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NON-THEMATIC REVIEW

Oxygen regulates molecular mechanisms of cancer progression and metastasis

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Abstract Oxygen is the basic molecule which supports life and it truly is “god's gift to life.” Despite its immense importance, research on “oxygen biology” has never received the light of the day and has been limited to physiological and biochemical studies. It seems that in modern day biology, oxygen research is summarized in one word “hypoxia.” Scientists have focused on hypoxia-induced transcriptomics and molecular–cellular alterations exclusively in disease models. Interestingly, the potential of oxygen to control the basic principles of biology like homeostatic maintenance, transcription, replication, and protein folding among many others, at the molecular level, has been completely ignored. Here, we present a perspective on the crucial role played by oxygen in regulation of basic biological phenomena. Our conclusion highlights the importance of establishing novel research areas like oxygen biology, as there is great potential in this field for basic science discoveries and clinical benefits to the society.

Keywords Oxygen biology · Cancer · Metastasis · Replication · Transcription · Translation · Protein folding · Cell motility · p53 · Cancer therapeutics · Oxygen therapy

1 Introduction

The emergence of an oxygen-based atmosphere altered the basic cellular molecular organization of primitive cells to their multicellular forms. With the emergence of multicellular life, our understanding of the role of oxygen became limited to its association with energy generating pathways. Modern science is not focusing on the involvement of oxygen in basic biological processes like DNA replication, transcription, translation, protein modifications, cellular signaling, and cell death.

In our opinion, oxygen-dependent regulation of the molecular machinery of cells is a central process in disease development and understanding these processes will result in novel therapeutic strategies. Diseased organs show altered oxygen signaling in comparison to healthy ones. Diseases of global concern including cancer, cardiac disease, and neurological disease have an underlying component of oxygen-based modifications of cellular and molecular machineries. However, there is a basic biology side to this research which again is ignored, and scientific efforts here have the potential to provide society with major scientific breakthroughs.

Interestingly, health benefits of ancient eastern practices of oxygen-based exercises, including pranayam, which is a practice of artificially controlling breathing rates have regularly been reported [1]. Again, in our opinion if oxygen has the potential to show health benefits, then the accompanying changes in cellular and molecular biology should

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be understood. The basic biological information of the impact oxygen has on cellular processes will help strategize present day therapies.

Several reports have focused on the role of oxygen availability in disease models such as hypoxic tumors [2], myocardial infarction [3], hyperoxic lung injury [4], and hyperoxic retinopathy [5], but the focus has remained on hypoxia and disease physiology. One single area which deals with hypoxia, cancer, and cardiovascular diseases in relation with HIF transcription factor has been researched in-depth manner at the molecular level.

Despite the fact that oxygen plays a critical role in the regulation of the molecular processes of life, there is no report which comprehensively presents and summarizes this point of view. The present review integrates existing research at the molecular level along with our understanding of the impact of oxygen on the basic biological processes of life such as transcription, DNA replication, cell cycle progression, protein folding, apoptosis, senescence, and cellular motility. This review highlights the importance of oxygen biology as a research area and the need for the recognition of this interesting yet untouched research field.

2 Role of oxygen in regulation of cellular transcription

The impact of oxygen on transcription holds significant importance as it facilitates a rapid, reversible, and well-controlled response to oxygen fluctuations. The survival of cells, whether physiological or pathological, depends on energy production through oxygen. Low oxygen availability is a cellular stress, and as a response to this, the cellular system evokes changes in transcriptional and signaling pathways to overcome stress-induced cell death. Although these systems are designed for the survival of non-pathological cells, several types of cancers that form solid tumors and metastasize exploit oxygen-dependent transcriptional machinery. Hypoxia-inducible factors (HIFs) play a central role in mediating these pro-survival changes by transcriptionally activating genes involved in the regulation of cellular metabolism, angiogenesis, cell proliferation, and viability [6] (Fig. 1). After activation, HIF1α moves to the nucleus and binds to the consensus DNA site BACGTSSK (B = G/C/T, K = G/T, and S = G/C), termed as the hypoxia response element (HRE) [7]. Several established targets of HIF1a (like VEGF, NOS2, EPO [8, 9]) help in maintaining the metabolic and divisional balance of the hypoxic cells. Emerging targets of HIF1α show its involvement in regulating cellular differentiation (ID2 and Gelactin-1), cell survival (cyclooxygenase-2), proliferation (c-myc), metastasis (snail), etc. [10–15]. Thus, HIFs-dependent regulation of transcription of these genes results in maintenance of cellular homeostasis, saving hypoxic cells from death or senescence. Maintaining cellular metabolism is a critical homeostatic response during oxygen shortage since the lack of oxygen limits oxidative phosphorylation (OXPHOS), pushing cells towards higher glycolysis. HIF1α increases glucose influx by inducing glucose transporters GLUT1 and GLUT3 [16, 17], and upregulates the expression of MCT-4 to help increase the efflux of lactic acid in hypoxic cells exhibiting higher glycolytic rates [18] (Fig. 1). Several enzymes of the glycolytic pathway like aldolase A, phospho glycerate kinase 1, and pyruvate kinase are directly induced by HIF1α [19]. HIF1α upregulates the expression of PFKFB3 gene and fructose-2,6-bisphosphate which allosterically activates 6-phosphofructo-1-kinase (PFK-1), thereby increasing rates of cellular glycolysis in hypoxic cells [20] (Fig. 1). Thus, pathways that ensure energy generation through glycolysis in hypoxic cell are transcriptionally induced by HIF1α. The reduction in OXPHOS is an expected outcome which is also achieved by alterations in the transcriptional machinery. HIF1α activates pyruvate dehydrogenase kinase-1 which phosphorylates pyruvate dehydrogenase resulting in lower acetyl-CoA and thus reduced input into the tricarboxylic acid cycle [21, 22]. Another mechanism to optimize oxygen usage includes a subunit switch in cytochrome c oxidase (COX) during hypoxia, which is driven by HIF1α-dependent transcription of COX4 and Lon which favors COX4-2 subunits over COX4-1 [23]. Thus, in rapidly dividing cancer cells which often experience low oxygen levels, there exists an intricate network of HIF1α-mediated transcriptional regulation which functions as elegant oxygen shortage-sensing machinery and alters cellular transcription to maintain homeostasis and fulfill critical energy demands of hypoxic cancer cells.

Oxygen alterations, especially hyperoxia-induce redox imbalance in cells and its maintenance is a crucial step in achieving cellular homeostasis. The NF-κB family of proteins are emerging as important transcription factors (TF) responsible for maintaining redox homeostasis. Disturbance of redox balance under hyperoxia [24] results in redox-dependent activation of NF-κB [25, 26], in addition to other transcriptional factors like AP-1, specificity protein-1 (SP-1), and p53 [27–29]. The NF-κB–thioredoxin system maintains oxygen-dependent homeostasis by regulating cellular expression of the proteins thioredoxin (Trx), thioredoxin reductase, and NADPH [30]. Trx reduces the oxidized cysteins of several transcription factors, thereby exercising a redox control on their function [31]. Furthermore, Trx functions as a peroxide scavenger through the peroxiredoxin (Prx) proteins which reduce peroxides like H_2O_2 [32]. Trx stabilizes NF-κB and increases transcription at HIF1α promoter to upregulate its expression [33, 34]. By reducing cysteine in HIF1α, Trx facilitates the co-activation of HIF1α by CBP/p300 and increases its transcriptional activity [33] (Fig. 2). Since ROS-quenching prevents dissociation of inhibitor of NF-κB (IκB) and NF-κB [35], Trx (by scavenging ROS) may also prevent the ROS-mediated degradation of IκB, in essence preventing NF-κB activation. In contrast, NF-κB-mediated nuclear

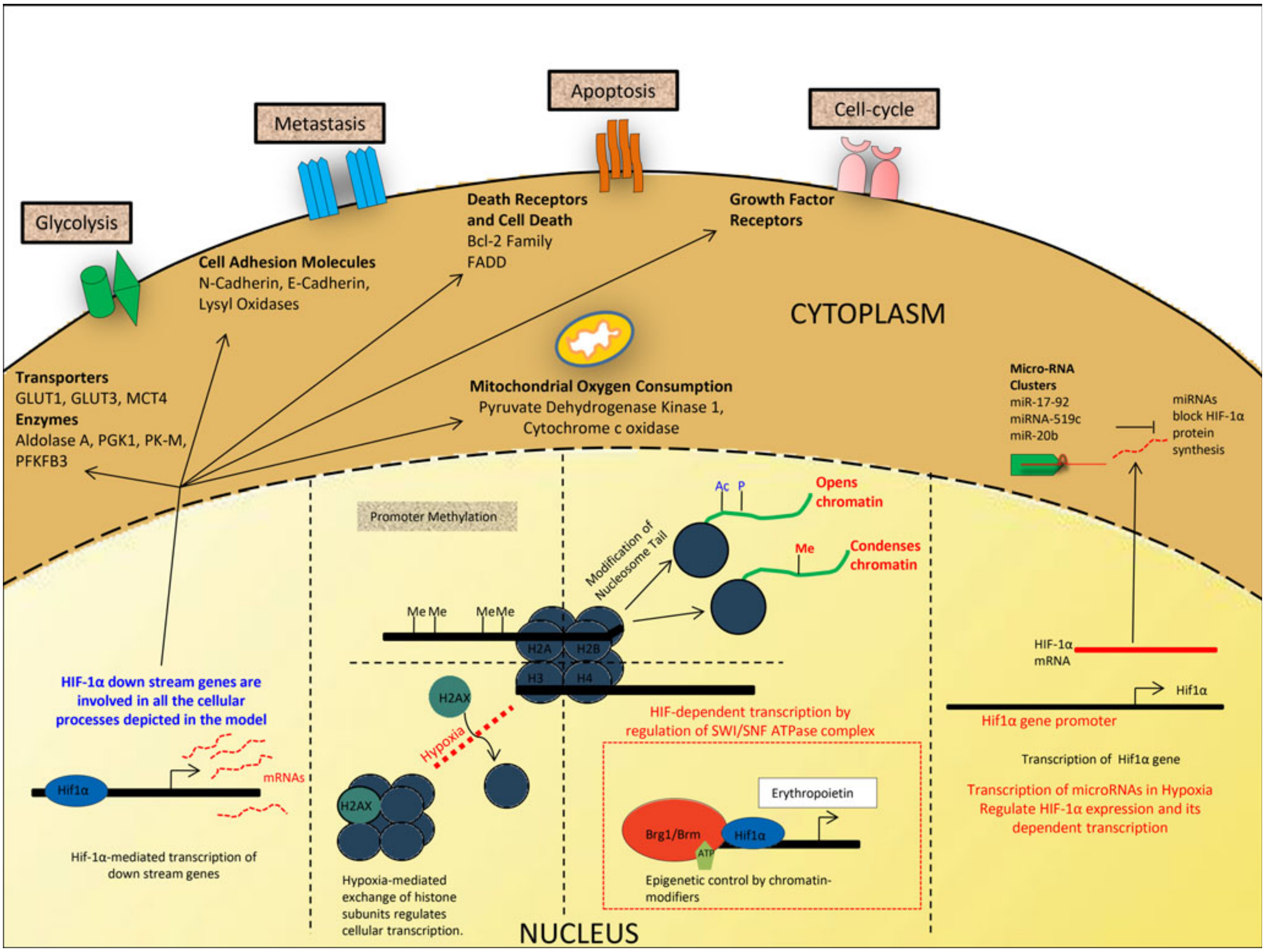
Apoptosis
Metastasis
Cell-cycle
Glycolysis
Death Receptors and Cell Death
Bcl-2 Family
FADD
Growth Factor Receptors
Cell Adhesion Molecules
N-Cadherin, E-Cadherin, Lysyl Oxidases
CYTOPLASM
Transporters
GLUT1, GLUT3, MCT4
Enzymes
Aldolase A, PGK1, PK-M, PFKFB3
Mitochondrial Oxygen Consumption
Pyruvate Dehydrogenase Kinase 1, Cytochrome c oxidase
Micro-RNA Clusters
miR-17-92
miRNA-519c
miR-20b
miRNAs block HIF-1α protein synthesis
Ac P
Opens chromatin
Me
Condenses chromatin
Promoter Methylation
Modification of Nucleosome Tail
Me Me
Me Me
H2A
H2B
H3
H4
H2AX
Hypoxia
HIF-1α mRNA
Hif1α
Hif1α gene promoter
Transcription of Hif1α gene
Transcription of microRNAs in Hypoxia Regulate HIF-1α expression and its dependent transcription
HIF-1α down stream genes are involved in all the cellular processes depicted in the model
Hif1α
mRNAs
Hif-1α-mediated transcription of down stream genes
H2AX
Hypoxia-mediated exchange of histone subunits regulates cellular transcription.
HIF-dependent transcription by regulation of SWI/SNF ATPase complex
Erythropoietin
Brg1/Brm
Hif1α
ATP
Epigenetic control by chromatin-modifiers
NUCLEUS

Fig. 1 Role of oxygen in regulating transient and permanent changes to transcription: transient transcriptional changes to low oxygen are mediated by HIF1α, causing activation of HIF1α-response genes that control glycolysis, metastasis, apoptosis, cell cycle, and mitochondrial oxygen consumption. Permanent transcriptional changes under low oxygen occur through direct modifications on DNA by methylation or through chromatin remodeling events that cause posttranslational histone modifications, alter histone subunits in nucleosome, or cause epigenetic remodeling through SWI/SNF complexes (here Brg1/Brm). Transcription of miRNA clusters control gene expression by silencing HIF1α, in effect changing transcriptional status of HIF1α response genes

translocation of Trx causes reduction of a cysteine in NF-κB, thereby increasing the transcriptional activity of NF-κB [34] (Fig. 2). The contrasting role of oxygen-dependent NF-κB activation through Trx in the cytoplasm and nucleus may indicate a stringent control over transcriptional activity of NF-κB. Since levels of Trx are upregulated in many cancers and correlate with increased growth, invasiveness, metastasis, and therapy resistance, it implicates oxygen's role in influencing carcinogenesis by modifying cellular redox environment.

In addition to NF-κB-mediated signaling, several other transcription factors are also known to respond to ROS availability, mostly facilitating an invasive and motile phenotype. ROS mediated activation of AP-1 and Smad, and transcriptional upregulation of Est-1 is known [36]. The activation of these transcription factors is pivotal in cancer metastasis and exemplifies how oxygen-dependent transcriptional changes influence the behavior of cancer cells by coordinated changes to gene expression.

