) Insulinlike growth factor receptor type 1 and type 2 are downregulated in the nitrofen-induced hypoplastic lung. J Pediatr Surg 45:1349–1353. https://doi.org/10.1016/j.jpedsurg.2010.02.111
84. Ruttenstock E, Doi T, Dingemann J, Puri P (2010) Insulin receptor is downregulated in the nitrofen-induced hypoplastic lung. J Pediatr Surg 45:948–952. https://doi.org/10.1016/j.jpedsurg.2010.02.018
85. Ruttenstock E, Doi T, Dingemann J, Puri P (2010) Downregulation of insulin-like growth factor binding protein 3 and 5

in nitrofen-induced pulmonary hypoplasia. Pediatr Surg Int 26:59–63. https://doi.org/10.1007/s00383-009-2509-5
86. Ruttenstock EM, Doi T, Dingemann J, Puri P (2011) IGFBP-4 gene overexpression in the nitrofen-induced hypoplastic lung. Eur J Pediatr Surg Off J Austrian Assoc Pediatr Surg Al Z Kinderchir 21:42–45. https://doi.org/10.1055/s-0030-1262851
87. Mižíková I, Palumbo F, Tábi T, Herold S, Vadász I, Mayer K, Seeger W, Morty RE (2017) Perturbations to lysyl oxidase expression broadly influence the transcriptome of lung fibroblasts. Physiol Genom 49:416–429. https://doi.org/10.1152/physiolgenomics.00026.2017
88. Chailley-Heu B, Boucherat O, Barlier-Mur A-M, Bourbon JR (2005) FGF-18 is upregulated in the postnatal rat lung and enhances elastogenesis in myofibroblasts. Am J Physiol Lung Cell Mol Physiol 288:L43–L51. https://doi.org/10.1152/ajplung.00096.2004
89. Kugler MC, Joyner AL, Loomis CA, Munger JS (2015) Sonic hedgehog signaling in the lung. From development to disease. Am J Respir Cell Mol Biol 52:1–13. https://doi.org/10.1165/rcmb.2014-0132TR
90. Ho UY, Wainwright BJ (2017) Patched1 patterns fibroblast growth factor 10 and Forkhead box F1 expression during pulmonary branch formation. Mech Dev 147:37–48. https://doi.org/10.1016/j.mod.2017.09.001
91. Weidenfeld J, Shu W, Zhang L, Millar SE, Morrisey EE (2002) The WNT7b promoter is regulated by TTF-1, GATA6, and Foxa2 in lung epithelium. J Biol Chem 277:21061–21070. https://doi.org/10.1074/jbc.M111702200
92. Keijzer R, van Tuyl M, Meijers C, Post M, Tibboel D, Grosveld F, Koutsourakis M (2001) The transcription factor GATA6 is essential for branching morphogenesis and epithelial cell differentiation during fetal pulmonary development. Dev Camb Engl 128:503–511
93. Wilson JG, Roth CB, Warkany J (1953) An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. Am J Anat 92:189–217. https://doi.org/10.1002/aja.1000920202
94. Thébaud B, Tibboel D, Rambaud C, Mercier JC, Bourbon JR, Dinh-Xuan AT, Archer SL (1999) Vitamin A decreases the incidence and severity of nitrofen-induced congenital diaphragmatic hernia in rats. Am J Physiol 277:L423–L429
95. Babiuk RP, Thébaud B, Greer JJ (2004) Reductions in the incidence of nitrofen-induced diaphragmatic hernia by vitamin A and retinoic acid. Am J Physiol Lung Cell Mol Physiol 286:L970–L973. https://doi.org/10.1152/ajplung.00403.2003
96. Takahashi YI, Smith JE, Goodman DS (1977) Vitamin A and retinol-binding protein metabolism during fetal development in the rat. Am J Physiol Endocrinol Metab 233:E263. https://doi.org/10.1152/ajpendo.1977.233.4.E263
97. Greer JJ, Babiuk RP, Thebaud B (2003) Etiology of congenital diaphragmatic hernia: the retinoid hypothesis. Pediatr Res 53:726–730. https://doi.org/10.1203/01.PDR.0000062660.12769.E6
