REVIEW

Oxygen toxicity: cellular mechanisms in normobaric hyperoxia

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Abstract In clinical settings, oxygen therapy is administered to preterm neonates and to adults with acute and chronic conditions such as COVID-19, pulmonary fibrosis, sepsis, cardiac arrest, carbon monoxide poisoning, and acute heart failure. In non-clinical settings, divers and astronauts may also receive supplemental oxygen. In addition, under current standard cell culture practices, cells are maintained in atmospheric oxygen, which is several times higher than what most cells experience in vivo. In all the above scenarios, the elevated oxygen levels (hyperoxia) can lead to increased production of reactive oxygen species from mitochondria, NADPH oxidases, and other sources. This can cause cell dysfunction or death. Acute hyperoxia injury impairs various cellular functions, manifesting ultimately as physiological deficits. Chronic hyperoxia, particularly in the neonate, can disrupt development, leading to permanent deficiencies. In this review, we discuss the cellular activities and pathways affected by hyperoxia, as well as strategies that have been developed to ameliorate injury.

Keywords Hyperoxia · Reactive oxygen species ·
Oxygen toxicity · Mitochondria · Cell death ·
Antioxidants

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Introduction

Oxygen toxicity was first systematically studied in animal species in the late nineteenth century. Paul Bert demonstrated oxygen toxicity in the central nervous system (CNS), manifesting as loss of consciousness, seizures, and even death in animals exposed to hyperbaric hyperoxia (100% O_2 at total pressures above 1 atm) (Bert 1878). James Lorrain Smith described pulmonary toxicity in animals exposed to normobaric hyperoxia (above 21% or 160 mm Hg, at 1 atm). Although mice, rats, and birds could tolerate moderately elevated O_2 levels (~40%) for over a week, higher levels (~80%) were shown to be lethal within several days. Inspection of deceased animals revealed injury and inflammation in lungs and other tissues (Smith 1899).

In humans, exposure to hyperoxic conditions is routinely encountered in supplemental oxygen therapy administered to patients to address blood hypoxemia and tissue hypoxia in a variety of pathological conditions. In the 1950s, for example, oxygen therapy became a common practice to treat underdeveloped and underweight premature newborns (Tin and Gupta 2007). Many neonates who received oxygen therapy in the following decades suffered serious complications as a result of chronic hyperoxia exposure, including blindness and abnormal brain and lung development (Tin and Gupta 2007). More recently, oxygen therapy has been widely employed to treat patients with severe COVID-19 who have

sustained significant lung injury that compromises gas exchange (Ospina-Tascón et al. 2021). Although this is acutely necessary and successfully addresses hypoxemia and tissue hypoxia, the detrimental effects of oxygen toxicity necessitate that it be administered with caution (Perrone et al. 2017). In non-clinical settings, examples of operational exposure to hyperoxia include military and recreational divers (van Ooij et al. 2016; Wingelaar et al. 2017) and astronauts (Thirsk et al. 2009). In these situations as well, the inherent toxicity of oxygen necessitates that exposure to hyperoxia be limited.

A very different and broad context in which hyperoxia is routinely encountered is mammalian cell culture (Abbas et al. 2021; Al-Ani et al. 2018). In mammals breathing atmosphere of 20–21% O_2, the O_2 levels in the alveolar airspace are only ~14% due to the dynamics of incomplete lung volume exchange and constant diffusion of O_2 into the pulmonary circulation. In mammalian tissues where cells are utilizing O_2 diffusing into the cell from extracellular fluid to support oxidative phosphorylation and other oxygen-requiring reactions, O_2 levels are even lower, ranging from 2 to 6% (Keeley and Mann 2019). Despite this, routine cell culture is performed in incubators that regulate CO_2 but not O_2, conditions in which O_2 equilibrates to 18–19%. Thus, cells cultured under standard conditions are in fact experiencing hyperoxia. Many cellular processes measured in "physioxia" (2–6% O_2) are different compared to 18–19% O_2, such as ROS production (Maddalena et al. 2017), proliferation and senescence (Packer and Fuehr 1977; Busuttil et al. 2003; Parrinello et al. 2003), mitochondrial function (Moradi et al. 2021b), and response to drugs (Yan et al. 2010; Fonseca et al. 2018; Otto-Ślusarczyk et al. 2021) and hormones (Moradi et al. 2021a). It is thus imperative to implement physiological O_2 conditions in cell culture, in order to improve the quality and validity of in vitro studies and avoid artificial outcomes.

In this review, we examine the cellular pathways and mechanisms of cellular damage and physiological dysregulation associated with normobaric oxygen toxicity. The effects of hyperbaric oxygen therapy and its associated toxicity have been reviewed elsewhere (Ciarlone et al. 2019; Doolette and Mitchell 2010).

Hyperoxia drives reactive oxygen species formation

The acute toxicity associated with hyperoxia arises due to increased rates of production of ROS from molecular oxygen, which results in macromolecular damage and dysregulated signaling pathways. The production and roles of ROS in biology have been extensively reviewed elsewhere (Brand 2010; Alfadda and Sallam 2012; Zorov et al. 2014); thus here, we provide only a brief summary and then focus on the sources of excess ROS production in hyperoxia specifically. Superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are produced in mitochondria, endoplasmic reticulum (ER), peroxisomes, and the cytosol by enzymes including respiratory complexes (Turrens 2003), NADPH oxidases (NOX) (Bedard and Krause 2007), uncoupled nitric oxide synthase (Montezano and Touyz 2012), monoamine oxidase (Pizzinat et al. 1999), and xanthine oxidase (Battelli et al. 2016). In most instances, $O_2^{\bullet-}$ is the progenitor ROS, produced by the single electron reduction of molecular O_2. However, it is rapidly dismuted into H_2O_2, either spontaneously or via enzymatic catalysis by superoxide dismutases (SOD) (McCord and Fridovich 1969). Three isoforms have been described: SOD1 in the cytosol (Crapo et al. 1992), SOD2 in the mitochondria (Weisiger and Fridovich 1973), and SOD3 in the extracellular space (Marklund 1984). H_2O_2 is relatively long-lived in cells and is also electrically neutral so it can diffuse across membranes. It has well-characterized roles as a signaling molecule (Sies 2017) and in pathological processes (Gough and Cotter 2011). In the presence of iron ions, H_2O_2 is further reduced through the Haber–Weiss and Fenton reactions to form the highly reactive and toxic hydroxyl radical ($HO^\bullet$) (Lipinski 2011). While $O_2^{\bullet-}$ and H_2O_2 preferentially react with Fe-S clusters and cysteine residues in proteins, ($HO^\bullet$) indiscriminately oxidizes lipids, proteins, and DNA (D'Autréaux and Toledano 2007). These chemical modifications can alter the composition, structure, and function of the affected molecules. H_2O_2 is neutralized to water by catalase, glutathione peroxidases (GPX), and thioredoxin (Kurutas 2016). Moreover, ROS can also react with nitric oxide to form reactive nitrogen species (RNS) like peroxynitrite, which further contribute to oxidative stress and disrupt nitric oxide-mediated signaling (Brown and Borutaite 2006).

Several decades ago, hyperoxia was shown to increase cyanide-resistant respiration—a proxy of ROS production—in lung slices from rats (Freeman and Crapo 1981). This has been reproduced in an isolated perfused rat lung preparation exposed to 95% O_2, using an Amplex Red-based assay to detect H_2O_2 (Audi et al. 2018). Yusa et al. observed a similar increase in H_2O_2 production in brain tissue of rats exposed to 99% O_2 (Yusa et al. 1987). Increased formation of ROS ($O_2^{\bullet-}$, $HO^{\bullet}$, and alkyl radicals) was also observed in sheep microvascular endothelial cell suspensions exposed to 100% O_2 (Sanders et al. 1993). In MCF-7 cells exposed to 95% O_2 for 44 h, mitochondrial $O_2^{\bullet-}$ levels, reported by the mitochondrial matrix-targeted fluorescent probe MitoSOX™, were elevated relative to 20% O_2 (Pinterić et al. 2018). In isolated porcine lung mitochondria, higher rates of ROS production (measured as H_2O_2) were observed with increasing O_2 levels above 20% (Turrens et al. 1982). Thus, there is evidence from multiple orthologous approaches that hyperoxia increases rates of ROS production.

NOX enzymes are also important sources of cellular ROS in hyperoxia. Genetic ablation/silencing or pharmacological inhibition of NOX1, NOX2, or NOX4 significantly decreases ROS production and prevents injury in a wide range of hyperoxia exposure models (Parinandi et al. 2003; Zhang et al. 2003; Brueckl et al. 2006; Usatyuk et al. 2007; Pendyala et al. 2009; Carnesecchi et al. 2009; Auten et al. 2009; Chan et al. 2013; Audi et al. 2018). Maddalena et al. showed a similar effect in cell culture, where increased H_2O_2 production at 18% O_2 versus 5% O_2 could be largely abolished by pharmacological inhibition of NOX activities (Maddalena et al. 2017). Thus, there is strong evidence for the participation of NOX enzymes in ROS production and oxidative damage in hyperoxia. The relative importance of mitochondrial versus NOX-derived ROS production may depend on cell type and associated differences in mitochondrial abundance and NOX expression. In addition, other ROS-producing enzymes likely also contribute, but have been less extensively studied and may be quantitatively less important.

Hyperoxia-induced ROS oxidize lipids, DNA, and proteins

Polyunsaturated fatty acids, such as arachidonic acid and linoleic acid, are particularly vulnerable to peroxidation by ROS, which can lead to the alteration of membrane structure and function (Catalá 2009). Lipid peroxidation involves a free radical mechanism that ends in the formation of lipid hydroperoxides and smaller end-products such as 4-hydroxynoneal (4-HNE), malonyldialdehyde (MDA), and 8-isoprostane, among others (Niki 2008). Some of these subproducts damage biomolecules and harm organelle function. For example, 4-HNE and MDA can form adducts with DNA and proteins (Guéraud et al. 2010). In vivo and in vitro studies have reported increased lipid peroxidation caused by hyperoxia, as measured by the formation of MDA, 4-HNE, and 8-isoprostane (Wispe et al. 1986; Block 1988; Vacchiano and Tempel 1994; Bandali et al. 2004; D'Agostino et al. 2009). Some of these have observed morphological and structural alterations to the plasma membrane, such as blebbing, and altered fluidity (Vacchiano and Tempel 1994; Wispe et al. 1986). More comprehensive lipidomic approaches reveal extensive phospholipid species changes associated with hyperoxia. For example, in lung tissue from mice exposed to 100% O_2 for 72 h and then allowed to recover for 4 days, extensive remodeling was observed in virtually all phospholipid species (Peterson et al. 2020). The enzyme peroxiredoxin 6 (Prdx6) seems to have an important role in the detoxification of lipid hydroperoxides and repair of phospholipid membranes in hyperoxia. Overexpression of Prdx6 decreased MDA production and prolonged survival of mice exposed to hyperoxia (Wang et al. 2004). In turn, Prdx6-deficient mice show a delayed recovery from lipid peroxidation following hyperoxia (Li et al. 2015a). Moreover, lungs from mice with a mutation that renders Prdx6 unable to bind phospholipids show no recovery posthyperoxic exposure, unlike control animals (Fisher et al. 2018). Phospholipid peroxidation is a cause of cell death, mainly via ferroptosis (Sharma and Flora 2021) and apoptosis (Nakagawa 2004).