While transcription factors enable transient control, a stable change in oxygen availability requires a more permanent response from stressed cells in order to support their survival. Knowledge on long-term changes to transcription is essential, since the multistep process of cancer and metastasis may be speculated to utilize long-term transcriptional changes that can be traced back to their oxygen "niches." Thus, long-term transcriptional changes by chromatin remodeling is influenced by altered reducing or oxidizing atmospheres present in the tumor tissues, infarct zones of the myocardium, and other disease models [37]. Oxygen regulates chromatin modifications through SWI/SNF proteins, histone modifications, incorporation of histone variants, and even DNA methylation

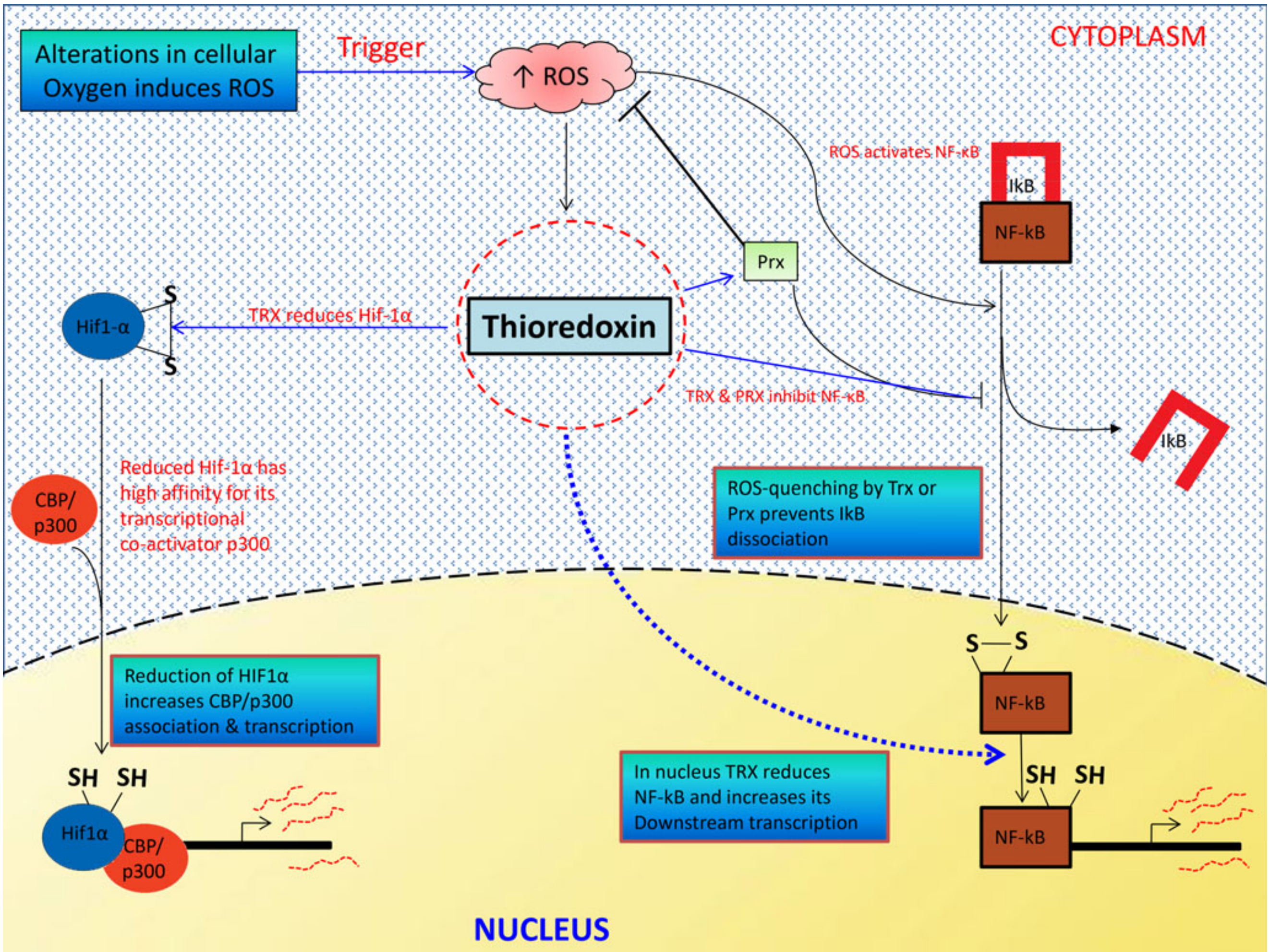
Alterations in cellular Oxygen induces ROS
Trigger
↑ ROS
CYTOPLASM
ROS activates NF-κB
IkB
NF-kB
Prx
Hif1-α
S
S
TRX reduces Hif-1α
Thioredoxin
TRX & PRX inhibit NF-κB
IkB
Reduced Hif-1α has high affinity for its transcriptional co-activator p300
CBP/ p300
ROS-quenching by Trx or Prx prevents IkB dissociation
S—S
NF-kB
Reduction of HIF1α increases CBP/p300 association & transcription
SH SH
Hif1α
CBP/ p300
In nucleus TRX reduces NF-kB and increases its Downstream transcription
SH SH
NF-kB
NUCLEUS

Fig. 2 Oxygen-induced redox changes regulate cellular transcription: high oxygen levels, reoxygenation, and acute hypoxia can trigger ROS. Production of ROS facilitates translocation of NF-κB into the nucleus by promoting dissociation of IκB from NF-κB, in effect stimulating NF-κB-mediated transcription. ROS can also activate the Thioredoxins (Trx) that negatively regulate NF-κB translocation either directly or by quenching ROS through Prx. In contrast, Trx reduce disulphide linkage in NF-κB localized in nucleus, causing higher NF-kB-mediated transcriptional changes. Trx also reduce the disulfide bonds in HIF1α, promoting its association with the co-activator CPB/p300 and increasing transcriptional activity

[37], all of which are observed during oncogenesis (Fig. 1). The SWI/SNF proteins utilize ATP for chromatin remodeling and altered oxygen levels are known to influence ATP generation (through OXPHOS). As a result, this might relate SWI/SNF-mediated chromatin remodeling to oxygen availability and cellular ATP levels in hypoxic cells. In addition, there exists direct evidence of SWI/SNF affecting transcriptional response during oxygen changes. For example, HIF1α was found associated with SWI/SNF at the enhancer region of its target gene erythropoietin (EPO) [38, 39]. Upon oxygen deprivation, EPO induction requires HIF1α binding and also the SWI/SNF association. This provides a link between oxygen availability (through HIF1α stabilization) and transcriptional activation by the SWI/SNF chromatin remodeling complex.

Histone proteins associate with DNA and prevent transcriptional activation by hindering the access of transcriptional machinery to these bound regions, whereas the posttranslational modifications of histones cause unwinding of target DNA to enable transcription. Since oxygen-mediated changes can affect histone modifications, they provide another tool to exercise long-term transcriptional control. For example, acetylation of histones during hypoxia is observed on cell survival genes such as VEGF and DNA-PK which is related to their activation [40, 41]. Similarly, oxygen governs the methylation of histones repressing tumor suppressor genes like BRCA1, RUNX3 [42, 43], probably based on the level of oxygen available [37]. Promoter methylation is also regulated by hypoxia and is found to reduce global methylation [44], probably to de-repress genes important for tumor progression. In addition to promoting acetylation and methylation, several other posttranslational modifications are carried out on histones in direct or indirect response to oxygen availability. As described previously, CBP/p300 is a HIF1α co-activator and itself a histone acetyl transferase (HAT). It may be speculated that under hypoxia, CBP/p300 availability governs both HIF1α-mediated cellular response (due to CBP/p300 co-activation) as well as histone modification (through CBP/p300 HAT activity). Oxygen deprivation leads to activation of other histone modifiers such as PCAF, Src-3, and even sirtuins—SIRT1, SIRT3, and SIRT6 [37], in effect, causing extensive transcriptional changes by utilizing different sensors of oxygen availability and responders which execute several combinations of posttranslational modifications. It is noteworthy that the extensive repertoire of posttranslational modifications (solely based on oxygen levels) adds to the advantage gained by cancer cells in oxygen niches, but at the same time, oxygen therapies may gain a firm scientific basis towards fighting cancer.

The transcription of noncoding RNA (ncRNA) expands the posttranscriptional regulatory potential of oxygen. Noncoding RNA may either be short (about 22 nucleotides) RNA molecules, as often found in miRNA or long (~200–100 kb) RNA molecules called long noncoding RNA (lncRNA). Under altered oxygen availability, miRNA can govern HIF1α expression and function. The miRNA cluster miR-17-92, MicroRNA-519c, and miR-20b cause silencing of HIF1α [45, 46] and affect the expression of HIF1α target genes (Fig. 1). One of the critical effects observed by miRNA-mediated HIF1α silencing is a significant decrease in the tissue angiogenesis. miR-20b [47] causes reduced VEGF levels, microRNA-519c reduces angiogenesis [46], and microRNA-107 (induced by p53) reduces tumor angiogenesis [48] (Fig. 1). These indicate that reduced oxygen levels affect transcription not only by stabilization of transcription factors such as HIF1α but also through the induction of several miRNAs. Constitutive HIF1α expression during prolonged hypoxia may be undesirable. Thus, microRNA-155 reduces the expression by silencing HIF1α during prolonged hypoxia [49]. The miRNA-mediated response to hypoxia has been well reviewed [50–52], but few reports exist on lncRNA-mediated response to hypoxia. In a recent study, it was shown that hypoxia leads to transcription of intragenic spacer chromatin, which act as lncRNAs and associate with proteins causing their nucleolar localization. This was demonstrated for the VHL protein where upon hypoxic stress, VHL was found to be sequestered in the nucleolus thus promoting HIF1α activity in the cytoplasm [53, 54]. Hypoxia can promote other posttranscriptional mechanisms such as alternate splicing of mRNA [55], in addition to ncRNA-mediated control. Clearly, hypoxia-mediated transcriptional changes play a pivotal in governing cellular response.

Molecular changes in response to oxygen availability seem to be critically dependent upon the transcription of target genes. While an overall reduction in protein synthesis is seen, specific genes may be expressed during particular oxygen levels. The role of HIF1α in mediating hypoxia-associated changes is well established. However, it seems that several other upstream molecules can control HIF1α-dependent response, for example Trx and ncRNA. Additionally, NF-κB-mediated response to oxygen equips the cell to execute wider homeostatic functions by utilizing the signaling role of NF-κB. Some of the notable responses include transcription genes which promote glycolysis and those which suppress OXPHOS, adding metabolic control which oxygen can exercise. Thus, transcriptional response represents a crucial cellular strategy to respond to chronic and acute oxygen changes by implementing multilayered integration and response molecules such as proteins and RNA.

3 Oxygen controls cellular DNA replication machinery

DNA replication is a coordinated event that is required to function in close association with the cell cycle. Eukaryotic cells respond to growth stimulatory factors by undergoing mitotic cell division which necessitates DNA duplication. Cells also ensure that commitment for division occurs under adequate supply of nutrients; incorrect replication of DNA is often associated with tumorigenesis. Therefore, DNA replication (which is a high energy-consuming process) seems to be well coordinated in response to oxygen availability in cells which eventually regulates the cellular OXPHOS and ATP levels. Recent research supports the school of thought where DNA replication functions downstream of oxygen signaling and as a result regulates the progression of cells through G1, S, and G2 check points of the cell cycle.

Oxygen controls the synthesis of deoxyribonucleotides that are obtained by the reduction of ribonucleotides in a reaction catalyzed by the enzyme ribonucleotide reductase [56]. Brischwein et al. showed that the enzyme responds to the partial pressure of oxygen (pO_2) and alters the levels of dCTP in cultured Ehrlich ascite cells. Decreased oxygen levels led to a reduction in dCTP levels and reduced frequency of the origin of replication (Fig. 3). Interestingly, this pathway does not affect the synthesis of other deoxyribonucleotide triphosphates. The mechanism of dCTP production seems to be dependent upon the M2-specific free-radical site in ribonucleotide reductase, which is controlled by oxygen. Interestingly, supplementing dCTP in the media of hypoxia-cultured cells restored the initiation of replication, suggesting that dCTP acts as an important mediator in the oxygen signaling and thereby establishes ribonucleotide reductase a potential oxygen-sensing enzyme in the hypoxic cells [57]. The members of the DNA replication machinery such as RPA70 show alterations in their cellular expression levels in response to varying levels of cellular oxygen [58]. Some proteins in the multi-protein DNA replication complex are also known to be regulated by oxygen (Fig. 3). For example, Replication Protein A (a eukaryotic single-strand DNA binding protein) which contains a zinc finger motif in a non-DNA binding domain was

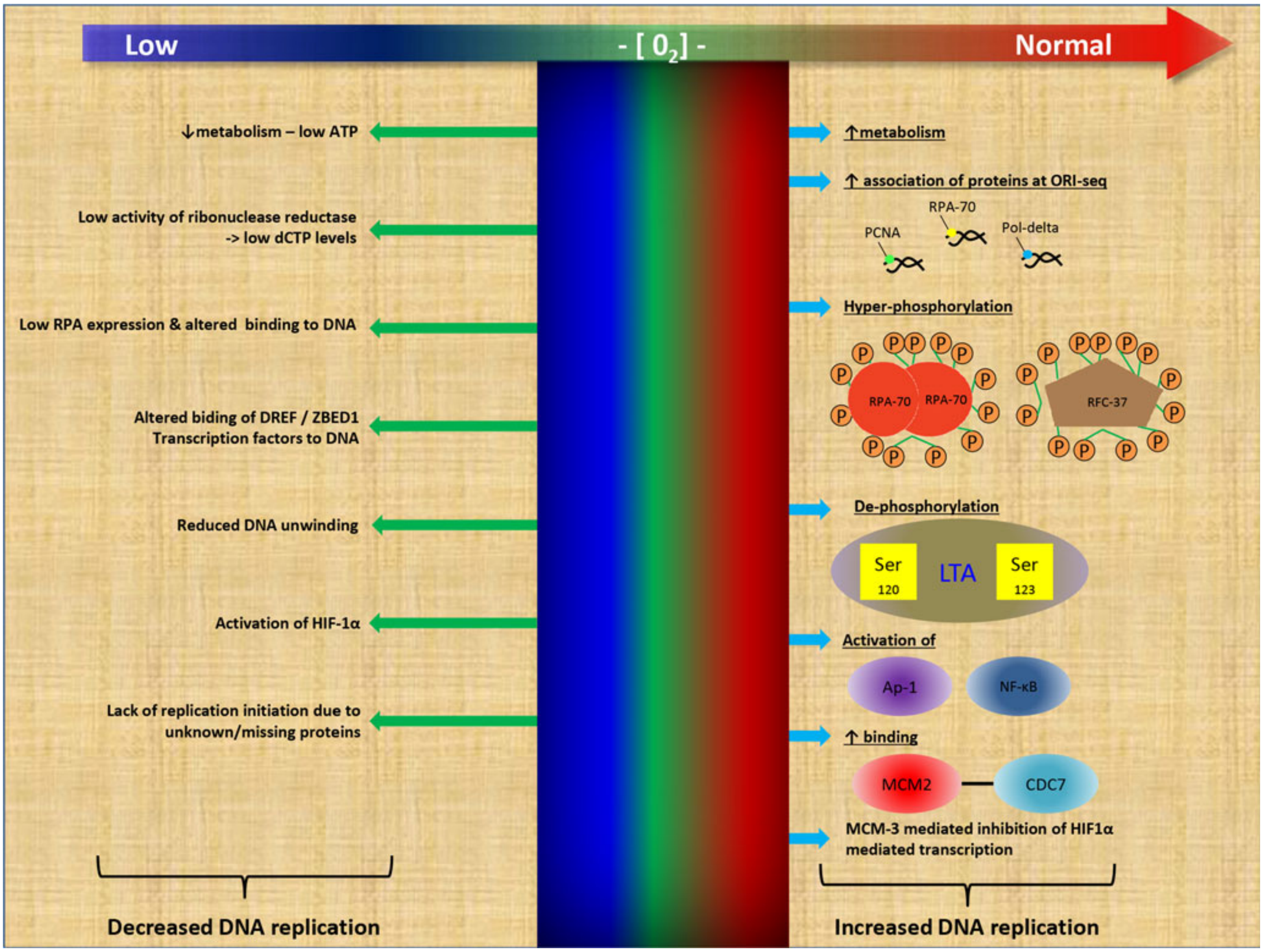
Low
- [0₂] -
Normal
↓metabolism – low ATP
Low activity of ribonuclease reductase
-> low dCTP levels
Low RPA expression & altered binding to DNA
Altered biding of DREF / ZBED1
Transcription factors to DNA
Reduced DNA unwinding
Activation of HIF-1α
Lack of replication initiation due to
unknown/missing proteins
Decreased DNA replication
↑metabolism
↑ association of proteins at ORI-seq
PCNA
RPA-70
Pol-delta
Hyper-phosphorylation
P
RPA-70
RPA-70
RFC-37
De-phosphorylation
Ser
120
LTA
Ser
123
Activation of
Ap-1
NF-κB
↑ binding
MCM2
CDC7
MCM-3 mediated inhibition of HIF1α
mediated transcription
Increased DNA replication

