FORUM REVIEW ARTICLE

The Thioredoxin System in Neonatal Lung Disease

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Abstract

Significance: Fetal lung development takes place in hypoxia meaning that premature birth is hyperoxia for the prematurely born infant. The most common respiratory morbidity afflicting premature infants is bronchopulmonary dysplasia (BPD). Pathophysiologically, BPD represents the impact of injury, including O₂ toxicity, to the immature developing lung that causes arrested lung development. **Recent Advances:** The thioredoxin (Trx) system, which is predominantly expressed in pulmonary epithelia in the newborn lung, acts as an antioxidant system; however, it is increasingly recognized as a key redox regulator of signal transduction and gene expression via thiol–disulfide exchange reactions. **Critical Issues:** This review focuses on the contribution of Trx family proteins toward normal and aberrant lung development, in particular, the roles of the Trx system in hyperoxic responses of alveolar epithelial cells, aberrant lung development in animal models of BPD, O₂-dependent signaling processes, and possible therapeutic efficacy in preventing O₂-mediated lung injury. **Future Directions:** The significant contribution of the Trx system toward redox regulation of key developmental pathways necessary for proper lung development suggests that therapeutic strategies focused on preserving pulmonary Trx function could significantly improve the outcomes of prematurely born human infants. *Antioxid. Redox Signal.* 21, 1916–1925.

Bronchopulmonary Dysplasia, a Chronic Lung Disease of Prematurity

PREMATURE DELIVERY OCCURS in ~12% of all births and is the leading cause of infant morbidity and mortality in the United States (6). Often requiring costly intensive medical care, premature infants are at high risk for persistent respiratory sequelae (54, 79, 80). The most common respiratory morbidity afflicting premature infants is bronchopulmonary dysplasia (BPD). The risk of developing BPD is inversely proportional to the gestational age and birth weight (23). BPD is clinically characterized by a prolonged need for assisted ventilation and/or O₂ therapy due to gas exchange abnormalities. Pathophysiologically, BPD represents the impact of injury, including O₂ toxicity, to the immature developing lung that causes arrested lung development and a histological pattern of alveolar simplification (fewer and larger alveoli) (77, 80). Lung development is divided into five overlapping stages. The first four stages, termed the embryonic, pseudoglandular, canalicular, and saccular, occur during gestation. Alveolarization represents the last stage in lung development, begins around 36 weeks of gestation, and

continues through the first 2–3 years of life (91). Infants at the greatest risk for BPD are born prematurely during the late canalicular or saccular stage of lung development (24–28 weeks) (79).

Fetal lung development takes place in hypoxia (3%) meaning that room air (21% O₂) is hyperoxia for the prematurely born neonate (4, 90). The fetal-to-neonatal transition from the hypoxic environment of the womb to the relatively hyperoxic extrauterine environment takes place in premature infants during a period of marked susceptibility to hyperoxia. Antioxidant systems, including superoxide dismutases, glutathione (GSH), glutaredoxin, and thioredoxin (Trx) systems, heme oxygenases, and small molecular weight antioxidants protect against and repair O₂-mediated injury (4, 5). Preterm neonates are more susceptible to the effects of O₂ due to developmental deficits in antioxidant defenses and an impaired ability to mount rapid antioxidant responses to hyperoxia (9, 38, 49, 74, 77). Although premature infants that develop BPD are often exposed to minimal amounts of supplemental O₂, they exhibit marked evidence of oxidant stress (74). The sudden and dramatic increase in lung O₂ levels upon preterm delivery and initiation of respiration

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likely perturbs the hypoxia-mediated signaling necessary for lung development. Multiple growth factors and signaling cascades play important roles in normal lung vascular development (16, 45). One of the most extensively studied endothelial growth factors is the vascular endothelial growth factor (VEGF). VEGF, a potent endothelial cell mitogen produced by type 2 alveolar epithelial cells (AECs), is significantly involved in alveolar development and its expression is regulated by hypoxia-inducible factors (HIF) (13, 73, 85). Numerous studies in newborn animal models have demonstrated the importance of normal VEGF signaling to lung alveolar development (30, 31, 39, 46, 47, 52, 80). Premature delivery has deleterious effects on the oxygen-dependent biological processes that mediate lung development, in particular, the HIF/VEGF pathway.

