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Review

To breathe or not to breathe: Understanding how oxygen sensing contributes to age-related phenotypes

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ABSTRACT

Aging is characterized by a progressive loss of tissue integrity and functionality due to disrupted homeostasis. Molecular oxygen is pivotal to maintain tissue functions, and aerobic species have evolved a sophisticated sensing system to ensure proper oxygen supply and demand. It is not surprising that aberrations in oxygen and oxygen-associated pathways subvert health and promote different aspects of aging. In this review, we discuss emerging findings on how oxygen-sensing mechanisms regulate different cellular and molecular processes during normal physiology, and how dysregulation of oxygen availability lead to disease and aging. We describe various clinical manifestations associated with deregulation of oxygen balance, and how oxygen-modulating therapies and natural oxygen oscillations influence longevity. We conclude by discussing how a better understanding of oxygen-related mechanisms that orchestrate aging processes may lead to the development of new therapeutic strategies to extend healthy aging.

1. Introduction

Oxygen is essential to maintain tissue homeostasis and promote organismal survival. Because of constant fluctuations in oxygen concentrations, sophisticated cellular and systemic sensors have evolved in essentially all metazoans to balance oxygen demand and supply (Kaelin and Ratcliffe, 2008). Not surprisingly, aberrations in oxygen-sensing regulatory pathways contribute to various diseases and eventually to an irreversible loss of tissue functions which leads to organismal death.

A progressive loss of tissue and organ functions over time is at the basis of the aging process (López-Otín et al., 2013). In recent years, several molecular and cellular contributors to organismal aging have been identified. These include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication (López-Otín et al., 2013). Interestingly, oxygen and oxygen-sensing pathways have been implicated in a number of these hallmarks. On one side, data obtained from model organisms have demonstrated the causal role of oxygen-associated pathways in the ageing process. On another side, studies of individuals exposed to chronic hypoxic conditions or intermittent hyperoxic environments have offered the opportunity to evaluate the role of oxygen perturbations and adaptation in human health.

2. Oxygen responsive pathways and their effects on cellular physiology

2.1. Hypoxia-responsive pathways

Hypoxia inducible factors are a family of evolutionary conserved transcription factors that orchestrate adaptation to fluctuating oxygen levels via regulation of oxygen homeostasis and metabolic reprogramming. Hypoxia inducible factor 1 (HIF-1) is composed of an α- and β-subunit which both contain a basic helix-loop-helix-PAS domain that facilitates heterodimerization and DNA binding (Wang et al., 1995). Both HIF-1α and HIF-1β contain a transactivation domain essential for the activation of target genes.

In normoxic conditions, the β-subunit is constitutively expressed while the α-subunit is continuously degraded via the proteasome. (Bruick and McKnight, 2001; Epstein et al., 2001; Ivan et al., 2001; Jaakkola et al., 2001). Decreased oxygen availability (defined as

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Fig. 1. The regulation of physiology, aging and disease by oxygen associated biological processes. Several conditions can alter oxygen and associated regulatory pathways. These pathways are implicated in biological processes that regulate aging including stem cells, the immune system, cellular senescence and mitochondria. When balances, these processes lead to tissue homeostasis and promote health. However when imbalances in these processes occur this can lead to the manifestation of diseases and aging. AMPK: AMP activated protein kinase, mTOR: Mammalian target of rapamycin, ROS: Reactive oxygen species, HIF-1α: hypoxia-inducible factor, 1α, HIF-2α: hypoxia-inducible factor, 1α, HIF-3α: hypoxia-inducible factor, 3α, HIF: Hypoxia inducible factors, HDACs: Histone deacetylases, HIF: Hypoxia inducible factors, mTOR: Mammalian target of rapamycin, ROS: Reactive oxygen species.

In vertebrates, both HIF and prolyl hydroxylase have three isoforms. HIF-2α, also known as epithelial PAS domain protein 1 (EPAS1), shows structural, functional and regulatory similarities to HIF1A but differs in its interactions with other proteins.

As result of metabolic reprogramming under hypoxic conditions, energy levels drop. These changes can be sensed by the AMP activated protein kinase (AMPK). AMPK consists of a catalytic α-subunit and two regulatory subunits, β and γ, forming heterotrimERIC complexes (Xiao et al., 2007). Binding of AMP and ADP to the γ-subunit allosterically activates AMPK and allows for phosphorylation on the threonine 172 (Thr172) residue by upstream kinases.

One of the central downstream effectors regulated by AMPK is mTORC1 (González et al., 2020). Raptor, a pivotal protein in the mTORC1 complex, is directly phosphorylated, and thus inhibited, by AMPK (Gwinn et al., 2008).

Oxygen has also been shown to regulate gene expression via various epigenetic mechanisms (Choudhry and Harris, 2018). Members of the 2-oxoglutarate-dependent dioxygenase family (2-OGDO), called histone lysine demethylases (KDMs) Jumonji-type, depend on 2-oxoglutarate for their enzymatic activity and are inactivated during hypoxic conditions. KDMs have both enhancing and repressing effects on gene expression via regulation of histone methylation. In cancer, KDM3A, 2B, 4B, 4C, 5B, and 6C are transcriptionally upregulated by HIF-1α under hypoxic conditions, leading to the enhancement of hypoxia associated gene expression ultimately contributing to tumor growth and metastasis (Beyer et al., 2008; Fu et al., 2012; Krieg et al., 2010; Lee et al., 2014; Pollard et al., 2008; Xia et al., 2009).

In addition, low oxygen levels can directly inhibit KDM6A and KDM5A leading to hypermethylation of histones, subsequent uncoupling of DNA from nucleosomes and thus transcriptional activation of KDM6A/KDM5A target loci involved in biological processes such as cell fate decision (KDM6A) (Chakraborty et al., 2019), proliferation, autophagy and apoptosis (KDM5A) (Batie et al., 2019). Besides methylation, oxygen has also been shown to affect Histone deacetylases (HDACs) mainly in the context of cancer (Wu et al., 2011).

Altogether, oxygen can affect tissue functions via regulation of four main branches including HIF factors, AMPK, mTOR and chromatin remodeling proteins (Fig. 1).

2.2. Oxygen-responsive pathways in aging

Alterations in oxygen availability can affect vital functions at the systemic and cellular level and potentially contribute to aging via various mechanisms (Fig. 1).

HIF is the major responder to oxygen perturbations, and its role in aging varies depending on the organism and on inter and intra-tissue differences. Pro- or anti-aging effects of HIF are mainly mediated by its interactions with other proteins.

