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The Role of Hyperoxia in the Pathogenesis of Experimental BPD

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Abstract

Supplemental oxygen is often used as a life-saving therapy in the treatment of preterm infants. However, its protracted use can lead to the development of bronchopulmonary dysplasia (BPD), and more recently, has been associated with adversely affecting the general health of children and adolescents born preterm. Efforts to understand how exposure to excess oxygen can disrupt lung development have historically focused on the interplay between oxidative stress and anti-oxidant defense mechanisms. However, there has been a growing appreciation for how changes in gene-environment interactions occurring during critically important periods of organ development can profoundly affect human health and disease later in life. Here, we review the concept that oxygen is an environmental stressor that may play an important role at birth to control normal lung development via its interactions with genes and cells. Understanding how changes in the oxygen environment have the potential to alter the developmental programming of the lung, such that it now proceeds along a different developmental trajectory, could lead to novel therapies in the prevention and treatment of respiratory disease, such as BPD.

Keywords

Bronchopulmonary dysplasia; hyperoxia; lung development

Preterm birth (defined as live birth prior to 37 weeks of completed gestation) affects an estimated 15 million infants worldwide every year, with over 1 million of these infants dying due to complications of being born too soon[1]. Although there has been a marked reduction in mortality associated with respiratory disease in this vulnerable population of infants due to improvements in perinatal care, the prevalence of respiratory morbidity has unfortunately not changed[2, 3]. Bronchopulmonary dysplasia (BPD) is a chronic respiratory disease that develops as a result of neonatal lung injury and is one of the most common

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complications of preterm birth[4, 5]. Although BPD is a multi-causal disease, its etiology is largely attributed to the premature exposure to oxygen and the production of cytotoxic reactive oxygen species (ROS) that injure or reprogram development of the lungs[6, 7]. In fact, children who develop BPD are often re-hospitalized following respiratory infection and are at increased risk for reduced lung function as they age[3, 8, 9]. Moreover, these children are also at higher risk for retinopathy of prematurity, impaired learning, and high blood pressure[10–12]. Nevertheless, as the number of surviving infants near the lower limit of viability continues to rise, the number of children with respiratory diseases is also likely to increase. Therefore, there is an urgent need to understand how prematurity and the early-life exposure to oxygen contribute to the pathogenesis of BPD and general health later in life.

Although the introduction of mechanical ventilation with high concentrations of oxygen (hyperoxia) to the neonatal intensive care unit (NICU) improved the survival of preterm infants with respiratory distress syndrome (RDS), it resulted in a new form of lung injury and disease, later termed BPD by Northway and colleagues in 1967[13]. BPD was initially described in a cohort of moderately preterm infants (mean gestational age of 34 weeks) in the late saccular stage of lung development who were treated with aggressive mechanical ventilation and high fractions of inspired oxygen as a result of severe respiratory distress[13]. Pathologic findings in the lung tissues of infants that died with BPD revealed extensive inflammatory and fibrotic changes in the airways and lung parenchyma. The availability of new therapies, such as exogenous surfactant, milder ventilation strategies, and antenatal steroids has increased the survival of infants of younger gestational age, thus changing the "classic" description of the BPD phenotype. The "new" BPD described today typically occurs in very preterm infants (mean gestational age of less than 28 weeks) in the early saccular stage of lung development and is characterized pathologically by alveolar hypoplasia and abnormal vascular organization[14, 15]. BPD is now considered to be a developmental disease resulting from interference, or an interruption, in the growth of the lung[14]. The disease is characterized by many as an "arrest" in lung development, which is misleading because it implies that the lung has stopped growing, when in fact the developmental programming of the lung has been altered in such a way that lung growth now proceeds along a different developmental trajectory. Putting semantics aside, the incidence of BPD remains high amongst preterm infants with extremely low birth weight and continues to be one of the most common morbidities associated with preterm birth[16]. As the number of surviving preterm infants continues to increase, it is critical that we advance our understanding of the developmental sensitivity of the lung and how disruption of the developmental programming of the lung increases the risk for BPD.