The Trx System

Trx is a dithiol-disulfide protein with a conserved -Cys-Gly-Pro-Cys- active site that is maintained in a reduced state by the enzyme Trx reductase (TrxR). TrxR reduces the active site of oxidized Trx (Trx-S₂) from a disulfide to a dithiol [Trx(SH)₂]. A recent comprehensive review extensively characterizes the biological functions of TrxR (1). Both Trx and TrxR are present in mammals in distinct isoforms and are either predominantly cytosolic (Trx1 or TrxR1) or mitochondrial (Trx2 and TrxR2). Knockout of the genes for any of the Trx or TrxR isoforms results in embryonic lethality in mice (15, 40, 53, 59). Trx can act as a direct antioxidant *via* reduction of hydrogen peroxide (H₂O₂) by enzymes known as Trx peroxidases, also called peroxiredoxins (36). Several proteins contain -SH groups that are susceptible to oxidative inactivation, typically through the creation of disulfide bond formation. Trx(SH)₂ can regulate protein activity *via* thiol-disulfide exchange reactions with protein mixed disulfides (PSSX) that, coupled with reduction by TrxR, result in net reduction of PSSX by NADPH (Fig. 1).

Other physiological functions performed by Trx include the donation of reducing equivalents to ribonucleotide reductase, control of protein folding, and regulation of apoptosis signaling kinase-1 (ASK-1) (2, 36, 37). Trx1 regulates the activity of enzymes such as apoptosis signal-regulating kinase-1 and protein kinase C. S-nitrosylation, the covalent attachment of NO to the -SH of cysteine, is an integral component of nitric oxide (NO)-mediated signaling. The Trx system indirectly regulates many NO-related cellular processes *via* its role as a major protein denitrosylase (10). Additional reversible modifications of cysteine-SH groups include sulfhydrylation and sulfenic acid and Trx facilitates the reduction of both (44, 57). Thiol-disulfide exchange reactions act as control mechanisms in signal transduction and gene expression that drive various cellular signaling events

FIG. 1. Trx-mediated reduction of protein mixed disulfides. Mechanism by which the thioredoxin (Trx) system (Trx + TrxR) can reduce protein mixed disulfides (PSSX) resulting in a net reduction of PSSX by NADPH.

(18). Oxidative stress induced by alterations in oxygen tension, ionizing radiation, or H₂O₂ causes nuclear accumulation of Trx1 (24, 48, 83). In the nucleus, Trx1 increases the DNA binding of many redox-sensitive transcription factors, including HIF-1 α , NF- κ B, Ref-1, AP-1, and p53, typically *via* PSSX thiol-disulfide exchange reactions as characterized above (35, 67, 83, 87, 89). Finally, Trx1 is secreted from cells in response to oxidative stress and is measurable in the plasma and bronchoalveolar lavage fluids. Extracellular Trx1 exists predominantly in the oxidized form and exhibits chemotactic properties for monocytes, polymorphonuclear leukocytes, and T lymphocytes (11). Trx is induced by oxygen, H₂O₂, x-ray irradiation, viral infection, and ischemia/reperfusion (56).

The Trx activity depends on the redox state of its active site. If the Trx-active site is inactivated by oxidation or is directly inhibited by endogenous inhibitors, then the Trx activity is lost. The thioredoxin interacting protein (Txnip) is the best characterized endogenous Trx inhibitor. Txnip inhibits Trx through the formation of a stable mixed disulfide bond between cysteine 247 of Txnip and cysteine 32 of the reduced Trx1 active site (62). Txnip does not react with oxidized Trx1. Txnip has a number of effects that appear to be independent of Trx1 binding. For a more extensive overview of Txnip functions, the reader is referred to a recently published comprehensive review by Spindel *et al.* (76). The hypothesized contributions of the Trx system toward lung development are shown in Figure 2.

Developmental Expression of the Pulmonary Trx System

In newborn human lungs and in fetal and newborn murine lungs, Trx1 and TrxR1 expression is predominant in airway epithelial cells and macrophages, which is similar to the expression in adult lungs (17, 32, 43, 50, 75). Soini *et al.* characterized the developmental distribution of Trx1 in human lungs from 17–40 weeks of gestation. Their data demonstrated that Trx1 is expressed in a normal bronchial epithelium beginning at 35 weeks of gestation. A similar

FIG. 2. Trx-mediated processes in neonatal lung development. Processes likely to be governed by the Trx system that are pertinent to lung development include transcription factor regulation, apoptosis, DNA synthesis, reduction of H₂O₂ and protein disulfides, and protein denitrosylation.

pattern of Trx1 expression was also seen in the lungs from patients with respiratory distress syndrome or BPD (75). Kobayashi *et al.* reported that Trx1 expression in developing mouse embryos was detectable in lung parenchyma and bronchial epithelium by E13.5. Bronchial epithelial expression increased to adult levels by E16.5 (43). Locy *et al.* recently investigated the expression of TrxR1 in 1-day-old term newborn human and murine lungs. TrxR1 immunostaining was most intense in both ciliated and nonciliated airway epithelial cells and the expression in the alveolar epithelium was comparatively less intense (50). The implications of differential TrxR1 expression in the airway and AECs are unclear; however, this suggests the possibility that perturbations in the Trx function could disproportionally affect bronchial epithelial cell function.