Upon the exposure to hypoxic oxygen conditions in human cells, mice (liver, brain, lung and kidney) and cerebral cortex of rats, the binding of HIF-1 to HRE elements and activation of HRE-responsive genes has been reported to decline with increasing age (Ahlulwalia et al., 2014; Frenkel-Denker et al., 1999; Ndubizu et al., 2009). In contrast, in other regions of the aging rat brain (Hippocampus, motor cortex and Purkinje cells in the first subfolium) (Wang et al., 2012), liver (Kang et al., 2005) and kidney (Tanaka et al., 2006) increased HIF basal activity with age has been reported, possibly due to declining vascular function and oxygen delivery to tissues.

HIF-1 interacts with several proteins associated with longevity. SIRT1, a NAD⁺ dependent deacetylase that decreases with age and which overexpression in the brain extends longevity (Satoh et al., 2013), is able to repress HIF-1α signaling by deacetylation at lys674 under normoxic conditions (Lim et al., 2010). However, this repression is lost during hypoxia, where a reduction in NAD⁺ concentration leads to SIRT1 inhibition and increased HIF-1α acetylation and transcriptional activity (Lim et al., 2010). Also in mice subjected to hypoxia, NAD⁺ levels and SIRT1 activity are reduced leading to increased HIF-1α activity and increased tumorigenesis in a xenograft model (Lim et al., 2010). In addition, re-establishment of SIRT1 levels and activity, either by genetic overexpression or resveratrol treatment, in hypoxic kidney cells, promotes HIF-1α deacetylation and a reduction in HIF-1-mediated transcriptional activity (Ryu et al., 2019). Interestingly, aged mice show a pseudo-hypoxic state associated with decreased NAD⁺ levels, compromised SIRT1 activity and increased HIF-1α expression (Gomes et al., 2013). For example, in aged mouse kidney, SIRT1 is decreased leading to the elevated acetylation and consequent transcriptional activation of HIF-1 target genes (Ryu et al., 2019). Activation of HIF-1α during aging caused repression of mitochondrial-encoded transcripts important for oxidative phosphorylation via interaction with C-MYC and downstream TMAF mitochondrial transcription factor A (Gomes et al., 2013). This suggests that the anti-aging functions of SIRT1 are at least partly mediated via HIF-1 regulation.

The fork head box O3 (FOXO3) transcription factor promotes longevity through its regulation of metabolism, cell survival, apoptosis and protein turnover (reviewed in Martins et al., 2016). FOXO3a is...
activated under hypoxia in a HIF-dependent (mostly HIF-1α and to a lesser extent HIF-2α) manner in normal mouse and human fibroblasts, and in various cancer cells (Bakker et al., 2007; Jensen et al., 2011). FOXO activation in response to hypoxia contributes to metabolic reprogramming, promotes (cancer) cell survival and limits ROS production under hypoxia (Bakker et al., 2007; Jensen et al., 2011). Similar to HIF proteins, FOXO3a has been shown to be a target for pro-oxidative degradation upon hydroxylation by PHD2, supporting a HIF-independent mechanism for FOXO3a regulation by oxygen (Zheng et al., 2014).

Chronic low grade sterile inflammation, also known as inflammaging, is a profound driver of aging and age-related diseases (Franceschi and Campisi, 2014). Several studies have reported a role of HIF-1α in regulating NF-κB-mediated cytokine production by macrophages and neutrophils, and promoting cellular survival under hypoxia (Peysson-naux et al., 2020; Walmsley et al., 2005). Similarly, a large body of evidence suggests that in malignant cells HIF-1 promotes cancer progression partially via NF-κB activation (reviewed in Balamurugan et al., 2017). Conversely, in fibroblasts, macrophages and mice, NF-κB has been shown to positively regulate the expression of HIF-1α, thus serving as a regulator of the response to oxygen (Rius et al., 2008).

mTOR suppression extends lifespan in a wide variety of organisms (Liu and Sabatini, 2020). Different studies have shown that HIF-1α can be upregulated by the PI3K/Akt/mTOR pathway in malignant (Erecinska and Silver, 2001) and normal cells (Yu and Cringle, 2005) Raptor interacts with HIF-1α through a mTOR signaling (TOS) motif located in the N terminus of HIF-1α, and mutants carrying a disrupted TOS motif show impaired HIF-1 activity during hypoxia (Land and Tee, 2007). In cancer, aberrant mTOR activation leads to the accumulation of HIF-1α and stimulates cancer cells growth. Pre-treatment of PC-3 cells with the mTOR inhibitor rapamycin inhibits both HIF-1α accumulation and HIF-dependent transcription induced by hypoxia, while transfection of these cells with wild-type mTOR enhances HIF-1 activation (Hudson et al., 2002).

A consistent impairment of AMPK activation in response to hypoxia upon aging has been reported (Mulligan et al., 2005). Genetic elevation of AMPK extends lifespan in C. elegans (Apfeld et al., 2004; Curtis et al., 2006) and Drosophila (Funakoshi et al., 2011). In addition, metformin, a drug that activates AMPK, has been shown to extend healthspan and mean lifespan in mice (Martin-Montalvo et al., 2013). This suggests that AMPK is a central regulator of pro-longevity pathway and several downstream interacting proteins have been identified.

HIF-1 and AMPK interact to regulate longevity. In C. elegans, life-span extension associated to mild oxidative stress is regulated by antagonistic feedback regulation between AMPK, which acts as an internal ROS quencher by upregulation of thioredoxin, and HIF-1, which promotes ROS via the regulation of iron uptake (Hwang et al., 2014). This suggests that a fine balance between HIF and AMPK is important to maintain optimal oxidative stress resistance to regulate lifespan in C. elegans.

AMPK signaling can activate effector proteins that are known to alleviate aging phenotypes such as SIRT1. Direct phosphorylation of SIRT1 by AMPK leads to the release of SIRT1 from its endogenous inhibitor, deleted in breast cancer 1 (DBC1) (Lau et al., 2014). Other reports have shown that AMPK activates SIRT1 via stimulation of cellular NAD⁺ levels by promoting the expression of Nicotinamide Phosphoribosyltransferase (NAMPT), an enzyme that catalyses the first step towards NAD⁺ production (Cantó et al., 2009). The activation of SIRT1 by AMPK leads to increased activity of downstream effector proteins including FOXO1 and FOXO3a that are known to regulate aging via cellular stress resistance, metabolism, cell cycle arrest and apoptosis (Cantó et al., 2009).

AMPK has also been linked to NF-κB regulation which has a profound impact on aging and age-related diseases via the regulation of inflammation (reviewed in Salminen et al., 2011).

Together, these studies suggest that AMPK regulates different health promoting biological processes via integration of diverse signaling networks mainly HIF-1, SIRT1 and NF-κB. The decline in AMPK activity over time accelerates aging and impair health.