Oxidative DNA damage can occur as direct base modifications or as strand breaks. One of the main products of the former type is 8-oxo-2′-deoxyguanosine (8-oxo-dG). Elevated levels of DNA strand breaks were observed in mouse HyHEL-10 cells exposed to hyperbaric hyperoxia (Cacciuttolo et al. 1993). Agarwal and Sohal observed increased 8-oxo-dG levels in houseflies exposed to 100% O_2 for 3 days (Agarwal, and Sohal 1994). Exposure of mice to 60% O_2 for longer than 2 h results in a significant

increase of 8-oxo-dG in lung tissue and urine samples (Kundumani-Sridharan et al. 2019). Fluorescence microscopy of lung tissue revealed that both nuclear and mitochondrial DNAs were affected by hyperoxia.

Proteins are also important targets of oxidation, with important repercussions in both physiological signaling and pathological processes. Due to its chemical reactivity and the ability of sulfur to adopt a variety of oxidation states, cysteine residues are a primary target of oxidation in hyperoxia (Paulsen and Carroll 2013). H_2O_2-mediated oxidation of cysteine results in the formation of distinct chemotypes such as sulfenic acids and disulfides, both of which are reversible (Poole and Nelson 2008). Reversible oxidation of cysteine residues of proteins can act as a molecular switch to upregulate or downregulate a variety of signaling pathways (Finkel 2011). However, further oxidation of sulfenic acid by ROS leads to the largely irreversible formation of sulfinic and sulfonic acids, which is associated with loss of function and degradation (Tomin et al. 2019). A thorough discussion of the mechanisms involved in redox signaling can be found elsewhere (Sies and Jones 2020; Go and Jones 2013).

Protein carbonylation is another irreversible form of oxidation that occurs mainly at aliphatic amino acid residues and has been associated with a variety of diseases (Davies 2005; Suzuki et al. 2010). Protein carbonyls are measured by their reaction with 2,4-dinitrophenylhydrazine (DNPH), producing the colored compounds hydrazones (Suzuki et al. 2010). A study by Sohal et al. was one of the first to observe protein carbonylation caused by hyperoxia (Sohal et al. 1993). They found that houseflies exposed to 100% O_2 for 3 days have an increased content of protein carbonyls that persists even after recovery at room air. Mori et al. detected increased levels of protein carbonyls in astrocytes treated with hyperbaric hyperoxia (Mori et al. 2007). Interestingly, they also showed that treating healthy astrocytes with either the supernatant of astrocytes cultured on hyperbaric hyperoxia or with the protein extract from this supernatant induces neuronal cell death. Another report showed that hyperoxia increases oxidation of thiol groups, formation of lipid peroxidation products and protein adducts, and formation of bityrosines, another marker of protein oxidation (Tatarkova et al. 2011). In summary, through the redox modification of biomolecules, hyperoxia induces a variety of adaptive and pathological changes in cellular macromolecules.

Hyperoxia injury in tissues and organs

Acute toxicity of hyperoxia was observed in the earliest recorded experiments (Bert 1878; Smith 1899). O_2 levels above 80% were fatal to mice within 3 days. Lung inflammation and edema were evident postmortem. Hyperoxic acute lung injury (HALI) has now been well characterized, and pathological characteristics include damaged pulmonary capillary endothelium, alveolar type I epithelial cell death, type II epithelial cell hypertrophy, interstitial edema, neutrophil accumulation, altered surfactant production, and decreased lung compliance (Kallet and Matthay 2013; Amarelle et al. 2021).

In addition to acute injury, long-term exposure to supplemental oxygen has been well characterized. In neonates, hyperoxia interferes with lung development, leading to developmental abnormalities that persist into adulthood. Animal models, particularly murine models, have been widely used to investigate the effects of hyperoxia in the lungs of newborns. This is due to the fact that lung development in rodents during postnatal days 1–5 is similar to that of preterm newborn infants (born before the 37th week) (Berger and Bhandari 2014; O'Reilly and Thébaud 2014). During the final 6 weeks of pregnancy, antioxidant enzymes are upregulated within the developing lung in order to prepare the fetus for respiration at extrauterine O_2 levels (Frank and Groseclose 1984). As such, preterm neonates lack sufficient antioxidant capacity and are more vulnerable to oxygen toxicity; this acute damage leads to long-term pathologies like bronchopulmonary dysplasia (BPD), which is characterized by inflammation, fibrosis, decreased alveolarization, disruption of the alveolar-capillary membrane, impaired surfactant production, and pulmonary microvascular dysplasia (Bhandari 2010; Wang and Dong 2018). Indeed, exposure of preterm newborn animals to hyperoxia impairs type II alveolar epithelial cell proliferation and differentiation, causes interstitial thickening, delays alveolar development, and increases immune cell reactivity (Bucher and Roberts 1981; Yee et al. 2006; Bouch et al. 2015). Many of these pathological changes persist even in adulthood.

More recent findings have revealed an important link between hyperoxia and COVID-19. The expression of the SARS-CoV-2 co-receptor TMPRSS11D, a transmembrane protease required for efficient viral entry into host cells, was found to be higher in lung tissue from experimental mice BPD models, human BPD patients, and human and mouse epithelial cells exposed to hyperoxia (Myti et al. 2020). Another study found that neonatal hyperoxia upregulates the expression of angiotensin converting enzyme 2 (ACE2) and TMPRSS2—the receptor and co-receptor of the virus, respectively—in type II alveolar epithelial cells from mice by 2 months of age and that these protein levels remained higher than in control animals even at 12 months (Yee et al. 2020). These results suggest that, although necessary to treat respiratory failure and hypoxemia, treatment with supplemental oxygen may facilitate and accelerate the SARS-CoV-2 infection cycle by upregulating the receptor and co-receptors of the virus. In addition, patients who were born preterm may be at greater risk for COVID-19.

Elevated pO_2 in the alveoli drive an increase in dissolved O_2 concentrations in blood, and this is rapidly communicated to all internal organs where it can cause oxidative damage and disrupt cell signaling processes. From the earliest studies of hyperoxia in animals (Bert 1878), it has been clear that the brain is highly susceptible to hyperoxia injury. This is particularly true in neonates progressing through critical developmental milestones. During embryonic brain development, neuronal migration is near complete by the 24th week of gestation; however, glial maturation continues to occur postnatally (Reich et al. 2017). In preterm animal models, hyperoxia causes neuronal cell death and disturbs glial maturation and neural connectivity in the cortex, basal ganglia, hypothalamus, striatum, hippocampus, and white matter (Felderhoff-Mueser et al. 2004, 2005; Dean et al. 2014). A proteomic study by Kaindl et al. revealed that treatment of newborn mice with hyperoxia alters the expression of proteins involved in vesicle trafficking, cell growth and differentiation, neuronal migration, and axonal arborization (Kaindl et al. 2008). Behavioral tests have revealed that postnatal exposure to hyperoxia leads to impaired motor coordination, spatiotemporal learning, and memory in rodents in their adolescence and young adulthood (Schmitz et al. 2012; Ramani et al. 2013; Serdar et al. 2016). In humans, clinical studies have reported detrimental effects of hyperoxia in patients with stroke and traumatic brain injury (Davis et al. 2009; Rincon et al. 2014).

Hyperoxia injury of the retina has been widely studied. Treatment of premature infants with supplemental oxygen leads to visual impairment and even blindness in adulthood (Saugstad 2006), a pathology termed retinopathy of prematurity (ROP). As such, experimental oxygen-induced retinopathy models have been used to investigate the mechanisms of oxygen toxicity in the retina. ROP is characterized by hyperoxia-mediated arrest of retinal vascularization, followed by hypoxia due to poor vascularization, which in turn induces vasoproliferation and subsequent retinal detachment (Hellström et al. 2013).

Heart function is also impaired in hyperoxia. In neonatal mice, sustained hyperoxia causes left ventricular dysfunction (Ramani et al. 2015). Similarly, right ventricular dysfunction has been observed in adult mice that were exposed to neonatal hyperoxia (Menon et al. 2018). Hyperoxia has been shown to alter the redox and metabolic state of mouse cardiomyocytes, impairing action potential generation, dysregulating the expression of several potassium and sodium channels, and ultimately leading to left-ventricular hypertrophy and decreased cardiac output (Panguluri et al. 2013; Chapalamadugu et al. 2015; Vysotskaya et al. 2018). In human cardiomyocytes, hyperoxia induces cell death, upregulates proinflammatory cytokines, and alters the expression of genes involved in cell-cycle regulation, metabolism, and signaling ex vivo (Hafner et al. 2017). Further, a meta-analysis revealed that heart failure patients are more susceptible to the hemodynamic effects of hyperoxia, such as cardiac output decline (Smit et al. 2018).

Virtually all organs and tissues have been shown to be targets of oxygen toxicity, though some are less well studied. Endocrine glands (Bean and Johnson 1954), the liver (Wong et al. 2001; Rogers et al. 2010; Zangl et al. 2014), the kidney (Hess and Menzel 1970), the gastrointestinal tract (Chen and Chou 2016; Liu et al. 2020; Li and Liu 2022), and adipose tissue (Soares et al. 2016) are all adversely affected by hyperoxia.

Hyperoxia directly and indirectly modulates diverse signaling pathways

Hyperoxia compromises cellular functions via macromolecular oxidative damage and by dysregulating cellular signaling processes. A wide range of signaling pathways is altered by ROS in hyperoxia (Fig. 1). In many instances, these are regulated by the oxidation of specific cysteine residues in key proteins, which can either activate or inhibit protein activities, in either case modulating signaling (Zhang et al. 2016). A central pathway that is regulated in this way involves the nuclear factor erythroid 2-related factor 2 (Nrf2) (Bellezza et al. 2018). When activated, Nrf2 modulates the intracellular oxidative stress response by binding the antioxidant response element (5′-TGACXXXGC-3′) of genes encoding antioxidant enzymes, enzymes involved in glutathione metabolism, and enzymes involved in the generation of reducing equivalents (Tonelli et al. 2018). The transcriptional activity of Nrf2 is regulated by its physical interaction with the cytosolic Kelch-like ECH-associated protein 1 (Keap1), which facilitates Nrf2's proteasomal degradation under normal oxidative conditions. Keap1 binding of Nrf2 is inhibited when excessive cytosolic ROS modify key sensor cysteines in Keap1 (Baird and Yamamoto 2020), leading to Nrf2's escape of degradation, translocation to the nucleus, and promotion of transcriptional activity on target genes.

