Epigenetic influences in the development of bronchopulmonary dysplasia

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Abstract

Lung development is orchestrated by highly integrated morphogenic programs of interrelated patterns of gene and protein expression. Both genetic and epigenetic influences may alter the developing lung in the canalicular and saccular phase of lung development that lead to the development of bronchopulmonary dysplasia (BPD). Maternal exposures to toxins, and especially tobacco smoke associated nicotine nitrosamine ketones, fetal and neonatal infections (with or without chorioamnionitis) and techniques of neonatal ventilator management including surfactant therapy in concert with innate genetic susceptibility have life-long consequences for the infant afflicted with BPD. Exposure to supplemental oxygen poses another threat to the prematurely newborn and increases the risk for BPD and retinopathy of prematurity, but other effects in later life have been note among infants given oxygen as newborns. Thus a greater focus on these epigenetic influences and novel strategies to care for the preterm infant will hopefully reduce the worldwide burden of BPD and increase awareness regarding epigenetic mechanisms that determine long term health and well-being.

Keywords: bronchopulmonary dysplasia, epigenetic effects, prematurity, tobacco smoke, chorioamnionitis, oxygen toxicity, CPAP and surfactant treatment

Considerable evidence has accumulated for heritable or familial components of disease susceptibility that is transmitted by non-genomic means. Environmental influences acting, even in past generations, and especially during early fetal development model diseases in infancy and later in life. The concept of "epigenetics" was introduced by Waddington [1], working with wing vein patterns in fruit flies, but his initial observations have now been appreciated as the genesis of many human diseases. Epigenetic mechanisms interacting with the intrauterine environment influenced by specific genetic polymorphisms, nutritional effects, inflammation (usually with infection), exposure to a variety of toxins (especially tobacco smoke), and needed ventilator treatments for critically ill infants all have profound effects on lung development that contribute to bronchopulmonary dysplasia (BPD), the most frequent chronic lung disease in infancy affecting 48-58% of infants born prior to 30 weeks gestational age [2]. Lung development, is orchestrated by highly integrated transcription factors, gene translation for protein expression, and epithelial-mesenchymal transformations in airway, alveolar and pulmonary vascular development. Improved understanding of "developmental plasticity" [3, 4] and influences of environmental factors (including maternal behaviors) on fetal and neonatal lung development offer insights to preventive strategies to potentially prevent or reduce the severity of BPD. Environmental influences including maternal tobacco smoking, intrauterine growth restriction, chorioamniotitis, and methods of mechanical ventilation, are factors known to be associated with chronic lung disease in premature infants and will be briefly discussed in this review.

Epigenetic inheritance is defined as biologic processes that regulate mitotically or meiotically heritable changes in gene expression without altering the DNA sequence [5]. Methylation of specific cytosines in CG dinucleotides in gene promoters, and alternations in DNA packaging arising from chemical modifications of the chromatin histone core around which DNA wraps are the primary epigenetic mechanisms of lung gene regulation. DNA methylation influences asthma associated genes [6, 7] and have been found to regulate pulmonary fibrosis [8]. Differential methylation of histones further modulate the expression of surfactant protein A by glucocoricoids [9]. Other modifications include acetylation, ubiquitination, and phosphorylation of histones that alter gene expression. Promoter methylation is also important

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for asymmetric silencing of imprinted genes and retrotransposons [10]. Epigenetic inheritance systems may be random (epimutations) or specific epigenetic changes can be induced by the environment [11], and especially the intrauterine environment [12]. DNA methylation serves to silence the expression of specific genes and alters the differentiation of specific tissues [13], and inheritance of tissue-specific DNA methylation patterns in monozygotic twins [14]. The potential role of non-coding RNA (e.g., post-transcriptional regulation by mircroRNA) in controlling epigenetic events has also been recently recognized. Although not all non-coding RNA are epigenetic in nature (e.g., post-transcriptional regulation by microRNA), some cooperate with chromatin remodeling and DNA methylation to causes epigenetic "gene-silencing" [15]. There remain substantial gaps in our understanding of how the intrauterine environment modifies lung development, and how our current treatments the newborn to epigenetic effects on the developing infant and child that may contribute to the development of early onset, as well as, later onset of chronic diseases, especially the lung and cardiovascular developments. Over the next decade we envision that that epigenetic, infectious, and environmental modifiers of intrauterine and postnatal development, including the development of BPD, will be a major focus of neonatal research.

