Hypoxia and Senescence: Role of Oxygen in Modulation of Tumor Suppression

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Abstract

Cellular senescence is a state of growth arrest implicated in both physiological and pathophysiological conditions. In aging cells, while senescence is induced via replicative exhaustion due to telomere shortening, in preneoplastic cells it emerges as a cellular failsafe program provoked by oncogenic activation and serves as an initial barrier constraining the malignant progression. Regulation of senescence is influenced by various intrinsic and extrinsic factors including tissue hypoxia, which apparently helps premalignant cells to evade instigation of "oncogene-induced senescence" (OIS). For a better understanding of the pathological consequences of senescence bypass, it is crucial and of great interest to explicit the hypoxia-related mechanisms and factors contributing to the modulation of oncogene-induced senescence. This chapter reviews the previous and recent data that contribute to the understanding of the fundamentals of cellular senescence as well as the mechanisms of hypoxia-induced modulation of OIS.

5.1 Introduction

Cellular senescence emerges as one of the major and indispensable cell behavior that has critical importance in the regulation of the life span of the living organisms.

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More than a half-century ago, cellular senescence was first described as an in vitro phenomenon by Hayflick and Moorehead, who demonstrated that primary fibroblasts grown in culture have a finite proliferative capacity. Hayflick's observation was intriguing as the primary fibroblasts in culture were initially able to divide rapidly, but progressively cell division slowed down and eventually stopped (Hayflick and Moorhead 1961; Hayflick 1965). Currently, Hayflick's "in vitro phenomenon" is acknowledged as "replicative senescence" defining a permanent cell growth arrest state and an ultimate cellular stress response to telomere shortening reflecting the cell aging (Hernandez-Segura et al. 2018).

In the last two decades, cellular senescence has gained its reputation by its key roles in various physiological processes such as embryogenesis, tissue renewal and homeostasis, aging, and tumor suppression (van Deursen 2019; Rhinn et al. 2019; Nehme et al. 2020). Although much progress has been made regarding the understanding of the physiological and pathological significance of senescence, still much remains to be done to clarify the cellular dynamics and processes contributing to the regulation of senescence. For example, hypoxia is a typical feature of almost all tissues and organisms that display varying levels of oxygen in different tissues (Pouyssegur and Lopez-Barneo 2016), though the influence of hypoxia on senescence is not completely understood (Otero-Albiol and Carnero 2021; Welford and Giaccia 2011). Considering the substantial role of hypoxia in promoting tumor progression, angiogenesis, and metastases, it is of great interest to understand whether hypoxia acts as a stress factor triggering senescence or whether it leads to bypass of senescence, and thus promotes malignant transformation.

In this chapter, we first describe the fundamentals of cellular senescence as well as the mechanisms involved in the activation and regulation of senescence, then we associate senescence and hypoxia together and examine their relationship in terms of tumor suppression or acquisition of malignant properties.

5.2   Fundamentals of Cellular Senescence

After Hayflick's observation in diploid fibroblasts, replicative senescence was shown in various types of cells, including vascular endothelial cells, epidermal keratinocytes, lymphocytes, adrenocortical cells, chondrocytes, and smooth muscle vascular cells (Blasco 2005). Replicative senescence occurs as a result of telomere shortening that resembles the structure of repetitive nucleotide sequence of "TTAGGG" in DNA including the accompanying proteins residing at the end of the chromosomes. Telomeres are responsible for protecting chromosomes from degradation and/or fusion with nearby chromosomes (Martinez and Blasco 2011; Masutomi et al. 2003; Greider and Blackburn 1989). As cells proliferate, telomeres shorten by each cell division, during the DNA replication process due to the incomplete replication of the end of DNA strands (Martinez and Blasco 2011; Martinez et al. 2009). Accumulations of short telomeres produce genomic instability, leading to a premature senescence phenotype, and (Greider 1993) thus, shorten life span (Blasco 2005; Olovnikov 1973). This phenomenon and its contribution to

cellular senescence were established in the 1990s in diploid fibroblasts and afterward were also proved in vivo in different tissues, including lymphocytes, liver, skin, blood, and colon (Greider 1993; Greider 1990; Harley et al. 1994).

Later on, evidence from in vitro studies suggests eukaryotic cells also possess an acute "stress-induced premature senescence phenotype" (SIPS) similar to the replicative senescence but acting as a cellular failsafe program that is induced by a variety of cellular stress factors, including DNA damaging chemotherapy agents, telomere dysfunction, mitochondrial dysfunctionality, oxidative stress or reactive, oxygen species, and cytokines (de Magalhaes et al. 2002; de Magalhaes and Passos 2018; Dierick et al. 2002). Induction of senescence in cancer cells by cytotoxic chemotherapy or radiotherapy is recognized as "therapy-induced senescence" (TIS), as well (Zhang et al. 2019; Gorgoulis et al. 2019).

Overexpression of oncogenes such as *RAS* or *BRAF* is also known as a potent inducer of cellular senescence. Initially, the constitutively activated oncogenes provoke a hyper-proliferative phase intrinsically, resulting in a DNA hyper-replication stress, employing DDR signaling and induction of senescence (Barradas et al. 2002; Collado and Serrano 2006; Pantoja and Serrano 1999; Dhomen et al. 2009). This type of oncogene-provoked senescence is currently well-known as "oncogene-induced senescence" (OIS) acting as an early tumor suppressor barrier against malign transformation (Collado and Serrano 2006; Collado et al. 2005). Initiation of senescence programs either by various stress factors or telomere shortening is triggered by the cell's intrinsic DNA damage-sensing ability eventually manifesting the activation of the DNA damage response (DDR) signaling (Hernandez-Segura et al. 2018; Di Micco et al. 2008; von Zglinicki et al. 2005).