Fig. 3 Oxygen controls DNA replication: low oxygen levels decrease ATP synthesis and reduce activity of ribonucleotide reductase thereby decreasing dNTP (mainly dCTP) production. RPA expression and binding to DNA is reduced, and transcription factors (DREF/ZBED1) that accumulate replication proteins bind less efficiently to DNA under low oxygen. These changes along with "as yet unidentified proteins" cause reduced DNA unwinding and reduced DNA replication. In contrast, the events observed in low oxygen are reversed in optimal oxygen levels, restoring protein–DNA interactions, modulating requisite phosphorylations as seen on RPA-70, RFC-37, MCM2, or de-phosphorylation of LTA at Ser 120 & 123, and causing increased DNA replication

recently shown to be regulated in hypoxic cancer cells by Madan et al. [58]. It was reported that the RPA protein responds to cellular redox environments via alteration of disulfide bonding in cysteine residues of the zinc finger motif. Since oxygen can modulate cellular ROS production, it executes redox signaling by altering redox environments. A relative abundance of oxidants (as found in endoplasmic reticulum) causes oxidation of disulfide bonds of proteins, while the opposite is observed under reducing environments usually in the cytoplasm. When the reducing environment for RPA is mimicked by the reducing agent DTT, about 10-fold higher ssDNA binding activity is seen. Also, a cysteine–alanine substitution causes a constitutive ssDNA binding version of RPA. On the other hand, diamide-mediated oxidation is shown to reduce DNA binding of RPA [59]. Another redox-modulated transcription factor is the DNA replication-related element binding factor (DREF), which was first identified in *Drosophila* and later its human homologue was discovered (ZBED1) [60] (Fig. 3). DREF controls DNA replication by regulating transcription of genes including PCNA and Polymerase-α-180 KDa subunit. The DREF consists of three critical redox-regulated cysteine residues in its DNA binding domain. Two out of these three cysteine residues, Cys^{59} and Cyst^{62}, are readily reduced and the reduced form prevents the DREF-DNA interaction, resulting in reduced transcriptional activation of the replication-associated genes [61]. This pathway might hold significant importance in cancer cells, since tumors exhibit redox-sensitive environments. Reactive oxygen species produced during hyperoxia [62] such as singlet oxygen is reported to directly damage PCNA and thus negatively affecting DNA replication [63]. The immediate effect of altering oxygen levels in regulating initiation of DNA replication at the ori-sequences has been observed to take influence as early as 3 min of the exposure time [64]. For preventing re-

replication and ensuring coordinated synthesis of DNA (origin firing), the transition from pre-replication to replication complexes is essential. Some of the proteins involved in regulating this transition respond to oxygen in such a way that a permissive oxygen level allows this transition (Fig. 3). Origin-firing follows phosphorylation of MCMs, Cdc6, and Cdt1 by S phase cyclin Cdks, association of RPA, Cdc45 and consequent unwinding of DNA followed by primer synthesis by Polymerase-α. Interestingly, low levels of oxygen prevent the transition from pre-replication to replication complex, possibly through one or more proteins in the complex [64] (Fig. 3).

The study conducted on the Simian Virus 40 origin of replication in CV1 cells by Riedinger et al. elucidated the presence of assembled replication origins in hypoxic cells (0.02 to 0.2 % pO2) [64] (Fig. 4). Interestingly, a synchronous initiation of replication was observed within 3 min of the process of reoxygenation of these hypoxic cells [64]. This observation clearly suggests a direct involvement of cellular oxygen in regulating the process of initiation of replication. In this study, the authors speculated presence of an oxygen-sensing mechanism and possibility of oxygen-regulated transcription factors in the regulation of proteins involved in DNA unwinding and initiation of replication [64]. Extending this study, Riedinger et al. identified that the T-antigens, 34 kDa RPA subunit, topoisomerase I, 48 kDa primase subunit, 125 kDa pol delta subunit, and 37 kDa RFC subunit are assembled at the origin of replication in hypoxic cells (Fig. 4). However, the initiation of replication was not observed [65]. Interestingly, upon reoxygenation, a significant increase in the association of 70 kDa RPA subunit, 180 kDa Pol delta subunit, and most importantly, PCNA was observed along with the initiation of DNA replication (Fig. 4). It is important to note that these proteins did not result from *de novo* synthesis given the short time frame of response to reoxygenation [65]. The hyper-phosphorylation of RPA-34 and RFC-37 and stabilization of the DNA-protein complex of Pol delta was observed upon reoxygenation of the hypoxic cells. The molecular mechanism behind these observations was not provided. However, protein sequestration, dephosphorylation of Ser^{120} and Ser^{123} of LTA, and binding of TFs like AP1 and NF-Kb were proposed as likely mechanistic links [65] (Figs. 3 and 4). Similar results were also shown by Nikoleit et al. where an increase in the PCNA nuclear localization was observed upon reoxygenation of the serum-deprived T24 human bladder carcinoma cells. The lack of nuclear-Cdc6 expression was highlighted as the critical factor regulating the delay in the initiation of DNA replication at the Ori sequences. On the other hand, lack of PCNA nuclear localization and a marked decrease in the association of the nuclear fraction of PCNA to the chromatin of the hypoxic cells was suggested as the critical factor for inhibition of DNA replication in cells experiencing low oxygen levels [66]. Interestingly, Riedinger et al. found that the hypoxia-induced arrest of DNA replication and decrease in the rate of propagation of replication forks at the SV40 origin sequences could be reversed upon glucose enrichment [67]. It is suggested that higher glucose concentration (by obviating a need for OXPHOS) could elevate cellular oxygen levels by Crabtree effect. Increased oxygen increases the rate of fork propagation but could not account for increased DNA unwinding required during initiation of replication. The mechanism responsible for immediate entry of cells into replication upon release from hypoxia remains unknown. However, oxygen may execute some immediate effects by controlling the binding of proteins responsible for replication to DNA. This speculation seems reasonable since redox-dependent binding of RPA to DNA has already been demonstrated [59].

The MCM group of proteins is emerging as newly identified targets of oxygen. MCM 2-7 are helicases responsible for DNA unwinding and phosphorylation of MCM2 by Cdc7 is essential for its helicase activity [68, 69]. Investigation of reoxygenation of T24 cells revealed that MCM2 association with Cdc7 increases after reoxygenation and this may be responsible for MCM2 phosphorylation, thereby activating its helicase activity [69] (Figs. 3 and 4). In reoxygenated cells, since MCM proteins exist at higher levels than required for their function, Hubbi et al. hypothesized that these proteins may be involved in functions other than DNA unwinding [70]. Indeed, it was found that cytosolic MCMs have a role in controlling HIF1α levels by interacting with them, and in situations requiring high HIF1a levels, MCM levels are reduced. MCM3 was observed to mediate the inhibition of HIF1α trans-activation along with inducing the HIF1α ubiquitination in a proline hydroxylation-dependent manner [70]. Thus, MCM3 serves as an important modulator of HIF1α functions and probably helps in overcoming the inhibition of DNA replication via a HIF1α-dependent transcription-driven mechanism. Therefore, MCM proteins might have the potential to serve as the reciprocal regulators of oxygen levels in cells. A moderate level of ROS accumulation is essential for several signaling activities such as cell proliferation and for cancers to accumulate multistep mutations [36] that often disobey proof-reading mechanisms. However, it has also been argued that too much ROS is dangerous even for the cancer cells, and that antioxidant systems in these cells are often advantageous [71]. Clearly, ROS accumulation is dangerous even in cancer cells, since it can cause DNA and protein damage that specifically stalls the replication processes. It may be interesting to observe how DNA replication rate correlates with the changing ROS environment at the origin and homing of metastatic cancer cell. Taken together, these findings suggest that a direct effect of oxygen level on DNA replication machinery is tightly regulated in mammalian cells via unidentified molecular pathways.

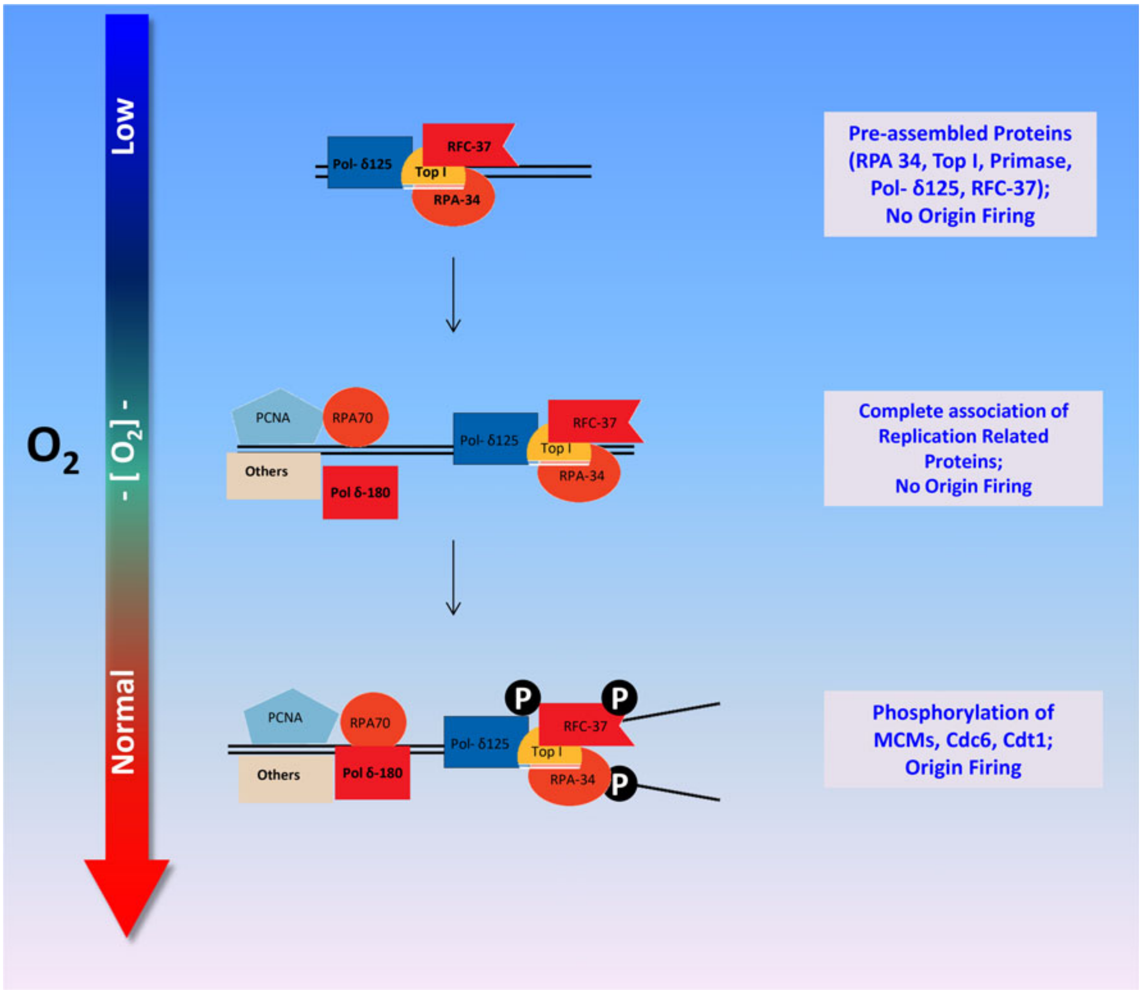
Low
O_2
- [O_2] -
Normal
Pol- δ125
RFC-37
Top I
RPA-34
Pre-assembled Proteins (RPA 34, Top I, Primase, Pol- δ125, RFC-37); No Origin Firing
PCNA
RPA70
Others
Pol δ-180
Complete association of Replication Related Proteins; No Origin Firing
P
Phosphorylation of MCMs, Cdc6, Cdt1; Origin Firing

Fig. 4 Oxygen promotes assembly of replication machinery and origin firing: under low oxygen, assembly of incomplete DNA replication machinery including Pol-δ125, RFC-37, Top-1, and RPA-34 proteins causes replication to remain arrested. Upon progressive increase in oxygen levels, the minimal or complete DNA replication machinery is assembled as association of PCNA, RPA-70, Pol-δ180, and others occurs, but DNA replication remains arrested. Further increase in oxygen levels induces phosphorylation of members of replication machinery and causes origin firing and DNA replication

4 Cellular oxygen regulates the progression of cell cycle

Cell cycle progression consists of an ordered and stringently regulated series of events undertaken by a cell to ensure error-free and regulated division. Each phase is characterized by synthesis, accumulation, and degradation of regulatory proteins called cyclins, which associate with their catalytic partners the "kinases." Cell cycle perturbations are important for oncogenic development. Oxygen participates in normal cell cycle functioning by ensuring adequate energy generation through regulation of OXPHOS [72]. The generation of oxygen radicals (ROS) in hyperoxic or hypoxic cells shows regulatory effects on cell cycle and suggests that its progression might be an oxygen-regulated process. In addition, a change in the level of HIF proteins in response to oxygen alterations regulates the cell cycle. In fact, the production of ROS and stabilization of HIFs are the major oxygen-governed processes that regulate the cell cycle. Cell cycle alters with a wide spectrum of oxygen availability termed as anoxia, hypoxia, and hyperoxia and thus reflects a compelling role of oxygen in the cell cycle process. Oxygen seems to cooperate with the cell cycle machinery as its complete absence (or anoxia) results in arrest of the cell cycle progression in various phases (Fig. 5). Since cell cycle requires energy and OXPHOS is the major pathway of ATP generation, it seems reasonable that absence of oxygen should immediately arrest cells due to the cessation of OXPHOS. However, the study of anoxia-mediated cell cycle arrest in organisms like *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Danio rerio* shows that the cells progress through the initial phases of cell cycle and the arrest is observed during the middle phases of the cell cycle [73, 74] (Fig. 5) Moreover, the phase in which cells get arrested is not the same but rather varies between different organisms. The embryos of *D. melanogaster* were found to show cell cycle arrest during all phases of the cell cycle except anaphase [75]. In contrast, the anoxia-induced arrest of the mitotic cycle was not observed in the zebrafish embryos. Recently, microRNAs such as microRNA-16-1 and microRNA-15a were found to target the 3′UTR of the cyclinD1 in turtles, resulting in the regulation of the cellular expression in cells exposed to anoxia [76]. These observations suggest that during anoxia, specific molecular mechanisms for achieving cell cycle arrest are activated and it appears that these pathways are essentially different from those activated during hypoxia exposure (Fig. 5). It is important to observe that anoxia-induced cell cycle arrest occurs in different phases in different species of *Drosophila* and zebrafish. This indicates that cellular response to anoxia is a process which is continuously evolving with different species. This is important, since it is clear that anoxic cells do not complete the cell cycle but rather get arrested. Oxygen appears to be an indispensable molecule for the cell cycle progression. Extensive research has centered on a physiologically common form of oxygen fluctuation, i.e., its shortage or hypoxia, yet from the standpoint of 21 % oxygen as physiologically normal, a number of organs and tissues function under hypoxia. However, a quantitative measure of the percentage and duration of oxygen deprivation better describes biologically relevant hypoxia. The cell cycle is affected under oxygen deprivation, and several reports suggest that HIFs and ROS mechanistically govern the arrest or progression of the cell cycle.