98. Mey J, Babiuk RP, Clugston R, Zhang W, Greer JJ (2003) Retinal dehydrogenase-2 is inhibited by compounds that induce congenital diaphragmatic hernias in rodents. Am J Pathol 162:673–679. https://doi.org/10.1016/S0002-9440(10)63861-8
99. Manson JM (1986) Mechanism of nitrofen teratogenesis. Environ Health Perspect 70:137–147
100. Toriyama K, Muramatsu H, Hoshino T, Torii S, Muramatsu T (1997) Evaluation of heparin-binding growth factors in rescuing morphogenesis of heparitinase-treated mouse embryonic lung explants. Differ Res Biol Divers 61:161–167. https://doi.org/10.1046/j.1432-0436.1997.6130161.x
101. Zhang H, Garber SJ, Cui Z, Foley JP, Mohan GS, Jobanputra M, Kaplan F, Sweezey NB, Gonzales LW, Savani RC (2009) The angiogenic factor midkine is regulated by dexamethasone and retinoic acid during alveolarization and in alveolar epithelial cells. Respir Res 10:77. https://doi.org/10.1186/1465-9921-10-77
102. Masumoto K, Teshiba R, Esumi G, Nagata K, Takahata Y, Hikino S, Hara T, Hojo S, Tsukimori K, Wake N, Kinukawa N, Taguchi T (2009) Improvement in the outcome of patients with antenatally diagnosed congenital diaphragmatic hernia using gentle ventilation and circulatory stabilization. Pediatr Surg Int 25:487–492. https://doi.org/10.1007/s00383-009-2370-6
103. Ruttenstock E, Doi T, Dingemann J, Puri P (2011) Prenatal administration of retinoic acid upregulates insulin-like growth factor receptors in the nitrofen-induced hypoplastic lung. Birth Defects Res B Dev Reprod Toxicol 92:148–151. https://doi.org/10.1002/bdrb.20293
104. Price WA (1999) Peptide growth factors regulate insulin-like growth factor binding protein production by fetal rat lung fibroblasts. Am J Respir Cell Mol Biol 20:332–341. https://doi.org/10.1165/ajrcmb.20.2.3304
105. Santos M, Nogueira-Silva C, Baptista MJ, Soares-Fernandes J, Moura RS, Correia-Pinto J (2007) Pulmonary epithelial cell differentiation in the nitrofen-induced congenital diaphragmatic hernia. J Pediatr Surg 42:1231–1237. https://doi.org/10.1016/j.jpedsurg.2007.02.014
106. IJsselstijn H, Perrin DG, de Jongste JC, Cutz E, Tibboel D (1995) Pulmonary neuroendocrine cells in neonatal rats with congenital diaphragmatic hernia. J Pediatr Surg 30:413–415
107. Yamataka T, Puri P (1996) Increased intracellular levels of calcitonin gene-related peptide-like immunoreactivity in pulmonary endocrine cells in an experimental model of congenital diaphragmatic hernia. Pediatr Surg Int 11:448–452. https://doi.org/10.1007/BF00180080
108. IJsselstijn H, Hung N, de Jongste JC, Tibboel D, Cutz E (1998) Calcitonin gene-related peptide expression is altered in pulmonary neuroendocrine cells in developing lungs of rats with congenital diaphragmatic hernia. Am J Respir Cell Mol Biol 19:278–285. https://doi.org/10.1165/ajrcmb.19.2.2853
109. Gosney JR, Okoye BO, Lloyd DA, Losty PD (1999) Pulmonary neuroendocrine cells in nitrofen-induced diaphragmatic hernia and the effect of prenatal glucocorticoids. Pediatr Surg Int 15:180–183
110. Santos M, Bastos P, Gonzaga S, Roriz J-M, Baptista MJ, Nogueira-Silva C, Melo-Rocha G, Henriques-Coelho T, Roncon-Albuquerque R, Leite-Moreira AF, De Krijger RR, Tibboel D, Rottier R, Correia-Pinto J (2006) Ghrelin expression in human and rat fetal lungs and the effect of ghrelin administration in nitrofen-induced congenital diaphragmatic hernia. Pediatr Res 59:531–537. https://doi.org/10.1203/01.pdr.0000202748.66359.a9