Greenough [16] has categorized prenatal factors predisposing to adverse influences on fetal lung development ranging from pulmonary hypoplasia to alveolar simplification that is most characteristic of BPD. To date, only antenatal administration of glucocorticoids (betamethasone) to accelerate or enhance lung maturation, and specific postnatal therapies including Vitamin A and caffeine citrate have reduced the occurrence of BPD [17, 18]. While these postnatal therapies offer limited protection for lung development for infants born prematurely fetal exposure to maternal smoking, fetal infection often resulting in chorioamniotitis (associated with the fetal inflammatory response syndrome), and "gentler" approaches to neonatal ventilation and modulation of "lung stretch" have been a focus of neonatal research as have attempt to greater antioxidant protection for these critically ill infants.

Genetic component

Several studies in monozygotic and dizygotic twins reveal a strong genetic component in the occurrence of BPD [19], and its documented higher prevalence among male infants [20]. To ascertain the genetic susceptibility and to examine the linkage between specific genes and BPD transmission disequilibrium testing has found that surfactant protein B (SP-B) intron 4 deletion significantly increased the risk of BPD in a Finnish cohort [21], and also among German preterm infants [22]. While several studies have examined the association between BPD and single nucleotide polymorphisms for gene expression, at this time it is unclear whether these mutations contribute much to the overall incidence of BPD worldwide.

Maternal smoking

Although lower gestation age and respiratory distress syndrome are strong predictors of BPD, maternal tobacco smoking has been reported to be an independent predictor of BPD [23]. Intrauterine exposure to carcinogens in tobacco smoke, and the associated effects including intrauterine growth restriction are important risk factors for respiratory morbidity in childhood. Antenatal nicotine exposure in fetal rhesus from day 26 to day 134 of pregnancy results in infants with enlarged airspaces, reduction in alveolar surface area and altered airway morphology and function, and a 16% decrease in lung weight [24]. Tobacco smoke is comprised of several carcinogens. Nicotine specific nitrosamines exposure to fetus by maternal smoking (or even exposure to secondhand smoke) leads to its higher levels in the fetus than the mother [25]. Nicotine nitrosamine ketones (NNK) cause alterations in DNA and induces epigenetic alternations by inhibiting tumor suppressor genes. Further nicotine specific nitrosamine ketones induce DNA methyltransferase 1 accumulation and tumor suppressor gene hypermethylation resulting in early cancer formation in mice [26]. Both maternal and fetal glutathione S transferases are reduced when mothers smoke and there is in utero exposure to tobacco smoke toxins. These enzymes are critical in detoxifying tobacco smoke and their deficiency lead to airway hyper-responsiveness manifested by many infants with BPD [27]. Peroxisome proliferator-activated receptor gamma (PPARγ) regulates both alveolar formation and surfactant synthesis. Exposure to tobacco smoke NNK reduce PPARγ signaling during lung development by alteration of histone modification (especially methylation of histone 5 lysine 20 associated gene expression) with impedes lung development in a gender specific fashion [28]. In the fetal rate model maternal tobacco smoke exposure from day 11.5 to day 22, decreased PPARγ expression and greater susceptibility in male off-spring with delays in and simplification of alveolar development [28]. NNK produced by tobacco smoke residues contamination of environmental surfaces or even in cigarette butts (so-called thirdhand smoke residues) have also been shown to

down-regulate surfactant synthesis caused by reduced leptin induced stimulation of the alveolar type II cell resulting in a reduction of surfactant secretion by the alveolar type II cell [29]. Given these toxicities to the fetus, clearly greater emphasis must be placed on eliminating maternal and fetal exposure to tobacco smoke because of potential lifelong effects (both directly toxic and epigenetic) not only by a mother who smokes but also by reducing tobacco smoke residues in the environment.

