

Review

Hypoxia and Senescence: The Impact of Oxygenation on Tumor Suppression

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Abstract

Cellular senescence has emerged as a biological response to two major pathophysiological states of our being: cancer and aging. In the course of the transformation of a normal cell to a cancerous cell, senescence is frequently induced to suppress tumor development. In aged individuals, senescence is found in cells that have exhausted their replication potential. The similarity in these responses suggests that understanding how senescence is mediated can provide insight into both cancer and aging. One environmental factor that is implicated in both of these states is tissue hypoxia, which increases with aging and can inhibit senescence. Hypoxia is particularly important in normal physiology to maintain the stem cell niche; but at the same time, hypoxic inhibition of an essential tumor suppressor response can theoretically contribute to cancer initiation. *Mol Cancer Res*; 9(5); 538–44. ©2011 AACR.

Introduction

Cellular senescence is a programmed response designed to remove damaged cells from the proliferative population as they age. Senescence can be induced by many stimuli, including telomere uncapping and shortening, DNA damage, oncogene activity, and oxidative stress, and is thought to provide a means to permanently arrest cells. As such, senescence plays a critical role as a tumor suppressor response that is mediated by classic tumor suppressor pathways and is necessarily inactivated early in the transformation process (1).

Signals leading to senescence depend primarily on the p53 and pRb pathways both for induction and maintenance of cell-cycle arrest (1). Seemingly different types of stresses lead to a common senescent phenotype through convergence upon these pathways. Telomere erosion, for example, is perceived by cells as DNA damage, and thus activates a typical DNA damage response involving p53 (2). Oncogene activity can affect both p53 and pRb through several mechanisms, including inducing a DNA damage response due to hyperreplication (3), or by inducing the expression of CDKN2A or CDKN2B (4, 5) that impinges upon pRb activity or p53 stability, respectively.

An underappreciated factor in the regulation of senescence is oxygen. Atmospheric pO₂, which is common in standard tissue culture incubators, is roughly 160 mm Hg. In contrast, the partial pressure of oxygen in the body is significantly lower, even as it varies widely in different organs (Fig. 1). The pO₂ in the alveoli of the lungs is 110 mm Hg, whereas the median pO₂ in the brain, liver, and heart is near 24 mm Hg (6). Thus, much of our understanding of senescence comes from studies that have been conducted in what is effectively hyperoxia, which itself can be a potent inducer of senescence (7). Intriguingly, various studies have shown that senescence from many different stimuli can be averted under lower levels of oxygen (8–10). This is true for replicative senescence and stress- and oncogene-induced senescence (OIS). Therefore, to understand how and when senescence is induced *in vivo*, it is critical to understand the role of oxygen in regulating senescence.

The goal of this review is to highlight recent and historical observations that contribute to the understanding of how hypoxia can modulate senescence. Insights into mediators of senescence in a number of different systems have recently come to light. Interestingly, independent connections to physiological hypoxia pull many of these novel mechanisms together onto a common playing field and will be discussed here.

Oxygen and oxidative stress

Supraphysiological levels of oxygen result in oxidative stress through the increased production of mitochondrial reactive oxygen species (ROS; ref. 11). The classic model of senescence from hyperoxia is that ROS play a causative role in inducing senescence (7, 12). Conversely, under lower oxygen, decreases in ROS rescue senescence (Fig. 2A; refs. 9, 10). There are several pieces of evidence that support this oxidative stress model. The first are the direct experiments