Txnip is highly expressed in lung tissue (27, 41). Filby *et al.* investigated the expression of Txnip in developing ovine lungs (Fig. 3A). Their findings demonstrated that Txnip is predominantly expressed in type 2 AECs *in utero* (27). We confirmed a similar pattern of Txnip by type 2 AECs in newborn human lungs (Fig. 3B) (81).

FIG. 3. Txnip localization in the developing lung. Txnip immunopositivity in (A) fetal ovine (TII=type 2 and TI=type 1 alveolar epithelial cells) and (B) newborn murine lungs (arrow, type 2 alveolar epithelial cells). From (A) Filby *et al.* (27) and (B) Farrell *et al.* (26). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Trx in AECs

The mechanisms that mediate oxygen-induced injury have been studied extensively. Oxygen toxicity is generally thought to be mediated through the actions of reactive oxygen species (ROS), such as superoxide, H_2O_2 , peroxynitrite, and hydroxyl radical, which are produced at rates greater than normal during hyperoxic exposure (60). GSH-dependent reduction of H_2O_2 and other substrate oxidants is catalyzed by glutathione peroxidases and produces GSH disulfide (GSSG). Glutathione reductase then catalyzes the reduction of GSSG back to GSH. This reduction is essential for the maintenance of high intracellular GSH:GSSG ratios, typically >99:1 (69, 92).

Although cytosolic concentrations of Trx family proteins are in the micromolar range (compared with millimolar concentrations of GSH), the Trx system confers additional intracellular reducing capacity. Shan *et al.* explored the effects of hyperoxia on Trx1 and TrxR1 in type 2 AECs isolated from the lungs of E19 rats. Their studies indicated that 95% hyperoxia substantially enhanced cellular ROS generation, increased apoptosis, induced Trx1 and TrxR1 expression, but decreased TrxR1 activity (71). These findings suggest that while hyperoxia potently upregulates Trx system expression, dysfunction of the Trx redox system, as evidenced by decreased TrxR1 activity, may contribute to enhanced cellular injury. Administration of recombinant human Trx1 (rhTrx1) has been shown to provide cytoprotection in various animal models of oxidant disease as reviewed by Nakamura *et al.* (55). Chen *et al.* investigated the effects of rhTrx1 on hyperoxia-induced type 2 AEC injury *in vitro* and demonstrated that rhTrx1 attenuated hyperoxia-induced cell injury through a reduction in ROS generation (12). Fetal rat lung type 2 AECs were exposed to 95% hyperoxia in the presence and absence of $2 \mu M$ rhTrx1. In hyperoxia, Trx1 significantly reduced ROS generation, increased cell viability, and increased manganese superoxide dismutase and GSH peroxidase activities (12).

Trx System in Endothelial Cells and Macrophages

Angiogenesis appears to be closely linked to the cellular redox status. In endothelial cells, Trx1 regulates a broad array of cellular functions that are important for angiogenesis. These functions include regulation of transcriptional activation (NF κ B, HIF-1 α , Ref-1), post-translational protein modifications (PSSX and PSNO), cellular migration and proliferation (NO), apoptosis (ASK-1), and intracellular signaling (20). Trx1 also exhibits classical antioxidant properties in endothelial cells by protecting against H_2O_2 -mediated toxicity (22).

Although the Trx system is abundantly expressed in macrophages, relatively little is known regarding its specific function, especially in the context of neonatal lung diseases. The Trx system appears to modulate T-cell and B-cell proliferation and differentiation, which is likely mediated through redox regulation of transcription factors (21). Cytokine features of extracellular Trx1 are likely elicited upon oxidative stress. Finally, the Trx system is likely to provide protection against oxidative stress associated with the respiratory burst after macrophage activation.