The Nrf2-Keap1 system appears to play an important role in hyperoxia. As early as 2002, genome-wide genetic linkage analysis had identified Nrf2 as a critical gene in the response to hyperoxia in two strains of laboratory mice (Cho et al. 2002b). Exposure of mice to hyperoxia (95–98% O_2) significantly increased Nrf2 mRNA levels and DNA-binding activity, with concomitant increases in mRNA levels of target genes (Cho et al. 2002a). Supporting this finding, Nrf2$^{-/-}$ mice were subsequently shown to be susceptible to lung injury resulting from exposure to 95–98% O_2. Similar results have been reported by Reddy et al. and Cho et al. (Reddy et al. 2009; Cho et al. 2012). In both studies, Nrf2 knockout mice showed increased lung injury and inflammation, while failing to recover normally upon return to normoxia. In contrast,
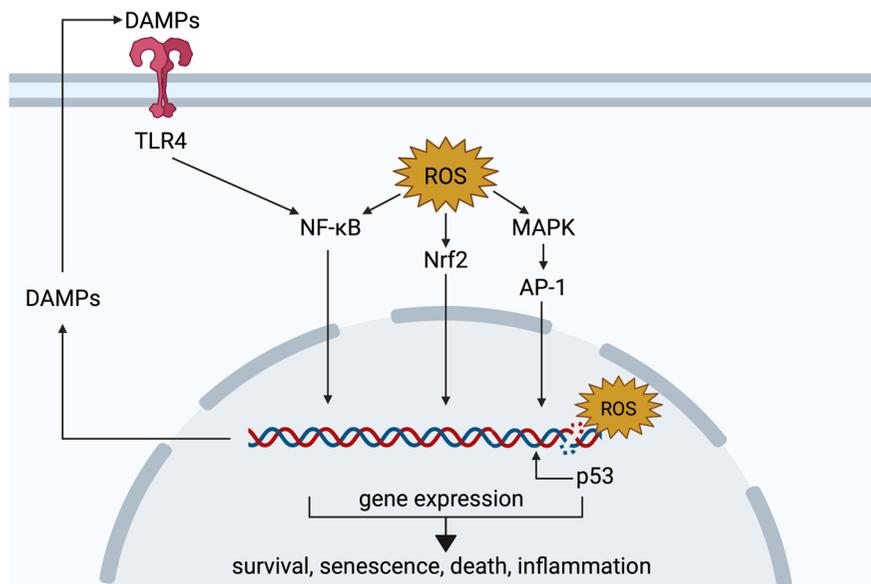

Fig. 1 Signaling pathways affected in hyperoxia. Excessive ROS modulate intracellular signaling, including via nuclear factor erythroid 2-related factor 2 (Nrf2), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and mitogen-activated protein kinase (MAPK) pathways. In parallel, oxidative damage to DNA activates p53, which induces the transcription of target genes. In turn, damage-associated molecular patterns (DAMPs) upregulated by these pathways are released into the extracellular space, where they can bind receptors such as the toll-like receptor 4 (TLR4) and further activate the NF-κB pathway. Signaling events orchestrated by these and other pathways determine the outcome of hyperoxia-mediated oxidative stress and may include cell survival, senescence, death, and inflammation. Created with BioRender.com

increasing Nrf2 activity by reducing Keap1 protein levels offers protection against the injurious effects of hyperoxia (Tamatam et al. 2020). Taken together, these studies indicate that the Nrf2-Keap1 pathway plays a key role in the response to oxygen toxicity, reducing tissue injury and improving recovery.

ROS also promotes the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor broadly involved in immune and inflammatory responses, via direct oxidation of regulatory cysteine residues (Lingappan 2018). However, the relationship between NF-κB and ROS is complex and bidirectional. Similar to Nrf2, under normal conditions, NF-κB remains bound by regulatory IκB proteins in the cytosol, preventing its translocation, DNA binding, and transcription regulation. Oxidative modifications to specific amino acids in IKB kinase (IKK) and IκBα can lead to NF-κB activation. NF-κB can induce the transcription of both antioxidant and pro-oxidant genes, suggesting a possible role in cell survival under oxidative stress (Morgan and Liu 2011); however, there is conflicting evidence regarding whether hyperoxia-mediated NF-κB activation is deleterious (Wright et al. 2010; Zara et al. 2013; Chou and Chen 2020; Li et al. 2020) or beneficial (Barazzone-Argiroffo et al. 2003; Franek et al. 2004; Yang et al. 2004; McKenna et al. 2014; Michaelis et al. 2014). This is likely due to the numerous genes regulated by NF-κB and to the multiplicity of NF-κB protein subunits and regulators that can be activated or inactivated through distinct pathways depending on the tissue and cell type.

Hyperoxia also appears to impact the Janus kinase-signal transducer and activator of transcription (Jak-STAT) pathway. STAT-induced gene expression regulates biological processes like proliferation, survival, and inflammation (Hu et al. 2021). Some evidence suggests a beneficial role of STAT proteins in hyperoxia through the induction of the protective enzyme heme oxygenase-1 (HO-1) and the inhibition of matrix metalloproteinase (MMP) upregulation (Lee et al. 2000; Lian et al. 2005). Likewise, deletion of STAT3 increased lung injury and alveolar capillary leak in mice exposed to hyperoxia (Hokuto et al. 2004). However, STAT3 activation in hyperoxic rats has also been linked to the pathogenesis of retinopathy of prematurity (Byfield et al. 2009; Ren et al. 2021).

Oxidative DNA damage driven by hyperoxia triggers activation and upregulation of p53, which orchestrates cell cycle arrest, decreased proliferation, senescence, or cell death (O'Reilly et al. 1998; Shenberger and Dixon 1999; Das et al. 2004; Das and Dashnamoorthy 2004; Maniscalco et al. 2005; Klimova et al. 2009; Parikh et al. 2019; You et al. 2019; Scaffa et al. 2021). Ataxia telangiectasia-mutated (ATM) and ATM-and-Rad3 related (ATR) are two kinases that are activated by damaged DNA. Both have also been shown to activate p53 in hyperoxia (Das and Dashnamoorthy 2004; Das et al. 2004; Kulkarni and Das 2008; Resseguie et al. 2015). Upregulation of p21 in hyperoxia and subsequent cell cycle arrest and induction of senescence has been demonstrated in multiple studies (Mcgrath 1998; Shenberger and Dixon 1999; Rancourt et al. 2001; Nyunoya et al. 2003; Das et al. 2004; Das and Dashnamoorthy 2004; Londhe et al. 2011; You et al. 2019; Parikh et al. 2019).

Receptors involved in proinflammatory pathways, such as toll-like receptor-4 (TLR4), are implicated in the inflammatory phase of hyperoxia injury. TLR4 can be activated by cytokines produced in a paracrine or autocrine fashion, and by damage-associated molecular patterns (DAMPs) like high mobility group box 1 (HMGB1) released by damaged cells. Indeed, TLR4 activation in hyperoxia promotes cell death and inflammation through the NF-κB-mediated induction of proinflammatory cytokines IL-6, IL-8, and TNF-α (Ogawa et al. 2007; Liu et al. 2015; Huang et al. 2016a). Contrastingly, TLR4 activation has also been associated with survival and protection against hyperoxia. Zhang et al. reported that TLR4-deficient mice are more susceptible to apoptosis induced by hyperoxia (Zhang et al. 2005). In another study with TLR4-deficient mice, reconstitution of endothelial TLR4 prolonged the survival of TLR4-KO animals post-hyperoxia (Takyar et al. 2016).

DAMPs are often upregulated by oxidative stress-mediated transcription factors (e.g., NF-κB) and are subsequently released into the extracellular space where they can activate pattern recognition receptors in immune cells and in the same cell, resulting in a positive feedback loop that further exacerbates damage (Roh and Sohn 2018). Accumulation of extracellular HMGB1 has been observed in hyperoxic mice, where treatment with neutralizing HMGB1 antibodies attenuated pulmonary edema, structural

alterations, and inflammation (Entezari et al. 2014; Yu et al. 2016).

The mitogen-activated protein kinase (MAPK) pathway leading to the transcriptional response mediated by activator protein-1 (AP-1) has also been implicated in hyperoxia-induced signaling. It has been demonstrated that apoptosis signal-regulating kinase 1 (ASK1), upstream activator of Jun N-terminal kinase (JNK) and p38, is activated upon oxidation of specific cysteine residues (Nadeau et al. 2009, 2007). In hyperoxia, extracellular signal-regulated kinase 1/2 (ERK1/2) has been mostly associated with survival and protective effects, while p38 and JNK activation has been mainly linked to cell death and injury (reviewed by Porzionato et al. 2015).

In conclusion, hyperoxia affects cellular signaling via a wide range of pathways resulting in numerous changes in cell behavior. This arises due to the preponderance of individual interactions of ROS with these signaling pathways and their interconnectedness. These wide-ranging effects likely underlie the observed impacts of hyperoxia on growth and development.

Hyperoxia drives cellular senescence

Replicative senescence is caused by DNA damage and/or telomere shortening leading to the induction of cell cycle arrest pathways and permanent exit from the cell cycle. Its initiation is regulated by the transcription factors p53, p21, and retinoblastoma protein (pRb) (Kumari and Jat 2021). Hyperoxia has been characterized as an initiator of cell cycle arrest and cellular senescence in mammalian cell lines. Early studies performed by Balin et al. showed that elevated O_2 levels shortened the replicative lifespan of WI-38 human fibroblasts (Balin et al. 1977). It was later observed that cell culture in 40% O_2 causes telomere shortening in these cells, halting their proliferation at the G_1 phase (von Zglinicki et al. 1995). Saretzki et al. demonstrated the elevation of gene expression markers of senescence in BJ human neonatal foreskin fibroblasts exposed to 40% O_2 for 4–6 weeks (Saretzki et al. 1998).