Role of HIF in oxygen-mediated regulation of cell cycle progression Oxygen shortage utilizes the transcriptional activity of HIFs to regulate cell cycle progression. Accumulation of HIF1a during hypoxia correlates with cell cycle arrest [77] (Fig. 5). HIFs are not known to directly regulate cell cycle progression and are found to function by upregulating classical cell cycle inhibitors like p21 and p27. However, there seems to be some dissent on how p21 and p27 are induced. While HIF1α induces p21 and p27 in primary differentiated cell types [78], a negative correlation exists in case of U2OS cell lines [79]. In normoxic NIH3T3 cells, overexpression of another isoform HIF2α (in addition to HIF1α) arrests the cell cycle in the G1 phase [80]. This indicates that HIF stabilization, even under normoxia, is sufficient to induce cell cycle arrest. As with HIF1α, the induction of p21 and p27 by HIF2α remains debatable in the different cell types studied. Despite the disagreement on the exact role of HIFs in p21 and p27 induction, reports show a predominantly G1-phase cell cycle arrest related to HIFs [78, 79]. Although commonly involved, p53 is not necessary for p21 induction as other proteins induced during hypoxia such as SP-1 may activate p21 [80]. HIFs are also known to affect Retinoblastoma (pRB) pathway and cyclins as a mechanism to induce cell cycle arrest in hypoxic cells. In the hypo-phosphorylated state, pRb sequesters the E2F-family of transcription factors [81, 82]. Since E2F1 activates several cell cycle promoting genes, its sequestration causes cell cycle arrest. Upon stimulation by growth factors, a series of phosphorylation events on pRb occur preventing pRb-E2F binding and associated cell cycle progression. These phosphorylations are carried out by several cyclin-Cdk kinases upon transition from one phase to another. HIF1a binds to cyclin D1 promoters in A549 pulmonary cells and results in transcriptional repression and downregulation of cyclin D1 (Fig. 5) [83]. In fact, a decrease in HIF1a level seems to correlate with increase in cyclin D1 and cyclin E levels. Reciprocally, cyclin levels have been reported to alter HIF expression or stability in the acinar growth model (of epithelial morphogenesis). Here, cyclin E dysregulation was reported to induce HIF1α expression [84]. On the other hand, cyclin E may cause increased HIF1α degradation by increasing prolyl hydroxylase expression through the VHL pathway [84] (Fig. 5). It seems that in order to let cells adapt to the hypoxic environments, HIFs cause cell cycle arrest in cancer cells. In contrast, the other facets of HIF activation on metabolism, cell survival, and angiogenesis seem to promote cancer progression, there are other players including ROS that regulate oncogenesis and metastasis.

Role of oxygen in ROS-mediated cell cycle arrest The progression of the cell cycle is affected by levels of ROS in a cell. In mitochondria, oxygen being the terminal electron acceptor produces H_2O as the major and H_2O_2 as the minor end product. In cells with low or high exposure to oxygen, the ROS production via the mitochondrial pathway may increase [85]. Early insights into ROS-mediated growth factor response came through studies on the platelet-derived growth factor (PDGF) receptors and epidermal growth factor (EGF) receptors on rat vascular smooth muscle cells and A431 epidermal carcinoma cells, respectively. It was reported that growth factor stimulation caused mitochondrial ROS generation in the form of H_2O_2 [86, 87]. The antioxidant catalase abolishes ROS-mediated phosphorylation of tyrosine in various proteins, including EGF receptor, PhospholipaseC1γ, and MAPK-stimulated pathways [88] (Fig. 5). Mitochondria act as the source of ROS upon growth factor binding, indicating that oxygen is not only essential to generate energy through OXPHOS but is purposely used as a precursor for ROS production and hence growth factor signaling. Supporting evidence indicates that human and rat fibroblasts progressively accumulate ROS upon cell cycle advancement [88], further emphasizing the role of oxygen as a signaling molecule during cell cycle. Recent reports suggest that ROS might be produced as a strategy to promote the cell cycle progression. For this reason, in situations such as hypoxia where ROS levels are likely to be altered, other ROS-generating mechanisms such as NOXs are employed [89]. It may be argued that the mitochondrial ROS produced during growth factor binding is merely a by-product but not an active participant in cell cycle

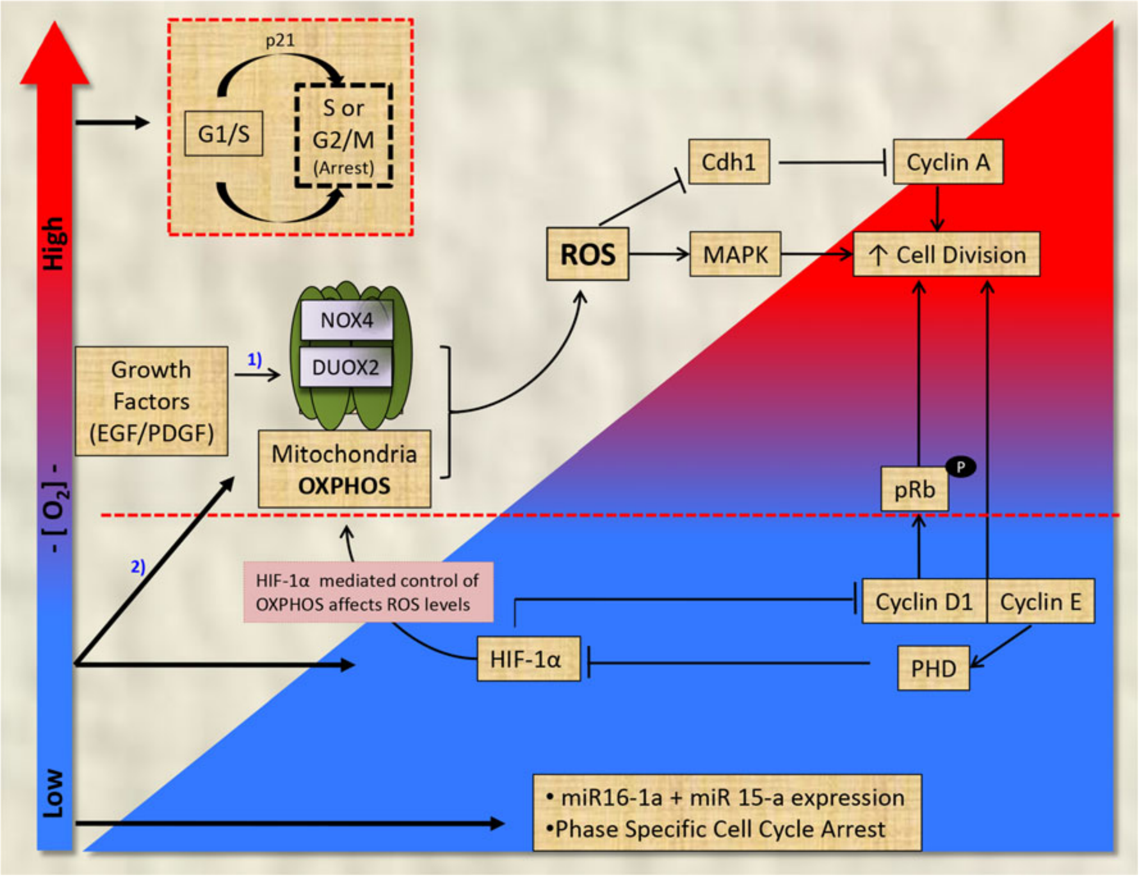
High
- [O$_2$] -
Low
p21
G1/S
S or G2/M (Arrest)
Cdh1
Cyclin A
ROS
MAPK
↑ Cell Division
NOX4
DUOX2
Growth Factors (EGF/PDGF)
1)
Mitochondria OXPHOS
2)
pRb
P
HIF-1α mediated control of OXPHOS affects ROS levels
Cyclin D1
Cyclin E
HIF-1α
PHD
• miR16-1a + miR 15-a expression
•Phase Specific Cell Cycle Arrest

Fig. 5 Oxygen controls the cell cycle: complete absence of oxygen causes cell cycle arrest in several species in a phase-specific manner. The arrest during anoxia is also mediated by microRNAs like miR16-1 and miR15-a. Low oxygen causes reduction in the levels of cyclin D1 and cyclin E through HIF1α, whereas HIF1α itself is negatively controlled by cyclin E through the prolyl hydroxylase. Accumulation of cyclins causes pRb hyper-phosphorylation and thus the cell cycle progression. ROS produced by mitochondria or NADPH oxidases/DUOX2 is induced through (*1*) growth factor signaling or (*2*) hypoxia. This causes cell cycle progression through MAPK pathway or by inhibiting Cdh-1-mediated cyclin-A degradation. High levels of oxygen cause S or G2/M phase cell cycle arrest which is primarily mediated by p21

progression. However, other non-mitochondrial mechanisms of producing ROS are activated under oxygen alteration. These include several NADPH oxidases (NOXs) which are present on the cell surface and catalyze the production of H_2O_2 [71, 90] (Fig. 5). Utilization of NOXs for ROS generation indicates that cells do not exclusively rely on mitochondrial ROS (the levels of which are tightly governed by oxygen availability), but rather ensure adequate ROS buildup for cell cycle progression through other mechanisms. Further, ROS derived from NOXs upon growth factor binding activates signaling pathways which promote cell division [91]. For example, growth stimulation by EGF and PDGF activates NOX to produce ROS [92]. The ROS activates the growth-promoting MAPK pathway to activate cell division (Fig. 5). Thus, oxygen can promote cell cycle by affecting ROS levels directly through the mitochondria or indirectly through NOX. It is also important to note that oxygen can induce or suppress ROS production through transcriptional changes by HIF1a proteins [21–23], thus regulating cell cycle progression. In addition to acting as a signaling molecule during the cell cycle progression, ROS has been reported to directly affect accumulation of cell cycle proteins [89]. The restriction point in G1 phase of the cell cycle is known to be the commitment step towards cell division. Growth factor signaling during G1 phase governs whether a cell undergoes division or not. In fact, during G1 phase, it seems a particular level of ROS helps pass the restriction point. Interestingly, it has been observed that levels of ROS alter synchronously with the cell cycle, steadily increasing from G1 and declining after mitosis [89]. The role of ROS is supported by the observation that antioxidant treatment on fibroblasts can cause late G1 phase arrest characterized by increased cell size. Interestingly, arrest occurs despite of active cyclin-cdk4/6 and cyclinE-cdk2 kinases and hyper-phosphorylated pRb [89]. The antioxidant quenching leading to arrest elucidated an interesting role of ROS. ROS causes transition from G1 phase by promoting cyclin-A accumulation and hence cell cycle progression. ROS achieves this by inhibiting the Cdh1 specificity factor, and hence preventing APC-mediated ubiquitination of cyclin-A [89] (Fig. 5). However, upon antioxidant-mediated quenching of ROS, the ROS-mediated Cdh1 inhibition is lost, and cyclin A gets ubiquitinated and degraded [88]. Thus, oxygen helps pass the restriction point by affecting ROS generation through OXPHOS. Similarly, antioxidant *N*-acetyl-L-cysteine (NAC) administered to mouse embryo fibroblasts shows a delay in G1/S phase transition, decreased cyclin D1 level, and about three to four times higher p27. This correlates with reduced Rb phosphorylation and thus a delay in cell cycle progression [93]. Considered together, the effect of oxygen seems to promote cell cycle through high, but physiological levels of ROS that are not obligatory, but essential. It remains an interesting question as to how some cancer cells, while utilizing ROS for boosted cell cycle progression, may also become metastatic. To further surprise, high ROS proves to be tumor suppressive [71]. A plausible explanation is that cells display a fine tuned and graded response to ROS levels, and may change behavior based on the duration of ROS exposure. Further research is warranted to understand ROS and cell cycle progression as promoters or repressors of cancers and metastasis.

The alteration in oxygen levels can also affect proteins which control the cell cycle, including p21, p27, p53, and pRb. Since oxygen can also influence ROS production, it is important to understand the interaction of these proteins with ROS. The ROS generated through PDGF-induced NOX4 and DUOX2 promotes pRb phosphorylation and thus cell cycle [92]. However, upon the knockdown of NOX4 and DUOX2, the levels of p53 and p21 increase, causing cell cycle arrest. A simultaneous knockdown of NOX4/DUOX2 with either p21 or p53 abolishes this arrest. This finding introduces another mechanism of ROS suppressing p53 and/or p21 accumulation and hence promoting cell cycle. But high levels of oxidative stress (like ROS) can activate p53/p21 as part of DNA damage response and contradict the findings [94]. The antagonistic choice between promoting cell cycle and causing DNA damage-mediated arrest may depend upon ROS concentration in the cells. In summary, oxygen levels regulate ROS production through several mechanisms, which influence both cell cycle progression and arrest (via DNA damage). This may be a strategy to ensure that only those cells capable of handling high ROS levels are selected to enter the cell cycle.

Hyperoxia and the cell cycle Hyperoxia and hyperbaric oxygen (HBO, higher than atmospheric pressure of oxygen), are usually non-physiological conditions (considering 21 % oxygen and 1 ATA as physiological levels) and can cause cell cycle changes. The effect of hyperoxia and HBO on cell cycle progression is well studied. Cell cycle changes are observed when prostate cancer cells are exposed to elevated concentration and pressure of oxygen. When exposed to 100 % oxygen at 6 ATA for 1.5 h, the G1/S phase senescent cells enter S phase and eventually arrest in G2/M-phase in a pressure-dependent manner [95], showing that pressurized oxygen can stimulate cell cycle progression although the mechanism of G2/M phase arrest is unknown. Human bronchial smooth muscle cells are observed to undergo the S phase arrest in hyperoxic conditions [96] (Fig. 5). In this study, where two different levels of oxygen (95 and 40 %) were administered with respect to room air, 95 % oxygen lead to decrease in cell proliferation within 24 h and reduced DNA synthesis to about 7 %. In the background of insignificant apoptosis, it was reported that the number of cells in G1 phase was decreased and instead arrested in the S phase by 72 h of exposure [96]. p21 also plays an important role in cell cycle arrest under elevated oxygen levels. It was observed that p21 levels remain constant in cells exposed to 40 and 21 % oxygen but are significantly increased in the cells exposed to 95 % oxygen (48 h) [96]. In this study, high levels of p53 expression were observed only around 72 h post-exposure [96]. In another study using 95 % oxygen on two adenocarcinoma cell lines Mv1Lu (p21 null) and A549, it was shown that A549 cells expressing p21 were arrested in the G1 phase of the cell cycle [97] (Fig. 5). Another school of thought suggesting that a drop in ATP levels could bring about the observed arrest was refuted by Rancourt et al. by providing evidence that ATP levels in hyperoxic and normoxic cells were comparable after 48 h of exposure. It was also observed that G1 arrest is transient and that the cells begin to move towards S phase after prolonged exposure to elevated oxygen [97].

Since p21 causes cell cycle arrest by binding Cdks and PCNA through its two binding domains, studies were carried out to elucidate the binding of p21 to Cdks and PCNA under elevated oxygen. It was shown that the oxidative stress did not abolish these binding activities of the two p21 domains and thus p21 retains its activity of sequestering Cdks and PCNA to bring about cell cycle arrest [98]. Despite these findings, some disagreement remains on whether p21 (which is a known target of p53) is solely dependent upon p53 for its expression during exposure to elevated oxygen. In A549 and HCT116 cell lines, p53 is not required for p21 induction during elevated oxygen. In contrast, H1299 cells, which were transfected by p53 cDNA constructs and exposed to 95 % oxygen, showed a p53-dependent expression of p21 [98]. Transfection of p53 in H1299 cells (p53 null) resulted in about sevenfold increase in p21 induction, which caused more cells to be arrested in G1 phase compared with p53-deficient cells arrested in S and G2 phases [98]. It was shown that p53-dependent p21 induction is responsive to elevated oxygen but other inhibitors like INK4, Cip/Kip, and Rb are not influenced. In alveolar cells, it was seen that p53-deficient cells do accumulate p21 but it took about 84 h for the p21 levels to reach levels comparable to p53Wt cells [99]. It must be mentioned that p21 has otherwise been established to be independently expressed by TGF-β and IL-6 induction independent of p53 [100, 101]. It is likely that p21 is induced in a less efficient manner by an oxygen-modulated factor, other than p53, e.g., SP-1 [102]. The cell cycle is a combined outcome of diverse molecular processes which involve direct and indirect intervention of oxygen. Thus, oxygen is emerging as a major molecule regulating the cell cycle through HIF1a and ROS, with implications of cell cycle dysregulation in disease.