Trx in the Airway

The airways and alveoli are covered with a thin layer of epithelial lining fluid (ELF). The ELF provides the first line

of defense from the environment and the composition of the ELF is most reliably detected by bronchoalveolar lavage. Approximately 10% of the protein composition of the ELF includes antioxidant proteins, which include Trx1 (88). Although the precise role of Trx1 in the ELF in neonatal lung diseases is unclear, Trx has been demonstrated to play a role in other lung diseases. It is unclear at present whether the contributions of the Trx system toward lung responses are related to the cellular or secretory components of the ELF.

Trx in Experimental Models of BPD

Term gestation newborn mice are born in the sacular stage of lung development, which is similar to premature human infants born at ~26 weeks of gestation. In contrast to premature infants, newborn mice have comparatively more mature antioxidant defenses. Originally characterized by Warner *et al.*, hyperoxic exposure of newborn mice causes lung developmental growth arrest similar to that seen in human infants with BPD (Fig. 4) (86). Newborn mice exposed to hyperoxia from birth exhibit evidence of arrested lung development by 7 days of exposure suggesting that the molecular mechanisms responsible for these hyperoxia-induced alterations occur early in the time course of exposure (61, 65, 86). In lungs from hyperoxia-exposed newborn mice, total Trx1 and TrxR1 protein levels are not different over the first 7 days of life (81). It is unclear if hyperoxia induces a change in Trx1 subcellular localization. Given that oxidative stress induces nuclear Trx1 accumulation, it is likely that even though total Trx1 levels are unchanged, there is a redistribution of Trx1 in response to hyperoxic exposure. The lack of an effect of hyperoxia on pulmonary Trx1 and TrxR1 protein levels in the lungs of hyperoxia-exposed mice is in contrast with the data presented by Das *et al.* in premature baboon models of BPD

that combined hyperoxic exposure and mechanical ventilation (19). Variable increases in Trx1 and TrxR1 gene expression were reported in the lungs of preterm baboons that were either continuously exposed to 100% O₂ and mechanical ventilation or received O₂ as needed to normalize arterial oxygenation for 6 or 10 days. Continuous exposure to 100% O₂ for 6 or 10 days resulted in increases in Trx1 protein levels *in vivo*, but the effect on TrxR1 protein levels was not reported (19).

Proper vascular development is necessary for normal lung development (3). VEGF is produced by alveolar type 2 cells in both the developing mouse lung and in the human fetal lung and plays a crucial role in angiogenesis (72). Lung VEGF expression is regulated through the activation of HIFs, consisting of an α and β subunit (14, 64). As previously stated, the Trx activity depends on the redox state of its active site. Land and Wilson demonstrated that the Trx1 nuclear activity and redox state are altered by changes in PO₂ in AECs (48). Their studies in A549 cells revealed that equilibration from ambient to fetal PO₂ was associated with nuclear Trx1 oxidation and HIF-1 α coimmunoprecipitation. Nuclear Trx1 oxidation did not resolve until steady-state conditions were reached. Furthermore, deactivation of HIF-1 α during a return to ambient PO₂ coincided with a transient nuclear Trx1 oxidation (48). Thus, these data suggest that transient oxidation of Trx1 in the nucleus may be required for initiation of HIF-dependent transcription. These findings must be balanced with the described increases in Trx function in A549 cells (25), and follow-up studies in AECs of noncancerous origin have not been reported.

Although total lung Trx1 levels were not altered by hyperoxia in newborn mice, the redox state of Trx1 was significantly changed (Fig. 5) (81). Whereas Trx1 was predominantly reduced in the lungs of control mice, pulmonary Trx1 was oxidized by 24 h and the extent of Trx1 oxidation in the lungs of hyperoxia-exposed newborn mice was similar after 3 and 7

FIG. 4. Effects of hyperoxic exposure on murine neonatal lung development. Histology of mouse pup lung tissues (H&E, 100 $\times$) following exposure to room air or 85% O₂ for 7 or 14 days. From Rogers *et al.* (65). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

FIG. 5. Effects of postnatal hyperoxic exposure on lung Trx1 redox state in newborn mice. Lung Trx1 redox state in newborn mice exposed to room air or 85% O₂ for 1, 3, or 7 days as assessed by redox Western blot. From Tipple *et al.* (81).