Although there are many risk factors that contribute to the pathogenesis of BPD, exposure to hyperoxia remains one of the principal factors, or conditions, responsible for its development[6, 7]. Regardless of gestational age, transitioning at birth from a relative hypoxic environment *in utero* into room air will always be a hyperoxic event and likely the greatest environmental exposure we will ever encounter in our life. Fortunately, evolutionary-dependent mechanisms, particularly those relating to the development of the respiratory system, have made this extreme environmental transition manageable. Unfortunately, the premature interruption of *in utero* lung development, such as that relating to preterm birth, may lead to unfavorable consequences affecting respiratory health postnatally. For example, the structurally and functionally immature lungs of preterm infants are often inadequately prepared to breathe oxygen, usually requiring the administration of therapeutically elevated levels of oxygen to prevent tissue hypoxia and respiratory distress (Figure 1). Hence, the lung is exposed to hyperoxia regardless of when it transitions to air at birth and the magnitude of the hyperoxic exposure can be enhanced in preterm infants who are treated with supplemental oxygen. Although the use of supplemental oxygen in the neonatal period is often necessary to support life, we have learned from newborn animal models that

exposure to hyperoxia alone acutely injures the lung during a period of rapid growth and development, resulting in pathologic findings similar to those observed in human BPD[17, 18].

Since the "fetal origins" hypothesis was first conceptualized by Barker *et al.*, there has been an increased appreciation for how the environment can alter organ development, and thus the occurrence of disease later in life[19]. In order to fully appreciate a discussion on the developmental impact of early-life exposure to oxygen and its contribution to oxidant-mediated diseases, such as BPD, a brief review of the evolutionary origins of the mammalian lung is essential. Lung development and the undefined use of oxygen in neonatal care will then be reviewed, followed by the vulnerability of the lung to oxygen toxicity and the development of suitable animal models to study oxidant-mediated lung disease. Lastly, the interplay between genes, cells, and the environment (principally exposure to oxygen) will be discussed, as well as the contribution of such interactions to the pathogenesis of BPD. These insights may help refine our view of how the premature exposure to oxygen in the neonatal period disrupts lung development, ultimately giving rise to disease later in life.

Evolution of the lung in response to changing atmospheric levels of oxygen

The geological record indicates that until the present atmospheric level of oxygen was reached, specifically 21%, there were cyclical episodes of low and high oxygen, ranging from as low as 15% to as high as 35%, respectively[20]. These fluctuating oxygen conditions have been suggested to play a substantial role in the development and evolution of cellular and organismal respiration[20, 21]. In fact, the transition from aquatic to terrestrial habitation by vertebrates likely occurred during a time when atmospheric levels of oxygen were high[22]. The cutaneous respiration and inadequate removal of carbon dioxide by aquatic species was incompatible with life on land, leading to the evolution of a more sophisticated circulatory and respiratory system[22]. As organismal size and complexity increased over time, these two systems became critical for the efficient uptake and transport of oxygen to tissues and organs, thus increasing chances for survival[23]. Generational studies in fruit flies (*Drosophilia melanogaster*) have demonstrated that under hyperoxic breeding conditions, defined as oxygen in excess of 21%, body weight and wing size are increased, whereas breeding under hypoxic conditions, defined as oxygen less than 21%, gives rise to the opposite[20]. Furthermore, oxygen concentration has been shown to negatively correlate with tracheal diameter and cell size in these insects[20, 24].

In light of the fluctuating oxygen conditions throughout evolution and the increasing dependency of many organisms on a constant supply of oxygen in order to effectively function, molecular pathways concurrently evolved to respond to conditions where oxygen demand exceeded supply (hypoxia)[25]. Physiological hypoxia plays an important role in the differentiation of cell types and the signaling of multiple cascades, including angiogenesis, for example. It is also associated with a range of pathophysiological processes, such as vascular disease, chronic inflammation, and cancer[26, 27]. One notable pathway that is highly conserved across species and is key to coordinating such responses involves the transcriptional regulator known as the hypoxia-inducible factor (HIF), consisting of HIF-1α, HIF-1β, and HIF-2α heterodimers[28]. The oxygen sensing properties of HIF and its ability to regulate cellular oxygen homeostasis have previously been reviewed[29, 30]. Briefly, under conditions of normoxia, when the supply of oxygen is sufficient to satisfy a cell's bioenergetic requirements, HIF, via activity of HIF hydroxylase enzymes, is repressed through the ubiquitin-proteosome degradation pathway. Conversely, under hypoxic conditions, HIF is induced, leading to nuclear localization and subsequent activation of target genes. These target genes include those that contribute to the control of angiogenesis,

metabolism, and erythropoiesis, resulting in increased blood and oxygen supply to hypoxic tissues[25].

Since fetal development occurs in a relative hypoxic environment, it is no surprise then that HIFs play an integral role in embryogenesis. The HIF system is in place as early as 8 weeks of gestation in humans, and HIF mRNA and protein levels are known to be quite high in the fetal lung, predominantly localizing to areas that give rise to the epithelium and vascular endothelium[31]. In fact, loss of HIF-1α, HIF-1β, or HIF-2α can be embryonic lethal, with survivors exhibiting severe cardiovascular malformations, impaired lung development, reduced production of surfactant, and postnatal respiratory distress[32, 33]. Furthermore, in a baboon model of prematurity, inhibition of HIF hydroxylase enzymes has been shown to increase HIF protein levels, resulting in improved lung growth and function[34, 35].