5 Effect of cellular oxygen on protein folding

The native protein conformation is necessary for proper function and nonnative, unfolded proteins may deleteriously aggregate with other proteins in a cell. A number of physical, chemical, and biological stresses are capable of affecting a protein's native conformation by altering the physical or biochemical milieu surrounding it. A growing body of research indicates the presence of oxygen-sensitive mechanisms that affect protein conformation resulting in significant implications in cardiovascular, neural, and cancer disease models [103]. Through some indirect mechanisms oxygen may affect protein folding in a reversible way; the incorrectly folded proteins under a particular oxygen level can undergo correct folding when introduced in an environment of different oxygen availability [104, 105]. The endoplasmic reticulum (ER) is crucial for protein folding and changes in cellular oxidative potential trigger the unfolded protein response (UPR) in the ER. The presence of chaperones like Grp78/BiP in ER facilitates protein folding during unstressed or mildly stressed conditions. BiP plays several roles based on its localization including signaling and apoptotic roles [106]. However, when present in ER, BiP interacts with the hydrophobic residues of nascent or misfolded proteins and facilitates their correct folding. Oxidative stress causes extensive protein misfolding which requires increased levels of BiP for refolding these proteins. Studies indicate that under oxygen fluctuations which generate oxidative stress, BiP levels are altered. For example, proteomic analysis shows that administering 5 % oxygen to cells increases the levels of BiP [107] (Fig. 6). Clearly, increased levels of BiP points to a homeostatic response evoked by hypoxic cells. A similar study in which BiP is induced by BiP inducer X (BIX), cells under ER stress survive better than those lacking BIX [108], showing that hypoxia-mediated BiP overexpression protects cells from UPR (Fig. 6). In contrast to rescuing proteins, there exist situations when acute stress-induced apoptosis benefits the organism, which otherwise can lead to diseases such as cancer [108]. It is for this reason BiP induction is not observed in cells exposed to less than 1 % oxygen (Fig. 6). BiP is also induced by the knockdown of another ER protein ERp57, which undertakes redox-dependent protein folding [109] and BiP induction results in protection of cells from hyperoxia-mediated apoptosis. At transcriptional level, BiP expression is controlled by the ER-associated protein ATF6 [110]. During ischemia, ATF6 induces BiP to protect cardiomyocytes from apoptosis, while reperfusion (restoration of blood supply) reverses this activation [111].

Another ER protein that links oxygen to protein folding is oxygen-regulated protein 150 kDa (ORP150). The protein was initially identified in the ER of mouse astrocytes subjected to hypoxia [112]. ORP150 is produced by *de novo* protein synthesis exclusively under oxygen deprivation and its activation cannot be mimicked by other stresses like heat, H_2O_2, and $CoCl_2$ [112]. The chaperoning activity of ORP150 is now identified [113]. Indeed, several studies establish ORP150 as a cytoprotective agent which reduces ER stress [114, 115] and possibly apoptosis in hypoxic cells [116]. ORP150 may even have beneficial roles in hypoxic tissues since it helps VEGF to attain proper folding, thus increases angiogenesis and wound healing associated with hypoxic tissues including hypoxic cancers [117, 118]. The copper chaperone for SOD is another chaperone which responds to oxidative stress and is thus related to cellular oxygen. The activation of Cu/Zn SOD1 requires the delivery of copper to SOD1 and this process is facilitated by CCS [119–121]. Copper delivery by CCS is specific to SOD1 and not to proteins found elsewhere [119, 121]. In *Drosophila*, CCS null flies resemble SOD1 null flies in terms of sensitivity to oxidative stress and show reduced adult life span [122]. In fact, it was recently reported that oxygen, copper, and CCS are required only for oxidation of disulfide bonds in SOD1. Under oxygen- and copper-deficient conditions, oxidation of SOD1 disulfide bond results in CCS-independent activation of SOD1 [123]. The protective role of many conventional and recently discovered heat shock proteins (Hsp) has been identified in relation with cellular oxygen changes. For example, hyperoxia-induced injury in newborn rat lungs and A549 cells is mediated by downregulation of Hsp27, and hyperoxia-induced apoptosis is reduced upon Hsp27 overexpression [124]. Similarly, oxygen–glucose deprivation followed by reoxygenation in N2A-neuroblastoma cells results in a decrease in Hsp20 levels followed by apoptosis. Interestingly, the overexpression of Hsp20 protects the N2A cells from reoxygenation-induced apoptosis [125]. The Hsp90- and Hsp70-mediated protection against hyperglycemia or ischemia-induced apoptosis in neuronal cells was observed by Leitch et al. [123] and Doeppner et al. [126, 127]. Further overexpression of Hsp90 was observed to prevent the neuronal cell lysis [128], providing insight into the cytoprotective role of these chaperones [129] (Fig. 6).

Some chaperones physically associate with HIFs and control their stability and degradation in response to varying cellular oxygen levels. Cells lacking Hsp90β show delayed accumulation of HIF1α and induction of Hsp90/Hsp70 helps in stabilization of HIF1α, suggesting that Hsp90 is required for HIF1α folding [130]. Zhou et al. showed that PI3K/Akt signaling is important for upregulating Hsp90/Hsp70

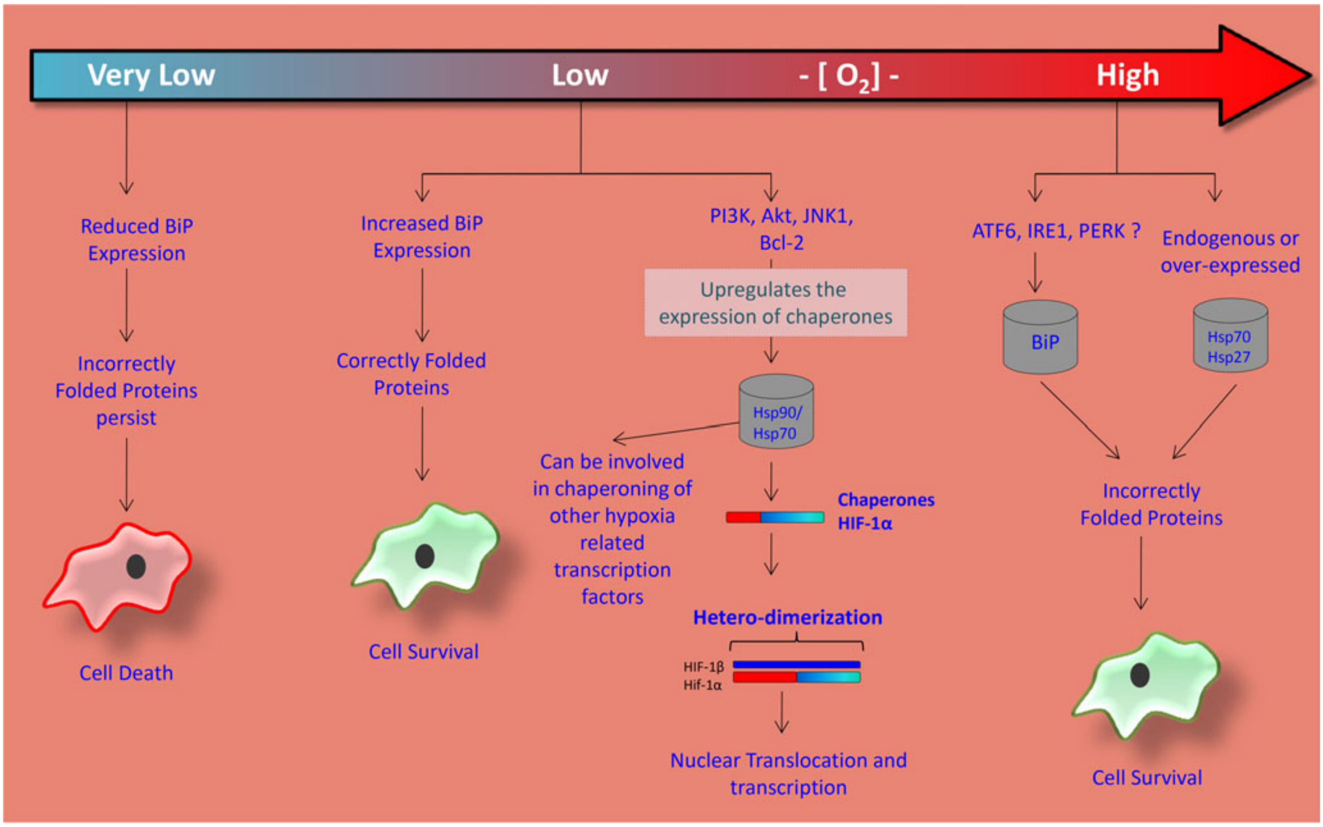
Very Low
Low
- [O$_2$] -
High
Reduced BiP Expression
Incorrectly Folded Proteins persist
Cell Death
Increased BiP Expression
Correctly Folded Proteins
Cell Survival
PI3K, Akt, JNK1, Bcl-2
Upregulates the expression of chaperones
Hsp90/ Hsp70
Can be involved in chaperoning of other hypoxia related transcription factors
Chaperones HIF-1α
Hetero-dimerization
HIF-1β
Hif-1α
Nuclear Translocation and transcription
ATF6, IRE1, PERK ?
Endogenous or over-expressed
BiP
Hsp70 Hsp27
Incorrectly Folded Proteins
Cell Survival

Fig. 6 Oxygen controls protein folding: the unfolded protein response (UPR) is triggered to correctly fold proteins upon stress. Very low oxygen levels fail to induce UPR as BiP expression is reduced, thereby causing accumulation of misfolded proteins and cell death. Under recoverable, low oxygen levels increased BiP expression aides correct protein folding and promotes cell survival. Low oxygen also causes increased Hsp90 and Hsp70 expression through P13K, Akt, JNK1, and Bcl-2 pathways, thereby chaperoning HIF1α and promoting its association with HIF1β, resulting in its nuclear translocation. Under high oxygen, it is unclear if ATF6, IRE1, and PERK induce BiP to correctly fold proteins. However, Hsp70 and Hsp27 expression aids correct protein folding and promotes cell survival

expression in hypoxic cells and this result in preventing HIF1α from VHL-independent degradation and correct folding of HIF1α [131]. Similarly, JNK1 also helps in HIF1a stabilization by upregulating the expression of Hsp90/Hsp70 proteins [132]. Bcl-2 also causes HIF1α stabilization by increasing the chaperone activity of Hsp90β [133]. Some VHL-independent mechanisms of HIF1α degradation compete with Hsp90/Hsp70 protein for binding at the NODDD and CODDD domains of HIF1α [134]. The RACK1 and COMMD1, for example, degrade HIF1α by competing with Hsp90 and Hsp90/Hsp70, respectively, in an oxygen-independent manner [135, 136]. A well-established inhibitor of HIF1α, the Kruppel-like factor 2, exerts inhibitory activity by disrupting HIF1α-Hsp90 association and hence disrupting the proper folding of HIF1α [137]. Another report shows that the VHL, which requires Fe(II) for activity, incorporates Fe(II) through a chaperone PCBP1, in the same way as CCS and HIF1α degradation is compromised upon PCBP1 depletion [138]. Taken together, the reports suggest that beyond the well-studied step of oxygen-dependent accumulation of HIF1α, the correct folding of this protein is another regulatory step in HIF1α signaling.

Hyperoxia induces extensive cell damage through oxidative stress and as a response cells activate molecular chaperones and protein degradation pathways to overcome hyperoxia-mediated cellular damage [139] (Fig. 6). Hsp70 overexpression results in hyperoxia-induced cytoprotection [140] and inactivation of HSPs in hyperoxic fibroblasts results in cellular injury, high membrane lipid peroxidation, and decreased ATP levels [141]. Similarly, expression of BiP protein and Hsp27 protect against hyperoxia-induced stress [124, 142]. However, a recent report shows that hyperoxia is insufficient to trigger UPR and does not activate ER stress receptors ATF6, IRE1, and PERK [143] (Fig. 6). In addition to indirectly controlling protein folding, oxygen may directly control protein conformation. Gogna et al. made a few important observations relating oxygen-dependent function of p53. It was reported that oxygen levels can directly control the conformation of p53 such that hypoxia induces a mutant p53 conformation [105]. Further, introduction of p53 in wild type conformation could promote the misfolded p53 to be correctly folded implying a chaperone-like activity of p53 [105] (Fig. 6). To further support the novel finding, this group showed that while hypoxia caused conformation-based p53 activation, elevated oxygen concentration and pressure could cause tumors to regress [104].

In conclusion, many molecules respond to oxygen levels and function as oxygen-dependent chaperones. These protein either directly affect protein folding or they induce other downstream chaperones. However, much needs to be researched upon the generic mechanisms or paradigms that dictate the folding of proteins in response to oxygen. It is an interesting area of therapeutic intervention and drug development where oxygen-deficient tumor areas can be targeted for oxygen therapies to restore protein-folding activities.

6 Oxygen controls cellular apoptosis

Oxygen supply through tissue vasculature defines the region in which cells can grow as oxygen limits the potential of cells to generate energy through OXPHOS. The vasculature is often dynamic causing alterations in oxygen levels, thus affecting cell survival. The tumor vasculature is more dynamic than normal tissues, often causing sudden changes to oxygen supplies within the tumors and probably influencing cancer cell survival and selection. Cells that encounter mild oxygen fluctuations may undergo cell cycle arrest (Fig. 7); however, acute stress is lethal and necessitates programmed cell death. Cancer cells not only adapt to evade cell death upon severe oxygen-based stress but often gain selective advantage by activating survival cascades, preventing apoptotic programs and gaining metastatic abilities in order to "find" favorable conditions. This has also led to the suggestion that metastasis might be an antioxidant strategy to favor the growth of cancer cells in these environments [71]. Interaction of oxygen with several intracellular signaling pathways determines whether a cell is fit to survive or not under oxygen fluctuation. These interactions are mediated by apoptosis-associated proteins, their posttranslational modifications and calcium signaling. Major signaling pathways including NF-κb, Wnt/β-Catenin, Notch, and PI3K pathways are involved in the oxygen-mediated control of apoptosis [144–147]. Posttranslational modifications such as acetylation, ubiquitination, and phosphorylation of apoptosis-associated proteins may also link oxygen fluctuations with programmed cell death [148–150]. Moreover, calcium-dependent phosphorylation of antiapoptotic proteins Bcl-2 and Bcl-xl in nuclei has also been shown to be effected by oxygen fluctuation [150] (Fig. 7). Oxygen response is thus ubiquitous and evolution of such a comprehensive system to control cell death could be an early event that has been fine-tuned to benefit complex organisms.

Oxygen fluctuations often cause accumulation of high levels of ROS which is lethal to a cell [151–153] and in fact inhibition of ROS correlates with a reduction in cell death [154, 155]. Since ROS can cause DNA damage and malignant transformations, stress-induced ROS initiates apoptosis. For example, the UPR in the endoplasmic reticulum triggers ROS which mediates apoptosis [156, 157] (Fig. 7) and has been shown to activate intrinsic mitochondrial death pathways [153, 156]. Even low glucose and oxygen can induce apoptosis through UPR-dependent ROS generation [158]. The sources of ROS may include the NOX family of NADPH oxidases [159, 160] in addition to the mitochondria [161] (Fig. 7). In fact, Ferber et al. showed that inhibition of over 2,700 mitochondrial genes leads to altered ROS [162] and cell death is brought about by mitochondria-derived superoxide radicals [163]. A cross talk between ROS, mitochondria, and NOXs also exists [164]. The role of mitochondria is imperative as it links oxygen utilization (through OXPHOS) with apoptosis (commonly initiated in the mitochondria). Mechanistically, the electron transport chain (ETC) leakiness in the mitochondria is a source of ROS, which is also influenced by oxygen levels [165]. It is believed that irreversible mitochondrial permeability and consequent cytochrome c release commit cells to undergo apoptosis. However, transient changes of the mitochondrial complex appear to drive periodic increases in superoxides called "superoxide flashes." These may be the markers indicating apoptosis or early events during apoptosis and even convergence points where signals for apoptosis integrate [166, 167]. Additionally, cytochrome c in its oxidized form is a superoxide scavenger and its loss during ischemia majorly determines ROS production during heart ischemia studies [168]. Since ROS can influence pathways such as ERK1/2 [155], PI3K-Akt [169], and AMPK [170] to modulate apoptosis, it would be interesting to link the temporal aspects ROS generation with these pathways which elicit apoptosis.