5.2.1   Morphological Alterations of Cellular Senescence

Cells undergoing senescence are defined as viable and metabolically active but stably arrested in the cell cycle and unable to respond to the mitogenic signals stimulating cell proliferation (Hernandez-Segura et al. 2018). Senescent cells undergo intense changes in gene expression displaying typically morphological and biochemical changes associated with induction of a secretory phenotype (SASP) with the involvement of extensive epigenetic regulations (Hernandez-Segura et al. 2018; Alster and Korwek 2014; Kosar et al. 2011). Under stress conditions, senescent cells acquire a unique morphology by exerting an abnormally enlarged, flattened cell structure as a result of the increased expression of Caveolin1, which is a part of the cholesterol-rich microdomains of the cell membrane (Dasari et al. 2006). Further, senescent cells display a disproportional increment in the cytoplasm-to-nucleus ratio concomitant with vacuole-rich cytoplasm and more granular appearing. This bulky state of the cytoplasm was initially defined as emerging with the manifestation of cell senescence, but recently has been proposed as it may exert a fundamental action in triggering the growth arrest in the course of senescence (Neurohr et al. 2019). Likewise, the generation of cytoplasmic chromatin fragments (CCFs) caused by the loss of nuclear filament protein lamin B1 (LMNB1)
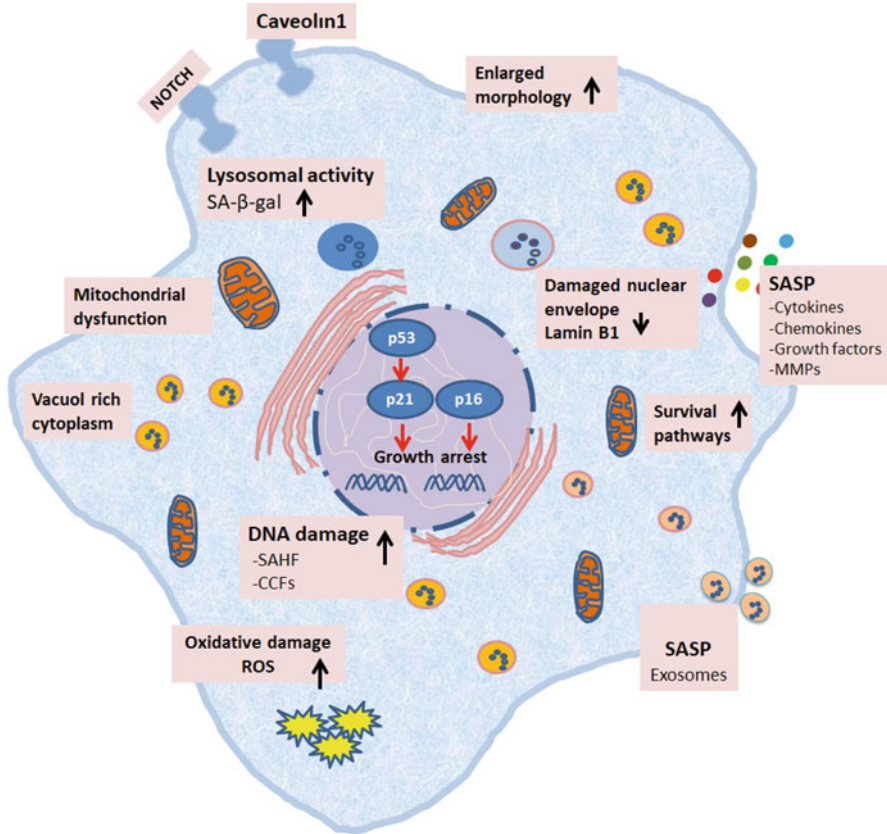
Fig. 5.1 Hallmarks of senescent cells. Senescent cells are viable and metabolically active but stably arrested in the cell cycle displaying morphological and biochemical and molecular changes including abnormally enlarged, flattened cell structure, a vacual-rich cytoplasm, dysfunctional mitochondria, and damaged nuclear envelope. Senescence is also associated with increased lysosomal β-galactosidase activity, SASP factors, epigenetic changes, including SAHFs, CCFs, as well as increased survival pathways

has been remarked as a characteristic of senescence and associated with the governing of the secretory phenotype of cells undergoing senescence (Fig. 5.1) (Freund et al. 2012; Wang et al. 2017).

Recent studies have provided substantial data that the increased activity of the lysosome-specific enzyme β-galactosidase (SA-ß-gal, senescence-associated ß-galactosidase) at pH 4.0–4.5 is the most distinguishing feature of senescent cells that also allows them to turn out to be blue when tested with a colorimetric assay using the substrate X-gal (Dimri et al. 1995; Dimri 2004; Debacq-Chainiaux et al. 2009). SA-β-gal activity test is the first accepted and currently valid gold standard test for detection of any type of senescent cells at suboptimal pH of 6.0, which generally is not present in pre-senescent, quiescent, immortal, or transformed cells

(Dimri 2004). Within the last decade, various methods have been developed for measuring SA-β-gal activity applying cytochemical or histochemical X-gal staining protocols (Debacq-Chainiaux et al. 2009; Hall et al. 2017). In addition, fluorescence-based methods employing the use of 5-dodecanoylaminofluorescein di-β-D-galactopyranoside (C12FDG), a florigenic substrate of SA-β-galactosidase, are also currently available for the quantitative measurement of senescent cells via flow cytometry, fluorescence microscopy, or microfluidic chip detection (Debacq-Chainiaux et al. 2009; Evangelou and Gorgoulis 2017). Lipofuscin accumulation has emerged as another feature of senescent cells and is accordingly used for the development of a new method established on the biotin-conjugated Sudan black B analog that has been recognized as a consistent test system for monitoring senescent cells in various cells and tissue types (Fig. 5.1) (Evangelou and Gorgoulis 2017).