It has been hypothesized that disrupted angiogenesis during lung development can impair alveolarization and contribute to the pathogenesis of BPD[36]. Vascular endothelial growth factor (VEGF), a transcriptional target of HIF, plays an important role in vascular development and maintenance and is essential for the formation of the embryonic vasculature[37]. However, its role in neonatal respiratory disease remains unclear, as current animal and human studies have yielded discordant findings. For example, while some animal studies have reported relative decreases in lung VEGF mRNA and protein in response to hyperoxia when compared to control animals, others have shown the opposite[38–44]. These conflicting results may partially be attributed to the developmental stage of the lung in the particular animal model used, the timing of VEGF mRNA and protein measurements, and variability of VEGF isoforms being measured[45]. Nevertheless, in various animal species that have reached full term, a trend toward decreasing levels of VEGF in response to hyperoxia has been observed when compared to age-matched controls of the same species[45]. Human studies examining levels of VEGF in response to hyperoxic exposure have also been variable. For example, some studies report decreased levels of VEGF in lung tissues from premature infants who develop BPD, whereas others report a bimodal distribution of VEGF over time, where levels are initially high during the first 12 hours of life, decrease over the next few days, and then increase[46–48]. Ultimately, while the oxygen-dependent loss of VEGF likely contributes to the abnormal microvasculature and alveolar hypoplasia found in BPD, further investigation into the role of VEGF in the pathogenesis of BPD is certainly needed[15].

Lung development and the undefined use of oxygen in neonatal care

At birth, sufficient development and function of the cardio-respiratory system, the digestive system, and the brain are required for survival. The lung, in particular, is an essential organ in this context, as the developmental maturity of the respiratory system is critical for surviving the transition into air at birth.

Human lung development is typically divided into five stages, including the embryonic (0–6 weeks gestation), pseudoglandular (6–16 weeks gestation), canalicular (16–24 weeks gestation), saccular (24–40 weeks gestation), and alveolar (36 weeks gestation to at least 2 years postnatally) periods, with some overlap of the beginning and end of each period[49]. Each of these developmental stages requires the careful orchestration of transcription and growth factors, morphogens, and extracellular matrix molecules in order to ensure the formation of a properly functioning lung that will be capable of performing the critical task of gas exchange upon the transition from fetal to postnatal life[50]. If any of these factors are not functioning at the appropriate time or location, as a result of preterm birth for example, then respiratory disease, or even death, can occur postnatally.

It is known that preterm birth interrupts the normal development of the lung. The lungs of infants born prematurely, particularly between 24–28 weeks gestation, are in the early saccular stage of development, and are required to function during a more primitive stage of maturation and under very different conditions compared to their term counterparts. During this time, division of alveolar saccules and ducts into true gas-exchanging alveoli has yet to occur, and proliferation of the capillary network is still incomplete[49]. Furthermore, the cortisol system and surfactant synthesis by alveolar type II epithelial cells are not yet mature. As a result, preterm infants are often unprepared to breathe on their own due to their underdeveloped lungs. Unfortunately, the aggregate of these pulmonary insufficiencies as a result of preterm birth can lead to respiratory distress. Although remarkable improvements in respiratory care of the preterm infant have improved survival, high rates of morbidity still persist in this fragile population[2, 3]. Thus, it is clear then that any injury to the lung, such as that caused by the premature exposure to oxygen, during a developmentally sensitive period of growth and maturation, may lead to pulmonary disease, such as BPD.

Oxygen has been given to more newborns in the world than any other neonatal treatment and is one of the most essential drugs used in neonatal care[2, 51]. The primary goal of oxygen therapy in the neonatal period is to achieve adequate blood oxygenation in an attempt to prevent tissue hypoxia (insufficient oxygen content of the blood), while at the same time minimizing the potential for oxygen toxicity. Despite its frequent, widespread use in the clinical management of newborns, very little is surprisingly understood regarding the appropriate dose of oxygen and its duration of use that is considered to be safe.