The activity of senescence associated (SA)-β-galactosidase, a well-recognized downstream senescence marker (Lee et al. 2006), is elevated in retinal pigment epithelial (RPE) cells cultured at 40%

O_2 (Honda et al. 2002). Parikh et al. also observed elevated SA-β-galactosidase activity, accompanied by increased p21, pRb, phosphorylated p53 levels, and DNA damage markers, in human fetal airway smooth muscle cells exposed to 40% O_2 for 7 days (Parikh et al. 2019). Similarly, primary human fetal lung fibroblasts cultured at 40% O_2 for 7 days showed increased SA-β-galactosidase activity, DNA damage markers, and G_2/M phase arrest, along with upregulation of p21 and p53. Additionally, hyperoxic fibroblasts had elevated expression of proinflammatory and profibrotic factors, as measured by RT-PCR (You et al. 2019). As mentioned above, standard cell culture conditions of 18–19% O_2 are hyperoxic relative to in vivo physioxia (2–6%). Parrinello et al. demonstrated that the onset of premature replicative senescence in mouse embryo fibroblasts cultured at 18% O_2 occurred much earlier than in 3% O_2 (Parrinello et al. 2003). This was associated with accelerated accumulation of DNA damage and mutations (Busuttil et al. 2003) and further exacerbated by knockout of DNA repair genes. Thus, hyperoxia drives replicative senescence in various cell types, apparently via the accumulation of DNA damage and mutations.

Mitochondria are key targets of oxygen toxicity

Mitochondrial respiration is both an important source of ROS in hyperoxia and a main target. Consistently, cells exposed to elevated O_2 levels exhibit reduced rates of respiration (Das 2013; Hals et al. 2017; Pinterić et al. 2018; Schoonen et al. 1990). Respiration-deficient (ρ°) HeLa cells tolerate hyperoxic conditions that are otherwise toxic to their wild-type counterparts. Moreover, the toxicity of hyperoxia is restored when these cells are repopulated with respiratory-competent mitochondria (Li et al. 2004). Similarly, uncoupling HeLa cell respiration with carbonyl cyanide m-chlorophenyl hydrazone (CCCP), thus lowering membrane potential, reduces mitochondrial ROS production rates in hyperoxia and confers tolerance to such conditions (Li et al. 2004).

Inhibition of oxidative phosphorylation in hyperoxia is associated with inhibition of several key enzymes at high O_2 levels. For example, pyruvate dehydrogenase (PDH) complex activity is reduced in lungs of mice and rats exposed to 95–100% O_2 (Kimura et al. 1983; Tanaka et al. 2020). Inactivation

of PDH complex limits pyruvate oxidation in the tricarboxylic acid (TCA) cycle and is in many cases partially compensated for by increased glycolytic rates (Tanaka et al. 2020). α-Ketoglutarate dehydrogenase (α-KGDH) is similarly inhibited by hyperoxia. In HeLa cells exposed to 98% O_2 for 4 days, α-KGDH activity was almost completely abolished, and this was accompanied by a fall in glutamine/glutamate utilization (Schoonen et al. 1990). The TCA cycle enzyme aconitase, which catalyzes the isomerization of citrate to isocitrate, is particularly sensitive to inhibition by $O_2^{\bullet -}$. Inactivation of mitochondrial aconitase in hyperoxia has been reported both in vivo and in vitro (Gardner et al. 1994; Morton et al. 1998). Additionally, hyperoxia inhibits the activities of mitochondrial respiratory complexes I and II (Das 2013; Schoonen et al. 1990). Proteomic analysis of hippocampal tissue of neonatal mice that were exposed to 85% O_2 until postnatal day 12 showed altered expression of subunits of several respiratory complexes, along with decreased ATP-linked oxygen consumption, even at 14 weeks of age—approximately 12 weeks after mice were returned to normoxia (Ramani et al. 2019). Thus, short-term exposure to hyperoxia both acutely and chronically inhibits mitochondrial bioenergetic function. The inhibition of mitochondrial bioenergetic function by hyperoxia results in mitochondrial dysfunction, evidenced by a reduced membrane potential (Audi et al. 2022) and increased fission of mitochondrial networks (Ma et al. 2018).

Mitochondrial DNA (mtDNA) is localized to the matrix (Richter 1995), which makes it proximal to several sources of excess ROS production during hyperoxia. Perhaps not surprisingly, increased levels of mtDNA oxidative damage are observed with hyperoxia exposure. Roper et al. reported elevated mtDNA oxidative damage in lung epithelial cells of mice breathing a 100% O_2 atmosphere for up to 72 h (Roper et al. 2004). In experiments with isolated mouse lung epithelial cells in culture, exposure to 60% O_2 for 24 h increased 8-oxo-dG by over fivefold (Kundumani-Sridharan et al. 2019). Treatment of rat lungs with a mitochondria-targeted DNA repair enzyme, endonuclease III, protects against hyperoxia-induced mtDNA damage ex vivo (Gebb et al. 2013). Similarly, Kim et al. reported that overexpression of another DNA repair enzyme, mitochondrial 8-oxoguanine DNA glycosylase (mt-OGG1), reduces oxidant-induced mtDNA lesions and apoptosis in

alveolar epithelial cells exposed to hyperoxia in vitro (Kim et al. 2014). Effects on non-lung tissues have also been reported. For example, rats housed in 60% O_2 for 21 days developed cataracts coincidentally with elevated mtDNA damage in lens tissue (Zhang et al. 2010). It is unclear the extent to which mtDNA damage and mutations can explain the phenotypic response to hyperoxia observed in vivo, since cells have a relatively high tolerance to mtDNA damage (Chomyn et al. 1992; Miyabayashi et al. 1992; Carelli and Chan 2014).

Cardiolipin (CL) is another mitochondrial target of hyperoxia. CL is a unique phospholipid with two phosphate groups and four acyl chains that in eukaryotic organisms is exclusively located in the mitochondria, mainly in the inner mitochondrial membrane (IMM). CL has an essential role in the stability of respiratory chain supercomplexes (often termed as respirosomes) (Pfeiffer et al. 2003) and dimerization of ATP synthase (Acehan et al. 2011). CL also interacts with cytochrome c (cyt c) on the outer surface of the IMM. ROS-mediated oxidation of CL causes its dissociation from cyt c, which then leads to the release of cyt c into the cytosol (Kagan et al. 2005; Shidoji et al. 1999; Polyak et al. 1997; Ott et al. 2002), a hallmark of apoptosis. Treatment of human lens epithelial B-3 (HLE B-3) cells with 80% O_2 for 48 h causes a reduction in CL content (Huang et al. 2006). An oxidative lipidomics study by mass spectrometry revealed CL peroxidation in mouse endothelial lung cells subjected to 72-h hyperoxia (95–100% O_2) both in vivo and in vitro. Lipid peroxidation was accompanied by apoptosis, measured by caspases 3 and 7 activity and TUNEL assay (Tyurina et al. 2010). It is evident that hyperoxia-mediated CL oxidation is a major trigger of mitochondrial toxicity and cell death. Further mechanistic details will be explored in the next section.

The uncoupling proteins UCP2 and UCP3 have been studied in the context of hyperoxia. These IMM proteins produce a relatively minor loss of membrane potential associated with decreased rates of ROS production in some experimental models. UCP3 overexpression in mouse C2C12 myotubes exposed to hyperoxia ameliorates protein carbonylation levels (Barreiro et al. 2009). Similarly, reduced UCP2 expression in MLE-12 cells and lungs from mice exposed to hyperoxia is accompanied by enhanced $O_2^{\bullet -}$ production and alveolar epithelial apoptosis. These effects

were abrogated by thioredoxin overexpression, which upregulated UCP2 via PGC-1α. This latter study also showed increased hyperoxic lung injury in UCP2-deficient mice (Raghavan et al. 2022). Together, these findings support the notion that UCP2/3 might decrease mROS production rates, providing additional targets for possible therapeutic intervention.

In summary, hyperoxia targets mitochondrial bioenergetics and function, mediates CL and mtDNA damage, and ultimately promotes the mitochondrial pathway of apoptotic cell death (Fig. 2).

Hyperoxia causes cell death via multiple pathways

Cell death is associated with oxygen toxicity. It occurs via multiple pathways, including apoptosis, necrosis, necroptosis, pyroptosis, and ferroptosis. Due to the
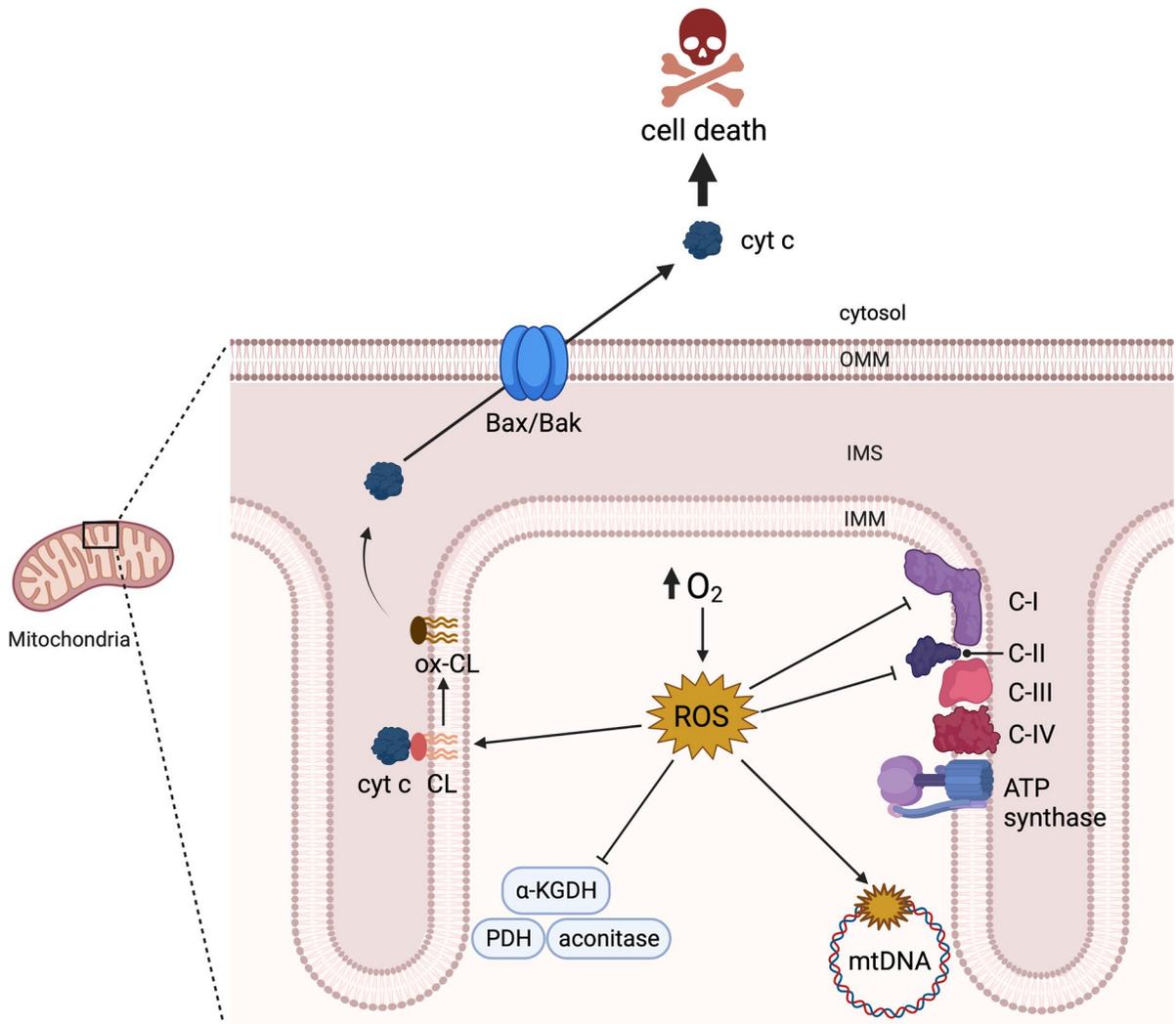