111. Asabe K, Tsuji K, Handa N, Kajiwara M, Suita S (1999) Immunohistochemical distribution of bombesin-positive pulmonary neuroendocrine cells in a congenital diaphragmatic hernia. Surg Today 29:407–412. https://doi.org/10.1007/BF02483031
112. Ijsselstijn H, Gaillard JL, de Jongste JC, Tibboel D, Cutz E (1997) Abnormal expression of pulmonary bombesin-like peptide immunostaining cells in infants with congenital diaphragmatic hernia. Pediatr Res 42:715–720. https://doi.org/10.1203/00006450-199711000-00026
113. Takayasu H, Nakazawa N, Montedonico S, Puri P (2007) Reduced expression of aquaporin 5 water channel in nitrofen-induced hypoplastic lung with congenital diaphragmatic hernia rat model. J Pediatr Surg 42:415–419. https://doi.org/10.1016/j.jpedsurg.2006.10.029
114. Takayasu H, Nakazawa N, Montedonico S, Sugimoto K, Sato H, Puri P (2007) Impaired alveolar epithelial cell differentiation

in the hypoplastic lung in nitrofen-induced congenital diaphragmatic hernia. Pediatr Surg Int 23:405–410. https://doi.org/10.1007/s00383-006-1853-y
115. Sugimoto K, Takayasu H, Nakazawa N, Montedonico S, Puri P (2008) Prenatal treatment with retinoic acid accelerates type 1 alveolar cell proliferation of the hypoplastic lung in the nitrofen model of congenital diaphragmatic hernia. J Pediatr Surg 43:367–372. https://doi.org/10.1016/j.jpedsurg.2007.10.050
116. Tovar JA, Qi B, Diez-Pardo JA, Alfonso LF, Arnaiz A, Alvarez FJ, Valls-i-Soler A, Morreale de Escobar G (1997) Thyroid hormones in the pathogenesis of lung hypoplasia and immaturity induced in fetal rats by prenatal exposure to nitrofen. J Pediatr Surg 32:1295–1297
117. Teramoto H, Guarino N, Puri P (2001) Altered gene level expression of thyroid hormone receptors alpha-1 and beta-1 in the lung of nitrofen-induced diaphragmatic hernia. J Pediatr Surg 36:1675–1678. https://doi.org/10.1053/jpsu.2001.27958
118. Lukošiūtė A, Doi T, Dingemann J, Ruttenstock EM, Puri P (2011) Down-regulation of lung Kruppel-like factor in the nitrofen-induced hypoplastic lung. Eur J Pediatr Surg Off J Austrian Assoc Pediatr Surg Al Z Kinderchir 21:38–41. https://doi.org/10.1055/s-0030-1262800
119. Ruttenstock EM, Doi T, Dingemann J, Puri P (2012) Prenatal retinoic acid upregulates connexin 43 (Cx43) gene expression in pulmonary hypoplasia in the nitrofen-induced congenital diaphragmatic hernia rat model. J Pediatr Surg 47:336–340. https://doi.org/10.1016/j.jpedsurg.2011.11.026
120. Ringman A, Zelenina M, Eklöf A-C, Aperia A, Frenckner B (2008) NKCC-1 and ENaC are down-regulated in nitrofen-induced hypoplastic lungs with congenital diaphragmatic hernia. Pediatr Surg Int 24:993–1000. https://doi.org/10.1007/s00383-008-2209-6
121. Losada A, Tovar JA, Xia HM, Diez-Pardo JA, Santisteban P (2000) Down-regulation of thyroid transcription factor-1 gene expression in fetal lung hypoplasia is restored by glucocorticoids. Endocrinology 141:2166–2173. https://doi.org/10.1210/endo.141.6.7522
122. Losada A, Xia H, Migliazza L, Diez-Pardo JA, Santisteban P, Tovar JA (1999) Lung hypoplasia caused by nitrofen is mediated by down-regulation of thyroid transcription factor TTF-1. Pediatr Surg Int 15:188–191