Until recently, few clinical trials have attempted to define the optimal dose of supplemental oxygen that maximizes efficacy and minimizes harm in the newborn. In an effort to assess the progression of retinopathy in oxygen-dependent preterm infants, the Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity (STOP-ROP) clinical trial randomized infants to 89–94% saturation or 96–99% saturation target groups[52]. Although the effects on the progression of ROP were mostly unremarkable, higher rates of pneumonia and BPD were observed in infants targeted to the higher oxygen saturation range. Furthermore, at 3 months, those infants with underlying lung disease targeted to the higher oxygen saturation range were more likely to be hospitalized and in need of oxygen and diuretic therapy. Similar results were noted in the Australian Benefits Of Oxygen Saturation Targeting (BOOST) trial, which revealed no evidence in favor of maintaining a higher oxygen saturation range (95–98%) in order to improve the growth and developmental outcome of oxygen-dependent preterm infants[53]. The study also showed that infants in the higher oxygen saturation range had more hospital readmissions and increased need for use of postnatal steroid and diuretic therapy, similar to findings in the STOP-ROP trial. Other, yet similar, clinical studies have suggested that chronic exposure to high oxygen saturations can injure the lungs and eyes of preterm infants[3, 54, 55]. Collectively, these studies provide evidence in favor of managing preterm infants with oxygen saturations less than 95%, which may reduce the incidence of oxidant-mediated injury, such as BPD and ROP. Confounding this conclusion are findings from the recent Surfactant, Positive Pressure, and Pulse Oximetry Randomized Trial (SUPPORT), which evaluated post-treatment effects of low (85–89%) and high (91–95%) oxygen saturation targets in preterm infants[56, 57]. Although the incidence of retinopathy was reduced in infants in the low oxygen saturation range, mortality was increased before discharge, and investigators were unable to define an optimal oxygen saturation target. An alternative approach to defining the positive and negative aspects of oxygen exposure has been to quantify oxygen exposure as an area-under-the curve (O_{AUC})[58]. Using this parameter, infants exposed to the top quartile of O_{AUC} in the first three days of life were significantly more likely to require medical care or be re-hospitalized in the first year of life. The Canadian Oxygen Trial (COT) and BOOST II trials

of the UK, Australia, and New Zealand are currently seeking to associate the short- and long-term health effects in preterm infants treated with supplemental oxygen.

Despite these recent and ongoing clinical efforts, the optimal concentration of oxygen considered safe in newborn infants remains unknown. Therefore, there is an urgent need to understand how the early-life exposure to oxygen reprograms lung development and affects general health beyond the neonatal period.

Vulnerability of the lung to oxygen toxicity

Due to its anatomical location, the respiratory tract epithelium is one of three tissues (including the cornea and skin) that are exposed to 21% oxygen (a partial pressure of about 160 mm Hg), while other tissues of the body are exposed to much lower oxygen tensions[59]. As a result, the cells that constitute the lung are primary targets for oxygen-induced injury. *In vivo* models of hyperoxia have demonstrated in several adult animal species that exposure to high levels of oxygen initially leads to lung endothelial and epithelial destruction, followed by pulmonary edema, hemorrhage, alveolar type II epithelial cell hyperplasia, and fibrosis ultimately compromising gas exchange[60–62]. Intriguingly, significant differences in the response to hyperoxia exist between newborn and adult animals of various species, suggesting the developmental regulation of this process[60, 63–65]. While some variability exists in the survival of newborn mice exposed to 100% oxygen, most adult mice die within 3–7 days when exposed to the same concentration of oxygen[17, 47]. Similarly, term infants, as well as some preterm infants, treated with high levels of supplemental oxygen for long periods of time may develop very few, if any, adverse side effects, whereas the normal adult human is known to be quite sensitive to prolonged exposure to high levels of oxygen[2]. These responses of the newborn may be unique to the developmental timing of the exposure and likely attributable, at least in part, to genes controlling cell growth and differentiation, cells that come together to form the morphological structure of the lung, and the environment, namely oxygen.

It is generally accepted that oxygen-induced injury, or oxygen toxicity, in the lung is mediated through reactive oxygen species (ROS), generated during normal mitochondrial respiration, the reperfusion of hypoxic tissue, and in association with inflammation and infection[66–68]. However, it has become increasingly evident that ROS participate in many intracellular signaling pathways, including those important for normal cell growth and differentiation, as well as inflammatory responses during host defense[69]. Nevertheless, when the production of ROS exceeds the antioxidant capacity of the cell, oxidative stress follows, and the potential for cellular and tissue injury via lipid peroxidation, DNA damage, and protein oxidation exists[67]. In fact, human studies have shown a quantitative increase in oxidative damage to pulmonary proteins and decrease in levels of antioxidants in the epithelial lining fluid or plasma of ventilated preterm infants in comparison to those infants who were not oxygen dependent[70–72].