Fig. 2 Mitochondrial targets of hyperoxia-mediated injury. Hyperoxia drives the over production of mitochondrial reactive oxygen species (ROS), which inhibit metabolic enzymes such as aconitase, α-ketoglutarate dehydrogenase (α-KGDH), and pyruvate dehydrogenase (PDH), and respiratory complexes I and II, leading to bioenergetic failure. mROS oxidize mito-chondrial DNA (mtDNA) and cardiolipin (CL), further promoting dysfunction and leading to the release of cytochrome c (cyt c) into the cytosol through Bcl-2-associated X protein/Bcl-2 homologous antagonist killer (Bax/Bak) oligomers to instigate apoptosis. Created with BioRender.com

complexity of cell injury caused by hyperoxia, there is no consensus regarding the relative contributions of each in hyperoxia, and they likely vary according to the experimental model used. A key event in the intrinsic pathway of apoptosis is the release of cyt c and other proapoptotic factors from the mitochondrial inter-membrane space into the cytosol, which occurs through two main mechanisms (reviewed by Garrido et al. 2006).

One mechanism is the permeabilization of the outer mitochondrial membrane (OMM) through the oligomerization of the proteins Bax and Bak. Apoptotic stimuli cause the cytosolic protein Bax to localize to the OMM, where it forms oligomers with itself and with the constitutively present Bak. Bax and Bak oligomers are then responsible for the release of proapoptotic factors into the cytosol. Activation and oligomerization of Bax/Bak is influenced by oxidative stress, seemingly via Cys-62 oxidation of Bax promoting its translocation to mitochondria (D'Alessio et al. 2005; Nie et al. 2008). Oxidation of Bax/Bak has not yet been demonstrated in a hyperoxic model, but the relationship between these proteins and excessive ROS production is clear. Treatment of rat alveolar epithelial cells with SOD/catalase mimetic EUK-134 prevented Bax activation, cyt c release, and apoptosis after hyperoxic exposure, indicating that ROS production occurs upstream of Bax activation in this model (Buccellato et al. 2004). Budinger et al. demonstrated that deficiency of Bax and Bak can protect against hyperoxia-induced apoptosis. There is thus a clear role of Bax/Bak in hyperoxia-mediated apoptotic cell death (Budinger et al. 2011).

A second mechanism underlying the intrinsic apoptosis pathway is the opening of the mitochondrial permeability transition pore (mPTP), which leads to mitochondrial swelling and rupture to release pro-apoptotic factors including cyt c (reviewed by Bernardi and di Lisa 2015; Bernardi et al. 2021). Oxidative stress is a key factor inducing mitochondrial permeability transition. mPTP opening is triggered by thiol oxidants and can be prevented by thiol reductants (Fagian et al. 1990; Lenartowicz et al. 1991; Valle et al. 1993; Bernardes et al. 1994; Kowaltowski et al. 1996). Addition of catalase, lipid peroxide inhibitors, iron chelators, and dietary and synthetic antioxidants similarly protect against mPTP opening (Castilho et al. 1995; Kowaltowski et al. 1996; Singh et al. 2013; Daniel et al. 2018; Teixeira et al. 2018;

Baburina et al. 2019). Moreover, oxidative modifications to specific cysteine residues of the adenine nucleotide translocator (ANT) (Costantini et al. 2000; McStay et al. 2002), cyclophilin D (CypD) (Nguyen et al. 2011), and the oligomycin sensitivity conferral protein (OSCP, a subunit of ATP synthase complex) (Carraro et al. 2020) seem to play a crucial role in mPTP assembly and opening. mPTP-mediated cell death has been linked to hyperoxic injury (Pagano et al. 2004). Cyt c release and mitochondrial swelling were observed in epithelial alveolar cells from mice exposed to 100% O_2 for 72 h in vivo. These changes were prevented by treatment with cyclosporine A (CsA), an inhibitor of mPTP opening. Thus, there is strong evidence hyperoxia causes cell death via the intrinsic apoptotic pathway, involving a variety of mechanisms.

Hyperoxia can also cause cell death through the extrinsic apoptosis pathway mediated through interaction of extracellular signaling molecules with cell death receptors such as Fas and CD40. Increased lung tissue Fas expression is seen in murine models of oxygen toxicity in vivo and in vitro (Barazzone et al. 1998; de Paepe et al. 2005). Similarly, protein and mRNA levels of Fas, FasL, and Fas-associated death domain (FADD) are increased in brain tissues from rats exposed to 80% O_2. In this study, trafficking of the Fas receptor to the plasma membrane was found to be increased in hyperoxia in vitro. In turn, lack of functional Fas receptors in mice provided neuroprotection against hyperoxic injury (Dzietko et al. 2008). Conversely, there is also evidence indicating that the extrinsic pathway is not required for hyperoxia-induced cell death, as genetic ablation of Fas and CD40 do not confer protection against lung injury in murine models (Barazzone et al. 1998; Barazzone Argiroffo et al. 2002).

A recent study by Tong et al. found that hyperoxia induces a non-canonical type of apoptotic cell death via ER stress (Tong et al. 2021). ER stress induces the unfolded protein response (UPR). Inositol-requiring enzyme 1α (IRE1α), one of the most conserved UPR signaling proteins, induces activation of the transcription factor X-box binding protein 1 (XBP1), which in turn induces the expression of ER chaperones and protein degradation components (Chen and Brandizzi 2013; Gong et al. 2017). However, overactivation of the UPR results in IRE1α-mediated JNK activation, which can promote both intrinsic and extrinsic

apoptotic pathways (Verma and Datta 2012). Tong et al. showed that prolonged exposure (7–14 days) of newborn rats to hyperoxia caused phosphorylation of IRE1α and JNK in lung tissues, indicating an involvement of the ER stress-associated apoptosis. There is also evidence of the activation of the ER stress-related caspase-12 (Lamkanfi et al. 2004) in murine hyperoxic models (Zhang et al. 2013; Huang et al. 2016b). Furthermore, treatment with ER-stress inhibitor 4-phenyl butyric acid protects against hyperoxic-cell death both in vivo and in vitro (Pao et al. 2021). Recent evidence showed role of the mitochondrial protein A-kinase anchoring protein 1 (Akap1) and ER stress in protection against hyperoxia; deletion of Akap1 resulted in increased ER stress-associated cell death (Sidramagowda Patil et al. 2022).

In many studies of hyperoxia, cell death resembles necrosis. Kazzaz et al. did not observe markers of apoptosis in A549 human lung adenocarcinoma cells cultured at 95% O_2 for 7 days (Kazzaz et al. 1996). Rather, morphological features of these cells were typical of necrotic cell death, including swelling and enlarged nuclei and mitochondria. However, this study did show apoptotic cell death in lungs from mice exposed to 100% O_2 for 48 h in vivo. Although contrasting, these results are not necessarily surprising. A direct exposure of cultured cells to 95% O_2 represents a more severe hyperoxia (perhaps $20\times$ physioxia) than would be experienced by alveolar cells of mice breathing 100% O_2 (approximately $5\times$ greater than in normoxia). Further, in this study, A549 cells were exposed to hyperoxia for 7 days, while animals were exposed for only 48 h.

Necroptosis is a form of programmed cell death that shares characteristics of both apoptosis and necrosis. In hyperoxia, the expression of receptor-interacting proteins (RIP) 1 and 3—key proteins in this pathway—was increased in the bronchoalveolar lavage fluid of rats exposed to hyperbaric hyperoxia (100% O_2 at 1875 mmHg) for 6 h. Inhibition of necroptosis by the RIP1 inhibitor necrostatin-1 and the ROS scavenger edaravone protected the animals against lung pathology (Han et al. 2018). Pyroptosis is a form of programmed necrotic and inflammatory cell death that appears to participate in hyperoxic injury. There is an increasing amount of recent evidence showing that inhibition of the pyroptosis pathway, either by pharmacologically or genetically targeting the inflammasome formation, or other elements of the

pathway, protects against hyperoxic injury (Fukumoto et al. 2013; Galam et al. 2016; Zhang et al. 2017b; Dapaah-Siakwan et al. 2019; Mendha et al. 2021; Wang et al. 2022a). A more recently described form of cell death is ferroptosis which, like pyroptosis and necroptosis, is a regulated cell death with necrotic phenotype. It is characterized by, among other things, lipid peroxidation (Cao and Dixon 2016), leading to loss of membrane integrity and rupture of the cell (Jiang et al. 2021). Two murine models have reported the involvement of ferroptosis in lung injury induced by hyperoxia (Jia et al. 2021; Chou and Chen 2022).

Taken together, the data indicate that multiple modes of cell death are involved in hyperoxic injury. The relative importance of any single pathway is likely dependent, in part, on the severity and duration of hyperoxia exposure. More research is needed to better understand these relationships.

Epigenetic responses to hyperoxia

Effects of hyperoxia exposure during the neonatal period can last into adulthood, suggesting that long-lasting epigenetic alterations might be involved. Epigenetic regulation of gene expression occurs via DNA methylation, covalent histone modifications, and the expression of non-coding RNAs (Aguilera et al. 2010). All these mechanisms are known to be affected by oxidative stress (reviewed by García-Guede et al. 2020). Unsurprisingly, both aberrant DNA methylation (Panayiotidis et al. 2004; Zhu et al. 2015; Chen et al. 2017; Bik-Multanowski et al. 2018) and histone modification have been identified in experiments with hyperoxia (Londhe et al. 2011; Zhu et al. 2015; Coarfa et al. 2020).