### 2.3. Mitochondria and reactive oxygen species

The free-radical theory of aging postulates that the production of ROS is a major determinant of lifespan (Harman, 1972). ROS include free radicals such as superoxide, hydroxyl radical, and singlet oxygen, as well as nonradical species such as hydrogen peroxide formed by the partial reduction of oxygen. ROS are thought to be involved in essential biological processes, including gene transcription, protein translation and protein-protein interactions, but also in damaging proteins and nucleic acids (Droge, 2002; Giorgio et al., 2009). In order to protect cells against excessive oxidative stress, ROS levels are maintained at physiological concentration by specific enzymatic and non-enzymatic mechanisms (Marengo et al., 2016).

The mitochondrial respiratory chain represents the major source of ROS and mitochondrial dysfunctions that are linked to aging and age-related diseases. Different studies show that excessive mROS-induced oxidative damage is found in atherosclerotic lesions of both animals and humans (Wang and Tabas, 2014). For example, ROS can oxidize low density lipoproteins (LDL), which accumulate in the artery wall, participating in the formation of atherosclerotic plaques (Maiolino et al., 2015). Moreover, multiple studies underline the pivotal role of mitochondria in the progression of several age-related neurodegenerative diseases, such as Alzheimer and Parkinson. It has been demonstrated that the Amyloid Precursor Protein (APP) has a mitochondrial-targeting sequence (Lin and Beal, 2006; Pera et al., 2017), while mutations in mitochondrial DNA have been associated with familial Parkinson’s disease (Bender et al., 2006).

How hypoxia affects ROS production remains controversial. Mitochondria are the largest consumers of cellular O₂ and accumulating evidences support the role of mitochondria as putative oxygen sensors (Waypa et al., 2001) but the nature of the hypoxic signal provided by these organelles is a contentious point. The major point of debate is that it seems paradoxical that a decrease of oxygen concentration could induce an increase of its bioproducts, as for example reported in pulmonary artery smooth muscle cells exposed to hypoxia (Rathore et al., 2008). Although controversial data exist (Mehta et al., 2008), there is evidence for a feedback regulation between ROS production and the HIF pathway. Chandel et al. (Chandel et al., 1998) reported that cells without electron transport chain are incapable of HIF-1α DNA binding activity and erythropoietin expression under hypoxia. ROS might directly and indirectly regulate HIF-1α by modifying aminoacidic sequences recruiting coactivators (Carrero et al., 2000; Lando et al., 2000).

In an attempt to clarify whether ROS levels increase or decrease during hypoxia, Waypa et al. reported that oxidation increases slightly in some compartments, whereas it decreases significantly in others at low oxygen tensions, indicating that ROS originate from different sources during hypoxia (Waypa et al., 2010). More recently, it has been shown that several cellular types respond to acute hypoxia with a transient increase in superoxide production that quickly runs out (minutes) and that can be sufficient to be translated into oxidative signals contributing to hypoxic adaptation (Hernansanz-Agustín et al., 2014).

While it remains clear that excessive levels of oxygen species are detrimental and can be a cause of disease, further studies will be necessary to clarify how hypoxia can influence such levels.

### 2.4. Cellular senescence

Cellular senescence was initially described as the finite lifetime of diploid cells mirroring the aging process at the cellular level (Hayflick, 1965). Many stresses can promote cells to enter a senescence state, including telomere shortening, genotoxic and mitochondrial damages, loss of proteostasis, mitochondrial dysfunctions, unbalanced metabolic
signal and pro-oncogenic stimuli. Major regulators of the senescence-associated growth arrest (SAGA) are the Cyclin-Dependent Kinases (CDKs) p16 and p21, which arrest cell cycle at the G1-S transition phase (Hernandez-Segura et al., 2018).

Cellular senescence can be described as a two-step process in which a temporary cell cycle arrest (also known as quiescence) is converted into a more stable and generally irreversible proliferative block. The step between temporary to stable growth arrest is defined byerossion and has been shown to be mainly dependent on mTOR activity (Blagosklonny, 2018). In contrast to proliferating cells, where mTOR-mediated cellular growth is balanced by cell division, in cells with disabled capability to activate proliferation it leads to cellular hypertrophy and compensatory alterations to various cellular compartments, such as induction of enhanced lysosomal activity (Blagosklonny, 2014). Interestingly, low oxygen concentrations are able to inhibit geronvico via downregulation of the mTOR pathway without altering p21 expression, thus preventing hypertrophy and maintaining a state of temporary growth arrest which can be overcome after switching off p21 expression (Leontieva et al., 2012).

Metabolic regulations are essential determinant of organismal life-span, and this can be partly explained by modulation of cellular senescence. Overexpression of glycolytic enzymes, similar to hypoxic conditions, avoids senescence in murine embryonic fibroblasts (MEFs) cultured at atmospheric oxygen, probably by decreasing mitochondrial activity and ROS production (Kondoh et al., 2005). In addition, Parrinello et al. demonstrated that primary mouse and human cells have a markedly different sensitivity to oxidative stress, with the first more sensitive to oxygen and senescence induction due to oxidative stress and the latter more able to prevent or repair oxidative DNA damage (Parrinello et al., 2003). Recently, our group has shown that culturing amniotic fluid stem cells at 1% O2 delays the onset of a senescent phenotype and results in improved functionality (Casciaro et al., 2020).

In addition to mTOR, also the NAD+-dependent deacetylases family of sirtuins (SIRT) seems to be involved in lifespan-extension through the modulation of oxidative stress and cellular senescence (Brunet et al., 2004). Chen et al. reported that knockdown of SIRT1 induces senescence in young mesenchymal stem cells (MSCs), whereas this phenotype is reversed when SIRT1 is overexpressed, suggesting that SIRT1 is functioning as a protective regulator against aging via reduction of senescence (Chen et al., 2014). SIRT3 enhances superoxide dismutase 2 (SOD2) activity ameliorating the capacity to counteract oxidative stress in mesenchymal stem cells (Ma et al., 2020). SIRT3 seems dispensable for HSC maintenance and tissue homeostasis at a young age, but becomes in older organisms. Notably, SIRT3 is suppressed during aging and its upregulation in both aged HSCs and MSCs improves their regenerative capacity (Brown et al., 2013; Denu, 2017).