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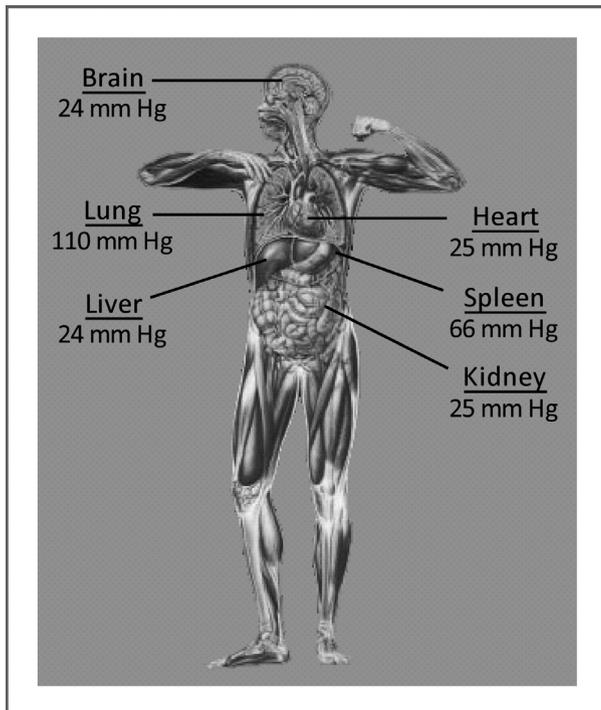


Figure 1. Levels of normal oxygen content in various organs throughout the body.

of adding potent oxidizing agents (such as H_2O_2) or reducing agents (such as N-acetyl cysteine) to cells and observing induction or inhibition of senescence, respectively (8, 13, 14). Second, other inducers of senescence, such as oncogene activity, have been shown to increase the intracellular ROS in a manner that is required to bring about senescence (8). Finally, senescence that is alleviated by culturing cells under low oxygen can be reinstated by inducing oxidative stress (e.g., with H_2O_2 or ionizing radiation; refs. 15, 16). Thus, one mechanism that explains the reduction of senescence under low oxygen may simply be a reduction in oxidative stress.

The modulation of ROS by oxygen levels and the effects of ROS on senescence, however, remain controversial topics. A host of recent reports challenge the notion that ROS decreases under hypoxia and, in fact, suggests that an increase in ROS is necessary for some of the classic molecular responses to hypoxia in mammalian cells (see next; refs. 17–21). Furthermore, Klimova and colleagues have observed an uncoupling of mitochondrial ROS and senescence (22). They found that decreasing mitochondrial ROS through the expression of antioxidant proteins was insufficient to inhibit senescence induced by hyperoxia in human lung fibroblasts (though inactivation of p53 and Rb did abrogate this response). What is unclear, though, is whether nonmitochondrial ROS are sufficient to induce senescence, highlighting the idea that the location of ROS is as important as overall levels. Along these lines, Yang and Hekimi have recently found that mutants in subunits of complexes I and III of the mitochondrial respiratory chain in *C. elegans*

lead to increases in mitochondrial superoxide but not overall ROS levels, and can increase the longevity. Mild treatment with the pro-oxidant paraquat also increases lifespan (23). Together these studies argue that the link among hyperoxia, ROS, and senescence is more complex than the classic model predicts, and suggest that low doses of ROS in the right place may even be beneficial to lifespan.

Oxygen and molecular adaptations

Hypoxia in cancer has been most well studied as a confounding factor in standard treatment regimens (24). Tumors that grow beyond a size of a few millimeters physically outgrow their normal blood supply and develop regions of hypoxia and hypoglycemia. This sets off a series of events that take advantage of normal cellular stress response machinery intended to relieve cells of their stressful environment through altered gene expression. The best understood of these responses is that of the hypoxia-inducible factors (HIF), which comprise a family of heterodimeric transcriptional regulators that target many genes associated with glucose metabolism, angiogenesis, and erythropoiesis, to name a few (25). The net result for normal tissues responding to hypoxia is increased tissue oxygenation and a return to homeostasis; neoplastic tissues, however, engage in a vicious cycle of growth and starvation. Hypoxia has been shown to increase tumor aggressiveness and metastasis, and is correlated with decreased survival in a host of different cancers (26).