123. Gonzalez-Reyes S, Martinez L, Martinez-Calonge W, Fernandez-Dumont V, Tovar JA (2006) Effects of antioxidant vitamins on molecular regulators involved in lung hypoplasia induced by nitrofen. J Pediatr Surg 41:1446–1452. https://doi.org/10.1016/j.jpedsurg.2006.04.022
124. Hösgör M, Ijzendoorn Y, Mooi WJ, Tibboel D, De Krijger RR (2002) Thyroid transcription factor-1 expression during normal human lung development and in patients with congenital diaphragmatic hernia. J Pediatr Surg 37:1258–1262
125. Mysore MR, Margraf LR, Jaramillo MA, Breed DR, Chau VL, Arévalo M, Moya FR (1998) Surfactant protein A is decreased in a rat model of congenital diaphragmatic hernia. Am J Respir Crit Care Med 157:654–657. https://doi.org/10.1164/ajrccm.157.2.9612072
126. Shima H, Guarino N, Puri P (2000) Effect of hyperoxia on surfactant protein gene expression in hypoplastic lung in nitrofen-induced diaphragmatic hernia in rats. Pediatr Surg Int 16:473–477. https://doi.org/10.1007/s003830000427
127. Van Tuyl M, Blommaart P, Keijzer E, Wert R, Ruijter SE, Lamers JM, Tibboel WH D (2003) Pulmonary surfactant protein A, B, and C mRNA and protein expression in the nitrofen-induced congenital diaphragmatic hernia rat model. Pediatr Res 54:641–652. https://doi.org/10.1203/01.PDR.0000086906.19683.42
128. Guilbert TW, Gebb SA, Shannon JM (2000) Lung hypoplasia in the nitrofen model of congenital diaphragmatic hernia occurs early in development. Am J Physiol Lung Cell Mol Physiol 279:L1159–L1171. https://doi.org/10.1152/ajplung.2000.279.6.L1159
129. Fox ZD, Jiang G, Ho KKY, Walker KA, Liu AP, Kunisaki SM (2018) Fetal lung transcriptome patterns in an ex vivo compression model of diaphragmatic hernia. J Surg Res 231:411–420. https://doi.org/10.1016/j.jss.2018.06.064
130. Guarino N, Oue T, Shima H, Puri P (2000) Antenatal dexamethasone enhances surfactant protein synthesis in the hypoplastic lung of nitrofen-induced diaphragmatic hernia in rats. J Pediatr Surg 35:1468–1473. https://doi.org/10.1053/jpsu.2000.16416
131. Cogo PE, Simonato M, Danhaive O, Verlato G, Cobellis G, Savignoni F, Peca D, Baritussio A, Carnielli VP (2013) Impaired surfactant protein B synthesis in infants with congenital diaphragmatic hernia. Eur Respir J 41:677–682. https://doi.org/10.1183/09031936.00032212
132. Thébaud B, Barlier-Mur AM, Chailley-Heu B, Henrion-Caude A, Tibboel D, Dinh-Xuan AT, Bourbon JR (2001) Restoring effects of vitamin A on surfactant synthesis in nitrofen-induced congenital diaphragmatic hernia in rats. Am J Respir Crit Care Med 164:1083–1089. https://doi.org/10.1164/ajrccm.164.6.2010115
133. Janssen DJ, Zimmermann LJ, Cogo P, Hamvas A, Bohlin K, Luijendijk IH, Wattimena D, Carnielli VP, Tibboel D (2009) Decreased surfactant phosphatidylcholine synthesis in neonates with congenital diaphragmatic hernia during extracorporeal membrane oxygenation. Intensive Care Med 35:1754–1760. https://doi.org/10.1007/s00134-009-1564-7
134. IJsselstijn H, Zimmermann LJ, Bunt JE, de Jongste JC, Tibboel D (1998) Prospective evaluation of surfactant composition in bronchoalveolar lavage fluid of infants with congenital diaphragmatic hernia and of age-matched controls. Crit Care Med 26:573–580