Chorioamnionitis and fetal systemic response to inflammation

Chorioamnionitis is the single most important cause of preterm birth, and severe chorioamnionitis is seen most frequently in preterm deliveries before 30 weeks of gestation [30]. Fetal systemic response to inflammation is associated with the BPD [31, 32], intraventricular hemorrhage, cystic periventricular leukomalacia and cerebral palsy [33, 34]. Watterberg [32] reported that ventilated preterm infants exposed to histologically confirmed chorioamnionitis had a lower incidence of RDS, but higher rates of BPD than did infants not exposed to chorioamnionitis. However, Van Marter et al. [35] found a lower incidence of BPD among infants delivered with chorioamnionitis, except among those with confirmed sepsis or those who required ventilation for 7 or more days. However, when Redline et al. [36] rigorously defined chorioamnionitis, using placental and fetal membrane using histo-pathologic techniques they found no associated elevated risk for BPD and chronic lung disease in very low birth weight infants. Similarly Kaukola et al. [37] after an extensive evaluation of cord blood immunoproteins and placental pathology found no association between chorioamnionitis and the "new" form of BPD. In animal models, intrauterine endotoxin exposure leads to disturbed alveolar growth and delayed vascularization mimicking the inflammatory challenge [38]. Within 1-4 days after preterm birth, inflammatory biomarkers (chemokines, adhesion molecules, pro-and anti-inflammatory cytokines, proteases and their inactivated inhibitors, and growth factors) have complex interactions that alters subsequent lung maturations. Neonates with inflammatory lesions of the placenta were more likely than their peers ($p < 0.01$) to have elevated blood concentrations of cytokines (IL-1β, IL-6, and TNF-α), chemokines (IL-8, MIP-1β, RANTES, and I-TAC), adhesion molecules (ICAM-1, ICAM-3, and E-selectin), matrix metalloproteinases (MMP-1 and MMP-9), the angiogenic inflammatory factor VEGF and its receptor VEGF-R2, and acute phase proteins (SAA and CRP) during the first 3 days after birth [39]. Infants with poor placental perfusion had lower levels of inflammatory proteins (IL-6, RANTES, ICAM-3, VCAM-1, E-selectin, MMP-1, MMP-9, MPO, and VEGF). Increased risk of BPD was associated with elevated blood concentrations of a variety of pro-inflammatory cytokines, adhesions molecules, and proteins, while reduced risk was associated with increased concentrations of RANTES in the first days after birth. While fetal growth restriction was also demonstrated to be a risk factor for BPD, it did not appear to be mediated by systemic inflammation in 932 extremely low gestational age newborns [40].

Goldenberg et al. reported 23% of infant born between 23 and 32 weeks of gestation have umbilical blood culture positive for genital mycoplasms (*Ureaplasma* and *Mycoplasma hominis*) [41]. Animal models of *in utero Ureaplasma* mediated inflammation produces a BPD phenotype, and Viscardi and Hasday [42] have persuasively argued that the in utero infection, augmented by postnatal exposure to volutrauma and oxygen elicits a sustained dysregulated inflammatory response that impairs alveolarization, and stimulates myofibroblast proliferation and excessive collagen and elastin deposition. Further, an analysis of over 23 studies including 2216 preterm infants found a strong association between *Ureaplasma* "colonization" and BPD [43]. The early administration of azithromycin influences the development of BPD has been addressed in a randomized, double-blind, placebo controlled trial among infants weighing < 1250 g at birth and required mechanical ventilation within 72 hours of birth. These results showed significant reduction in BPD in the group of infants in whom *Ureaplasma* was identified and treated. The authors, however, caution that larger trials are needed to definitively address of whether treatment with this or similar antibiotics should be "routine" [44].

Infant ventilation strategies

Emphasis on "gentle ventilation" strategies, including non-invasive ventilation modalities, presents unique challenges. Studies comparing ventilation techniques (synchronized intermittent mandatory ventilation versus high frequency ventilation), when ventilation is delivered through endotracheal tubes have generally failed to demonstrate the superiority of one device or strategy over another. As shown by Cohen et al. [45] even at birth, cord blood RNA histone acetyltransferase binding activity and chromatin remodeling pathways were differentially expressed among infants later developing "new"

BPD. In Utah, Albertine's group have reported that nasal continuous positive airway pressure ventilation of fetal lambs preserved histone acetylation pathways in fetal lung tissue [46]. They also reported that inhibitors of histone deacylatase such as valproic acid or trichostain A provide "protection" against fibrosis during periods of nasal ventilation contrasted to endotracheal tube positive pressure ventilation [47].