Important endogenous, enzymatic antioxidant defenses in the lung include the manganese, copper-zinc, and extracellular superoxide dismutases (MnSOD, CuZnSOD, ECSOD), catalase (CAT), and glutathione peroxidase (GPx). The regulation of pulmonary antioxidants in hyperoxia differs between animal species[63]. For example, newborn mice, rats, and rabbits are capable of increasing antioxidant levels when exposed to hyperoxia, whereas newborn guinea pigs and hamsters are unable to do so, ultimately compromising their ability to survive[63]. Indeed, numerous animal models have investigated the utility of antioxidants in response to early-life exposure to oxygen. For example, overexpression of MnSOD or ECSOD in the respiratory epithelium of newborn mice exposed to hyperoxia improved survival and preserved alveolar type II epithelial cell proliferation during the first

three days of exposure, respectively[73, 74]. Since antioxidant defenses in humans do not mature until late in gestation, infants born prematurely are particularly at risk for sustaining lung injury attributable to oxidative stress. In fact, multiple studies have reported a positive correlation between oxygen supplementation in the neonatal period and decrements in lung function later in life[75, 76]. In a study conducted by Davis *et al.*, premature infants treated with recombinant human CuZnSOD (rhSOD) had improved pulmonary outcomes at one year of corrected age, but did not reduce the incidence of BPD[77]. While it has been suggested that rhSOD may reduce the risk of developing retinopathy of prematurity in preterm infants, the long-term efficacy of such treatment in improving lung function remains unknown[78].

Although experimental and clinical data provide evidence suggesting that antioxidants may be helpful in mitigating oxidant-mediated injury to the developing lung, the tolerance of newborns to oxygen is mechanistically more complicated than just changes in levels of antioxidants[59]. It has previously been shown that the nutritional state of the newborn and exposure to antenatal corticosteroids also modulate early-life sensitivity to oxygen[79–84]. Intriguingly, pre-treatment with sub-lethal endotoxin conferred protection to newborn rats exposed to 95% oxygen for 14 days[85]. In comparison to control rats, the lungs of the endotoxin-exposed animals had improved lung alveolarization and pulmonary microvasculature. While the mechanisms by which endotoxin preserves development of oxygen-exposed lungs remains unknown, these observations emphasize the complexity of the problem.

Thus, it is evident that modulation of oxygen toxicity in the developing lung is a complex process, requiring adaptive response mechanisms in both place and time in order to properly function. It is conceivable then that disruption of these mechanisms during a critical period of lung development, as a result of preterm birth for example, coupled with the early-life exposure to ambient or therapeutically elevated levels of oxygen may have unpredictable consequences affecting health later in life. A review of the pulmonary outcomes in BPD during infancy and beyond is discussed elsewhere in this issue by Bhandari and McGrath-Morrow.

Suitable animal models

Much of what is currently understood about lung development and the pathogenesis of BPD has arisen from animal models of oxidant-mediated lung injury[2]. Such models, some better than others, include the rodent, rabbit, lamb and non-human primate. Large animal models, such as the non-human primate, have been extensively studied since postnatal lung development in non-human primates' parallel human lung development. Preterm ventilated baboons delivered at 75% of gestation, approximately equivalent to 30 weeks gestation in humans, and exposed to only enough oxygen to maintain normal arterial concentrations of oxygen, had significantly less lung injury after 11 days in comparison to baboons treated with 100% oxygen[86]. Since both groups of animals were ventilated similarly in the study, oxygen was considered to be the primary cause of lung injury in the high exposure group. In a similar study, preterm ventilated baboons, also delivered at 75% of gestation, were exposed to either only enough oxygen to maintain normal arterial concentrations of oxygen for 21 days, or 100% oxygen for 7 days followed by 80% oxygen for 14 more days[87]. Examination of the animals' lungs eight months later revealed minimal lung injury in the low oxygen exposure group, whereas animals in the high oxygen exposure group had larger alveoli and reduced alveolar numbers. Interestingly, alveolar hypoplasia was shown to develop in preterm baboons delivered at 67% of gestation, comparable to 26 weeks gestation in humans, and treated with the minimum necessary mechanical ventilation and oxygen, suggesting the importance of lung maturity in response to oxygen-mediated injury[18].

Although the non-human primate model has taught us a lot about the effects of prematurity, ventilation, and hyperoxia on lung development, it is not always practical for many investigators[88, 89].