135. Shima H, Ohshiro K, Taira Y, Miyazaki E, Oue T, Puri P (1999) Antenatal dexamethasone suppresses tumor necrosis factor-alpha expression in hypoplastic lung in nitrofen-induced diaphragmatic hernia in rats. Pediatr Res 46:633–637. https://doi.org/10.1203/00006450-199911000-00023
136. Ohshiro K, Miyazaki E, Taira Y, Puri P (1998) Upregulated tumor necrosis factor-alpha gene expression in the hypoplastic lung in patients with congenital diaphragmatic hernia. Pediatr Surg Int 14:21–24
137. Schaible T, Veit M, Tautz J, Kehl S, Büsing K, Monz D, Gortner L, Tutdibi E (2011) Serum cytokine levels in neonates with congenital diaphragmatic hernia. Klin Padiatr 223:414–418. https://doi.org/10.1055/s-0031-1295436
138. Guarino N, Solari V, Shima H, Puri P (2004) Upregulated expression of EGF and TGF-alpha in the proximal respiratory epithelium in the human hypoplastic lung in congenital diaphragmatic hernia. Pediatr Surg Int 19:755–759. https://doi.org/10.1007/s00383-003-1052-z
139. Kakiashvili E, Dan Q, Vandermeer M, Zhang Y, Waheed F, Pham M, Szászi K (2011) The epidermal growth factor receptor mediates tumor necrosis factor-alpha-induced activation of the ERK/GEF-H1/RhoA pathway in tubular epithelium. J Biol Chem 286:9268–9279. https://doi.org/10.1074/jbc.M110.179903
140. Wang Y, Huang Z, Nayak PS, Matthews BD, Warburton D, Shi W, Sanchez-Esteban J (2013) Strain-induced differentiation of fetal type II epithelial cells is mediated via the integrin α6β1-ADAM17/tumor necrosis factor-α-converting enzyme (TACE) signaling pathway. J Biol Chem 288:25646–25657. https://doi.org/10.1074/jbc.M113.473777
141. Towne JE, Krane CM, Bachurski CJ, Menon AG (2001) Tumor necrosis factor-alpha inhibits aquaporin 5 expression in mouse lung epithelial cells. J Biol Chem 276:18657–18664. https://doi.org/10.1074/jbc.M100322200
142. Candilera V, Bouchè C, Schleef J, Pederiva F (2016) Lung growth factors in the amniotic fluid of normal pregnancies

and with congenital diaphragmatic hernia. J Matern Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet 29:2104–2108. https://doi.org/10.3109/14767058.2015.1076387
143. Fleck S, Bautista G, Keating SM, Lee T-H, Keller RL, Moon-Grady AJ, Gonzales K, Norris PJ, Busch MP, Kim CJ, Romero R, Lee H, Miniati D, MacKenzie TC (2013) Fetal production of growth factors and inflammatory mediators predicts pulmonary hypertension in congenital diaphragmatic hernia. Pediatr Res 74:290–298. https://doi.org/10.1038/pr.2013.98
144. Friedmacher F, Hofmann AD, Takahashi H, Takahashi T, Gosemann J-H, Puri P (2014) Disruption of THY-1 signaling in alveolar lipofibroblasts in experimentally induced congenital diaphragmatic hernia. Pediatr Surg Int 30:129–135. https://doi.org/10.1007/s00383-013-3444-z
145. Friedmacher F, Fujiwara N, Hofmann AD, Takahashi H, Alvarez LAJ, Gosemann J-H, Puri P (2014) Prenatal retinoic acid increases lipofibroblast expression in hypoplastic rat lungs with experimental congenital diaphragmatic hernia. J Pediatr Surg 49:876–881. https://doi.org/10.1016/j.jpedsurg.2014.01.017 **(discussion 881)**
146. Gosemann J-H, Doi T, Kutasy B, Friedmacher F, Dingemann J, Puri P (2012) Alterations of peroxisome proliferator-activated receptor γ and monocyte chemoattractant protein 1 gene expression in the nitrofen-induced hypoplastic lung. J Pediatr Surg 47:847–851. https://doi.org/10.1016/j.jpedsurg.2012.01.038