Table 1. A comparison of three randomized trials of CPAP versus intubation and prophylactic surfactant administration or INSURE strategy

Author	Strategy	Surf. prophylaxis	Surf. rescue	Death < 36 wk	0_2@36 wk
Morley	Intubation	0	78%	5.9%	33.9%
2008	CPAP	0	37%	6.5%	29.3%
Ref (50)				OR	OR
				1.10 (.57-2.12)	0.76 (.54-1.09)
NICHD	Intubation	51.4%	47.5%	17.5%	44.3%
2010	CPAP	14.1%	67.1	14.2%	40.6%
Ref (51)			(ODDS RATIOS)	OR	OR
				0.81 (.63-1.03)	0.94 (.82-1.06)
Dunn	Intubation	99%	NA*	7.2%	29.3%
2010	INSURE	100%	NA	7.0%	21.5%
	CPAP	0%	46%	4.1%	25.4%
Ref (52)			RELATIVE RISK of CPAP to Intubation & Prophylactic Surfactant		
				0.57(.25-1.27)	0.83(.64-1.09)

Noninvasive ventilation is assumed to reduce the "stretch" on the developing lung airways and immature alveolar structures. Parathyroid hormone-related protein (PTHrP) is a highly conserved lung "stretch" regulated protein [48]. Although PTHrP is expressed in the endoderm and binds to mesoderm to upregulate inflammation, PTHrP also acts to regulate surfactant synthesis and alveolar-capillary interactions. Further, the nuclear transcription factor PPARγ has also been shown to promote lung development. It has been proposed that use of PPARγ agonists (i.e. rosaglitazone and other agonists such as prostaglandin J1) may reduce lung stretch induced injury and prevent BPD. This concept is currently being tested in animal models of BPD; however, translational research to the NICU is still lacking.

Popularized in Scandinavia, the strategy of intubation, surfactant administration, and prompt extubation (i.e. INSURE) minimizes the duration of endotracheal intubation [49]. Case control trials have established that this technique appears to have significantly reduced BPD in selected centers [50]. Unfortunately, use of delivery room CPAP alone, or the use of INSURE technique compared to early intubation and surfactant administration and ventilation failed to reduce the incidence of bronchopulmonary dysplasia in a large randomized trial reported by Dunn and coworkers [51]. Furthermore, use of delivery room CPAP contrasted to intubation and selective surfactant administration in the SUPPORT trial in which infants were randomized to CPAP or early intubation with surfactant administration failed to demonstrate any significant impact on the occurrence of BPD at 36 weeks [51-53]. Noteworthy, was the very high proportion of infants in whom CPAP was deemed a failure (67.1%) in the SUPPORT trial, thus requiring eventual intubation (delivery room or NICU 67.1%) with surfactant administration [52] and the three fold increase in the rate of pneumothorax [53]. The outcomes have been summarized in Table I and clearly demonstrate that a strategy of management with CPAP alone failed to significantly lower the incidence of BPD or chronic lung disease. In CURPAP trial BPD outcomes were no different among infants 25-28 gestation receiving either prophylactic or early selective surfactant or nasal CPAP within the first 5 minutes after birth [54]. These authors suggest that nasal CPAP should be started soon after birth in spontaneously breathing infants and surfactant reserved for the approximately 50% of infants with evidence of progressive respiratory syndrome. However other innovative strategies to noninvasively administer surfactant as an aerosol [53] or via a laryngotracheal mask followed by CPAP [54] offer important advantages to reduce the inflammation associated with routine neonatal intensive care.

Conclusion

Both genetic and epigenetic mechanisms play significant roles in the development of BPD. Ongoing basic research into these fundamental mechanisms that influence lung development in the human fetus and newborn will yield dividends that will influence the clinical translation into methods of neonatal care that with disturb normal development as little as possible and, hopefully, reduce the toll of this chronic lung disease.

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