There are many advantages to using small animal models to study lung oxidant injury and BPD, including cost, maintenance, number of animals per study, and the capacity for genetic manipulation[88]. In fact, Northway et al. demonstrated in 1976 that mice exposed to 100% oxygen develop a BPD-like phenotype[17]. More recently and analogous to children born prematurely, adult mice exposed to 100% oxygen at birth and recovered in room air exhibit persistent alveolar simplification, increased lung compliance, increased sensitivity to viral infection, and pulmonary vascular disease[90–94]. Given the similar lung pathology between autopsy samples from individuals with BPD and newborn rodents exposed to hyperoxia, many investigators have since used newborn rodents to study lung oxidant injury and BPD[88, 93]. Similar to preterm infants born during the saccular stage of development, term rodents are also born during this period, with most alveolar development occurring postnatally. This criterion makes the newborn rodent well suited for studying early-life oxidant lung injury. However, we are to remain cautious in using this model to study preterm disease. While the saccular lung of a term newborn rodent is programmed for postnatal function and survival, the saccular lung of a preterm infant is not. As a result, certain adaptive mechanisms initiated in response to hyperoxic exposure may be developmentally intact in the term newborn rodent, but are lacking in the preterm infant. Nevertheless, chronic exposure of rodents to high levels of oxygen is known to cause anatomic changes similar to those observed in BPD[14, 95, 96].

Gene-cell-environment interactions

As mentioned earlier, there has been an increased appreciation for the impact of environmental change during development on the occurrence of disease later in life[19]. In the context of the lung, orderly development, beginning during embryogenesis and continuing through adolescence, requires the timely expression of key genes and their interaction with specific cell types, as represented in Figure 2. Understanding how the oxygen environment influences these complex developmental interactions has been challenging[59, 97]. For example, there are instances, as mentioned earlier, when term, as well as preterm, infants treated with therapeutically elevated levels of oxygen develop few, if any, adverse side effects[2]. This response is likely attributed to individual variation in interactions between genes, cells, and the environment. It is thought that variations in the expression of certain genes controlling cell growth and differentiation by different cell types may contribute to the genetic predisposition of sensitive populations to develop lung disease[98, 99]. The clinical manifestation of these diseases may therefore be dependent upon interactions between these genes and cells with environmental factors, such as exposure to hyperoxia at birth[100].

The identification of key genes and their contribution to the development of the lung has been possible due to the utility of various molecular techniques and the use of various transgenic mouse models. For example, a molecular signature for birth has been identified through the use of expression profiling during lung development in mice[101]. Overrepresented groups of genes contributing to this molecular signature included those involved in oxygen and gas exchange (SP-B, Hb, Pld1, and Ppp3cb) as well as those involved with the initiation of alveolarization (Tcf21, Gata6, Lgals1, and rbp1). Additional studies have identified thyroid transcription factor-1 (TTF-1; NKx2.1) and Forkhead box A transcription factor Foxa2 as important contributors to the production of pulmonary surfactant and lung morphogenesis[102–104]. Lung-specific overexpression of TTF-1 in transgenic mice has been shown to result in pulmonary fibrosis and eosinophilia, associated with increased expression of eotaxin and interleukin-6 (IL-6)[105]. Moreover, overexpression

of a mutant form of TTF-1 that cannot be phosphorylated in the lung causes severe lung hypoplasia in mice at birth[106]. Similarly, deletion of Foxa2 leads to air-space enlargement, goblet cell metaplasia, and infiltration of neutrophils into the lungs of mice[107]. Additional genes and regulatory pathways contributing to lung development have been extensively reviewed elsewhere[102, 108].

While genes contributing to the development of the lung ensure that it will function upon birth, genes contributing to the defense of the lung against environmental exposures, such as high levels of oxygen, ensure that it will continue to mature and grow postnatally as developmentally intended. Since exposures to high levels of oxygen are often associated with oxidative stress, oxidant injury, and inflammation, a great deal of emphasis has been placed on understanding the molecular pathways controlling these processes. For example, the transcription factor nuclear factor, erythroid 2-related factor 2 (Nrf2) is known to respond to hyperoxia in the lung, and regulates the inducible gene expression of antioxidant enzymes, which are critical in detoxifying oxygen-mediated generation of ROS[109, 110]. Mice deficient in Nrf2 exhibit aggravated lung injury and fail to upregulate antioxidant enzymes in response to hyperoxia[111]. In fact, in human subjects, a single nucleotide polymorphism in the Nrf2 promoter was identified that increases the risk of acute lung injury[112]. Nuclear factor kappa B (NF-κB) is activated in response to hyperoxia, and activates genes that regulate apoptosis, inflammation, and oxidative stress[113, 114]. In fact, clinical studies have shown that enhanced NF-κB activation contributes to an increased risk of developing BPD in preterm infants[115, 116]. Furthermore, increased levels of the inflammatory cytokine IL-6, a downstream target of NF-κB, have been reported in a premature baboon model of BPD, as well as in neonatal mice and rats exposed to hyperoxia at birth[18, 64, 117]. Whether NF-κB is protective or harmful is not fully known. However, newborn mice lacking the p50 subunit of NF-κB exhibit increased mortality when exposed to hyperoxia[118]. Increased levels of the tumor suppressor p53 have also been reported in preterm baboons, mice and cell lines exposed to hyperoxia[41, 119, 120]. P53 is perhaps best known as a tumor suppressor that is mutated in cancer. However, it accumulates and becomes transcriptionally competent in cells with DNA damage or under conditions of stress. It protects cells and organs from further damage by controlling the transcription of genes that inhibit cell proliferation, promote DNA repair, and facilitate apoptosis (for review see[121]). Recent studies have revealed that p53 can also affect the maintenance of mitochondrial homeostasis[122]. Defects in mitochondrial respiration can promote alveolar simplification in newborn mice, much like the effects of exposure to hyperoxia[123]. Whether p53 signaling contributes to neonatal lung disease remains to be determined.