Noncoding RNA molecules, including miRNAs (19–25 nucleotides) and long non-coding RNAs (lncRNAs; > 200 nucleotides) are also affected by hyperoxia. miRNA molecules regulate gene expression by silencing specific mRNAs. On the other hand, lncRNAs are a highly heterogenous class of RNAs that act through a wide variety of mechanisms. For instance, lncRNA can bind specific miRNAs and inhibit their function by "sponging" them (reviewed by Panni et al. 2020). Using miRNA microarray, Zhang et al. identified 21 miRNAs that are differentially expressed in lungs from neonatal mice exposed to hyperoxia versus control mice (Zhang et al. 2011).

Table 1 Evidence of the roles of noncoding RNA molecules in hyperoxia

RNA molecule	Type of RNA	Hyperoxic model	Outcome	Reference
miR-150	miRNA	Lung injury/newborn mice/95% O_2/3–10 days	• Downregulated in hyperoxia • KO decreased lung injury	(Narasaraju et al. 2015)
		Lung injury/primary mouse lung epithelial cells, BEAS-2B and A549 cells/95% O_2/12–72 h	• Cytoprotective effect	(Zhang et al. 2017a)
miR-876-3p	miRNA	Lung injury/newborn mice/85–100% O_2/4–14 days Normal human bronchial epithelial/85% O_2/24 h	• Downregulated in hyperoxia • Predicted protective effect against injury	(Lal et al. 2018)
miR-16	miRNA	Lung injury/isolated T2AECs/60% O_2 /24 h	• Downregulated in hyperoxia • miR-16 mimics inhibited apoptosis and the TGF-β/Smad2 and Jak/STAT3 pathways	(Li et al. 2018)
miR-34a	miRNA	Lung injury/newborn mice/100% O_2/4 or 7 days Isolated T2AECs and MLE-12 cells/40–95% O_2/4–48 h	• Silencing ameliorated apoptosis in vitro and in vivo • Overexpression aggravated injury	(Syed et al. 2017)
		Lung injury/newborn mice/85% O_2/14 days MLg cells/85% O_2/unspecified duration	• Upregulated in hyperoxia • Deletion protected against injury	(Ruiz-Camp et al. 2019)
miR-17	miRNA	Lung injury/newborn mice/70% O_2/4–14 days	• Downregulated in hyperoxia • Downregulated STAT3 • Upregulation relieved pulmonary injury	(Zhang et al. 2020)
		Lung injury/newborn mice/85% O_2/14 days	• Downregulated in hyperoxia • Downregulation was associated with lung injury	(Wang et al. 2020)
miR-185-5p	miRNA	Lung injury/mice/95% O_2/24–72 h MLE-15 cells/95% O_2/24–48 h	• Upregulated in hyperoxia • Upregulated RIP1 and RIP3 • Induced necroptosis and apoptosis	(Carnino et al. 2020)
miR-96	miRNA	OIR/newborn rats/cycling 10–50% O_2 every 24 h/14 days Retinal vaso-obliteration/newborn rats/80% O_2/5 days HRMECs/80% O_2/1–48 h	• Downregulated in hyperoxia • Overexpression promoted vascular repair in vivo and protected against endothelial dysfunction in vitro	(Desjarlais et al. 2020)
miR-101-3p	miRNA	Lung injury/newborn mice/65% O_2/7–14 days	• Overexpression mitigated injury • Downregulated HMGB3 and TGF-ß1/Smad3 axis	(Yuan et al. 2020)
miR-18a	miRNA	Lung injury/mouse/95% O_2/7 days MLE-12 cells/95% O_2/12–48 h	• Downregulated in hyperoxia • Overexpression prevented pyroptosis and relieved lung injury	(Zou et al. 2020)
miR-29b	miRNA	Plasma from preterm infants lung injury/newborn mice/85% O_2/14 days	• Downregulated in hyperoxia • Improved alveolarization and decreased expression of ECM proteins	(Durrani-Kolarik et al. 2017)
miR-29a	miRNA	Lung injury/newborn mice/> 90% O_2/4 days	• Upregulated in hyperoxia • Inhibition alleviated injury	(Hu et al. 2020)

Table 1 (continued)

RNA molecule	Type of RNA	Hyperoxic model	Outcome	Reference
miR-199a-5p	miRNA	Lung injury/newborn mice/100% O_2/7 days Mouse MLE-12 cells and RAW264.70 cells/95% O_2/4–24 h	• Upregulated in hyperoxia • Mimic treatment worsened injury	(Alam et al. 2019)
miR-20b	miRNA	Lung injury/rats/95% O_2/48 h	• Downregulated in hyperoxia • Overexpression downregulated Mfn1/2 and reduced apoptosis	(Mu et al. 2021)
miR-214	miRNA	Lung injury/newborn rats/95% O_2/7 days Alveolar epithelial cells/85% O_2/24 h	• Downregulated in hyperoxia • Overexpression restored alveolarization in vivo and decreased apoptosis in vitro	(Zhang et al. 2021b)
miR-421	miRNA	Lung injury/newborn mice/85% O_2/7 days MLE-12 cells/85% O_2/6 h	• Upregulated in hyperoxia • Downregulation was associated with alleviated injury • Mimic treatment abrogated Rian-mediated protection	(Tao et al. 2021)
miR-194-5p	miRNA	Lung injury/newborn mice/ > 90% O_2/4 days BEAS-2B cells/95% O_2/48 h	• Mediated hyperoxic injury • Upregulation blocked CASC2-mediated protection	(Ji et al. 2021)
miR-181c-5p	miRNA	HLMECs/80% O_2/12–24 h	• Upregulated in hyperoxia • miR-181c-5p mimic downregulated NCAPG and enhanced apoptosis	(Wu et al. 2021)
miR-342-5p	miRNA	Lung injury/newborn mice/100% O_2/4–7 days T2AECs and MLE-12 cells/95% O_2/2–48 h	• Downregulated in hyperoxia • Overexpression and mimic treatment ameliorated injury	(Wen et al. 2021)
miR-299-3p	miRNA	OIR/newborn mice/75% O_2/5 days	• Downregulated in hyperoxia • Overexpression reduced apoptosis	(Wang et al. 2022b)
FOXD3-AS1	lncRNA	Lung injury/primary mouse lung epithelial cells, BEAS-2B and A549 cells/95% O_2/12–72 h	• Upregulated in hyperoxia in vivo and in vitro • Deletion is cytoprotective in vivo and in vitro	(Zhang et al. 2017a)
Xist	lncRNA	Lung injury/newborn mice/65% O_2/7–14 days	• Upregulated in hyperoxia • Silencing protects against injury	(Yuan et al. 2020)
H19	lncRNA	Lung injury/newborn mice/70% O_2/4–14 days	• Upregulated in hyperoxia • Silencing upregulated miR-17, downregulated STAT3, and relieved injury	(Zhang et al. 2020)
MEG3	lncRNA	Lung injury/mice/95% O_2/7 days MLE-12 cells/95% O_2/12–48 h	• Upregulated in hyperoxia • Knockdown inhibited NLRP3 inflammasome, caspase-1, and pyroptosis	(Zou et al. 2020)
MALAT1	lncRNA	BPD patients A549 cells 92% O_2/48 h	• Upregulated in BPD • Downregulated in hyperoxia in vitro • Silencing promoted apoptosis	(Zhang et al. 2021a)
Rian	lncRNA	Lung injury/newborn mice/85% O_2/7 days MLE-12 cells/85% O_2/6 h	• Downregulated in vivo and in vitro • Overexpression downregulated miR-421 and alleviated injury	(Tao et al. 2021)

Table 1 (continued)

RNA molecule	Type of RNA	Hyperoxic model	Outcome	Reference
CASC2	lncRNA	Lung injury/newborn mice/ > 90% O$_2$/4 days/10 days recovery in normoxia BEAS-2B cells/95% O$_2$/48 h	• Poorly expressed in hyperoxic mice • Overexpression ameliorated lung injury in vivo • Inhibited apoptosis of epithelial cells in vitro	(Ji et al. 2021)
DLEU2	lncRNA	HLMECs/80% O$_2$/12–24 h	• Downregulated in hyperoxia • Overexpression inhibited miR-181c-5p and hyperoxic damage	(Wu et al. 2021)
TUG1	lncRNA	OIR/newborn mice/75% O$_2$/5 days	• Upregulated in hyperoxia • Knockdown reduced pathological alterations, apoptosis, inflammation, and miR-299-3p expression	(Wang et al. 2022b)

Abbreviations: *CASC2*, cancer susceptibility candidate 2; *DLEU2*, deleted in lymphocytic leukemia 2; *ECM*, extracellular matrix; *FOXD3-AS1*, FOXD3 antisense RNA 1; *HMGB3*, high mobility group box 3; *HLMECs*, human lung microvascular endothelial cells; *HPMECs*, human pulmonary microvascular endothelial cells; *HRMECs*, human retinal microvascular endothelial cells; *Jak*, Janus kinase; *KO*, knockout; *MALAT1*, metastasis-associated lung adenocarcinoma transcript; *MEG3*, maternally expressed 3; *Mfn1/2*, mitofusin 1/2; *NCAPG*, non-SMC condensin I complex subunit G; *NLRP3*, NLR family pyrin domain containing 3; *OIR*, oxygen-induced retinopathy; *RIP1/3*, receptor-interacting protein 1/3; *Smad2*, small mothers against decapentaplegic 2; *STAT3*, signal transducer and activator of transcription 3; *TGF-β*, transforming growth factor-β; *TUG1*, taurine up-regulated 1; *T2AECs*, type II alveolar epithelial cells; *Xist*, X-inactive specific transcript

Similarly, Bao et al. identified approximately 2000 differentially expressed lncRNAs in newborn mice exposed to hyperoxia compared to wild-type mice (Bao et al. 2016). Many subsequent studies have shown the roles of different lncRNA and miRNA molecules in hyperoxia (Table 1).

Therapeutic approaches to ameliorating hyperoxia injury

Preventing or mitigating oxidative damage caused by increased ROS levels produced by cells during supplemental oxygen therapy (i.e., hyperoxia) can allow hypoxemia and tissue hypoxia to be corrected while avoiding or reducing oxygen toxicity. Strategies to reduce hyperoxic injury have been investigated in humans and rodent models and address key aspects including ROS production/neutralization, apoptotic cell death, and the inflammatory response, among others (Table 2).

Mitochondrial oxygen toxicity in hyperoxia has been targeted using synthetic $O_2^{\bullet-}$ scavengers like Mito-TEMPO and Mito-TEMPOL. Both molecules have a triphenylphosphonium (TPP) moiety with a single, shielded, positive charge that drives accumulation in the matrix in proportion to membrane potential. Mito-TEMPO has been shown to ameliorate lung damage and injury in several models of hyperoxia injury (see Alva et al. in press).