Importantly, it has been found that SIRT1 deacylates and inhibits p53 (Vaziri et al., 2001) by allowing its MDM2-mediated ubiquitination and degradation (Li et al., 2002). In most cases, p53 is the principal mediator of stress-induced senescence, in particular in response to acute DNA damage (Serrano et al., 1997). SIRT3 can also target p53, preventing neuronal damage and mitochondrial dysfunction both in a mouse cortical primary neurons model and in the cortex of AD patients (Lee et al., 2018). More controversial is how oxygen can regulate p53 activity. Hypoxia has frequently been described to be a p53 inducer via HIF-1α-mediated suppression of MDM2, with consequent prevention of p53 degradation (Chen et al., 2003), increased p53 mRNA translation (Gavrilova et al., 2003) or elevation in p53 mRNA level (Wang et al., 2004). However, p53 regulation during hypoxia remains contradictory, with several reports showing that low oxygen can reduce p53 protein level (Sermeus and Michiels, 2011). These discrepancies might be due to cell type-intrinsic differences in adapting to low oxygen, but also to more technical variables such as the severity and the duration of hypoxia (Pan et al., 2004; Sermeus and Michiels, 2011).

Senescent human cells secrete many biologically active proteins, such as cytokines, chemokines, and proteinases, collectively known as the senescence-associated secretory phenotype (SASP) (Coppe et al., 2010a), which can have both beneficial and detrimental functions (Freund et al., 2010). On one side, SASP factors reinforce the tumor suppressive function of the SAGA and participate in tissue repair and remodeling. On the other side, SASP interleukins and chemokines generate low grade chronic inflammation and are associated to various age-related pathologies, including tumorigenesis. Coppe et al. observed that senescent mouse fibroblasts do not express a human-like SASP when cultured at 20% O2, but only when maintained at 3% O2 – a more physiological condition particularly for dermal cells (Coppe et al., 2010b).

However, further reduction of O2 levels might reduce the SASP. SASP cytokines secreted by osteoclasts enhance osteoclastogenesis and bone resorption, but hypoxic conditions were shown to reduce SASP production and delaying osteoclastogenesis (Gorissen et al., 2018).

Even if cellular senescence is only one of the hallmarks of the aging process, understanding its mechanisms is essential to improve pathological conditions that are often characterized by both altered gene expression and/or oxygenation.

2.5. Stem cell function

Stem cells are implicated in embryonic development and in preserving tissue homeostasis throughout life. An appropriate balance between self-renewal and differentiation is crucial for stem cell function during both early and adult life (Morrison et al., 1997).

Embryonic compartments and adult tissues experience different oxygen tensions compared to atmospheric oxygen (21%–160 mmHg). For example, the partial oxygen pressure in the placenta has been measured to be less than 20 mmHg at 8–12 weeks of pregnancy and tends to increase along the gestation up to 60 mmHg at 12–13 weeks of gestation (Rodesch et al., 1992). Also in the majority of adult tissues, the oxygen pressure is not superior to 9% (65 mm Hg) (Brahimi-Horn and Pouysségur, 2007) and in some tissues it can drop to 1% (7.2 mm Hg) (Erecinska and Silver, 2001; Yu and Cringe, 2005).

Different studies indicate that stem cell niches are hypoxic (Parmar et al., 2007) offering a selective advantage by reducing aerobic metabolism and protecting from excessive oxidative damage. It has been reported that low oxygen tension reduces the rate of double strand breaks and telomere shortening per cell division together with an overall change in gene expression with significant transcriptional upregulation of glycolytic and carbohydrate metabolism genes in MSCs (Estrada et al., 2012). In hypoxic MSCs, the expression of AIM3, an aminoacyl-tRNA synthetase associated to aging, is repressed in a HIF-1α dependent manner. As result, stem cells with repressed AIM3 are able to upregulate autophagy and reduce OXPHOS activity, improving their functionality (Kim et al., 2019).

Stemness maintenance is controlled by multiple pluripotency transcription factors. In particular, Oct-4 is essential for preserving an undifferentiated state in embryonic stem cells (ESCs), embryonic epiblast and primordial germ cells (PGCs) (Nichols et al., 1998; Scholer et al., 1990). More recently, it has been shown that cells expressing Oct-4 are present also in different embryonic annexes (Prusa et al., 2003) as well as among adult stem cells (Greco et al., 2007; Jiang et al., 2002; Kerks et al., 2006). HIF-2α, has been found to be expressed coincidentally with Oct-4 in multiple embryonic tissues, directly regulating its transcriptional activity. Covello et al. proposed that the Oct-4 promoter is maintained in an open conformation in embryonic stem cells and PGCs and is thereby sensitive to regulation by HIF-2α. According to this model, the Oct-4 locus adopts a closed conformation in differentiating embryonic somatic cells, making it refractory to regulation by HIF-2α, which can nevertheless continue to regulate other important target genes (Covello et al., 2006). In addition, cross talk between HIF-2α and Oct-4 could maintain hypoxic tumor cells in a more undifferentiated state, since it has been previously demonstrated that teratomas over-expressing HIF-2α presented both an upregulation of Oct-4 and NANOG

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and contained undifferentiated cell types (Covello et al., 2005).

Recently, it has been demonstrated that HIF can modulate the stem cell functionality also indirectly, through a IGF-1R-HIF-2α-Oct-4-CXCR4 signaling loop important to maintain Oct-4 expression, cell proliferation and migration of mouse germline stem cells (Kuo et al., 2018). Importantly, the stabilization of Oct-4 at low oxygen tensions induces a delay in the expression of senescence associated markers, such as p21 (Tsai et al., 2011), while maintaining higher cyclin B (Choi et al., 2017) than the normoxic condition. Oct-4 achieves these functions through multiple mechanisms. For example, downregulation of p21 is achieved by enhancing the expression of different DNA methyltransferases (Li et al., 2019). Furthermore, in ESCs it has been reported that Oct-4 can bind to the promoter region of mT-302a and in this way it can induce cells to enter S phase through cyclin D1 (Greer Card et al., 2008).

Thus, it seems clear that hypoxia preserves stemness. However, controversies exist about the role of oxygen tension in controlling stem cell differentiation. For example, several studies investigated the impact of hypoxia on MSCs differentiation in vitro showing contrasting results, in part due to the different cellular sources and the differentiation protocols used (Lennon et al., 2001; Yang et al., 2011). Standardization of differentiation procedures should be implemented for a better understanding of the mechanisms involved in stem cells aging and for the application of new approaches in cellular therapy.

2.6. Immune system and immunosenescence

Immunosenescence is defined as the progressive deterioration of the immune system and a decline in immune efficacy with age which leads to vulnerability to infectious diseases, blunted vaccination immunogenicity, and susceptibility to inflammatory diseases (Fulop et al., 2018). In part, this is due to hematopoietic stem cell exhaustion which is driven by oxidative stress and can be prevented by antioxidant treatment (Ito et al., 2004). However, other disturbances in anti-and pro-inflammatory immune responses are reported.