5.2.2 Senescence-Associated Metabolic Changes

Although senescent cells do not proliferate, they retain an elevated metabolic activity due to an increased need for energy and other constituents to maintain the senescent phenotype (Hernandez-Segura et al. 2018; Kwon et al. 2019; Nacarelli and Sell 2017). Thus, ongoing senescence catabolism in cells mainly depends on elevated glucose consumption to yield ATP (Birsoy et al. 2015). Consequently, an increase in glycolysis ensues in oncogenic or stress-induced senescence as well as in replicative senescence (Kwon et al. 2019; Nacarelli and Sell 2017). Additionally, mitochondrial dysfunction that is a characteristic of senescence impacts on ATP generation and the NAD+/NADH ratio, leading to growth arrest (Kwon et al. 2019; Passos et al. 2007). The catabolic signaling pathways such as AMPK and NFκB are also often changed in senescent cells (Kwon et al. 2019; Nacarelli and Sell 2017; Birsoy et al. 2015). Conversely, anabolism of senescent cells is mostly concentrated on protein and lipid synthesis because of the factors participating to SASP and lipids that are used to constitute new organelles containing membranes and to provoke an autophagy response (Kwon et al. 2019; Nacarelli and Sell 2017). The most frequently altered signaling pathways related to anabolic processes for the period of cellular senescence include GSK3, ATM, SREBP1, and mTOR pathway (Nacarelli and Sell 2017; Nacarelli et al. 2018). Additionally, an increase in glycogen synthesis has been reported in senescent human fibroblasts and various aging tissues (Kim et al. 2010; Seo et al. 2008).

5.2.3 Senescence-Associated Mitochondrial Dysfunction

Senescent cells encompass a number of modifications in mitochondrial mass, membrane potential, and mitochondrial morphological structure (Nacarelli and Sell 2017). Dysfunction of mitochondria is characterized by membrane potential changes, resulting in proton leakage and excessive production of ROS, which in turn leads to oxidative damage and senescence (Fig. 5.1) (Kwon et al. 2019; Passos

et al. 2007). Elevated levels of oxidative stress in cells' ongoing senescence have been associated with dysfunctional mitochondria. During senescence, the antioxidant defense mechanisms are also incapable of neutralizing the effects of excessive ROS production (Kordowitzki 2021; Mancini et al. 2021). Several studies have shown that telomeres are the most sensitive regions of the DNA affected by the adverse effects of ROS. Whereby ROS can result in DNA breaks inducing telomere dysfunction and premature senescence (Kordowitzki 2021; Wang et al. 2021; Duan et al. 2005). Conversely, agents reducing mitochondrial ROS, for example, nicotinamide and MitoQ, have been reported to prolong the replicative life span of cells in culture by hindering the telomere dysfunction (Chapman et al. 2019).

Further studies have shown that senescence and ROS are also closely related in terms of senescence signaling as ROS plays a significant role in downstream regulatory pathways of senescence. Excessive ROS production has been shown during replicative, oncogene, and stress-induced senescence.

Moreover, experimental activation of main signaling molecules regulating senescence, including p16, p21^{CIP1}, and p53, has been associated with elevated ROS (Passos et al. 2007; Passos et al. 2010; Ogrunc et al. 2014). These findings supported the notion that ROS may act as a signaling molecule to help the stabilization of senescence-induced growth arrest in cells undergoing senescence. Hence, it was suggested that the storage of ROS in senescent cells participates in the maintenance of DDR, instigating the growth arrest (Passos et al. 2010; Ogrunc et al. 2014; Parrinello et al. 2003; Nassrally et al. 2019; Stockl et al. 2006).

5.2.4 Main Effectors of Senescence: DNA Damage Responders and Cell Cycle Regulators

Senescent cells are eminent with their permanent growth arrest state. Eukaryotic cell cycle progression is maintained via the activation of cyclin-dependent kinase (CDK) complexes that coordinate and ensure the timely transition between cell cycle phases (Hernandez-Segura et al. 2018; Kastan and Bartek 2004). Cell cycle arrest occurring in the progression of cellular senescence is mostly mediated by activation of tumor suppressor pathways, namely, p53-p21^{CIP1} and/or p16^{INK4A}-pRB (Fig. 5.1) (Gorgoulis et al. 2019; Bai et al. 2007; Kim et al. 2015; Yano et al. 2021; Zhu et al. 2002; Zheng et al. 2006). Both signaling pathways are highly complex and require upstream regulators and downstream effectors to fully function in the execution of senescence. In most of the somatic cells, various pro-senescent stress stimuli causing DNA breaks converge on activation of DNA damage response mainly involving ataxia-telangiectasia mutated (ATM) and Rad3-related (ATR) kinases to initiate senescence (Di Micco et al. 2008; von Zglinicki et al. 2005; Efeyan et al. 2009; Bartek and Lukas 2007). ATM and ATR kinases are recognized as sensor kinases and start a downstream phosphorylation cascade initiated with phosphorylation of histone H2AX located in DNA breaks. The phosphorylated form of H2AX, known as the γ-H2AX foci, is required for enrolling and anchoring the other DDR players. Activation of ATM and ATR kinases results in phosphorylation

of checkpoint kinases CHK1 or CHK2, respectively which results in stabilization of p53. Further, p53 transactivates the various genes, including the cyclin-dependent kinase complexes inhibitor p21^{CIP1} (also known as *CDKN1A*) halting the cell in G1 or G2/M phases by inhibiting the activity of CDK2 or CDK1, respectively (Bartek 2011; Bartek et al. 2007a; Brazina et al. 2015; Falck et al. 2001).