HIFs exert a protective role on cells, for example through regulation of the expression of genes like erythropoietin, suppression of TGF-β signaling, and expression of survivin and Bcl-2 [171–173] (Fig. 7). The negative regulators of HIF1α increase cell death sensitivity [174]. Several physiological and pathological conditions indicate that HIF1α exert protective roles. Induction of HIF1α through mTOR in the developing rat brain correlates with increased survival [175]. In fact, stabilization of HIF1α in epithelial cells causes suppression of Fas-associated death domain [176]. Conversely, suppression of HIF1α increases susceptibility to apoptosis as seen during simultaneous inhibition of GSK-3β and CDK1 which help overcome resistance to apoptosis in hypoxic cells [177, 178]. Cells are also more susceptible to radiation-induced cell death when HIF1α is knocked down [179]. HIF1α expression during ischemia–reperfusion also displays beneficial roles in cardiomyocyte, post-ischemic renal injury, and hyperthermic preconditioning [180–182]. Emerging targets of HIF1α, such as the ARC (apoptosis regulator with caspase recruitment domain) may also be cytoprotective as they exert anti-apoptotic activity during hypoxia [183] (Fig. 7). An interaction between ROS and HIFs is also capable

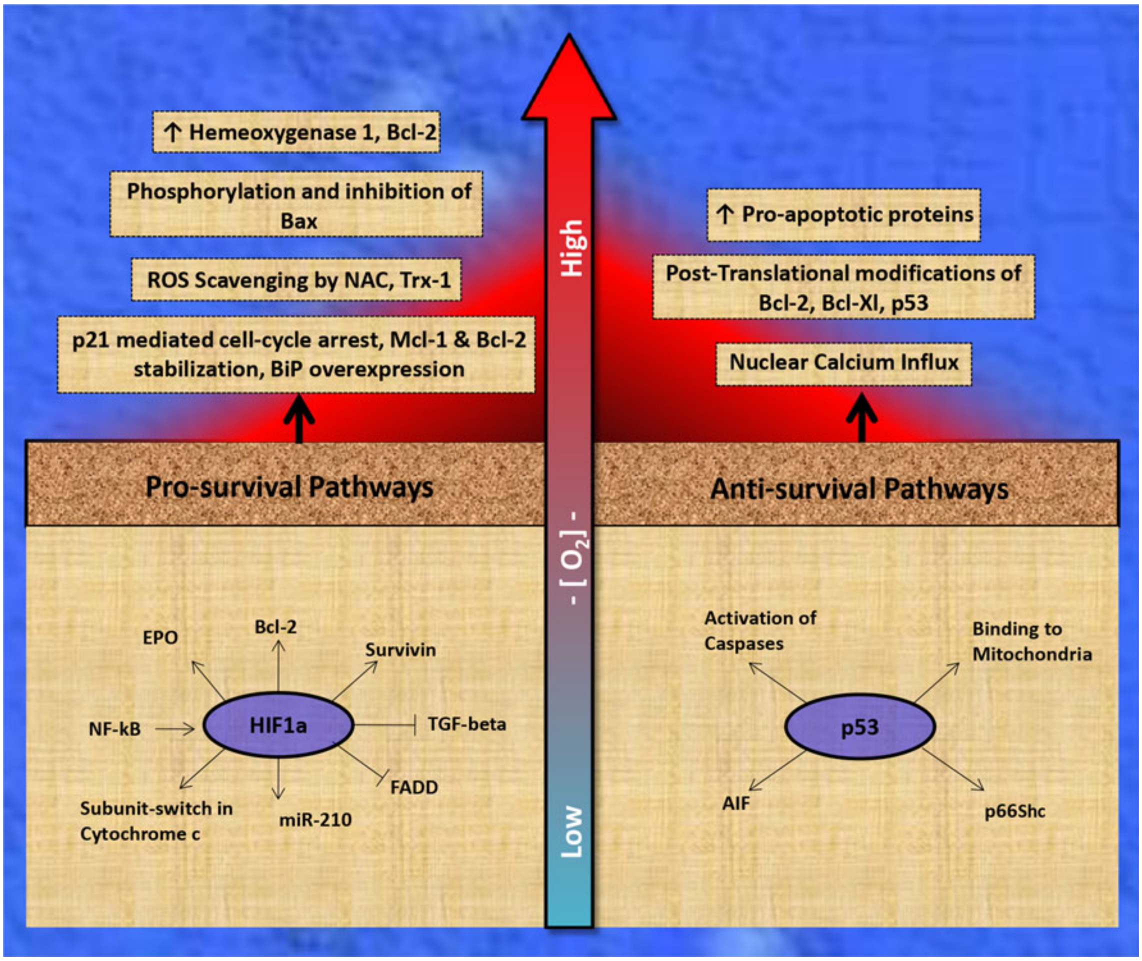
↑ Hemeoxygenase 1, Bcl-2
Phosphorylation and inhibition of Bax
ROS Scavenging by NAC, Trx-1
p21 mediated cell-cycle arrest, Mcl-1 & Bcl-2 stabilization, BiP overexpression
↑ Pro-apoptotic proteins
Post-Translational modifications of Bcl-2, Bcl-Xl, p53
Nuclear Calcium Influx
High
- [O2] -
Low
Pro-survival Pathways
Anti-survival Pathways
EPO
Bcl-2
Survivin
NF-kB
HIF1a
TGF-beta
FADD
Subunit-switch in Cytochrome c
miR-210
Activation of Caspases
Binding to Mitochondria
p53
AIF
p66Shc

Fig. 7 Oxygen controls cellular apoptosis: high and low oxygen levels promote cell survival or death pathways, respectively. Low oxygen levels promote survival through HIF1α-mediated gene expression and cell death through p53-mediated pathways. High oxygen induces pro-survival pathways through increased levels of anti-apoptotic proteins, ROS scavenging, cell cycle arrest, and inactivation of pro-apoptotic pathways. High oxygen also induces increased pro-apoptotic proteins, inactivation of anti-apoptotic proteins, and nuclear influx of calcium. The outcome is decided by the strength of the two opposing signals

of controlling cell death during hypoxia and in fact ROS can stabilize HIF1α [184], while HIF1α can control mitochondrial activity during hypoxia [185] through events such as subunit switch in cytochrome c, miR-210 expression, and others [178] (Fig. 7). HIF1α responds to diverse agents such as UVB radiation damage in connection with ROS [186].

Oxygen fluctuations are cellular stressors which can activate p53, the well-known modulator of apoptosis during stress. Recently, the role of p53 in oxygen-dependent apoptosis has been highlighted (Fig. 7). p53 fusion protein targeting to hypoxic non-small cell lung cancer resulted in apoptosis [187]. p53 can utilize the signaling from ROS inducing proteins such as p66Shc and cytochrome c to generate apoptotic signals [188, 189]. Evidence suggests that interactions between p53 and mitochondria may be required for inducing apoptosis as seen in neonatal ischemic brains [190]. In particular, inhibition of p53 association with mitochondria using pifithrin-μ is a neuroprotective strategy, as it prevents cytochrome c release, reduces oxidative stress, and consequently reduces the lesion size in ischemic brain [190]. A critical role of p53 in apoptosis upon oxidative stress is implied by the functional redundancy of p53; certain mutations in p53 are tolerated due to activation of alternate mechanisms. Functional p53 induces a caspase-dependent apoptotic response while mutantp53 induces caspase-independent, AIF-dependent response in neuroblastoma cells under hypoxia-mimetic agent $CoCl_2$ [191]. Functional p53 is also reported to induce AIF through caspase 2 [192]. Along with these well-known targets, new pro-apoptotic targets of p53 like PERP and Siva have been reported to be activated during ischemia [189, 193]. It is clear that oxygen–p53 connection has an indispensable role in apoptosis and they function in close association with the mitochondrial pathways (Fig. 7).

Hyperoxia is also reported to be well-connected to apoptosis. The common proteins involved in apoptosis include Bcl-2 family members of apoptosis-signaling proteins of which BAX, BID, BAK, and BAD are pro-apoptotic while Bcl-Xl and Bcl-2 are anti-apoptotic. Hyperoxia is deleterious to cells as it induces ROS production, although mitochondrial ROS may be dispensable [194]. For example, exposure of neonatal rat lung cells to hyperoxia induces apoptosis by activation of pro-apoptotic protein BAX [195]. Similarly, hyperoxia treatment (100 % O_2) given during perinatal hypoxia–ischemia leads to BAX-mediated neuronal cell death [196]. During hyperoxia, posttranslational modifications such as phosphorylation of Bcl-2 and Bcl-xl can result in loss of their anti-apoptotic potential [197] (Fig. 7). Investigation on whether ROS acts upstream of death pathways or is produced as a result of mitochondria permeability led to the conclusion that ROS can indeed initiate apoptotic signaling through BAX in hyperoxic cells [198]. In alveolar epithelial cells, oxidative stress activates BAX and BAK for cell death as SOD overexpression or loss of BAX and BAD can prevent apoptosis [199]. Cells overexpressing anti-apoptotic Bcl-2 have reduced mitochondria-mediated apoptosis and higher antioxidant compounds during hyperoxia [200]. In addition to ROS, nuclear calcium influx has been reported during hyperoxia of cortical neurons, which specifically affects CREB protein-mediated transcription of pro-apoptotic genes [201]. These reports indicate that hyperoxia, in general, has pro-apoptotic roles.

However, the cell does not always succumb to hyperoxia-mediated apoptosis. In fact, inhibitory posttranslational phosphorylation can prevent apoptosis. For example, during hyperoxic acute lung injury IL-6 induces phosphorylation of BAX, rescuing apoptosis via PI3K/Akt-mediated pathway [202]. Also, overexpression of hemeoxygenase-1 can prevent cell death through increased production of carbon monoxide, which inhibits apoptosis in endothelial cells by decreasing caspase 3/9 activation and cytochrome c release [203]. NAC can prevent the ROS-mediated activation of JNK pathway that induces apoptosis in type II alveolar epithelial cells by scavenging ROS [204]. Another radical scavenger, thioredoxin 1, exerts a cytoprotective role by, in part, upregulating Bcl-2 protein [205] (Fig. 7). Cells may use these proteins to restore cellular homeostasis during hyperoxia. Although p53 is pro-apoptotic, it can also activate p21 to arrest cells giving them a chance to recover from oxidative stress. p21 prevents induction of apoptosis by utilizing various mechanisms; while p21, p53, and BAX seem to be induced under hyperoxia, p21-deficient mice showed less survival [206]. The cytoprotection by p21 may be due to reduction in proliferation, increase in pro-survival proteins, or induction of senescence [99]. In fact, p21 overexpression causes a delay in the loss of anti-apoptotic proteins Mcl-1 and Bcl-Xl and protects against hyperoxia [207]. One investigation has suspected the role of p21 (with deleted NLS) as an oncoprotein by showing that its cytoplasmic localization, possible association with mitochondria, and p21-dependent delay in the loss of anti-apoptotic proteins exerted cyto-protective effects during hyperoxia [206]. The same study elucidated another role of p21 that it causes increase in BiP chaperone in ER, leading to correct protein folding which suppressed UPR-mediated induction of apoptosis during hyperoxia [206]. Thus, p21 is not just an essential mediator of cell cycle arrest during oxygen changes, but it also exerts cytoprotection by delaying apoptosis through numerous mechanisms.

In summary, elevated oxygen levels appear to induce ROS-mediated cell death while low oxygen promotes HIFs, in addition to ROS generation. Recent reports have expanded our knowledge on the mediators and effectors of oxygen-mediated apoptotic pathways and suggest unconventional roles of known proteins in modulating these intricate processes. Tumor environments, stem cells niches, pulmonary damage, and cardiovascular models, among others, have provided insights into how apoptosis can benefit or damage cells or tissues. Aspects of physiologically low-oxygen or elevated oxygen as well as of reoxygenation emphasize oxygen's pivotal contribution to apoptosis. Although both apoptotic and survival signaling ensues upon hypoxic or hyperoxic/HBO exposure, the relative balance might dictate the ultimate outcome. Clearly, cancers utilize these homeostatic mechanisms in hypoxia (through HIFs) and in a less direct manner during elevated oxygen exposure to gain survival advantages.

7 Oxygen mediates cellular senescence

In the natural process of aging, a cell accumulates several abnormalities of which genomic insults are noteworthy. Although these abnormalities do not affect the functioning of the cell, the division can predispose cells towards malignancies. Senescence is a form of quiescence that preserves the functional competence of cells while prohibiting their entry into cell division. Senescence can be induced naturally due to shortening of telomeres (called replicative senescence) or may be induced due to high oncogene activity (oncogene-induced senescence, OIS).

Oxygen and senescence are connected in a number of ways. Oxygen stress is capable of cellular damage through generation of ROS and a general disturbance of redox homeostasis, both of which induce senescence. Also, since oxygen supply can affect cellular energy balance, the rate at which a cell divides (often limited by nutrient availability) and reaches senescence may also be an oxygen-governed process. The relationship between ROS and senescence was reported in fibroblasts where oncogenes such as Ras-generated excessive H_2O_2 which led to cellular senescence [208], possibly due to growth factor signaling. Interestingly, hypoxia was found to inhibit senescence by reducing cellular ROS levels [208]. Extending the correlation of ROS with senescence, Leikam et al. showed that strong growth factor signaling triggers ROS-mediated senescence [209], supporting the observation of ROS-driven cell cycle progression (Fig. 8). These findings support a model in which growth factor-induced ROS must be present within a range to promote cell cycle. A low level of ROS signifies absence of growth stimulus whereas an excess of ROS signifies rapid growth, as observed during oncogenesis. Other studies support ROS-dependent senescence by reporting that mitochondrial mass increases during OIS and that interference with mitochondrial OXPHOS and electron transport chain is sufficient to induce senescence [210]. In addition to the mitochondria, the ROS produced by NOXs can induce senescence by causing DNA damage [211] (Fig. 8). Irrespective of the source of ROS, it has been shown that antioxidants can prevent senescence in cells where ROS is derived from mitochondria or photochemical treatment [212, 213]. In addition, inhibitory pathways that contribute to ROS production have also been shown to reduce senescence. For example, PTEN-mediated inhibition of superoxide production in PI3K-mediated pathway can inhibit UVB-induced senescence in human keratinocytes [214].

The hematopoietic stem cells (HSCs) residing in acute hypoxic areas require high rates of proliferation and self-renewal, and provide a model to study the significance of hypoxic niches and senescence. It is emerging that hypoxia causes decreased ROS levels in HSCs and is thus responsible for delaying senescence. This correlation was observed by Jang and Sharkis showing that low oxygen levels select for primitive HSCs, and that their self-renewal ability is diminished upon introduction to higher oxygen levels during serial transplantation in mice [215] (Fig. 8). Interestingly, ROS utilizes the p38/MAPK pathway to cause senescence indicating a specific response of cells to oxygen levels and thus ROS [215, 216]. In contrast to these findings, Hole et al. reported that ROS in CD34+ hematopoietic progenitor cells promotes proliferation [217], probably further supporting the model where ROS levels can decide cell cycle progression or senescence.

p53 and pRb are capable of keeping cells in a senescent state and can function cooperatively to induce senescence. However, whether *ROS* triggers p53-mediated senescence remains unknown, although p53 utilizing ROS to induce senescence (and apoptosis) is a known phenomenon [218]. In contrast, some studies show senescence mediated by p53 without any report of ROS change. For example, oncogenic Ras and p53 cooperate to induce senescence, although no ROS change was reported [219]. However, since Ras itself is known to produce ROS for senescence [208], it is likely that ROS plays an essential role. ROS, by causing DNA damage, can activate ATM pathways and thus elicit p53-mediated senescence [220–222] (Fig. 8). This is similar to activation of p53 during telomere shortening, a situation similar to DNA double-stranded breaks [223] (Fig. 8). Activation of several Cdk inhibitors like p21, p15, p16, and p27 is seen during senescence [224, 225] (Fig. 8). The role of pRb in sequestering E2F family of cell cycle proteins is known, and through its interaction with p16, pRb is involved in senescence [226].