Immune cells differentiate, mature, migrate and act in different parts of the body and are therefore exposed to various oxygen tensions during their lifetime. For example, studies using non-invasive methods in mice revealed that parts of the bone marrow, the primary site of immune cell production, are hypoxic (app. 1.3%) (Spencer et al., 2014). However, in the spleen, an organ pivotal for immune cell maturation and expansion, oxygen gradients exist, reaching from 0.5% to 4.5% (Braun et al., 2001).

A number of genetic mouse models have shown that HIF-1α is an essential player in innate immunity. Walmsley and colleagues were the first to discover that hypoxia increases neutrophil survival in a HIF-1α-NF-kB dependent manner. Patients with normally occurring mutations in VHL, leading to constitutively active HIF-1α, show delayed neutrophil apoptosis suggesting that HIF-1α regulates their survival (Walmsley et al., 2005, 2006). Myeloid-specific deletion of HIF1A in mice inhibits the invasion of neutrophils and macrophages in a model of skin inflammation (Cramer et al., 2003). More recently, it was discovered that upon systemic inflammation, HIF-2α becomes activated in blood neutrophils and promotes their survival (Thompson et al., 2014).

Macrophages have two activated forms: M1 and M2. M1 macrophages clear pathogens in a ROS-dependent manner, whereas M2 macrophages resolve inflammation and favor oxidative metabolism. Imbalance between these two states is associated with inflammatory disease and represent a hallmark of immunosenescence. Oxygen responsive pathways play a key role in macrophage polarization and hence influence the M1/M2 ratio of macrophages. In a model for ischemia reperfusion, Takeda et al. identified that PHD2 haptodefiency drives macrophage polarization towards M2 leading to increased arteriogenesis via NF-kB-mediated expression of arteriogenic factors (Takeda et al., 2011). In addition, HIF-1α and HIF-2α have opposing roles in macrophage subtypes: HIF-1α becomes activated in M1 macrophages whereas HIF-2α in M2 macrophages (Takeda et al., 2010). AMPK drives metabolic alterations in macrophages leading to polarization into the M2 state and promoting an anti-inflammatory functional phenotype (Sag et al., 2008). In addition, AMPK activation suppresses macrophage proliferation (Ishii et al., 2009).

Also cells of the adaptive immune response have been shown to be affected by oxygen and oxygen responsive pathways. A specific set of T-cells called Th17 cells secrete pro-inflammatory cytokines (mainly IL17) and therefore promote inflammation, whereas Treg cells contradict this reaction by the secretion of anti-inflammatory cytokines and suppression of a variety of immune cells. An imbalance between Treg cells and Th17 cells is reported to occur in aging and immunosenescence (Schmitt et al., 2013).

HIF-1α is activated under normoxic conditions in Th17 cells via the transcription factor STAT3. In these cells, HIF-1α interacts with p300 and RORγt, a key transcriptional regulator important for Th17 development, to regulate IL17 transcription (Dang et al., 2011). Similarly, exposure of T-cells to hypoxic conditions leads to increased numbers of IL17+ cells, suggesting that oxygen can influence T-cell development and inflammatory responses (Dang et al., 2011). In contrast, constitutive activation of HIF-1α in thymocytes (lymphocytes in the thymus) by VHL inactivation leads to increased apoptosis which is reversed upon subsequent HIF-1α inactivation (Riju et al., 2004).

Exposure of Cytotoxic T-cells (CD8+) to hypoxic conditions show increased lytic capacity and secretion of cytokines compared to cells in 20% oxygen (Caldwell et al., 2001). Interestingly, Lucashev et al. proposed that a specific short isoform of HIF-1α inhibits secretion of pro-inflammatory cytokines by T-cells hence controlling the immune response via a negative feedback loop (Lukashev et al., 2006).

mTORC1 has been shown to play a key role in cytotoxic T-cell differentiation by controlling metabolism via HIF-1α. Finlay and colleagues identified that mTORC1 is essential to activate and sustain a HIF-1α-dependent transcriptional program that includes the upregulation of glycolytic enzymes, cytolytic effector molecules, and essential chemokine and adhesion receptors. This mTORC1-HIF-1α dependent program is essential for T-cell effector function and migratory properties (Finlay et al., 2012).

B-cells are, among other stimuli, activated by antigens and helper-T-cells and regulate antibody production and immune memory. It has been shown that HIF1α deficiency in mice results in lineage-specific defects in development of B lymphocytes and autoimmunity (Kojima et al., 2002). B-cells and B-cell lymphoma cells exposed to hypoxia upregulate chemokine receptor 4 (CXCR4) in a HIF-1α-dependent manner which is essential for B-cell organ specific homing and lymphoma invasion (Piovan et al., 2007). Recently, Abbott et al. showed that exposure of cells to hypoxia promotes plasma cell formation and antibody production which were severely restrained in vivo after application of respiratory hyperoxic (60%) oxygen (Abbott et al., 2016).

In summary, oxygen and oxygen responsive pathways have profound roles in the normal function of both innate and adaptive immunity, and disturbances in these pathways contribute to immunosenescence and inflammatory diseases.

3. Effects of oxygen and oxygen-related pathways in disease

Different disease states including COPD, ischemia-reperfusion injury and anemia are linked to inadequate tissue oxygenation. Oxygen associated pathways play a profound role in these pathologies and their dysregulation might be targeted for therapies. Below, pathological states deriving from aberrations in oxygen homeostasis are described and the roles of different isoforms of HIF, AMPK and mTOR in these diseases are reviewed.

3.1. COPD

Chronic obstructive pulmonary disease (COPD) is a complex disorder characterized by a progressive and persistent limitation of pulmonary
Polosukhin et al., 2011). In addition, HIF-1α in the heart which is lost in animals with only one copy of the HIF1A gene (Cai et al., 2003). In addition, HIF-1α is activated in renal I–R and regulates survival of tubular epithelial cells, inhibits the expression of NF-kB mediated pro-inflammatory cytokines and concomitant immune cell infiltration (Conde et al., 2017). Contrasting studies show that HIF-1α upregulation is detrimental by promoting necrosis, inflammation and oxidative stress via the direct activation of MiR21a which regulates several downstream effectors important for these processes (Shen et al., 2019). In support to a detrimental role of HIF-1α in this context, NOX4, a major source of ROS in I–R, is a direct target of HIF-1α (Diebold et al., 2010).

AMPK might play a protective role during I–R events. Macrophage Migration Inhibitory Factor (MIF) is induced during cardiac I–R and activates AMPK through stimulation of its receptor CD74, leading to increased glucose uptake and protection of cardiomyocytes from cell death (Miller et al., 2008). In addition, treatment with pharmacological activators of AMPK before renal I–R reduces tubular injury and improves organ functionality (Lieberthal et al., 2016).