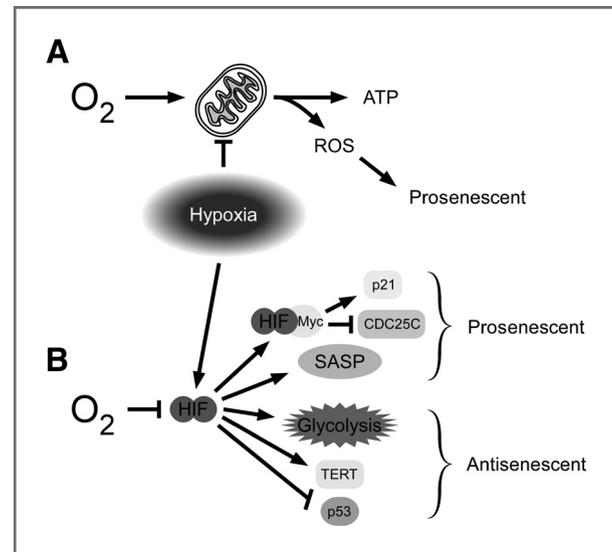


Figure 2. Hypoxia modulates senescence in a variety of ways. A, excess oxygen feeds the mitochondria and produces ROS that can promote senescence. Reduced oxygen can reduce damaging ROS. B, hypoxia leads to the stabilization of HIF, which impacts many pathways that can affect senescence. Binding to Myc can induce p21 expression and inhibit CDC25C, thereby promoting cell-cycle arrest. HIF transcriptionally controls a number of genes in the SASP that can promote senescence in an autocrine or paracrine fashion. In contrast, HIF also promotes glycolysis and reduces oxidative phosphorylation through transcriptional regulation of glycolytic enzymes. HIF can induce the expression of the telomerase active subunit (TERT) and negatively regulate p53 at several levels. These latter effects are all inhibitory to senescence.

Among the many genes regulated by the HIF proteins, a large subset is surprisingly associated with senescence (Fig. 2B). Such genes include cell-cycle regulators, glycolytic enzymes, and a variety of secreted molecules. Interestingly, in spite of the observations that low oxygen reduces senescence, both genes that can promote and inhibit senescence are present in this group, suggesting that cell-type and context-specific responses are involved in determining whether certain signals will lead to senescence within a particular biological environment.

Hypoxia and cell-cycle regulation

Hypoxia can induce cell-cycle arrest in a variety of cell types. As for many stresses, though, it is the degree of hypoxia that is critical in determining the outcome. For example, reducing from atmospheric oxygen levels, primary fibroblasts undergo increased cell-cycle progression (9, 10), until oxygen levels reach physiological hypoxia (on the order of 7.6 mm Hg). At that point and below, hypoxia induces a G1/S arrest that is dependent on HIF-1 α -mediated expression of the cell-cycle regulators CDKN1A (p21; ref. 27) and CDKN1B (p27; refs. 28, 29), and HIF-1 α -mediated repression of CSC25A (30). Under anoxic conditions (<0.08 mm Hg), cell-cycle arrest occurs in an HIF-1 α -independent manner and at multiple points in the cell cycle (31, 32).

Interestingly, HIF-1 α expression of many target genes occurs robustly at levels of oxygen (e.g., 15–50 mm Hg) that do not induce p21 or p27 (33), at which there are decreased levels of basal p53 compared with atmospheric oxygen (16) and at which cells show improved proliferation (9, 10). How then does HIF decide which target genes to activate and when (and thus whether to induce cell-cycle arrest or not)? One possible mechanism to explain this dichotomy is the mechanism of gene regulation that HIF-1 α employs on different genes. For example, expression of classic HIF-1 α target genes (VEGF, GLUT1, PGK1, etc.) is dependent on sequence-specific binding of HIF-1 α to hypoxia-responsive elements within promoter regions (34). Regulation of p21, however, has been attributed to a nonclassical mechanism involving binding and displacement of Myc that acts as a silencer in this case (27). Another possibility is that HIF-1 α may target different genes through different mechanisms in different cell types. For example, recent evidence suggests that HIF-1 α can activate TWIST in human mesenchymal stem cells (MSC), leading to inhibition of p21 expression by displacement of E2A from the p21 promoter (35). Like primary fibroblasts, therefore, hypoxia in MSCs leads to evasion from senescence albeit via a different mechanism. Thus, different gene regulation strategies may be employed by HIF-1 α depending on the gene, the level of hypoxia, or the presence or absence of other cofactors within a given cell.