days of continuous hyperoxic exposure. These data suggest that Trx1 function is likely to be severely attenuated in the lungs of hyperoxia-exposed newborn mice through oxidation of the Trx1 active site as early as 1 day of exposure. This increase in Trx1 oxidation precedes the detection of hyperoxia-induced lung morphological alterations (65, 81). Murine Trx1 redox findings are consistent with the data presented by Das *et al.* in premature baboons (19). Hyperoxic exposure increased the relative proportion of oxidized Trx1 in the lungs of premature baboons exposed to hyperoxia either continuously or as needed (Fig. 6). Increased Trx1 oxidation was also seen in newborn baboon lung explants exposed to 95% hyperoxia *in vitro*. The most significant Trx1 oxidation in baboon lung tissues *in vivo* and *in vitro* was detectable at the highest O₂ concentration (19). Although hyperoxia likely causes nuclear Trx1 accumulation, sustained hyperoxia impairs resolution of Trx1 oxidation. Persistent Trx1 oxidative inactivation is likely to have devastating effects on nuclear and cytosolic Trx1-dependent processes in the lung.

Both murine and baboon models are designed to investigate the effects of hyperoxic exposure on developmentally immature lungs. Differences in species, mode of ventilation (mechanical ventilation *versus* spontaneously breathing), and oxygen concentration (100% *versus* 85%) likely explain the discrepancies between murine and baboon data. Whereas collective data from newborn mouse and baboon models suggest the potential importance of Trx1 in the pathogenesis of BPD, the inherent difficulties in measuring the protein redox state in human tissues combined with the paucity of appropriate human samples make Trx1 redox determination virtually impossible to verify in newborn human infant lungs.

FIG. 6. Effects of hyperoxic exposure on Trx redox state in baboon lungs. Effect of O₂ on baboon lung Trx redox state *in vivo* and in explant culture as assessed by redox Western blot. From Das *et al.* (19).

Trx, NO Signaling, and Lung Development

Impaired NO production may contribute to the lung developmental deficits seen in infants with BPD (8). NO, which is produced by epithelial and endothelial cells, significantly contributes to lung vascular and alveolar development. Disruption of NO signaling resulted in alveolar simplification in animal models of BPD (34). Physiologically, the effects of NO are primarily exerted *via* the post-translational modification of proteins. Furthermore, treatment with the NO precursor L-citrulline or with inhaled ethyl nitrite preserved alveolar growth in hyperoxia-exposed newborn rat pups (7, 84).

The Trx system is a key regulator of protein denitrosylation and, therefore, an important component of NO-mediated signaling (10, 28, 70). Given the significant alterations in Trx1 functional capacity in hyperoxia, it is likely that the impaired Trx function would negatively impact NO-regulated signaling in the developing lung. To date, there are no reports of the effects of altered Trx function on NO-mediated signaling in lung development.

TrxR1: A Novel Therapeutic Target to Prevent BPD?

Induction of antioxidant response genes *via* antioxidant response element (ARE) activation in the promoter/enhancer regions of target genes enhances survival from oxidative insults (42, 58). ARE activation is primarily mediated by nuclear factor E2-related factor 2 (Nrf2) leading to increased transcription of genes that provide direct antioxidants, including Trx and TrxR, increase glutathione (GSH) levels, and stimulate NADPH synthesis (58). A growing body of evidence suggests that TrxR1 disruption may directly or indirectly influence antioxidant responses *via* Nrf2 activation and that TrxR1 inhibition may be a common feature of Nrf2 agonists (1, 29, 51, 78). Aurothioglucose (ATG) and auranofin (AFN) potentially inhibit TrxR1, are used clinically to treat rheumatoid arthritis, and are used experimentally to inhibit TrxR1 (33). In adult mice, ATG treatment was well tolerated, preserves GSH levels, and attenuates hyperoxic lung injury (82). In murine transformed Clara cells (mtCCs) *in vitro*, TrxR1 inhibition protected against the effects of hyperoxia *via* Nrf2-dependent mechanisms. Furthermore, TrxR1 knockdown increased Nrf2 activation, while AFN treatment of mtCCs increased Nrf2-mediated antioxidant responses (50). In AFN-treated mtCCs, GSH depletion markedly enhanced hyperoxic susceptibility (50). The protective effects of TrxR1 inhibition *in vivo* appear to be mediated *via* Nrf2 activation and require GSH. Cross talk between the Trx and GSH systems most reasonably explains the tolerance of TrxR1 inhibition *in vivo* and *in vitro* (1, 50, 63, 66, 68, 82). The utility of TrxR1 inhibition to elicit Nrf2-dependent antioxidant responses in the newborn lung is an area of active investigation in our group (Fig. 6).