3.3. Anemia

Another context in which hypoxia-responsive pathways are dysregulated is anemia, a condition characterized by a reduced quantity of red blood cells (RBCs). Low number of RBCs impairs the transport of oxygen to tissues and causes impaired physical and cognitive capacity and increased risk for co-morbidities. Anemia arises during a wide variety of pathological conditions including RBC intrinsic disorders, hemoglobin alterations, malnutrition, chronic inflammation, chronic kidney disease and cancer (reviewed in Sankaran and Weiss, 2015).

The HIF pathway is a profound regulator of erythropoiesis through its regulation of Erythropoietin (EPO), a cytokine that is predominantly produced by cells in the kidney and stimulates erythroid precursor cells to differentiate into RBCs in the bone marrow. Although HIF-1α was discovered for its binding and regulation to the HRE element in the EPO gene in vitro (Semenza and Wang, 1992), it became clear that HIF-2α is the dominant regulator of EPO during adulthood (Gruber et al., 2007). In line with this, naturally occurring loss-of-function and gain-of-function mutations in the HIF2 gene affect RBC counts in humans (Lee and Percy, 2011). Furthermore, genetic augmentation of HIF-2 in progenitor osteoblasts in mice leads to a significant expansion of erythrocyte progenitor cells via the stimulation of EPO secretion in these cells (Rankin et al., 2012). Mice with increased levels of HIF-2 in osteoblasts also showed protection from stress-induced hemolytic anemia (Rankin et al., 2012). Because of this crucial role of HIF-2 in EPO production and erythropoiesis it is not surprising that HIF activation by treatment with propyl hydroxylase inhibitors shows promising results for the treatment of anemia in humans (Maxwell and Eckardt, 2016).
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Taken together, alterations in oxygen homeostasis can contribute to different aspects of COPD, ischemia reperfusion (injury) and anemia (Fig. 2).

4. Oxygen perturbations and effect on lifespan of model organisms

Environmental oxygen levels modulate health and lifespan in different experimental model organisms. Below we will review the effects of oxygen on lifespan and age-related conditions in *D. Melanogaster*, *C. elegans* and *M. musculus*.

4.1. *D. Melanogaster*

Although Drosophila is resistant to acute anoxia, several studies revealed that chronic exposure to hypoxia (<2%) or hyperoxic oxygen conditions (>20%) during adulthood impairs lifespan, most likely because of an increase in protein carbonylation and lipid peroxidation (Rascón and Harrison, 2010; Zhao et al., 2012).

In contrast to severe hypoxia, moderate hypoxia (10 kPa, app. 9.8% O₂) increases maximum, but not median, lifespan (Rascón and Harrison, 2010), and improves age-related cardiovascular and musculoskeletal dysfunctions (Li et al., 2020).

The mechanistic understanding of how hypoxia mediates lifespan extension remains limited, but might be related to modulation of oxidative stress as mild mitochondrial distress. Activation of the mitochondrial Unfolded Protein Response (UPR) (Copeland et al., 2009; Owusu-Ansah et al., 2013), or moderate hypoxia induces relatively mild levels of ROS leading to accumulation of fat droplets in stem cell niches of the brain maintaining glial progenitor cell proliferation, a cell type that is important for the flies health (Bailey et al., 2015). In addition, exposure of flies to hypoxia leads to TSC2-mediated TORC1 inhibition, a mechanism associated to lifespan extension in flies and other organisms (Lee et al., 2019).

4.2. *C. Elegans*

*C. elegans* is resistant to extremely high or low oxygen concentrations. It shows no signs of mortality or changes in metabolic rates even when continuously exposed to 100% O₂ for more than 35 generations (6 months) (Van Voorhies and Ward, 2000), and recovers in 3–4 h after a 24 -h exposure to anoxic conditions (Van Voorhies and Ward, 2000). Despite showing a preference for conditions in a range between 5% and 12% O₂ (Gray et al., 2004), worms exposed to hypoxic oxygen conditions (0.5%) have a significantly increased lifespan (Leiser et al., 2013).

The mechanisms regulating lifespan extension under hypoxia are largely unknown, but the effect has been shown to depend on HIF and DAF16 (FOXO ortholog) activation (Leiser et al., 2013; Zhang et al., 2009). In accordance, VHL inactivation – which results in constitutive HIF-1 activation – increased lifespan via reduction of proteotoxic stress (Mehta et al., 2009). In addition, lifespan extension was shown in worms with genetic disruption of the electron transport chain, similar to observations in *D. Melanogaster* (Mishur et al., 2016; Yang and Hekimi, 2010; Yee et al., 2014). These data emphasize that HIF can also have pro-longevity functions and thus plays a dual role during aging.

4.3. *M. musculus*

Exposure of mice to hyperoxia or hypoxic oxygen conditions has been shown to impair healthspan and accelerates a wide variety of diseases. For example, exposure to hyperoxia (100%) at birth shortens lifespan and increases pulmonary disease and cardiac failure at later age (Yee et al., 2011).

Similar to observations in *C. elegans* and *D. Melanogaster*, intermittent mild hypoxia (90mmHG, app. 11.8%) in combination with hypercapnia (50mmHG, app 6.5%), a state of increased CO₂ has been reported to increase median lifespan (16%) (Kuklikov et al., 2019). In addition, intermittent exposure to hypoxia/hypercapnia improves reproduction, grip strength, locomotor activity and reduction in physical stamina in old mice (Kuklikov et al., 2019). A gradual decrease (1% per day) in oxygen tension to hypoxia (7%) for 2 weeks after myocardial infarction significantly decreases mitochondrial oxidative damage and re-entry of adult cardiomyocytes into the cell cycle resulting in functional improvement and heart regeneration (Nakada et al., 2017). In contrast, longer exposure of mice to hypoxic oxygen conditions can result in increased mortality (Nakada et al., 2017).

Reduction of respiration and mitochondrial function could explain the lifespan extension observed in mice under mild/intermittent hypoxia. Heterozygous loss of Mclk1, a mitochondrial hydroxylase of the electron transport chain, leads to a pronounced increase in lifespan (Liu et al., 2005). The increase in lifespan seen in mclk1 +/- mice is associated with decreased mitochondrial function and decreased TCA cycle activity (Lapointe and Hekimi, 2008). However, complete knockout of Mclk1 is lethal suggesting that stress resistance only occurs when primed with mild pre-exposure to stress (Liu et al., 2005; Wang et al., 2015).