HIF-1 α and HIF-2 α can have opposing effects on the cell cycle and cellular proliferation. The clearest example of this is in clear cell renal carcinoma in which HIF-2 α has been found to be a driver of tumor growth, while HIF-1 α may restrict proliferation (36, 37). At least one mechanism

to explain this is that the HIF- α subunits differentially regulate the activity of c-Myc; HIF-1 α antagonizes Myc, whereas HIF-2 α enhances Myc activity. It is unclear, however, whether these differences result in differences in, for example, p21 expression under hypoxia, or on the overall regulation of senescence in primary cells.

Hypoxia and metabolism

One of the classic adaptations that cells undergo when faced with decreased oxygen is the metabolic shift from aerobic to anaerobic metabolism. At the heart of this, adaptation is HIF-1 α -dependent transcriptional regulation of virtually all of the genes in the glycolytic pathway. These genes include the glucose transporters GLUT1 and GLUT3, genes involved in glycolysis [PGI (phosphoglucose isomerase), PFK1, aldolase, TPI, glyceraldehyde 3 phosphate dehydrogenase, PGK, PGM (phosphoglycerate mutase), enolase, pyruvate kinase, and PFKFB1–4], and genes involved in decreasing mitochondrial function (PDK1 and MXI1; for review, see ref. 38). In tumors, subjugation of this pathway even under nonhypoxic circumstances (the Warburg effect) is a common and tumor-promoting event (38).

In addition to providing an anaerobic source of ATP, glycolysis can also increase lifespan by decreasing oxidative stress. In support of this concept, both PGI and PGM have been found in overexpression screens for genes that can bypass senescence, and depletion of these genes induces premature senescence (39). Thus, another way that moderate hypoxia can suppress senescence is through the HIF-1 α -mediated activation of the glycolytic pathway and concurrent inactivation of oxidative metabolism.

Hypoxia and SASP

Upon entering senescence, cells show a number of characteristic phenotypic changes, including changes in cellular morphology and gene expression. These changes are what are commonly used to define the very state of senescence. One recent addition to the characterization of senescent cells is the observation that senescent cells display a unique secretory phenotype. This senescence-associated secretory phenotype (SASP) includes the altered secretion of numerous inflammatory and immunomodulatory cytokines [such as interleukin (IL)-6, IL-7, and IL-8], growth and survival factors [such as *GRO*, HGF (hepatic growth factor), and IGF1s (insulin-like growth factor binding proteins)], and shed cell surface molecules [such as urokinase-type plasminogen activator (uPAR); ref. 40]. The effects of these changes are complex and can conceivably promote both promalignant and prosenescent paracrine and autocrine responses.

The significance of a number of the proteins present in the SASP has been independently validated recently through genetic screens and other studies. For example, to identify the mechanisms of oncogene-induced and replicative senescence, Acosta and colleagues conducted a functional shRNA (short hairpin RNA) screen on human fibroblasts and looked for genes whose loss promoted bypass

of senescence. They identified the chemokine receptor CXCR-2 and its ligands IL-8 and GRO α as critical mediators of senescence (41). A second group performed similar experiments to identify molecules essential for oncogenic B-Raf–induced senescence and found an inflammatory network involving IL-6 and IL-8 (42). Finally, the plasminogen activator inhibitor (PAI1), which has been used as a marker of senescence for several years, has recently been causatively linked to the induction of senescence downstream of p53 (43). PAI1 serves to inhibit the function of the urokinase-type plasminogen activator signaling through its receptor uPAR (44), and thus may be functionally similar to shedding of the receptor. All of these pathways have proven to be sufficient to induce senescence when activated.

Quite intriguingly, several of these genes are regulated by hypoxia in various systems. In particular, IL-8 and CXCR-2 have been found to be HIF-1 α targets in prostate cancer cells (45), and GRO α expression is increased in hypoxic pulmonary arteries (46). IL-6 is upregulated in chronically hypoxic carotid bodies (47), and PAI1 is a direct target of HIF-1 α in many different cell types (48–51). Thus, once again, numerous pro-senescent pathways may be regulated by hypoxia and HIF-1 α , depending on the proper context.