Txnip-Dependent Regulation of Lung Development

Filby *et al.* reported a highly significant inverse relationship between Txnip mRNA levels and the rate of lung cell proliferation during fetal lung development suggesting that Txnip may be an important endogenous modulator of fetal lung growth and lung cell proliferation (27). Inhibition of VEGF expression in animal models alters lung vascular and alveolar development resulting in a phenotype similar to human BPD (30, 31, 39, 80). We found that lung Txnip mRNA levels increase ~20-fold between E19 and day-of-life 1 in murine lungs (unpublished observation). Furthermore, Txnip protein expression is 3.5 times greater on day-of-life 1 than at E19 and Txnip and VEGF expression is inversely correlated (Fig. 7A) (26). Pulmonary Txnip and VEGF protein levels are highly inversely correlated in hy-

FIG. 7. Relationship between lung Txnip and VEGF expression in embryonic and newborn murine lungs. (A) Regression analyses of lung Txnip and vascular endothelial growth factor (VEGF) contents in embryonic day 19 and 1-day-old newborn mice. From Farrell *et al.* (26). (B) Regression analyses of lung Txnip and VEGF contents in newborn mice exposed to room air or 85% O₂ for 1, 3, or 7 days. From Tipple *et al.* (81).

peroxia-exposed newborn mice (Fig. 7B) (81). Developmentally, Txnip protein levels decreased over the first week of life in the lungs of newborn mice raised in room air. Conversely, Txnip protein levels remained elevated in the lungs of newborn mice raised in hyperoxia (85% O₂). These and other data led us to conclude that in the newborn mouse lung, the net effect of hyperoxic exposure on Trx1-dependent functions, including VEGF expression, was most reasonably correlated with Txnip protein expression (81).

Farrell *et al.* determined the role of Txnip on HIF activation *in vitro* in SV-40 transformed murine type 2 (MLE-12) AECs (26). In these studies, Txnip suppressed basal HIF activation and the effect of Txnip on HIF activation appeared to be independent of binding to the Trx active site, although the findings did not exclude a possible Trx1/Txnip interaction beyond that described by Patwari *et al.* (62). Thus, in a setting of Trx1 oxidation, free Txnip levels are likely to increase resulting in increased Trx-independent Txnip-mediated effects.

Conclusions

In this review, I have highlighted a number of studies that implicate the Trx system as a significant contributor to both normal lung development and neonatal lung disease. Although classically described for its antioxidant function, the Trx system exerts effects that go far beyond simple ROS detoxification. Indeed, given its predominant expression by pulmonary epithelia, the Trx system is ideally suited as a key redox sensor of altered O₂ tension such as that which occurs upon preterm birth. As outlined in Figure 8, upon preterm delivery into a relatively oxygen-rich environment, the redox balance Trx is shifted toward the oxidized form resulting in disruption of protein function, disrupted redox-dependent transcription factors, decreased DNA synthesis, enhanced apoptosis, Txnip release, and Trx secretion. Thus, therapeutic strategies focused on preserving Trx function during the

FIG. 8. Hypothesized effects of preterm birth on lung Trx-dependent processes. Upon premature delivery into the relatively hyperoxic extrauterine environment, the Trx pool becomes oxidized. The resultant impairment in Trx-mediated antioxidant function results in elevated H₂O₂ levels. Increased protein oxidation and nitration lead to impaired protein function resulting in disruption of protein-mediated signaling pathways. Disruption of redox-dependent transcription, decreased DNA synthesis, enhanced apoptosis, Txnip release, and Trx secretion are also likely to occur.

transition from a relatively hypoxic environment *in utero* to the comparatively oxygen-rich extrauterine environment, if successfully executed, could significantly improve the outcomes of prematurely born infants.

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Abbreviations Used

AEC = alveolar epithelial cell
AFN = auranofin
ARE = antioxidant response element
ASK-1 = apoptosis signaling kinase-1
ATG = aurothioglucose
BPD = bronchopulmonary dysplasia
ELF = epithelial lining fluid
GSH = glutathione
GSSG = glutathione disulfide
H₂O₂ = hydrogen peroxide
HIF = hypoxia-inducible factor
mtCC = murine transformed Clara cell
NO = nitric oxide
Nrf2 = nuclear factor E2-related factor 2
PSSX = protein mixed disulfides
rhTrx1 = recombinant human thioredoxin-1
ROS = reactive oxygen species
Trx = thioredoxin
TrxR = thioredoxin reductase
Txnip = thioredoxin interacting protein
VEGF = vascular endothelial growth factor