Initiation of the mitogen-activated protein kinase p38/MAPK signaling by ATM/ATR also increases the expression of *CDKN2A* encoded CDK4/6 inhibitor P16^{INK4A}, acting upstream of the other tumor suppressor protein pRB and facilitating its hypophosphorylation together with p21^{CIP1} (Bartek 2011; Bartek et al. 2007a). Hypophosphorylated Rb protein binds to E2F and thereby cell cycle's progression is halted and senescence is instigated. Irrespective of specific triggers, DDR signaling cascades converge on both p53-p21^{CIP1} and p16^{INK4A}-Rb tumor suppressor pathways to activate senescence (Bartek and Lukas 2007; Bartek 2011; Bartek et al. 2007a; Brazina et al. 2015; Evangelou et al. 2013). Activation of the other product of *CDKN2A* gene *ARF* known as "tumor suppressor ARF" through epigenetic de-repression mediates the inhibition of MDM2 and thus prevents p53 degradation and promotes cellular senescence via p53-p21^{CIP1} axis (Brookes et al. 2004, 2002). Long-term overexpression of any of the key elements p53, pRB, p16^{INK4A}, and p21^{CIP1} was competent to initiate senescence (Pantoja and Serrano 1999; Brookes et al. 2004; Lin et al. 1998). The senescence-associated cell cycle arrest was initially thought to be permanent and irreversible; however, growing evidence supports the reversibility of senescence by showing early-senescent or pre-senescent cells able to return the cell cycle under specific genetic or transcriptomic conditions (Beausejour et al. 2003; Katoh et al. 2021; Lee et al. 2016; Walters et al. 2016).

5.2.5   Senescence-Associated Epigenetic Regulations

Cells require intense epigenetic regulations and chromatin changes, resulting in senescence-associated heterochromatic foci (SAHF), a gene repressing mechanism employed to induce senescence, yet another peculiarity of senescence (Hernandez-Segura et al. 2018; Kosar et al. 2011). SAHF encompass distinct histone modifications including trimethylated Lys9 or Lys20 (H3K9me3, H3K20me3), and acetylated Lys27 (H3K27ac) on histone H3, as well as increased activity of chromatin repressors heterochromatin protein 1 gamma (HP1γ), high mobility group protein A (HMGA), histone variant macroH2A, histone co-chaperones HIRA, and ASF1A49–51.a (Kocylowski and Halazonetis 2011; Sanders et al. 2013; Zhang et al. 2007; Zhang et al. 2005; Zhang et al. 2014). Importantly, SAHF formation has been originally related to senescence-associated proliferation arrest due to the presence of downstream targets of E2F involved in cell cycle execution (Kocylowski and Halazonetis 2011; Zhang et al. 2014). SAHF can be observed as 4,6-diamidino-2-phenylindole (DAPI)-positive nuclear assemblies in cells undergoing senescence. However, SAHF is not accepted as a widespread marker of senescence due to its dependence on ATR activation and DNA replication stress as well as oncogene

activation (Kocylowski and Halazonetis 2011). SAHF execution of a DDR-resistant heterochromatin structure also hinders DDR signaling (Di Micco et al. 2011). Not surprisingly, therefore, histone deacetylase (HDAC) inhibitors induce chromatin loosening to enhance DDR signaling and consequently induce cell death via apoptosis (Di Micco et al. 2011; Abramova et al. 2011; Kochetkova et al. 2017).

5.2.6 Senescence-Associated Changes in Cell Survival Pathways

Senescent cells display upregulated survival signaling such as PI3K/, leading to upregulation of antiapoptotic proteins of the Bcl-2 family, including Bcl-2, Bcl-xl, Bcl-w, and p21^{CIP1}, which encourages the resistance against apoptosis (Hernandez-Segura et al. 2018; Di Micco et al. 2021). In physiological environments, various stress factors inducing oxidative damage or intense DNA damage can activate p53 via DDR, which in turn can promote apoptosis by initiating the transactivation of the pro-apoptotic participants of the Bcl-2 family Noxa and Bax (Yin 2000). Nevertheless, senescent cells display upregulated activity of p21^{CIP1} protein promoting survival signaling and preventing induction of apoptosis (Yosef et al. 2017). Moreover, downregulated Bcl-w and Bcl-xl can lead to abolishment of senescent cells in the lung and epidermis as shown in vivo (Yosef et al. 2016). However, whether the sustained viability of senescent cells is due to the selection against apoptosis sensitivity or whether it is an internal outcome of the senescence process remains to be examined.

5.2.7 Senescence-Associated Secretory Phenotype

Senescence-associated secretory phenotype (SASP) delineates the other intriguing function of senescent cells engaging several pro-inflammatory factors comprising the cytokines, chemokines, growth factors, angiogenic elements, proteolytic enzymes, lipids, components of extracellular matrix, and matrix metalloproteinases (MMPs), and extracellular vesicles (Malaquin et al. 2019). SASP is composed of soluble proteins released into the extracellular milieu, cleaved trans-membrane proteins, and the factors transported in exosome-like vesicles (Maciel-Baron et al. 2016; Tanaka and Takahashi 2021).

Interestingly, SASP is not an essential characteristic of senescence and some types of cells experiencing senescence do not necessarily associate with inflammatory secretory phenotype as exemplified in human primary fibroblasts induced to senesce by ectopic expression of p16^{INK4a} and cyclin-dependent kinase inhibitor, p21^{CIP1} (Faget et al. 2019). The SASP can operate in a cell-autonomous (autocrine) or non-cell-autonomous (paracrine) manners, and takes the responsibility for supporting and disseminating senescence phenotypes and maintaining tissue homeostasis (Maciel-Baron et al. 2016).

SASP is strictly regulated in a complicated way encompassing diverse signaling molecules such as nuclear factor-kappa light-chain-enhancer of activated B cells

(NF-κB) CCAAT/enhancer-binding protein beta (C/EBPβ), mammalian target of rapamycin (mTOR), and NOTCH1. NF-κB and C/EBPβ are the two main transcription factors responsible for the transcriptional regulation of SASP proteins, including IL-1A, IL-6, and IL-8, that are employed in inflammation (Maciel-Baron et al. 2016; Salminen et al. 2012). The cytosolic DNA-sensing GMP-AMP synthase stimulator of interferon genes (cGAS-STING) pathway and the senescence-associated Alarm in high mobility group box 1 (HMGB1) protein are also involved in governing of SASP and secretion of pro-inflammatory (Davalos et al. 2013; Loo et al. 2020).