Together, current literature indicates that the correlation between oxygen and life- and healthspan is non-linear, showing detrimental effects of severe hypoxic and hyperoxic conditions, but beneficial effects of mild hypoxia. This effect is possibly explained by the concept that mild stress during life triggers survival and stress resistance pathways that protect organisms from other stresses later in life (hormesis).

5. Health- and lifespan of people living at high altitude

Approximately 140 million people worldwide populate geographical regions at high altitude (>2500 m above sea level) from which the most well-studied are people living at the Tibetan Plateau in Asia (3000–4500 m) and the Andean Altiplano in South America (2500–4500). Less extreme altitudes can be found in Southern Europe (and parts of the USA). Among other conditions, oxygen tensions are significantly lower in these regions and hence providing a useful study population to investigate the relation between hypoxia and human health and longevity.

Acute exposure of non-adapted individuals to hypoxia due to short-term residence in high altitude environments (>2500 m) can lead to the development of a set of symptoms including vomiting, insomnia, dizziness, and lassitude or fatigue, commonly referred to as altitude sickness (Hackett and Roach, 2001). In some rare cases, altitude sickness can progress into pulmonary and cerebral edema, which can be life-threatening, showing the detrimental health effects of acute hypoxia (Hackett and Roach, 2001).

In the case of chronic exposure to hypoxic conditions as a result of living at extreme high altitudes, health benefits and reduction of mortality from different diseases have been reported. Studies performed in ethnic homogenous groups in the Himalaya have reported a lower incidence of hypertension, obesity and diabetes in individuals living at higher altitude (>3500 m) (Negi et al., 2012). Recently, a cross-sectional survey study among >5000 people living at different altitudes in Derong, China, found a lower risk of dyslipidemia (elevated blood lipid levels) and obesity in individuals residing at >3000 m of altitude (Huang et al., 2020). Even though these two studies report health benefits of extreme high altitudes, a substantial amount of data shows that living at extremely high altitudes might also have negative effects on human physiology and pathology. A subset of adapted highlanders lost tolerance to chronic hypoxia over time, resulting in increased RBC production (erythrocytosis) and higher risk to develop cardiovascular and metabolic dysfunctions such as hypertension, elevated fasting serum glucose, insulin resistance, and elevated fasting serum triglycerides (Corante et al., 2018). A cross-sectional study on students living at different altitudes (3500–4500 m) in Tibet found a higher incidence of congenital heart disease in subjects living at the
highest elevations (Chun et al., 2019). Lifespan studies on people living at extreme altitudes are limited but Tibetan highlanders have typical shorter lifespan compared to people living at lower altitudes (Li et al., 2017). In contrast, the number of centenarians is significantly higher at extreme high altitudes in China, compared to other regions located at lower elevations (Li et al., 2017).

One explanation for these opposing results is that the ability to survive at extreme high altitudes is the result of exceptional genetic and physiological evolutionary adaptation which differs between geographical distinct populations (reviewed in Witt and Huerta-Sánchez, 2019). Tibetan highlanders have reduced hemoglobin (Hb) concentration, decreased arterial oxygen pressure, increased resting ventilation and increased blood flow (Beall, 2007; Chen et al., 1997; Groves et al., 1993; Wu et al., 2005). These adaptations are most likely explained by a unique set of SNPs in genes of the HIF pathway such as EGLN1 (Coding for PHD2) and HIF-2α (Beall et al., 2010). Several studies have shown that loss-of-function mutations in the EGLN1 gene can explain lower hemoglobin expression in highlander Tibetans (Huerta-Sanchez et al., 2014; Lorenzo et al., 2014; Simonson et al., 2010; Yi et al., 2010). Different from people that populate the Tibetan plateau, Andean highlanders show increase HB levels, normal resting ventilation and higher arterial oxygen content compared to native Americans living at low altitudes (Beall, 2006). Although positive selection of SNPs in genes related to the HIF pathway have been identified in these populations (Bigham et al., 2010), adaptations to low oxygen levels are, at least in part, explained by genetic variants leading to differences in cardiovascular system development (Crawford et al., 2017). These results suggest that living at extreme high altitudes has a negative effect on lifespan except for individuals that show distinct evolutionary-selected mechanisms to cope with such extreme environmental conditions.

Living in less extreme altitudes, but still in environments with lower oxygen pressure, is also correlated to health benefits. A study conducted among individuals living in Switzerland showed a significant reduction in mortality from coronary heart disease and stroke with the increase of altitude (Faeh et al., 2009). In addition, being born at higher altitude has additional and independent beneficial effects on mortality from coronary heart disease in comparison to spending shorter periods of residency (Faeh et al., 2009). A clinical study of arteriosclerotic patients living at different altitudes revealed lower plaque burden and less arterial calcification in patients living at >2000 m of altitude compared to patients living at sea level (Cao et al., 2020). Lopez-Pascual et al. conducted a study among graduate students in Spain living at different altitudes and defined a decreased risk for developing metabolic syndrome with increased altitude (Lopez-Pascual et al., 2017). In the USA, an epidemiological study showed extended life expectancy and reduced mortality due to ischemic heart disease in individuals at altitudes higher than 1500 m (Ezzati et al., 2012). Another recent study in the USA revealed an inverse correlation between the development of diabetes type 2 and living at high altitude (between 1500 m and 3500 m) (Woolcott et al., 2014). These data suggest that living at higher altitude leads to protective adaptations and a potential consequent decreased risk to develop different age-associated morbidities.

Together, epidemiological studies show controversies in health effects of high altitudes. It seems clear that living at moderate altitude is beneficial for health mainly due to a reduced risk for the development of cardiovascular and metabolic conditions. Regarding extreme high altitudes, health benefits do not outcompete negative effects that can lead to shorter lifespan. The underlying molecular mechanisms remain largely unknown, and the studies summarized here are mostly correlational. Also, it should be taken into account that the phenotypes described above are most likely multifactorial and should be investigated in more detail to elucidate causal relations.

6. Oxygen therapies

Oxygen-related pathways could represent a potential target for medical interventions in various diseases.

Hyperbaric oxygen therapy (HBOT), where patients are treated with 100% oxygen at pressures greater than 1 atm, results in attenuation of HIF-1α-mediated effects (Terraneo et al., 2014), enhanced angiogenesis (Hopf et al., 2005), reduced inflammation, increased regenerative capacity after injury (Oyaizu et al., 2018) and increased antimicrobial activity (Memar et al., 2019).

HBOT might increase the production of reactive oxygen species (ROS) and thus promote the expression of cytotoxic antioxidant responses (Simsek et al., 2011). For example, molecular chaperones including the Nrf2-regulated antioxidant genes are upregulated in endothelial cells exposed to HBOT and contribute to protect from oxidative stress (Godman et al., 2010). In addition, evidence of beneficial effects of HBOT are in the skin, where it can improve ischemic wound healing via the ROS/MAPK/MMP signaling axis (Zhang and Gould, 2014), and significantly reduce apoptosis and proliferation upon exposure to UV-A (Fuller et al., 2013).