Hypoxia and p53

A central mediator of senescence is the p53 tumor suppressor, a multifunctional transcription factor that is responsible for effecting cell-cycle arrest and cell death programs downstream of a host of stimuli. One of these stimuli is hypoxia, although the level of hypoxia required to activate p53 is generally significantly more severe than that which is sufficient to induce HIF-1 α (near anoxia for p53; ref. 52). Even under mildly hypoxic conditions, however, when a cell is exposed to a p53-inducing genotoxic stress, an interaction between HIF-1 α and p53 would have the potential to regulate senescence. Indeed, there are numerous reports that describe means by which components of these two pathways interact.

At the direct level, protein–protein interactions between p53 and HIF-1 α have been described, which, in general, seem to promote p53 stabilization or HIF-1 α degradation (53–58). Likely, maintaining p53 expression would have a pro-senescent effect. Indirectly, targets of each of p53 and HIF-1 α have been found to cross-regulate each other. For example, the p53 target PML, which itself is both necessary and sufficient to induce senescence (59, 60), has been found to negatively regulate the translation of HIF-1 α through the suppression of mTOR (61). Conversely, the HIF-1 α target MIF can bind to p53 directly (62, 63) and inhibit many p53-dependent responses (64–67), senescence included (15). Collectively, p53 and HIF-1 α show a number of mechanisms in which they act in opposing manners.

Hypoxia and telomerase

Senescence was, of course, first appreciated by Hayflick and colleagues who observed that primary human cells proliferate for a finite period of time before arresting (68, 69). It was not until decades later though it became clear

that the root of this arrest was the shortening of telomeres that induced senescence (reviewed in ref. 70). In this light, the observation that low oxygen can increase cellular lifespan raises the issue of the regulation of telomere length. Indeed, another HIF-1 α target is the telomerase active subunit hTERT (human telomerase reverse transcriptase; refs. 71, 72); and this mode of regulation has been hypothesized to be involved in cancer development. Recently, Bell and colleagues showed that upregulation of telomerase by hypoxia could extend the lifespan of human fibroblasts by 10 population doublings (73), which is in accordance with the early observations by Packer and Fuehr that hypoxia can extend the lifespan of primary fibroblasts (9). Here, again, hypoxia acts to inhibit the senescent response.

Hypoxia and OIS

Perhaps, the most important implication of regulation of senescence by hypoxia is the impact on tumor suppression. OIS plays a critical role in tumor suppression, acting as a fail-safe mechanism to avert inappropriate proliferation. Senescent cells remain permanently in a state of cell-cycle arrest that is enforced by the p53 and pRb tumor suppressor pathways. In primary human fibroblasts, for example, OIS by overexpression of H-Ras is induced rapidly by following oncogene expression and is associated with the accumulation of p53 and p16 (74). Results such as these have been reported for many different oncogenes in different cell types.

As mentioned, however, senescence due to H-Ras expression is dependent on ROS production and rescued under low-oxygen conditions (approximately 23 mm Hg; ref. 8) that interestingly are within the range of normal for many tissues (Fig. 1). So, how relevant is a tumor suppressor response that may be inhibited under certain *in vivo* conditions due to a tolerable oxygen environment? Is there tissue specificity to senescence due to tissue oxygenation? Are there microenvironments within tissues that are permissive to cells that would otherwise senesce? The answers may be drawn from the fact that the stress associated with culturing cells in atmospheric conditions (and other effects of "culture shock;" ref. 75) contributes to the *in vitro* promotion of senescence. The same oncogenic mutation therefore may not induce senescence under physiological conditions *in vivo* in the absence of other signals or stresses (Fig. 3).

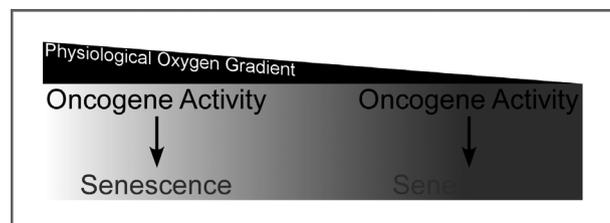


Figure 3. Oxygen gradients regulate senescence such that within the physiological range of oxygen, a similar oncogenic stimulus may or may not induce senescence. As these gradients are different in different tissues, oxygen levels may contribute to tissue selectivity of oncogenesis.