SASP is categorized into two secretory components, namely the inflammatory components, mainly regulated by IL1, and the TGFβ-related components, relying on NOTCH signaling (Acosta and Gil 2012; Hoare et al. 2016). The SASP can act as a double-edged sword and may exert both beneficial and deleterious effects that may be predisposed by various factors including its composition, intensity, and the microenvironment (Coppe et al. 2010a). Of note, the composition of SASP is considered rather heterogeneous and may vary among the cell types and may also change with the stimuli triggering senescence (telomere loosening, oncogene activation, oxidative stress, etc.) (Loo et al. 2020). The pro-inflammatory cytokines such as IL-1, IL-6, and IL-8 are the most common elements of SASPs and do not vary by senescence stimuli or cell types (Faget et al. 2019). The beneficial effects of SASP mainly depend on its paracrine functions contributing to maintenance of senescence implicated in the immune surveillance, tumor suppression, cell-to-cell communication, clearance of senescent cells, and wound healing (Malaquin et al. 2019). Remarkably, pro-inflammatory factors play a role in the promotion of tumor progression and thus resemble detrimental functions of SASP (Coppe et al. 2010b). It can obstruct tissue repair and regeneration and support organismal aging through the storage of senescent cells and attenuation of stem/progenitor cell components (Tanaka and Takahashi 2021)

5.3 Oncogene-Induced Senescence (OIS) and Tumor Suppression

5.3.1 Mechanisms of OIS

Eukaryotic cells have evolved genetically encoded cellular failsafe programs such as induction of apoptosis or senescence to overcome the potentially hazardous events that may lead to malign transformation (Lee and Schmitt 2019; Lowe et al. 2004)

In pre-neoplastic cells, once activated p53 mainly stimulates the cell cycle arrest over transactivation of $p21^{CIP1}$ and promotes the DNA repair response temporarily. However, when DNA damage persists, due to extensive damage p53 induces mitochondria-mediated apoptosis via transactivating numerous pro-apoptotic participants of the Bcl-2 family genes comprising Puma (p53 upregulated modulator of apoptosis), Bax, Bak (Bcl2 antagonist/killer) (Lowe et al. 2004; Galluzzi et al. 2018). Activation of senescence through $p53$-$p21^{CIP1}$ corporation inducing insistent cell cycle arrest establishes the alternate mechanism for elimination of the
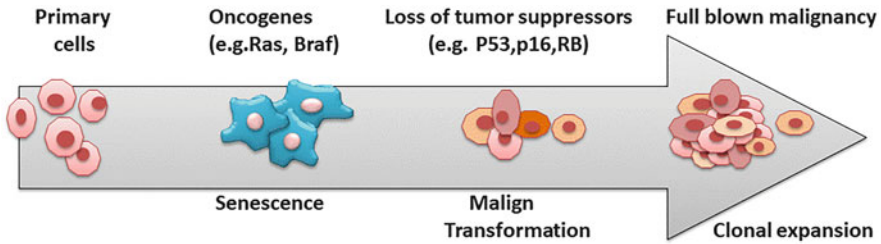
Fig. 5.2 OIS acts as an intrinsic tumor-suppressor mechanism. OIS is acutely induced in primary cells via activation of mitogenic oncogenes such as Ras/BRAF and can be bypassed by loss of tumor suppressors, e.g., P53, p16, and RB. Bypass of senescence promotes progression into the full malignancy

precancerous lesions (Collado et al. 2005; Collado and Serrano 2005). Although both processes share a common activating mechanism involving DDR pathway and p53, the decision of cells whether to activate senescence or apoptosis mainly depends on the characteristics of the activated oncogene, cell type, or environmental factors forcing the cell into malignant transformation (Lowe et al. 2004).

In mammalian cells, when excessive mitogenic signals are perceived through activated oncogenes, such as *RAS* or *BRAF,* accumulation of oncogene-induced ROS produced by NADPH oxidases may boost up the initial hyperproliferative phase accompanied with increased DNA replication that eventually results in replication stress and telomeric dysfunction, leading to DNA damage and induction of cellular senescence (Di Micco et al. 2011, 2021, 2006; Bartkova et al. 2010). Evidently, in hyperplastic cancerous regions in human tissues, accumulation of oncogene-induced dysfunctional telomere, and marks of activated DDR has been detected (Bartkova et al. 2005).

This phenomenon is clearly represented by the ectopic overexpression of the oncogenic *HRAS* (HRasG12v) in human diploid fibroblasts, proving that oncogene activation is a potent stimulator of cellular senescence triggering a hyperproliferative response associated with exhausted DNA replication machinery, ultimately leading to activation of DDR signaling and of senescence (Ogrunc et al. 2014; Di Micco et al. 2006; Serrano et al. 1997). This course is known as "oncogene-induced senescence" (OIS), mainly signified by halted cell proliferation and higher expression of p16^{INK4a} and p53-p21^{Cip1} (Collado and Serrano 2006, 2005) (Fig. 5.2).

Besides prolonged DDR activation, OIS display similar characteristics as of the other types of senescence including p21^{CIP1}-mediated cell cycle arrest and p16^{INK4A} increased ROS levels and thus oxidative damage, upregulated survival proteins (including anti-apoptotic Bcl-2 family proteins), metabolic alterations, and accumulation of SA-β-gal and SASP as well as SAHF (Collado and Serrano 2006, 2005; Di Micco et al. 2006).