The HIF1α protein seems relatively less studied in relation to senescence, but reports indicate a protective role of HIF1α on cells. For example, it was reported in primary human lung fibroblasts that increased ROS correlated with increased HIF1α, and the replicative life span of cells was increased due to HIF1α-mediated telomerase induction [227] (Fig. 8). Similarly, HIF1α seems to activate telomerase in murine embryonic stem cells as identified by a RNAi screen [228, 229]. Also, the role of pVHL was implicated in senescence where it was shown that pVHL pathways can function with or without HIF1α to influence senescence [230–232].

Elevated oxygen also seems to have some relation with senescence since high pressure (2 ATM) or concentration (40 %) of oxygen can induce senescence in human diploid fibroblasts [233]. Hyperoxia can reduce telomerase activity which correlates with reduced telomere lengthening in endothelial cells [234] (Fig. 8). In addition to this, hyperoxia decreases histone deacetylase (HDAC) activity and increases p53 and p21 levels causing the cells to enter senescence [235]. In another study using hyperoxia (70 % oxygen), ROS independent mechanisms were reported to be responsible for senescence [236] (Fig. 8) involving p53-mediated p21 and pRb-mediated p16 upregulation [236].

8 Oxygen regulates cellular motility and cancer metastasis

Oxygen contributes to cell motility in a number of events which indicate that oxygen acts like a chemotactic molecule. The processes of HSC migration, neutrophil recruitment and extravasation, wound healing, as well as cell invasion and metastasis are all events where oxygen gradients guide cellular movement. Cellular motility is associated with changes in expression of cell adhesion molecules, often described as restoring to mesenchymal phenotypes. From the perspective of cellular motility, the process of metastasis involves dissolution of the basement membrane, detachment from primary site, and intravasation into the blood through endothelium. This is followed by extravasation from the vessels and colonization the secondary tissue, homing, and proliferation. Many of these molecular processes are influenced by oxygen levels.

The events in metastasis can be viewed as those occurring at the level of tissue stroma, yet gaining support from the complex intracellular signaling cascades. In order to sustain these signals over the long duration of metastasis, ROS production has emerged as a crucial strategy utilized by these motile cells. The activation of the MAPK pathway, that is indispensable to support cytoskeletal changes, is one such signaling cascade activated by ROS. The long-term activation of MAPK pathway occurs through the ROS-mediated inactivation of the paxillin-associated protein–tyrosine phosphatase (that inhibits RTK signaling), as well as direct activation of the

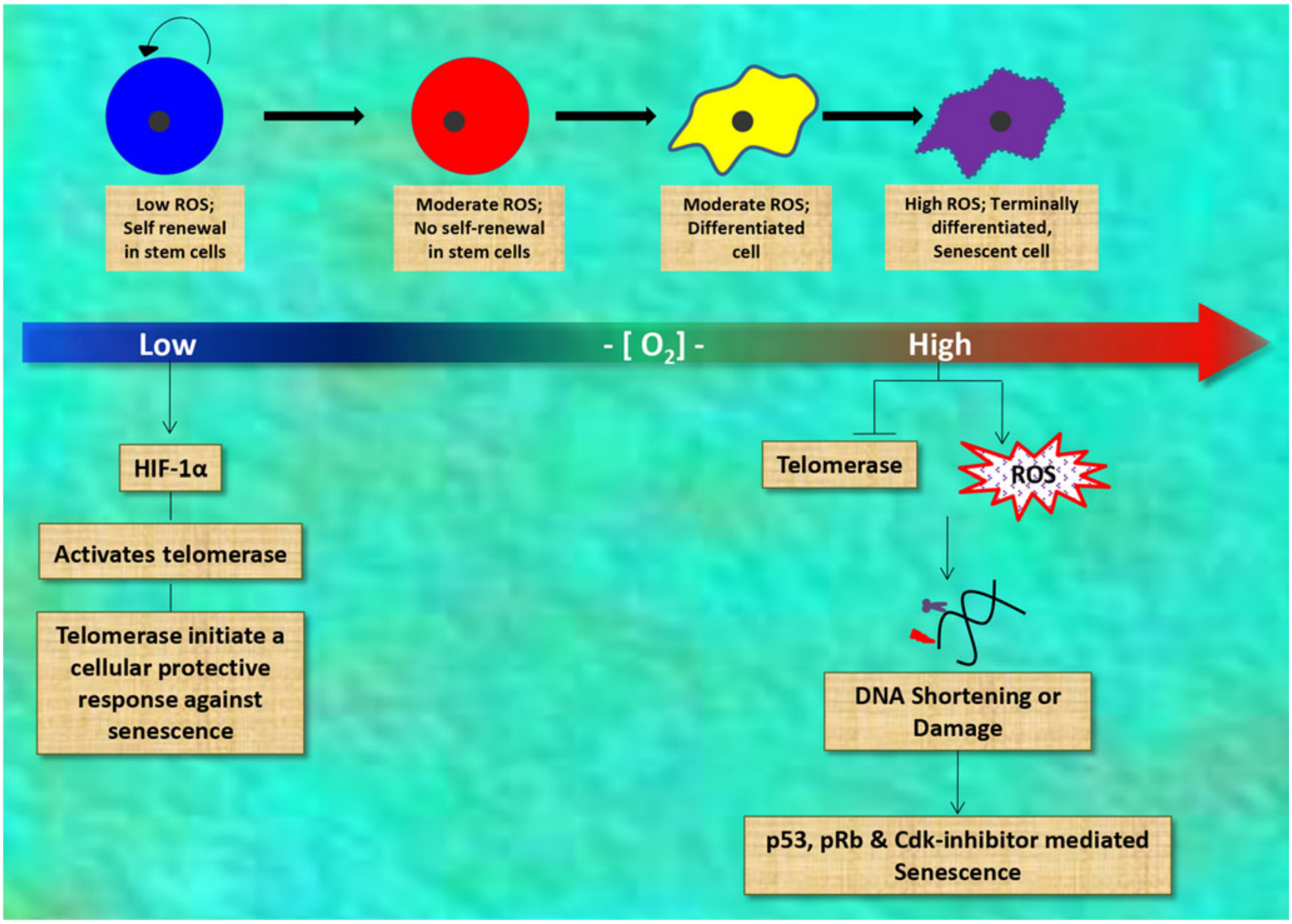
Low ROS;
Self renewal
in stem cells
Moderate ROS;
No self-renewal
in stem cells
Moderate ROS;
Differentiated
cell
High ROS; Terminally
differentiated,
Senescent cell
Low
- [O$_2$] -
High
HIF-1α
Activates telomerase
Telomerase initiate a cellular protective response against senescence
Telomerase
ROS
DNA Shortening or Damage
p53, pRb & Cdk-inhibitor mediated Senescence

Fig. 8 Oxygen regulates cellular senescence: (*top*) the senescence model of stem cell differentiation where low oxygen levels correlate with low ROS and increased self-renewal potential. Upon increasing oxygen levels, ROS accumulation causes reduced self-renewal and increased senescence. (*bottom*) The molecular basis of low senescence in hypoxia is based on HIF1α-dependent activation of telomerase to prevent replicative senescence. High oxygen levels promote senescence by inhibiting telomerase activity, and by ROS mediated DNA damage; the resulting damage or shortening of DNA causes DNA damage signaling and p53, pRb, and Cdk inhibitor-mediated senescence

PKC pathway [237]. The resulting effect of ROS is thus the activation of RTK and PKC that maintain MAPK activity, in effect promoting motility. Another target of ROS is PAK that is an effector of Rac-mediated cytoskeletal remodeling. The activity of PAK is responsible for cytoskeletal remodeling that is utilized to gain motility and to support angiogenesis [36].

Hypoxic regions within tumors not only exploit the cytoprotective role of HIF1α but also utilize this transcription factor to increase the expression of metastasis-associated genes, including matrix metalloproteinases (MMPs) [238]. In general, MMPs are responsible for the dissolution of the basement membrane. While this is a part of normal developmental processes in HSC migration, it is also the primary step towards metastasis [239] implicated in several cancers, including lung and breast tumors [240, 241]. MMP expression is also seen in cell lines of breast, lung, pancreatic, and cervical cancers [242]. Under hypoxia, HIF1α overexpression results in an increase in the cellular levels of MT1-MMP in HSCs which play an important role in regulating their migration patterns [239]. HIF1α also regulates the gelatinase activity of MMP-9 in breast cancer cells [240] and transcriptionally targets MT2-MMP through HREs on its promoter [242]. In fact, a critical balance of MMPs and tissue inhibitors of MMPs seems to be disturbed during hypoxia in favor of MMPs, which leads to an increase in invasion and metastasis potential of hypoxic cells [243] (Fig. 9).

Cellular motility, facilitated upon transition of epithelial cells to mesenchymal cells (the EMT transitions), is well known during metastasis. EMT transitions include altered expression of cell adhesion molecules—while E-cadherin is associated with retention of adhesion, N-cadherin promotes migration [244] (Fig. 9). Accumulating evidence shows a potent role of oxygen in acting as a switch for EMT by affecting HIF1α and redox environment, for example. It has been observed that lower oxygen levels can induce EMT transitions; however, it is surprising to note that higher oxygen concentrations can reverse EMT transitions [244]. This observation is a phenomenon of immense therapeutic significance.

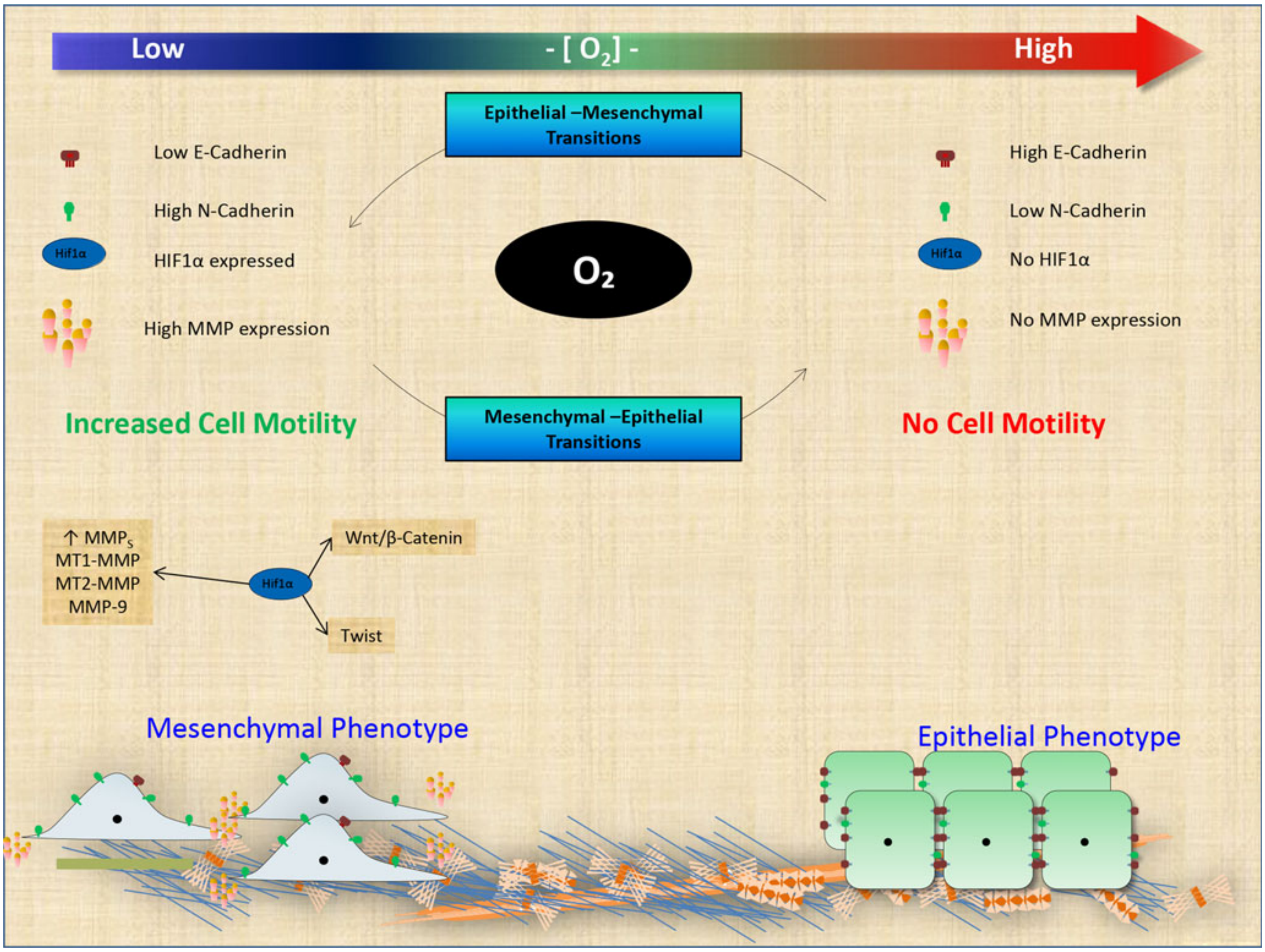
Low
- [O_2] -
High
Epithelial –Mesenchymal Transitions
Low E-Cadherin
High N-Cadherin
Hif1α
HIF1α expressed
High MMP expression
O_2
High E-Cadherin
Low N-Cadherin
Hif1α
No HIF1α
No MMP expression
Increased Cell Motility
Mesenchymal –Epithelial Transitions
No Cell Motility
↑ MMP_S
MT1-MMP
MT2-MMP
MMP-9
Hif1α
Wnt/β-Catenin
Twist
Mesenchymal Phenotype
Epithelial Phenotype

Fig. 9 Oxygen governs cellular motility and metastasis: high oxygen levels induce epithelial-like phenotypes, displaying higher E-cadherin and low N-cadherin levels resulting in low cell motility and metastasis. Lower oxygen levels cause epithelial to mesenchymal transitions displaying high N-cadherin, low E-cadherin, and HIF1α expression. At a molecular level, HIF1α mediates increase in expression of N-cadherin, decrease in expression of E-cadherin, basement membrane dissolution through matrix-metalloproteinases (MMPs), increased expression of Twist and Wnt/beta-catenin signaling, and causes increased cellular motility. Oxygen may reverse cell motility based on its relative levels

The reversal of EMT (called MET) is seen and is characterized by increased E-cadherin expression and decreased N-cadherin expression along with reduced expression of pro-angiogenic proteins like VEGF, FGF, and PDGF [244]. Thus, it is interesting how complex phenotypes can be reversed by simple alteration in oxygen levels (Fig. 9).

The Wnt/β-catenin pathway is utilized by HIF1α to signal EMT transition as knockdown of β-catenin leads to E-cadherin expression in human prostate cancer cells LNCaP providing an essential link between HIF1α and E-cadherin expression [245] (Fig. 9). However, Schietke et al. report that lysyl oxidases, which are regulated by HIF1α during hypoxia, are sufficient to induce E-cadherin downregulation [246]. It remains elusive whether β-catenin signaling is utilized for this downregulation. HIF1α can also cause increased expression of transcription factors involved in cellular motility. For example, it has been shown that Twist expression, which in turn causes N-cadherin expression, is controlled by HIF1α and is induced by thrombin [247] providing another example of oxygen levels regulating cellular motility (Fig. 9).