147. Doi T, Lukosiūte A, Ruttenstock E, Dingemann J, Puri P (2010) Disturbance of parathyroid hormone-related protein signaling in the nitrofen-induced hypoplastic lung. Pediatr Surg Int 26:45–50. https://doi.org/10.1007/s00383-009-2506-8
148. Carroll JL, McCoy DM, McGowan SE, Salome RG, Ryan AJ, Mallampalli RK (2002) Pulmonary-specific expression of tumor necrosis factor-alpha alters surfactant lipid metabolism. Am J Physiol Lung Cell Mol Physiol 282:L735–L742. https://doi.org/10.1152/ajplung.00120.2001
149. Okawada M, Kobayashi H, Tei E, Okazaki T, Lane GJ, Yamataka A (2007) Serum monocyte chemotactic protein-1 levels in congenital diaphragmatic hernia. Pediatr Surg Int 23:487–491. https://doi.org/10.1007/s00383-006-1858-6
150. Khoshgoo N, Kholdebarin R, Pereira-Terra P, Mahood TH, Falk L, Day CA, Iwasiow BM, Zhu F, Mulhall D, Fraser C, Correia-Pinto J, Keijzer R (2017) Prenatal microRNA miR-200b therapy improves nitrofen-induced pulmonary hypoplasia associated with congenital diaphragmatic hernia. Ann Surg. https://doi.org/10.1097/SLA.0000000000002595
151. Santos M, Moura RS, Gonzaga S, Nogueira-Silva C, Ohlmeier S, Correia-Pinto J (2007) Embryonic essential myosin light chain regulates fetal lung development in rats. Am J Respir Cell Mol Biol 37:330–338. https://doi.org/10.1165/rcmb.2006-0349OC
152. Chen G, Qiao Y, Xiao X, Zheng S, Chen L (2010) Effects of estrogen on lung development in a rat model of diaphragmatic hernia. J Pediatr Surg 45:2340–2345. https://doi.org/10.1016/j.jpedsurg.2010.08.028
153. Xu C, Liu W, Chen Z, Wang Y, Xiong Z, Ji Y (2009) Effect of prenatal tetrandrine administration on transforming growth factor-beta1 level in the lung of nitrofen-induced congenital diaphragmatic hernia rat model. J Pediatr Surg 44:1611–1620. https://doi.org/10.1016/j.jpedsurg.2008.09.021
154. Schaible T, Reineke J, Gortner L, Monz D, Schaffelder R, Tutdibi E (2016) Are cytokines useful biomarkers to determine disease severity in neonates with congenital diaphragmatic hernia? Am J Perinatol 34:648–654. https://doi.org/10.1055/s-0036-1597133
155. Dingemann J, Doi T, Ruttenstock E, Puri P (2010) Abnormal platelet-derived growth factor signaling accounting for lung hypoplasia in experimental congenital diaphragmatic hernia. J Pediatr Surg 45:1989–1994. https://doi.org/10.1016/j.jpedsurg.2010.06.014
156. Gosemann J-H, Friedmacher F, Hunziker M, Alvarez L, Corcionivoschi N, Puri P (2013) Increased activation of NADPH oxidase 4 in the pulmonary vasculature in experimental diaphragmatic hernia. Pediatr Surg Int 29:3–8. https://doi.org/10.1007/s00383-012-3209-0
157. Aras-López R, Tovar JA, Martínez L (2016) Possible role of increased oxidative stress in pulmonary hypertension in experimental diaphragmatic hernia. Pediatr Surg Int 32:141–145. https://doi.org/10.1007/s00383-015-3826-5
158. Cigdem MK, Kizil G, Onen A, Kizil M, Nergiz Y, Celik Y (2010) Is there a role for antioxidants in prevention of pulmonary hypoplasia in nitrofen-induced rat model of congenital diaphragmatic hernia? Pediatr Surg Int 26:401–406. https://doi.org/10.1007/s00383-010-2552-2