Hyperactive mutated members of the MAPK signaling pathway, including RAS, RAF, MEK, BRAF, and various other mitogenic response players such as PI3K, AKT, MYC, ERBB2, and p38MAPK exerting an oncogenic potential have been

reported to induce OIS (Astle et al. 2012; Braig et al. 2005; Damsky and Bosenberg 2017; Ferbeyre 2018). Likewise, loss of tumor suppressor functions of PTEN, NF1 can also initiate cellular senescence due to hyperproliferation and DDR activation (Jung et al. 2019; Larribere et al. 2015). Of note, activation of PI3K/Akt pathway promotes p53-dependent senescence without inducing hyperproliferating signals or excessive DNA damage, suggesting a different mechanism. OIS is induced as a telomere-independent mechanism incorporating the p53-p21^{CIP1} and p16^{INK4A}-RB axis (Fig. 5.2) (Aoki and Fujishita 2017). Different studies have revealed that the accumulation of RB, the downstream partner of p16^{INK4A}, is particularly important in pursuance of senescence due to its influence on the suppression of E2F-target genes connected to DNA replication (Di Micco et al. 2021; Dimri et al. 1996). P53 emerges as another indisputable component of OIS as reports have indicated that loss of p53 or its regulator p19^{ARF} triggers RAS-persuaded cancer cell invasion in mice, while its recurrence suppresses tumor growth along with signs of senescence (Serrano et al. 1997). Although p53 and p16^{INK4A} are widely accepted as well-established key players of OIS, under certain circumstances depending on cell type, neither p53 nor p16^{INK4A} is required for the induction or execution of OIS (Cipriano et al. 2011). For example, Ras-induced initiation of senescence does not involve p16^{INK4A} or p53 in human mammary epithelial cells, reflecting the disagreement between cell types in the activation of OIS (Cipriano et al. 2011).

In fact, studies have reported other inconsistencies related to OIS. For example, loss of tumor suppressor protein PTEN has been described to instigate cell cycle arrest and thus senescence recognized as "PTEN loss-induced cellular senescence (PICS)," which was further shown to involve hyperproliferation and DDR for induction of cellular senescence in vivo (Jung et al. 2019; Chen et al. 2005). However, activation of PI3K−AKT oncogenic signaling has been reported to provoke a p53-dependent senescence response without substantial hyperproliferation and DNA damage accumulation unlike oncogenic *RAS* or *BRAF* (Astle et al. 2012; Aoki and Fujishita 2017). These studies suggest that there may be different underlying mechanisms implicated in the induction of senescence driven by activated oncogenes or loss of tumor suppressors.

Recently, NOTCH signaling, which has been identified as a highly conserved key signaling pathway involved in cell development, differentiation, and also cancer, was implicated in the regulation of OIS (Bray 2016). NOTCH signaling is instigated by ligand-dependent proteolytic cleavage processes in that Notch intracellular domain (NICD) unbound from the plasma membrane and translocates into the nucleus in order to cooperate with the DNA binding protein CSL to trigger transcription (Bray 2016). Studies revealed that NOTCH1 is upregulated during OIS concomitant with the induction of transforming growth factor-beta (TGFβ) (Hoare et al. 2016; Ito et al. 2017). Remarkably, NOTCH1 activity seems to be elevated transiently only in the early stage of OIS as it returns to its basal levels in the later stages of OIS (Hoare et al. 2016). Most interestingly, overexpression of N1ICD (the active intracellular form of the Notch1 receptor) in human fibroblasts drives cell-autonomous senescence identified as "Notch-induced senescence" (NIS) or primary NIS, which considerably differs from the "Ras-induced" senescence by an altered

secretory phenotype and chromatin structure (Hoare et al. 2016; Ito et al. 2017). SAHF formation is excluded in NIS cells comprising reduced amounts of the pro-inflammatory cytokine IL-8 and stimulation of the well-known ligand of NOTCH JAG1 as well as TGFβ1 (Hoare et al. 2016). Considering NOTCH's key roles in important physiological and pathological processes, understanding its functional implication in the course of senescence is of major attention.

5.3.2   OIS and Tumor Suppression

Functional studies have been identified in the biological role of OIS as an explicitly tumor-suppressive mechanism, an early intrinsic cellular barrier against tumorigenesis, as shown in both human pre-neoplastic lesions and animal models (Collado et al. 2005; Collado and Serrano 2005, 2010). In numerous studies including premalignant lung and pancreatic tumors, it was demonstrated that conditional expression of oncogenic *KRASV12* leads to neoplastic transformation that is concomitant with senescence characteristics (Collado et al. 2005; Efeyan et al. 2009). In mouse lymphoid cells, ectopic expression of *NRAS* activated OIS, preventing full-blown malignancy (Fig. 5.2) (Braig et al. 2005). The role of OIS in preventing tumor suppression has been also shown in a mouse model of prostate cancer where the loss of *PTEN* is utilized as an inducer (Chen et al. 2005). Remarkably, one of the most fascinating evidence showing OIS as an intrinsic barrier against tumorigenesis comes from the study conducted on human melanocytic naevi both in vitro and in vivo settings (moles) (Dhomen et al. 2009; Michaloglou et al. 2008; Michaloglou et al. 2005). This study has shown that 80% of melanomas harbor *BRAFV600E* mutation and melanocytes expressing *BRAFV600E-* are arrested in cell cycle and display numerous characteristics of OIS, including increased expression of p16^{INK4A} and SA-β-gal positivity (Fig. 5.2) (Michaloglou et al. 2008, 2005). The naevi are capable of transforming into malignant tumors upon interruption or reversal of BRAF-induced senescence, suggesting that they are benign lesions remaining stably arrested for years before the onset of melanoma carcinogenesis that also resembles the OIS at best in vivo (Michaloglou et al. 2008, 2005). The previous in vitro studies have shown that OIS can be circumvented by disabling RB and p53 tumor suppressors. Subsequently, induction of senescence cannot be accomplished if any of p53 and/or RB are abrogated in a cell prior to an oncogenic activation (Fig. 5.2) (Serrano et al. 1997).

Hence, OIS can serve as a break in response to excessive proliferation, providing an initial barrier, and eventually protecting cells against tumorigenesis. OIS has been widely studied, and there are various examples as shown in vivo (Collado and Serrano 2010; Kuilman et al. 2010).