In addition to fluctuations in oxygen levels, reoxygenation can also affect cellular motility. For example, reoxygenation of human pancreatic cancer cells (PANC-1) after hypoxia causes invasion mediated by increased production of MMP-2 [248]. Supporting evidence shows that E-cadherin expression is transiently reduced after hypoxia and reoxygenation of human colon cancer cell lines [249] indicating a mesenchymal phenotype. Sprague et al. observed that plasminogen activator inhibitor-1 (PAI-1, a serine protease inhibitor) (involved in pre-adiposite migration) [250] is upregulated and secreted from hypoxic head and neck cancer cells 24 h following reoxygenation [251]. The levels of PAI-1 correlate with metastatic ability of cells [251]. Similarly, Postovit et al. observed that re-oxygenation promotes metastasis by lysyl oxidase's catalytic activation [252]. These findings suggest that reoxygenation can promote metastasis by diverse pathways.

These reports suggest that the movement of cells either along or away from an oxygen gradient follow a molecular reprogramming, mediated predominantly by HIF1α and ROS but utilizing several downstream pathways. In fact, it has been suggested that some of the mechanisms that drive aerotaxis towards oxygen and nutrients in *Escherichia coli* are similar to those involved in redox-driven movement during metastasis [71]. As reports on oxygen-dependent motility accumulate, our understanding of compromised cell motility during embryonic development and immune activation on one hand, and aberrant motility during cancer-metastasis on the other will increase, thereby also opening up oxygen therapies as likely additions to ongoing administration of drugs.

8.1 p53 and oxygen

The connection of oxygen with p53 is an example of the extent to which oxygen impinges upon cellular mechanisms and influences cancers. A fluctuation in oxygen levels activates p53 through posttranslation modifications by upstream proteins. Interestingly, these posttranslational modifications are specific for a particular form of oxygen-mediated activation (such as through ROS or HIFs). Besides this, direct interaction of p53 and hypoxia-specific proteins (such as HIF1α), modulation of ROS levels through p53, and even oxygen-dependent chaperoning activities of p53 are ways by which oxygen is linked to p53 and thus cancers. Fluctuation of oxygen levels disturb cellular homeostasis which results in activation of p53. Posttranslational modifications are critical component of p53 activation and they regulate the stability, protein–protein interactions, and transcriptional function of p53. Several posttranslational modifications of p53 are linked to altering oxygen levels. p53 activity is now shown to be related to HIF1α in hypoxic cells, further the involvement of p53 in regulation of cellular metabolisms suggests its ability to sense cellular oxygen levels.

Since HIF1α is essential in hypoxic responses, its interaction with p53 is interesting given that both these transcription factors are involved in certain cooperative as well as antagonistic roles. It remains unclear whether HIF1α is necessary for p53 activation under hypoxia [253, 254] or the hypoxic environment causes p53 activation. HIF1α indirectly stabilizes p53 by binding to MDM2, and thereby reduces the MDM2-mediated p53 degradation [255]. However, in agreement with its tumor suppressive role, p53 indirectly causes degradation of HIF1α probably to limit the pro-survival effects [255] (Fig. 10).

Under oxygen fluctuations, the accumulation of ROS causes DNA damage and leads to a p53-mediated DNA damage response (Fig. 10). The accumulation of ROS causes oxidation of nucleotides and RNA damage [256, 257], while DNA instability and compromised repair are seen under hypoxia [258, 259]. Although moderate levels of ROS help cancer cells acquire new mutations, it has recently also been suggested that cancers that activate antioxidant mechanisms to fight ROS accumulation survive better. As a deduction, ROS can actually help suppress tumorigenesis through an interplay with tumor suppressor proteins including p53 [71]. Reoxygenation and hyperoxia, like hypoxia, can cause extensive DNA damage which often triggers p53-mediated apoptosis [260, 261]. Like other stresses, oxygen fluctuations utilize conserved pathways involving ATM, ATR, and p53 to sense DNA damage. ATM responds to double-strand damage and involves Chk2 while ATR gets activated by single-strand damage and replication stalling, activating the Chk1 pathway (Fig. 10). Under hyperoxia, the extent of p53 activation seems to vary in different cell types. For example, in bronchial smooth muscle cells, 95 % oxygen does not cause significant p53 accumulation until 72 h [96], while A549 and Mv1Lu cells under 95 % oxygen show high p53 expression and p53-Serine15 phosphorylation [97]. The ATR pathway, but not the ATM pathway, seems to be required for signaling initial damage through critical p53 phosphorylations as ATM(−/−) cells have no effect on p53-Ser15 phosphorylation [262] and ATR alone mediates p53-Ser(-6, -15, -37, and -392) phosphorylations [263]. An emerging view of hyperoxia-mediated damage suggests that ATR is initially required for DNA damage-mediated p53 phosphorylation but sustenance of phosphorylation status involves ATM. Another family member of ATM/ATR called SMG-1, a RNA surveillance protein, can also activate p53 upon DNA double-strand breaks [264]. Collectively, hyperoxia-mediated p53 activation seems to utilize ATR/ATM proteins but follows a unique ATR/ATM-mediated signaling pathway for eliciting DNA damage response (Fig. 10).

It may appear that ROS acts upstream of p53 while p53 acts as a bystander only responding to DNA damage. However, it has been observed that p53 modulates cellular susceptibility to ROS by dramatically influencing cellular ROS levels by enhancing pro-oxidant genes to promote apoptosis under stressed conditions [265, 266]. For example, p53 utilizes p66Shc to induce oxidative stress, cytochrome c oxidation, and apoptosis [188] (Fig. 10). In addition, p53 can cause uncoupling of ETC in mitochondria to generate ROS through its targets BAX and PUMA [267]. This observation reflects back to the conundrum where ROS, in normal and cancer cells, can prove to be deleterious. It has been suggested that the accumulation of ROS, while causing apoptosis and senescence in non-transformed cells, can be equally lethal to the cancer cells by causing senescence and apoptosis mediated by tumor suppressor proteins [71].

The metabolic control by p53 can determine ROS levels as p53 can increase ROS through OXPHOS and through suppression of glycolysis. Two recently identified targets of p53 are utilized for this balance and include SCO2 [268] and TIGAR [269]. p53 can increase ROS levels by targeting cytochrome c oxidase through SCO2, or utilize TIGAR to

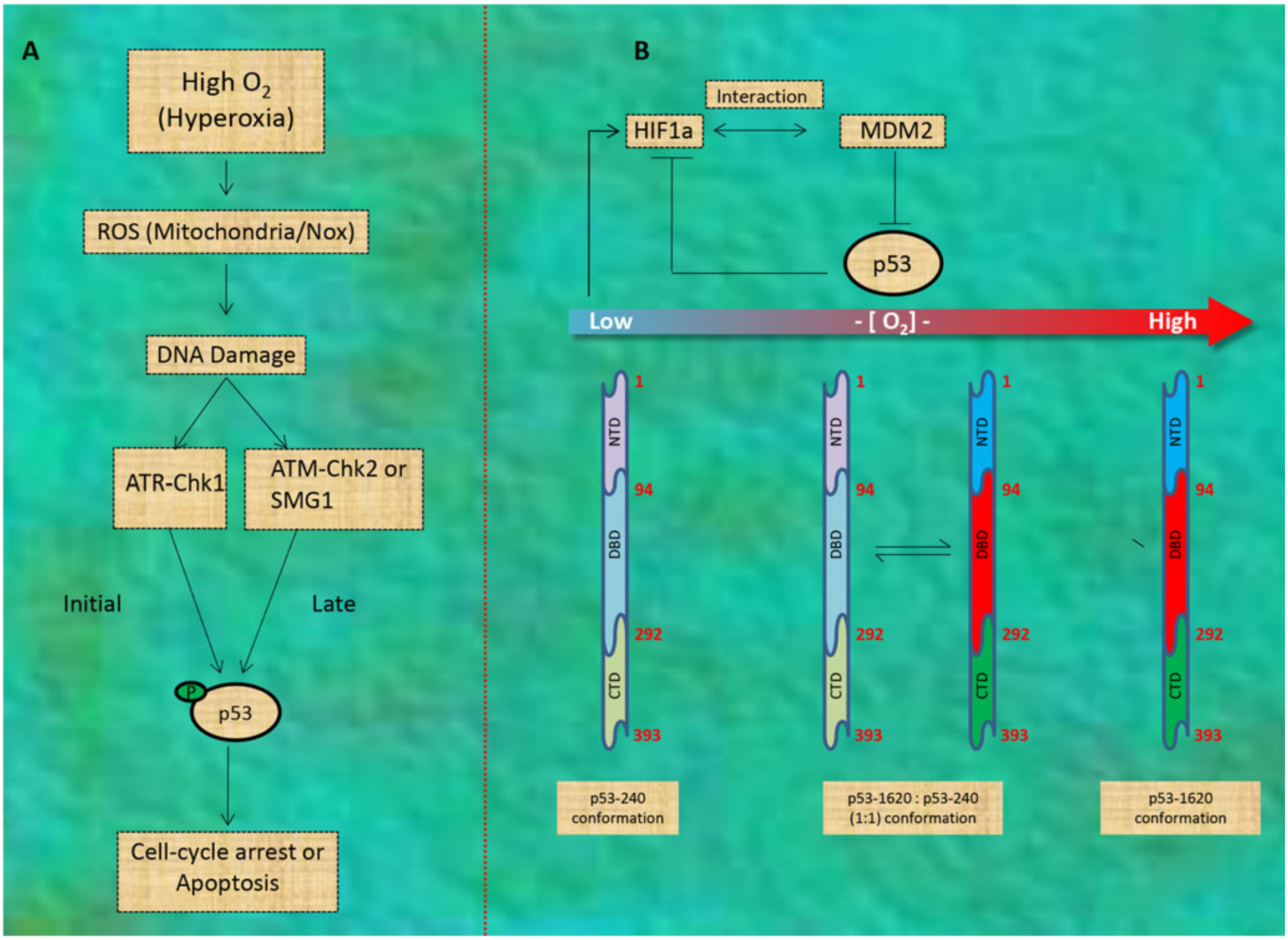

Fig. 10 Oxygen controls p53 activity: **a** high oxygen levels increase cellular ROS levels through mitochondria or Nox causing DNA damage, thereby initially activating ATR-Chk1 mediated, and later ATM-Chk2-mediated phosphorylations on p53, that in turn promote cell cycle arrest or apoptosis. **b** (*top*) Under low oxygen, HIF1α increases p53 accumulation by interacting with MDM2, while p53 negatively regulates HIF1α resulting in a negative feedback loop. (*bottom*) p53 conformation is controlled by oxygen. Low oxygen results in the mutant conformation of p53 assayed by conformation specific antibody (p53-240 conformation) while high oxygen results in predominantly wild-type p53 conformation assayed by conformation specific antibody (p53-1620 conformation). Normal oxygen levels have equal levels of normal and mutant p53 conformations

suppress glycolysis. In cancers carrying mutated p53, SCO2 cannot be induced and hence cancer cells are protected against ROS [270]. TIGAR activation has dual effects such that by suppression of glycolysis, TIGAR prevents oncogenic transformations, but by itself can suppress ROS and offer protection to a cancer cell. However, Madan et al. have shown that TIGAR is induced by p53 under mild stress [271], thereby choosing one of its antagonistic functions described above.

In addition to signaling pathways, p53 responds to oxygen availability through redox modulations, conformational changes and by inducing chaperones. Redox modulation of p53 involves the thiol groups of cysteine present in two regions of the p53 DNA-binding domain which help it to bind to its consensus sequences [272]. Previously, p53 was described as a metallo-protein that binds to zinc [272] and mutations in the zinc-binding pocket of p53 compromise the DNA-binding activity [29].

Reports also suggest that preservation of conformation of p53 is essential in its functioning as p53 forms aggregates during oxygen deprivations such as ischemia, and aggravates diseases such as coronary heart disease [273]. In itself, p53 conformation is seen altered upon decreasing oxygen level as assayed by an antibody raised against the mutant conformation of p53 (Mt-p53, the p53-240 conformation) which recovers upon normoxic conditions to wild-type conformation (Wt-p53) as assayed by another antibody (p53-1620 conformation). In the study, p53 was assigned the role of a molecular chaperone, such that its mutant conformation is rescued by wild-type p53 protein [105] (Fig. 10). As a follow-up of the study, it was observed that while hypoxia leads to a mutant p53 conformation [105], treatment of tumors in mice xenografts with oxygen in a cyclic manner caused tumor regression in a p53-dependent manner [104], indicating a new oxygen-dependent conformational regulation of p53.

Higher oxygen levels were able to bring about a specific set of posttranslational modifications, significantly different from those observed under hypoxia or normoxia [104].

Various posttranslational modifications of p53 have led to the emergence of "smart-p53" versus "dumb-p53" model [274]. In the former view, p53 undergoes these posttranslational modifications to intelligently select its interaction with promoters (to induce transcription) and other proteins to exert tumor suppression [274] while in the latter view, the modifications of p53 by various proteins exclusively dictate its function. It has been observed that stabilization, promoter specificity, subcellular localization, transactivation functions, etc. [275, 276] are mediated by the posttranslational modifications of p53.

Hypoxia is capable of regulating p53 ubiquitination through regulation of HIF1a–MDM2 interaction [277] and ubiquitination of p53 affects its localization (in addition to controlling its levels) [278]. Mono-ubiquitinated p53 is exported out of the nucleus [279] and localizes in the mitochondria [278]. Another oxygen-dependent posttranslational modification of p53 occurs by the p300/CBP protein, which is the common co-activator of p53 and HIF1α (Fig. 10). It has been proposed that p300/CBP is under limited availability during hypoxic stress [280], and HIF1α and p53 both compete for p300 [281]. Another mechanism utilized by HIF1α is the activation of cockayne syndrome B protein, which competes for p53 binding with p300, making more p300 available for HIF1α binding [281].

In addition to ubiquitination and acetylation, p53 activation by phosphorylation is well known [282]. As described above, p53 Ser15 is an important site for phosphorylation, and ATM/ATR cooperate with Chk2-Chk1 for these phosphorylations. Under hypoxia, DNA damage can lead to p53 phosphorylation. However, it is interesting to consider that protein phosphatase recruitment might also be affected by hypoxia, for example by recruiting PNUTS (protein phosphatase-1 nuclear targeting subunit) [277].

Recent reports show that sirtuins, a family of HDACs target p53 [283] for deacetylation. The fact that sirtuin activity itself depends on NAD+ (a redox cycler) causing deacetylation of p53 itself links cellular redox status with posttranslational modification of p53. It has been shown that SIRT inhibitors that target SIRT1 and SIRT2 cause p53 acetylation, leading to cell death [284]. Similarly, expression of SIRT1 under H_2O_2 or UV stress exerts cytoprotective effects by reversing stress-induced p53 acetylation [285, 286]. Thus, changes in cellular redox environment often found during altered metabolism in cancer cell or aged cells can have implication in cell survival [286] through sirtuins. This has been shown in aging Wistar rats where an age-dependent decline in NAD+/NADH level correlated with decreased SIRT1 activity and hence increased p53 acetylation and cell death [286]. In addition to the previously mentioned posttranslational modifications in response to oxygen, several other modifications of p53 are known. For example, acetylation of p53 occurs by PCAF, hMOF, Tip60, etc. in addition to SIRT1 and p300/CBP [287]. Similarly, lysine residues of p53 undergo modifications such as methylation, sumoylation, and neddylation [278, 288]. As scientific findings accumulate, the role of oxygen in these posttranslational protein modifications will be better understood.

8.2 Summary

The importance of oxygen in regulation of cellular and molecular biology of life is a very complex research area. It appears that altering oxygen levels cannot be considered as a single parameter for analyzing its impact. The review suggests that it is accompanied by other phenomena like redox-homeostasis response, DNA damage, and ROS among others. One thing is clear: oxygen impacts basic biological processes and scientists have observed them at both the physiological and molecular level. Yet somehow the role of oxygen as a comprehensive research field is not in sights of the research leaders. With this review, we wish to broaden the horizon and spread awareness regarding the existence of an interesting and untouched research area with immense opportunities of basic science and therapeutic discoveries. We would like to coin the terms like oxy-genomics, oxy-therapy, and oxy-biology for the field leaders to notice and exhaust the great potential of this field for achieving gain of knowledge and benefits to patients.

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