Because of the increased ROS production, HBOT has been successfully tested for antimicrobial activity in the treatment of Pseudomonas aeruginosa (Kolpen et al., 2017; Lima et al., 2015).

Hyperoxegenation also promotes a more rapid recovery from muscle injuries. On one side, it reduces the volume of contused limbs during the acute phase of the repair (Oyaizu et al., 2018). On the other side, it activates earlier the IL-6/STAT3 pathway, stimulates the production CD163+ and CD206+ cells anti-inflammatory macrophages, and promotes satellite cell proliferation and differentiation, resulting in an increased regeneration of muscle fibers and muscle strength (Oyaizu et al., 2018). More recently, a clinical study has shown a potential rejuvenation effect of intermittent HBOT (Hachmo et al., 2020). Repeated short daily HBOT sessions increased PBMC telomere length by more than 20% and decreased the number of senescent cells by 10–37%, particularly T helpers cells, in aged individuals (Hachmo et al., 2020).

Despite the benefits listed above, HBOT has known side effects. Physiological responses to higher oxygen pressure of body air cavities lead to difficulty in middle ear equalization (O’Neill and Weitzner, 2015), paranasal sinuses pressure sensation (Camposori, 2014), dental barotrauma (Heyboer et al., 2017a) and emphysematous bulla (Heyboer et al., 2017a).

Hyperoxia-induced antioxidant mechanisms can promote seizures partly via excessive lipid peroxidation of plasma membrane (Torbati et al., 1992). This event is quite rare but recent evidences suggest that its incidence increases in patients with comorbidities (Heyboer et al., 2017a). Continuous exposure of the lungs to elevated levels of oxygen results in severe pulmonary damage with decreased lung compliance, decrements in inspiratory and expiratory lung volumes and rates, and decreased CO2 diffusing capacity. Symptoms of pulmonary oxygen toxicity are chest discomfort and dyspnea (Claireaux, 1975). Similarly, there is a theoretical risk of pulmonary edema in patients with compromised left ventricular function who are undergoing HBOT. HBOT can also cause an increase in blood pressure both in hypertensive and normal patients, even if the effects on blood pressure seem mild (Heyboer et al., 2017a). Lastly, increased ROS concentrations can have harmful effects on ocular functions leading to myopia and cataract (McMonnies, 2015). Together, these evidences suggest that HBOT could represent an interesting strategy to reduce disease burden, but more research is required to determine its dosing and indications to limit toxicity.

Recently, a class of drugs known as hypoxia mimetic agents has been developed. Most drugs of this family activate hypoxia-responsive pathways via inhibition of PHD2, the enzyme responsible for HIF degradation (Yeh et al., 2017).

Most research on these molecules has been conducted in models of chronic kidney disease (CKD)-induced anemia. Anemia is a common
complication of CKD, mainly due to a decreased production of renal EPO, and includes iron deficiency, blood loss, reduced erythrocyte survival duration and inflammation (Babitt and Lin, 2012). By stabilizing HIF, hypoxia mimetics induce the activation of multiple pathways, and promote EPO production. Furthermore, these molecules are able to counteract the effects of hepcidin, which levels increase in CKD, altering iron metabolism. The most advanced PHDs inhibitors in the clinic are Roxadustat (Dhillon, 2019), Daprodustat (Bailey et al., 2019), Molidustat (Akizawa et al., 2019) and Vadadustat (Pergola et al., 2016). In particular, Roxadustat received approval in China for the treatment of CKD-induced anemia in patients who are dialysis-dependent, but more studies are ongoing to assess the efficacy in not dialyzed patients and the risk of adverse events such as hyperkalaemia and metabolic acidosis (Chen et al., 2019). All the other drugs have shown efficacy in phase 2 trials and are under investigation in phase 3 trials.

Several studies have also been conducted to elucidate the effects on ischemia prevention both through mechanisms dependent or independent from PHDs inhibition (reviewed in Davis et al., 2019). These compounds include DMOG, a classical non-specific 2-oxoglutarate analogue (a tricarboxylic acid cycle intermediate), which represents a cofactor of PHDs (Davis et al., 2019).

Due to their ability of increasing erythropoiesis and tissue resistance to hypoxia, these molecules have gained attention of the scientific community also because of their use in sports doping. For example, the novel hypoxia mimetic candidate GSK360A, which has been shown to have beneficial effects not only on cardiac function but also on skeletal muscle by increasing PDK1 and transferrin, has been questioned by anti-doping authorities (Bao et al., 2010; Beuck et al., 2012). Furthermore, cobalt, an essential trace element, has been added to the doping agents list in 2017 due to its ability to mimic hypoxia (https://www.wada-ama.org/sites/default/files/resources/files/2016-09-29_wada_prohibited_list_2017_eng_final.pdf). The majority of biological effects of cobalt are mediated through HIF-1 activation which results in increased EPO production, up-regulation of intestinal divalent metal transporter 1 (DMT1) and hepatic ferritin and transferrin expression, thus increasing erythropoiesis and oxygen-transport function (Skalny et al., 2018).

Together, these evidences suggest that both HBOT and hypoxic mimetics could represent new drug classes, but more research is required to determine their dosing and collect more indications to assess side effects to use them safely for treating more diseases in the future (Fig. 3).

7. General considerations and concluding remarks

Oxygen is an essential driver of a multitude of cellular processes in most animals. Multiple molecular systems have been developed to quickly adapt to oxygen oscillations, and HIF factors represent their major regulators. Exposure to severely hypoxic or hypoxic environments seems to accelerate age-related diseases in animal models. In contrast, mild hypoxia conditions promote longevity (Rascon and Harrison, 2010), and intermittent hypobaric therapy has been demonstrated to be a potential efficient treatment for inflammatory-driven conditions (Tibbles and Edelsberg, 1996). These apparent controversial outcomes could be due to the different ability of cells to adapt at oxygen fluctuations depending on the exposure length and severity, together with the initial health state. For example, it has been reported that low oxygen tension is able to inhibit geroconversion by downregulating the mTOR pathway (Blagosklonny, 2013), while some other studies report that HIF-1α can activate the pro inflammatory NF-kB pathway, thus accelerating cancer progression (D’Ignazio and Rocha, 2016). So, to breathe or not to breathe? Further investigation should be conducted to clarify the complicated relationship among oxygen and longevity, but the data collected until now suggest an enormous potential for the use of oxygen modulation as a therapeutic option for extending healthy aging.

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