One clinical manifestation that would support this concept is the melanocytic nevus, a benign tumor of melanocytes that is filled with cells that frequently harbor a mutation in the BRAF gene (76). BRAF, much like H-Ras and other potent oncogenes, induces senescence in cells in culture (77). In addition, BRAF mutations can induce senescence in a physiological setting in which Michologlou and colleagues have observed robust senescent staining throughout specimens of nevi. This phenotype has also been modeled in mice (78). What is relevant about this example is that parts of the skin are mildly to severely hypoxic with oxygen tensions in the dermis near 76 mm Hg, the epidermis ranging down to roughly 3.8 mm Hg, and portions of the sebaceous glands and hair follicles near anoxia (79). Melanocytes normally reside at the epidermal/dermal junction, and nevi often grow into the epidermis. So, in this context, if a BRAF mutation occurs and induces senescence, how do nevi manifest fields of senescent cells? One possibility is that melanocytes with active BRAF continue to proliferate for some time (potentially promoted by the low-oxygen environment in which they reside) before some other signal induces them to senesce. In this respect, *in vivo* conditions support the propagation of oncogene-activated cells.

An experimental model that provides another example is the case of loss of the VHL tumor suppressor. Recent studies have shown that loss of VHL in mouse fibroblasts induces senescence in a pRB-dependent manner (80). VHL is particularly relevant for this discussion, as it is the primary regulator of the HIF- α subunits under oxidic conditions, directing proteasome-mediated degradation that inactivates HIF function (81). Loss of VHL has been found to induce senescence in an HIF-independent manner through the regulation of p400 and p27, which promote the ability of pRB to affect cell-cycle arrest and senescence (80). Furthering these studies, however, we have recently shown that moderate hypoxia can again deactivate this senescence response (16). In addition, loss of VHL in renal epithelia *in vivo*, where oxygen tensions are mildly to moderately hypoxic (approximately 10–50 mm Hg; 82) is insufficient to induce senescence, but does increase the sensitivity to oxidizing agents such as paraquat, resulting in a robust senescent response when VHL loss is coupled with oxidative stress. These findings reinforce the idea that what has been typically been shown to induce senescence in atmospheric conditions *in vitro*, may be insufficient to do so *in vivo* due to the lower oxygen tension.

Summary

In addition to the well-established impact of hypoxia on tumor aggressiveness and therapy, recent findings suggest that normal tissue hypoxia can impact a primary response to

tumor initiation, namely, cellular senescence. These observations underscore the importance of studying biological processes in appropriate physiological conditions. At the molecular level, hypoxic activation of the HIF pathway can affect several mediators of senescence in complex ways such that conflicting signals conceivably can be sent simultaneously. This is probably best shown by the apparent opposing induction of p21 by HIF in numerous transformed or tumor cell lines, versus the downregulation of p53 and thereby p21 in primary cells. What distinguishes these two responses is critical for understanding the complexities of HIF biology and stress responses. At a larger level, however, these observations challenge the established models of what is sufficient to induce senescence in a physiological setting and remind us that experimental interpretations need to take into consideration the various contextual influences that may affect the senescence/survival balance. Moderate hypoxia, which is common throughout the body, has the potent effect of preventing the onset of senescence due to many well-studied stimuli.

An interesting corollary to this is that within tissues, oxygen gradients often exist with stem cells residing in the most hypoxic regions (83). It is tempting to think then that these cells, which are known to be more resistant to oxidative stress as a mechanism of self-preservation (84, 85), benefit from their hypoxic environments by avoiding senescence, which would be detrimental to the regenerative capacity of the tissue. These regions would also then be hypothetically more susceptible to oncogenic transformation in support of some aspects of the tumor stem cell theory. From a therapeutic point of view, modulating tumor hypoxia may benefit not only classical treatment approaches, but may also allow reinstatement of the tumor suppressor function of senescence. Hopefully, further investigations along these lines will help illuminate what mechanisms drive cells to overcome this barrier to tumor development, and also aid in attempts to curb tumor progression through improved therapeutic approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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