Lung development, as mentioned earlier, is divided into five stages of morphological development, with each stage requiring a distinct set of developmental factors. Specifically, the pseudoglandular stage of development marks the beginning of cellular differentiation within the lung, which can be divided into four primary groups of cells, including the proximal airway epithelium, distal airway epithelium, proximal mesenchyme, and distal mesenchyme. Each of these groups of cells give rise to specific cell types, of which over forty have been identified in the lung[97]. Critical to pulmonary differentiation, as well as branching morphogenesis, are interactions between the epithelial and mesenchymal tissue layers. Using tissue grafts, early studies have shown that distal mesenchyme can induce epithelial budding from the trachea when removed of its own mesenchyme[124, 125]. In contrast, tracheal mesenchyme was unable to induce epithelial budding when grafted onto distal lung epithelium. These studies underscore the physiological complexity of pulmonary differentiation, as well as the importance of establishing specific developmental factors in both space and time in order to properly develop a lung. Understanding the types of cells present at specific developmental periods is critical for understanding how exposure to oxygen can be toxic because individual cell types exhibit different sensitivities to hyperoxia.

As briefly discussed earlier in the section on the vulnerability of the lung to oxygen toxicity, oxygen sensitivity appears to be cell-type specific and age related. For example, several species of adult animals are more sensitive to hyperoxia than their newborn counterparts, and this sensitivity has been attributed, at least in part, to respiratory insufficiency caused by death of microvascular endothelial and alveolar type I epithelial cells[126, 127]. In contrast, alveolar type II epithelial cells are more tolerant of hyperoxia and participate in repair of the injured lung[128]. Further, increased 8-oxoguanine and TUNEL staining has been observed in the airway epithelium of adult mice exposed to hyperoxia[129]. However, these observations may reflect DNA damage and not solely apoptosis, since significant airway epithelial cell proliferation was not seen following recovery in room air. On the other hand, newborn animals are typically much more tolerant to hyperoxia-induced cell death, albeit at the expense of altered lung development. Such tolerance has been partly attributed to higher levels of anti-oxidant enzymes present in newborn lungs[130]. Indeed, over-expression of MnSOD or ECSOD in alveolar type II epithelial cells can preserve postnatal lung development of newborn mice exposed to hyperoxia[73, 74]. Whether over-expression of these same enzymes in other cell types would be equally protective, remain to be determined. More importantly, we need a better understanding of how specific cell types differentiate, proliferate, and apoptose during the different periods of lung development in order to critically investigate how those processes are affected by exposure to hyperoxia, either occurring at the time of birth or from therapeutic intervention.

Summary

Lung development is a complex process that has evolved over time and has been optimized to ensure compatibility with postnatal survival. The purposeful orchestration, or programming, of transcription factors, morphogens, and extracellular matrix molecules in both space and time ensure the formation of a properly functioning lung that will be capable of performing the critical task of gas exchange upon the transition to newborn life. However, factors that interfere with the developmental programming of the lung will likely lead to altered lung development, thus increasing the risk for respiratory disease in susceptible individuals later in life. One such factor, of which there are many, includes hyperoxia and its association with the development of BPD has long been recognized. Since birth in itself will always be a hyperoxic event, lung maturity plays an important role postnatally in managing exposure to higher levels of oxygen compared to those experienced *in utero*. If we consider that oxygen will continue to be used therapeutically to treat those infants unprepared to breathe oxygen on their own, then we must understand the dose that is safe to use at any age and the windows of developmental sensitivity. Limited progress in the prevention and treatment of BPD over the last several decades may be attributed to, at least in part, our narrow-minded approach to understanding the pathogenesis of the disease in the context of individual factors or mechanisms. Rather, if novel therapies or preventative measures are to be developed moving forward, we must collectively consider the roles of genes, cells, and the oxygen environment and their integrative contribution to the multi-causal development of BPD.