Likewise, other types of senescence, cells undergoing OIS also retain a SASP that provides OIS cells to intermingle with each other (Acosta et al. 2013). Various studies have shown that OIS cells include SASP components such as IL-6 and IL-8 together with other inflammatory molecules, which in turn provoke a self-amplification action in an autocrine fashion via augmented expression of n NF-κB

and CCAAT/enhancer-binding protein beta (C/EBPβ) (Coppe et al. 2010b; Acosta et al. 2013; Chien et al. 2011; Sebastian et al. 2005). OIS cells also exert paracrine functions that are involved in cell to cell transmission and alteration of their extracellular milieu, ultimately inducing a senescence phenotype called "secondary senescence" in their proliferating neighboring cells through secreted SASP elements (Acosta et al. 2013). Studies conducted with omics technologies such as quantitative proteomics have further identified the TGFβ (transforming growth factor-beta), VEGF (vascular endothelial growth factor), and CCL2 (chemokine CC motif ligand 2) as the main players of paracrine senescence (Acosta et al. 2013; Coppe et al. 2008). Besides the secretion of IL-1, TGFβ has been also shown to mediate the relationship concerning oxidative stress-mediated DNA damage, and paracrine senescence, along with DDR signaling persuading senescence in bystander cells (Di Micco 2017). The paracrine activities of OIS are mainly driven by the SASP secretome that represents a multifaceted and dynamic character and in part regulated by NOTCH signaling (Acosta et al. 2013). While NOTCH-primed secondary senescence mainly depends on growth factors, TGF-β and the expression of fibrillary collagens, SASP-primed secondary senescence is determined by the secretion of pro-inflammatory cytokines, C/EBPβ manifestation, and SASP dissemination (Acosta et al. 2013). Nevertheless, secondary senescence is manifested by the contribution of NOTCH-primed and SASP-primed secretome profiles (Faget et al. 2019).

Paracrine senescence is evidently shown in experimental models of human and mouse cells displaying OIS. In fact, an important connection between the manifestation of SASP factors and the stimulation of paracrine senescence has been established by non-cell-autonomous actions of OIS in vivo, which may have comprehensive biological meanings in terms of tumorigenesis (Acosta et al. 2013). Cells undergoing OIS release SASP factors that have been shown to recruit immune cells for eradication of tumor cells concomitant with an unappreciated tumor-promoting activity modulated mainly by the physiological conditions (Kang et al. 2011; Yevsa et al. 2012). In various studies utilizing cancer models, including skin, prostate, and liver, SASP has been shown to enable movement of tumor cells or provoke an immunosuppressive milieu endorsing proliferation, angiogenesis, and metastasis potential of cells (Greten and Eggert 2017; Alimirah et al. 2020; Guccini et al. 2021). In conclusion, it is broadly recognized that OIS may function in dual ways in cancer progression that is mainly influenced by the genetic background of the tissue, the content of SASP, and the extent of senescence (Acosta et al. 2013). Thus, while OIS exerts a cancer-preventive effect in the early phase of tumorigenesis, over time it may change to a deleterious cancer-promoting precancerous stage that appears as an in vivo antagonistic pleiotropy phenomenon (Coppe et al. 2010b).

Hence, it is of significant biological interest to investigate the tumor-preventive process and its genetically programmed dynamics in advance to cells' evasion of senescence and expansion into full-blown malignancy.

5.4   Intersection of Hypoxia and Senescence

Although atmospheric oxygen levels are roughly 20%, the received levels of oxygen by organisms are different. The amount of oxygen in the body is significantly lower and highly variable within the different organs and tissues. The oxygen concentration is approximately 4% in human brain tissue, in skeletal muscle, or in the liver, while it is near 5 or 6% in lung alveoli, and reaches only 1% in the skin (Vaupel et al. 1990, 1989). If an oxygen deficiency occurs under certain conditions or pathology, mammalian cells retain a systemic and molecular adaptive response to compensate for the hypoxia (Pouyssegur and Lopez-Barneo 2016; Semenza 1999). This adaptive response can be induced in all cell types under lack of oxygen and mainly relies on the stabilization of hypoxia-inducible factors (HIFs), HIF1α, HIF2α, or HIF3α (Wang et al. 1995; Semenza 2012; Semenza 2014). Under hypoxic conditions, a dimerization occurs between HIFα and HIF1β (ARNT) subunits and subsequently the heterodimer complex translocate to the nucleus, thereby binding to hypoxia response elements (Wang et al. 2005) in DNA and inducing the transcriptional activation of the target genes. HIFα is mainly regulated in a post-translational manner by prolylhydroxylases (PHDs) that are highly susceptible to oxygen levels. PHDs hydroxylate HIFα from the proline residues and thereby target these proteins to the von Hippel–Lindau protein (VHL) complex, which mediates the ubiquitination and degradation in the proteasomal complex. Another factor, known as the factor inhibiting HIF1α (FIH), is also an oxygen-regulated protein that works at lower oxygen levels than that of PHDs, and mediates the hydroxylation of HIFα by asparagine residues. However, FIH-mediated inhibition is exclusive to HIF1α because HIF2α is resistant to FIH-mediated modifications (Semenza 2012, 2014, 2007).

Notably, HIF1α and HIF2α isoforms differ in function. HIF1α is ubiquitously expressed, whereas expression of HIF2α is restricted to some cell types, such as endothelial cells, hepatocytes, cardiomyocytes, or glial cells (Semenza 2012, 2014). Moreover, HIF1α has been mostly involved in the acute response to hypoxia, whereas HIF2α has been associated with the chronic response to hypoxia or hypoxic response occurs at geographical altitude. Also, a third HIFα subunit, namely HIF3α, is expressed in different tissues such as the thymus, lungs, brain, heart, and kidneys that comprise alternative splicing variants and differ from the two other isoforms by lacking the transactivator domain. Eventually, HIF3α can act as an inhibitor of HIF-dependent transcription particularly via the splicing variant IPAS that can bind and constitute a heterodimer complex with HIF1α incapable of activating the transcription of HRE comprising genes (Semenza 2012, 2014).