159. Hirako S, Tsuda H, Ito F, Okazaki Y, Hirayama T, Nagasawa H, Nakano T, Imai K, Kotani T, Kikkawa F, Toyokuni S (2017) Role of catalytic iron and oxidative stress in nitrofen-induced congenital diaphragmatic hernia and its amelioration by Saireito (TJ-114). J Clin Biochem Nutr 61:176–182. https://doi.org/10.3164/jcbn.17-17
160. Sluiter W, Bos AP, Silveri F, Tenbrinck R, Kraakslee R, Tibboel D, Koster JF, Molenaar JC (1992) Nitrofen-induced diaphragmatic hernias in rats: pulmonary antioxidant enzyme activities. Pediatr Res 32:394–398. https://doi.org/10.1203/00006450-199210000-00005
161. Taira Y, Oue T, Shima H, Miyazaki E, Puri P (1999) Increased tropoelastin and procollagen expression in the lung of nitrofen-induced diaphragmatic hernia in rats. J Pediatr Surg 34:715–719
162. Mychaliska GB, Officer SM, Heintz CK, Starcher BC, Pierce RA (2004) Pulmonary elastin expression is decreased in the nitrofen-induced rat model of congenital diaphragmatic hernia. J Pediatr Surg 39:666–671
163. Takahashi T, Friedmacher F, Zimmer J, Puri P (2018) Decreased expression of integrin subunits α3, α6, and α8 in the branching airway mesenchyme of nitrofen-induced hypoplastic lungs. Eur J Pediatr Surg Off J Austrian Assoc Pediatr Surg Al Z Kinderchir 28:109–114. https://doi.org/10.1055/s-0037-1604022
164. Burgos CM, Nord M, Roos A, Didon L, Eklöf A-C, Frenckner B (2010) Connective tissue growth factor expression pattern in lung development. Exp Lung Res 36:441–450. https://doi.org/10.3109/01902141003714056
165. Ruttenstock EM, Doi T, Dingemann J, Puri P (2011) Prenatal administration of retinoic acid upregulates connective tissue growth factor in the nitrofen CDH model. Pediatr Surg Int 27:573–577. https://doi.org/10.1007/s00383-010-2833-9
166. Pereira-Terra P, Kholdebarin R, Higgins M, Iwasiow BM, Correia-Pinto J, Keijzer R (2015) Lower NPAS3 expression during the later stages of abnormal lung development in rat congenital diaphragmatic hernia. Pediatr Surg Int 31:659–663. https://doi.org/10.1007/s00383-015-3703-2
167. Doi T, Hajduk P, Puri P (2009) Upregulation of Slit-2 and Slit-3 gene expressions in the nitrofen-induced hypoplastic lung. J Pediatr Surg 44:2092–2095. https://doi.org/10.1016/j.jpedsurg.2009.02.068
168. Akpinar İ, Korgun D, Çetin A, Yesilkaya A, Karaguzel G, Boneval C, Melikoglu M (2014) Epimorphin expression in a rat model of pulmonary hypoplasia associated with congenital diaphragmatic hernia. Pediatr Surg Int 30:1037–1043. https://doi.org/10.1007/s00383-014-3579-6
169. Friedmacher F, Fujiwara N, Hofmann AD, Takahashi H, Gosemann J-H, Puri P (2014) Evidence for decreased lipofibroblast expression in hypoplastic rat lungs with congenital diaphragmatic hernia. Pediatr Surg Int 30:1023–1029. https://doi.org/10.1007/s00383-014-3549-z

170. Chao C-M, Moiseenko A, Zimmer K-P, Bellusci S (2016) Alveologenesis: key cellular players and fibroblast growth factor 10 signaling. Mol Cell Pediatr. https://doi.org/10.1186/s40348-016-0045-7
171. Gouveia L, Betsholtz C, Andrae J (2018) PDGF-A signaling is required for secondary alveolar septation and controls epithelial proliferation in the developing lung. Dev Camb Engl. https://doi.org/10.1242/dev.161976
172. Keijzer R, Liu J, Deimling J, Tibboel D, Post M (2000) Dual-hit hypothesis explains pulmonary hypoplasia in the nitrofen model of congenital diaphragmatic hernia. Am J Pathol 156:1299–1306. https://doi.org/10.1016/S0002-9440(10)65000-6