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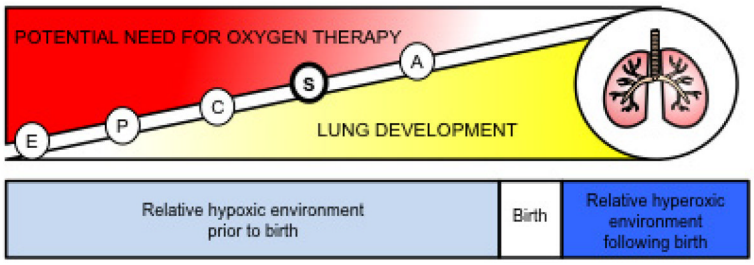

Figure 1.

Lung development is divided into five different stages, including the embryonic (E), pseudoglandular (P), canalicular (C), saccular (S), and alveolar (A) periods. With the exception of the alveolar period, lung development primarily occurs in a relative hypoxic environment *in utero* until it transitions to a relative hyperoxic environment at birth. Lung development is disrupted when preterm infants, whose lungs are often in the saccular stage (bold) and developing under low oxygen conditions, transition into room air or are exposed to therapeutically elevated levels of oxygen.

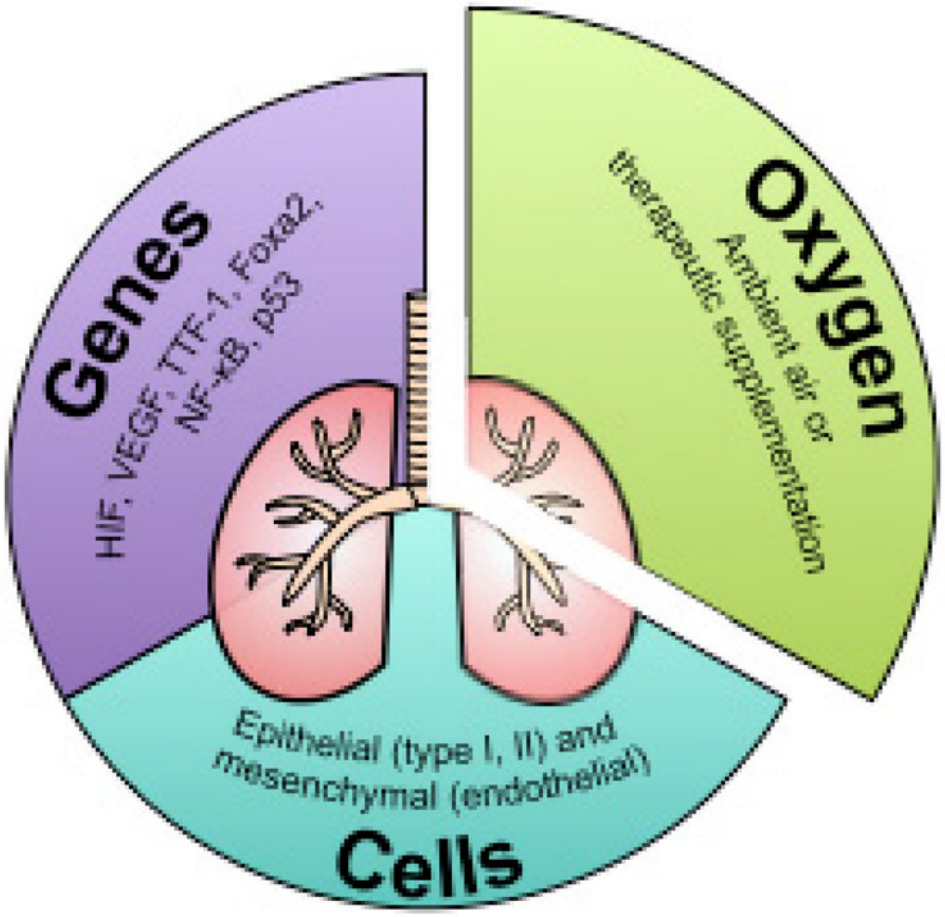

Figure 2.
The integration of a specific oxygen environment with genes and cells is required for proper development of the lung. Representative genes and cell types that may be affected by exposure to hyperoxia, as described throughout this chapter, are noted in the figure.