Non-mitochondria-targeted antioxidants can also reduce the extent of hyperoxia injury. Ascorbic acid is effective in reducing the levels of the oxidative damage biomarker 8-isoprostane in young men breathing 100% O$_2$ (Fernandes et al. 2021). The antioxidant N-aceylcysteine reduces lung injury in rats exposed to 90% O$_2$ (Qiao et al. 2019). Similarly, tocopherol, retinol, and coenzyme Q have all shown efficacy in various models of hyperoxia injury.

Therapeutic strategies targeting the response to oxidant injury have also been investigated. As outlined above, elevated cytosolic ROS levels generate a compensatory response via the Nrf2-Keap1 system. A variety of phytochemicals have been shown to ameliorate hyperoxia-mediated injury via this signaling pathway. For example, the plant polyphenolic compound curcumin activates Nrf2 indirectly (Park et al. 2021). Both curcumin (Sakurai et al. 2013) and its synthetic analogues (Stamenkovska et al. 2020) reduce lung damage due to hyperoxia in neonatal rats. Similar results have been obtained with resveratrol (Yang et al. 2022). Another phytochemical,

Table 2 Therapeutic candidates studied to treat hyperoxic injury

Antioxidants

Candidate	Mechanism of action	Models	Outcomes	References
MitoTEMPO/MitoTEMPOL	Mitochondrial $O_2^{\bullet-}$ scavenger	Murine BPD, various in vitro models	↑Alveolarization ↓Right ventricular hypertrophy ↓ACE2 and TMPRSS2 expression ↓Proinflammatory cytokines ↓Cell death ↓Mitochondrial fragmentation	(Datta et al. 2015; Forred et al. 2017; Ma et al. 2018; Yee et al. 2020)
Ascorbate (vitamin C)	Alkyl hydroperoxide scavenger, regenerates reduced tocopherol	Healthy patients, congestive heart failure patients, Murine HALI, various in vitro models	↑Left ventricular function ↓Hyperventilation ↓Oxidative biomarkers in blood ↓HMGB1 levels ↓Leukocyte infiltration ↓Lipid and protein oxidation ↓Vasoconstriction	(Mak et al. 2002; Al-Shmgani et al. 2012; Gao et al. 2012; Patel et al. 2020; Fernandes et al. 2021)
Tocopherol (vitamin E)	Alkyl hydroperoxide scavenger	In vitro OIR, neonatal rabbits, other in vitro models	↓Lipid and protein oxidation ↓Vascular cell injury ↓Surfactant system impairment	(Tripathi and Tripathi 1984; Ward and Roberts 1984; Wispe et al. 1986; Al-Shmgani et al. 2012)
Retinol/retinoic acid (vitamin A)	ROS scavenger, retinoid X receptor agonist	Murine BPD	↑Surfactant protein levels ↑Alveolar maturation ↓Lung damage ↓Growth retardation ↓MIP-2 expression	(Zimová-Herknerová et al. 2008; James et al. 2010; Gelfand et al. 2020)
Coenzyme Q_{10}	ROS scavenger	Murine neonatal organ injury	↑Antioxidant enzyme activity in heart, kidney, and brain ↓Oxidative stress in liver	(Lee et al. 2022)
N-acetylcysteine	L-cysteine prodrug, replenishes GSH	Murine HALI, in vitro models	↑Mitochondrial membrane potential ↓Lung damage ↓Cell death ↓Cyt c release ↓HGMB1 and RAGE expression ↓TLR2/4 and NF-κB activity ↓Proinflammatory cytokine secretion	(Huang et al. 2016a; Qiao et al. 2019; Zou et al. 2019)
Curcumin and analogs	ROS scavenger, multiple molecular targets	Murine BPD	↑Relaxation of tracheal smooth muscle ↑Lung maturation ↑Alveolarization ↑PPAR-γ activation ↑Catalase activity ↓Apoptosis ↓ERK1/2 activation ↓TNF-α expression ↓TGF-β signaling	(Sakurai et al. 2011, 2013; Stamenkovska et al. 2020)
Sulforaphane	ROS scavenger, Nrf2 inducer, NF-κB inhibitor, other targets	Murine BPD and HALI	↑Nrf2-mediated transcriptional response ↑Macrophage function ↓Inflammatory cell infiltration ↓LDH levels ↓Mucous hypersecretion	(McGrath-Morrow et al. 2014; Cho et al. 2019; Patel et al. 2020)

Table 2 (continued)

Candidate	Mechanism of action	Models	Outcomes	References
Resveratrol	ROS scavenger, SIRT1, multiple molecular targets	Murine brain injury, murine HALI and BPD	↑SIRT1/PGC-1α signaling ↑PGC-1α, NRF1, and TFAM expression ↑Mitochondrial biogenesis ↑SOD and GSH ↓Alveolar simplification ↓Lung fibrosis ↓Apoptosis ↓Mitochondrial dysfunction ↓p53 expression ↓Proinflammatory cytokine release ↓Wnt/β-catenin signaling	(Özdemir et al. 2014; Xu et al. 2015; Zhu et al. 2020, 2021; Kang et al. 2021; Yang et al. 2022)
Quercetin	ROS scavenger, multiple molecular targets	Murine BPD, fetal airway smooth muscle cells	↑Alveolarization ↓Inflammation ↓NF-κB levels ↓Lipid peroxidation ↓Senescence	(Maturu et al. 2018; Parikh et al. 2019)
Anthocyanins	ROS scavenger	Murine OIR, HUVECs	↑Nrf2 gene targets ↑Cell viability ↓Mitochondrial dysmorphology ↓Endothelial cell proliferation	(Cimino et al. 2013; Ercan et al. 2019)
Caffeine	ROS scavenger, $A_{2A}R$ antagonist, multiple molecular targets	Murine BPD, neonatal murine brain injury	↑Alveolar development ↑Weight gain ↓DNA damage ↓$A_{2A}R$ expression ↓Proinflammatory cytokines ↓Inflammatory infiltration ↓Apoptosis ↓ER stress ↓NLRP3 inflammasome expression ↓NF-κB activation ↓MMP2 levels	(Endesfelder et al. 2017, 2019; Teng et al. 2017; Chen et al. 2020c)
Indole-3-carbinol	ROS scavenger, AhR agonist/inducer, other targets	Murine BPD	↑AhR gene targets ↑Alveolarization ↑NF-κB target genes ↓Fibrosis	(Guzmán-Navarro et al. 2021)
Tetrandrine	ROS scavenger, multiple molecular targets	Murine BPD	↑Antioxidant enzymes ↓Apoptosis ↓Inflammation ↓Fibrotic markers ↓NF-κB and ERK1/2 signaling	(Jiao et al. 2020)
Antiapoptotic				
Cyclosporin A	Cyclophilin D inhibitor; delays mPTP opening	Murine	↓Cyt c release ↓Mitochondrial swelling ↓Lung damage	(Pagano et al. 2004)

Table 2 (continued)

Candidate	Mechanism of action	Models	Outcomes	References
Anti-inflammatory				
TRP601	Caspase inhibitor	Murine brain injury	↓Apoptosis ↓Neurodegeneration	(Sifringer et al. 2012)
Interleukin-10	Anti-inflammatory cytokine	Murine HALI, fetal alveolar cells (in vitro)	↑Survival ↑VEGF release ↑Proliferation ↑Jak1 and TYK2 phosphorylation ↓Lung injury ↓Cell death ↓NF-κB activation ↓Proinflammatory cytokines ↓iNOS and NO levels ↓MMP2 and MMP9 activities	(Lee and Kim 2011; Li et al. 2015b; Lee and Lee 2015)
Interleukin-1 receptor antagonist	Anti-inflammatory cytokine	Murine BPD, murine BPD-pulmonary hypertension	↑Pulmonary small vessels ↑Immune cell viability ↓Pulmonary vascular resistance ↓Lung structural disintegration ↓Cardiac fibrosis ↓Immune cell activation ↓Proinflammatory cytokines	(Nold et al. 2013; Bui et al. 2019)
Acetylsalicylic acid	COX inhibitor	Murine HALI	↓NF-κB activation ↓ROS ↓Proinflammatory cytokines ↓Macrophages ↓Neutrophil infiltration ↓Lung edema	(Chen et al. 2020b; Tung et al. 2022)
Ibuprofen	COX inhibitor	Murine OIR	↓Retinopathy score ↓Extra-retinal nuclei count per section	(Sharma et al. 2003)
HIF-1 upregulators				
FG-4095	PHD inhibitor	Fetal baboon lung explants, primate BPD, distinct cell lines	↑HIF-1/2α target genes ↑Angiogenesis ↑Alveolar surface area ↑Lung compliance	(Asikainen et al. 2006, 2005)
Dimethyloxalylglycine	PHD inhibitor	Murine OIR	↑Peripheral vascularity ↓Neovascularization ↓Ischemia	(Sears et al. 2008; Trichonas et al. 2013)
Roxadustat	PHD inhibitor	Murine BPD	↑Survival ↑Alveolarization ↑eNOS expression ↑VEGF expression	(Huang et al. 2021)
Others				
Memantine	NMDA receptor antagonist	Murine brain injury	↑Neuron viability ↓Apoptosis	(Polat et al. 2020)
Lacosamide	Enhances slow Na$^+$ channel inactivation	Murine brain injury	↑Neuron viability ↓Apoptosis	(Polat et al. 2020)

Table 2 (continued)

Candidate	Mechanism of action	Models	Outcomes	References
Vitamin D	Vitamin D receptor agonist	Murine BPD	↑Alveolarization ↑VEGF and VEGFR2 expression ↑HIF-1α expression ↓Alveolar simplification ↓Apoptosis ↓TLR4 expression ↓IFN-γ and IL-1β expression ↓Neutrophil extracellular traps ↓Proinflammatory cytokines	(Kose et al. 2017; Yao et al. 2017; Chen et al. 2020a; Wang and Jiang 2021)
Metformin	AMPK activator	Murine BPD, HUVECs	↑Radial alveolar count ↑Vascular proliferation ↑ATP levels ↑Lung capillary number ↓Mortality ↓Inflammation ↓Fibrosis	(Chen et al. 2015; Yadav et al. 2020)
Rosiglitazone	PPAR-γ agonist	Murine BPD and HALI, preterm rabbits	↑Radial alveolar count ↑Alveolar sacculation ↑Lung maturation ↑Surfactant proteins ↑VEGF expression ↓Wnt and TGF-β signaling ↓Neutrophil influx	(Richter et al. 2016; Rehan et al. 2010; Dasgupta et al. 2009)
Alda-1	ALDH2 activator	Murine HALI, HMVECs	↑Mitochondrial membrane potential ↑Akt/mTOR signaling ↓Alveolar damage ↓Inflammation ↓Immune cell infiltration ↓Bax and cyt c levels ↓4-HNE levels	(Sidramagowda Patil et al. 2019, 2021)

Abbreviations: *ACE2*, angiotensin converting enzyme 2; *AhR*, aryl hydrocarbon receptor; *ALDH2*, aldehyde dehydrogenase 2; *Alda-1*, ALDH2 activator 1; *AMPK*, adenosine monophosphate-activated kinase; *ATP*, adenosine triphosphate; *A$_{2A}$R*, A2A adenosine receptor; *BPD*, bronchopulmonary dysplasia; *COX*, cyclooxygenase; *cyt c*, cytochrome c; *ER*, endoplasmic reticulum; *ERK1/2*, extracellular signal-regulated kinase 1/2; *GSH*, reduced glutathione; *HALI*, hyperoxic acute lung injury; *HIF-1/2*, hypoxia-inducible factor 1/2; *HMGB1*, high mobility group box 1; *HUVECs*, human umbilical vein endothelial cells; *Jak*, Janus kinase; *IFN-γ*, interferon γ; *IL-1β*, interleukin-1β; *LDH*, lactate dehydrogenase; *MIP-2*, macrophage inflammatory protein 2; *MMP-2*, matrix metalloproteinase 2; *mPTP*, mitochondrial permeability transition pore; *mTOR*, mammalian target of rapamycin; *NF-κB*, nuclear factor kappa-light-chain-enhancer of activated B cells; *NLRP3*, NLR family pyrin domain containing 3; *NMDA*, N-methyl-D-aspartate; *NRF1*, nuclear respiratory factor 1; *Nrf2*, nuclear factor erythroid 2-related factor 2; *NO*, nitric oxide; *eNOS*, endothelial NO synthase; *iNOS*, inducible NO synthase; *OIR*, oxygen-induced retinopathy; *PGC-1α*, peroxisome proliferator-activated receptor-gamma coactivator 1 α; *PHD*, prolyl hydroxylase domain; *PPAR-γ*, peroxisome proliferator-activated receptor γ; *RAGE*, receptor for advanced glycation end-products; *ROS*, reactive oxygen species; *SIRT1*, sirtuin 1; *SOD*, superoxide dismutase; *TMPRSS2*, transmembrane protease, serine 2; *TGF-β*, transforming growth factor β; *TFAM*, transcription factor a, mitochondrial; *TLR2/4*, toll-like receptor 2/4; *TNF-α*, tumor necrosis factor α; *TYK2*, tyrosine kinase 2; *VEGF*, vascular endothelial growth factor; *VEGFR2*, VEGF receptor 2; *4-HNE*, 4-Hydroxynoneal

sulforaphane, ameliorates hyperoxia-induced lung injury in Nrf2$^{+/+}$ but not Nrf2$^{-/-}$ mice (Cho et al. 2019). Taken together, these studies suggest that administration of select phytochemicals might be beneficial in treating hyperoxia injury.

Strategies for avoiding apoptotic cell death have also been investigated. The mPTP inhibitor cyclosporin A has successfully reduced hyperoxic injury in a murine model (Pagano et al. 2004). Similarly, the caspase inhibitor TRP601 reduces hyperoxia injury in mice (Sifringer et al. 2012).

Although the overproduction of ROS appears to be responsible for acute hyperoxia injury, the disruption of O$_2$-dependent cellular signaling is clearly important in the long-term manifestations of oxygen toxicity. Prolyl hydroxylase inhibitors that stabilize HIF-1 ameliorate hyperoxia injury in a wide range of experimental models. This relates to the fact that HIF-1α degradation is not complete under physioxic conditions in vivo (i.e., 2–6% O$_2$) (Yan et al. 2010; Bracken et al. 2006; reviewed by Stuart et al. 2019), and it can therefore play a role in growth and development. In contrast, hyperoxia that increases tissue pO$_2$ will be associated with reduced HIF-1α levels and concomitant loss of HIF-1 signaling activity. Further research is needed to determine how all of these studies can be
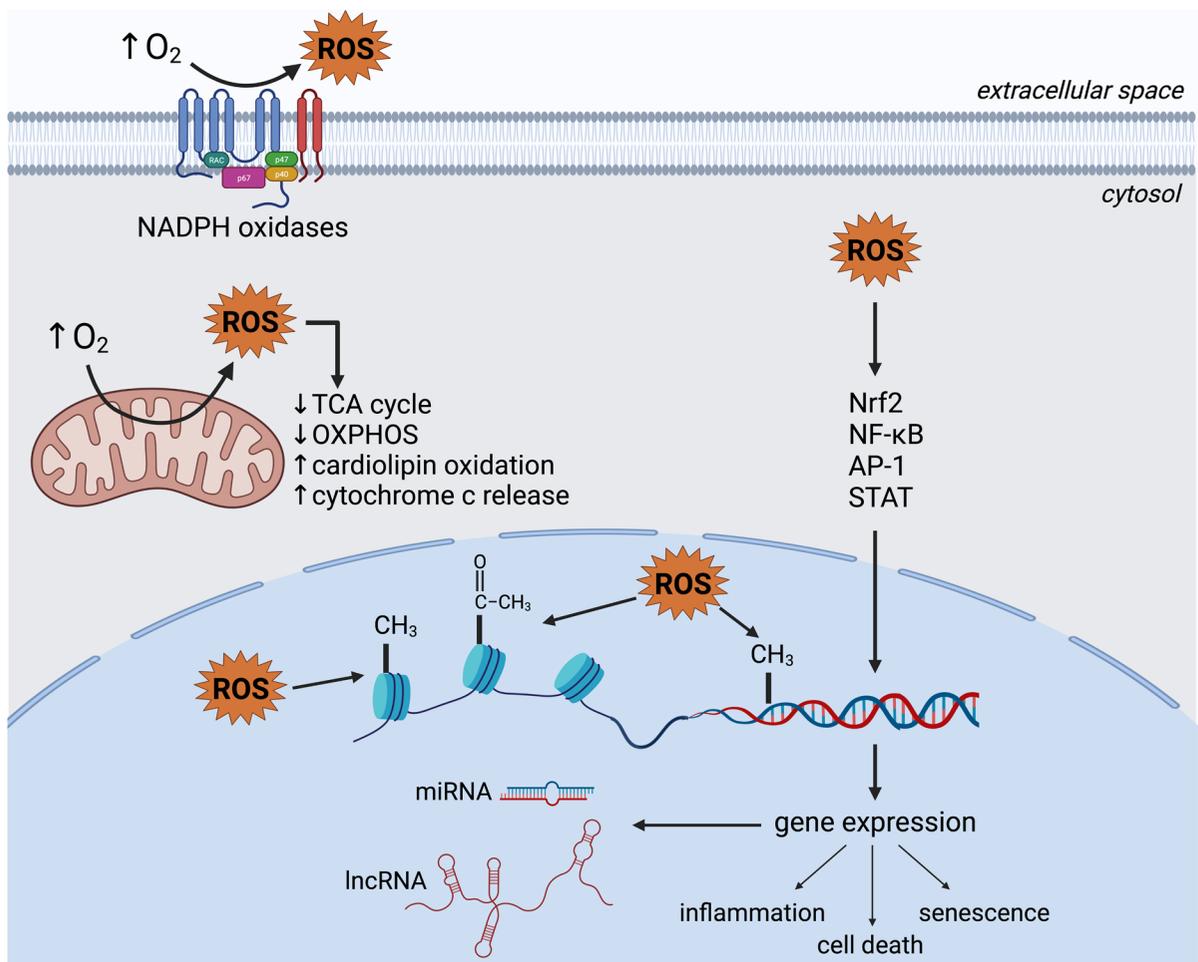

Fig. 3 Molecular mechanisms and cellular pathways of hyperoxia. Through an increased production of reactive oxygen species, hyperoxia dysregulates signaling pathways and promotes epigenetic modifications, resulting in altered gene expression, and ultimately leading to senescence, inflammation, and death. In the mitochondria, hyperoxia inhibits respiration and promotes cardiolipin oxidation and cytochrome c release, further contributing to the induction of cell death pathways. Created with BioRender.com

translated in human patients in order to evaluate their potential efficacy in clinical practice.

Conclusions and future directions

Hundreds of studies with isolated proteins and organelles, cultured cells, animal models, and humans indicate widespread oxidative injury and dysregulation of O_2-dependent signaling processes in hyperoxia (Fig. 3), leading to serious and sometimes permanent pathologies. Virtually all experiments have been of relatively short duration (< 1 week); however, oxygen supplementation in severe COVID-19 and COPD can be needed for longer periods. Furthermore, the vast majority of studies have focused on the developmental effects of oxygen toxicity in the neonate. Future studies should focus on improving our understanding of long-term effects of exposure in adults.

Since there is no apparent alternative to addressing the tissue hypoxemia associated with oxygen supplementation in patients experiencing reduced lung function, effective strategies must be aimed at ameliorating the negative effects of hyperoxia. A wide variety of approaches has been studied in pre-clinical models, and there is no shortage of candidate molecules showing some efficacy. However, given the vast range of oxygen effects on cells, this is a complex problem. Nonetheless, establishing these strategies is of immediate importance, given the ongoing need for oxygen therapy related to the COVID-19 pandemic and beyond.

Equally important, in terms of scientific data quality, is addressing the issue of oxygen toxicity in mammalian cell culture. Virtually all cell culture is performed under substantially hyperoxic conditions, with important effects on cellular activities. It would be a reasonable assumption that cells grown in standard conditions ($\sim 18\%$ O_2) may be somewhat preconditioned and thus less sensitive to the toxicity of severe hyperoxic environments ($> 60\%$ O_2) compared to cells grown at physioxia. This underlines the need for revisiting previous results obtained from cell culture-based research, including studies using in vitro hypoxia and hyperoxia models. It is thus imperative that cell culturists be aware of how the hyperoxia of cell culture affects their experiments and make adjustments to avoid this problem. While the elevated cost of commercially available O_2-regulating incubators may seem like a barrier to implement physioxia in cell culture workflows, we have recently developed an inexpensive cell culture incubator capable of maintaining physioxia (Samokhin et al. 2022) that can be employed for this purpose. Given the pervasive nature of oxygen's effects on cellular function, it is a key parameter to regulate in vitro.

Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Ethics approvals Not applicable.

Consent to participate Not applicable.

Consent for publication All authors consent to publish this manuscript.

Competing interests The authors declare no competing interests.

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