Both HIF1 and 2α share some mutual transcriptionally regulated targets including GLUT1 or VEGF, though both differ in some others like LDHA and PGK1, which are specifically regulated by HIF1α or, EPO, MMP9, and OCT4 that are the cases for HIF2α. These differences in target preferences are mainly determined by the cell type or the level of oxygen concentrations (Semenza 2012, 2014)

Hypoxia is one of the major characteristic features of solid tumors and has been associated with poor prognosis or poor response to chemotherapy (Schito and

Semenza 2016; Semenza 2003; Kilic et al. 2007). However, its importance in the regulation of senescence is not well recognized. Senescence can be controlled by a plethora of factors; among those, the level of oxygen in the tissues emerges as particularly important (Kilic Eren and Tabor 2014). Intriguingly, as mentioned earlier, in living organisms O_2 levels vary between the tissues and also present as significantly lower than that of the in vitro cell culturing conditions achieved under 20% of O_2 at atmospheric levels. Thus, most of our understanding of cellular senescence has been defined by studies obtained in hyperoxic conditions that itself can potentially induce senescence (von Zglinicki et al. 1995).

During the last years, various studies have demonstrated that hypoxia can revise the life span of cells by modulation of replicative, therapy, or oncogene-induced senescence, in human or mouse cells (Parrinello et al. 2003; Kilic Eren and Tabor 2014; Chen and Ames 1994; Chen et al. 1995; Yuan et al. 1995)

Low levels of oxygen prolonged the life span of human fibroblasts 20%, whereas bovine and mouse fibroblasts are 80% and 500%, respectively (Packer and Fuehr 1977; Saito et al. 1995). In hypoxia, mouse embryonic fibroblasts (MEF) pursue proliferation despite an intact wt $p19^{ARF}$/p53 pathway and expressed $p16^{INK4a}$, which is indispensable for evasion of immortalization (Parrinello et al. 2003; Welford et al. 2006; Betts et al. 2008). Hypoxia-mediated suppression of replicative senescence is mainly related to decreased amount of DNA damage or reactive oxygen species (ROS) in mouse cells. Accordingly, normoxia has been suggested to induce senescence in MEFs due to accumulated DNA damage whereby alterations of $p19^{ARF}$/p53 pathway enable these cells to become unresponsive to DNA damage and thus immortalization occurs (Parrinello et al. 2003; Welford et al. 2006). Furthermore, the prolonged life span has been related to various signaling molecules, in particular, the HIFα comes forward (Welford et al. 2006). The regulators of cell cycle $p21^{Cip1}$ and Myc have been identified as the two target genes of HIF1α and HIF2α, respectively (Tsai et al. 2011; Gordan et al. 2007). HIF1α has been also suggested to activate the hTERT (human telomere reverse transcriptase) that is associated with mitochondrial ROS (Bell et al. 2007). In another study, HIF-1α was shown to display a crucial role in retardation of senescence in MEFS via transactivation of MIF and inhibition of p53-governed pathways (Fig. 5.3) (Welford et al. 2006). Akin to replicative senescence, hypoxia decreased the levels and the amount of chemotherapy-induced senescence in cancer cells, depending on HIF-1α activity (Sullivan et al. 2008). Accordingly, targeting of HIF-1α in human breast and colon cancer cells averted senescence and, eventually, bypassed the resistance to chemotherapy under hypoxic conditions (Sullivan et al. 2008). Furthermore, HIF-1α was shown to be capable of suppressing drug-induced senescence under normoxic conditions (Rohwer et al. 2010).

Hence, these studies underline the implication of hypoxia and HIF-1α in the modulation of replicative as well as therapy-induced senescence.
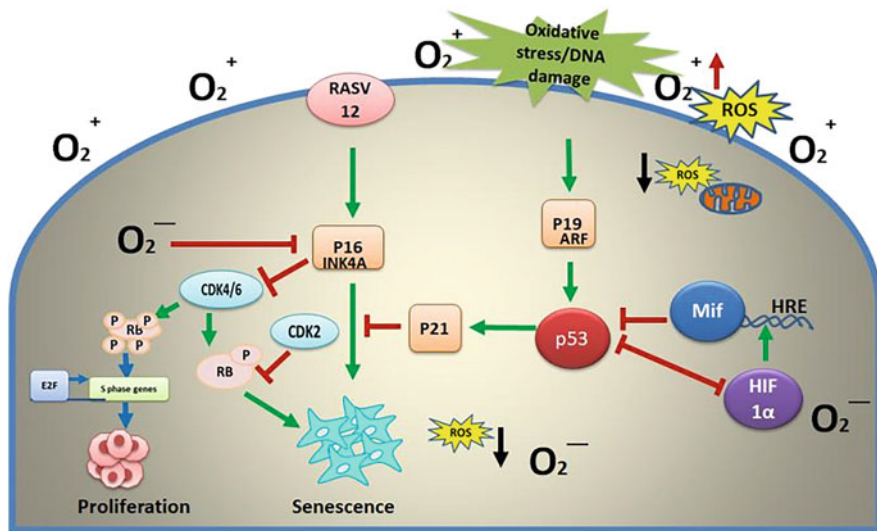
Fig. 5.3 Impact of hypoxia on senescence. Normoxia induces senescence in MEFs due to accumulated DNA damage via activation of p19ARF/p53 pathway. MIF as a target of HIF-1α directly binds and inhibits p53 and p21[CIP1]. In hypoxia, Ras provoked senescence (RASV12) in human diploid fibroblasts (HDFs) involves HIF1α-independent p16[INKA], but dependent p53 and p21[CIP1] downregulation. Inactivation of either p16-Rb and/or p53-p21 tumor suppressor pathways is essential for inactivating OIS

5.4.1   Impact of Hypoxia on Regulation of Cell Cycle

It is frequently shown in various cell types that hypoxia itself can induce cell cycle arrest (Goda et al. 2003; Hammer et al. 2007). When oxygen levels drop from the atmospheric ranges (21%= of 159 mmHg) to the 7.6 mmHg, hypoxia induces an arrest at the G1/S phase of the cell cycle via HIF1α-induced activation of cell cycle regulators such as p21[CIP1] or p27[KIP] (Goda et al. 2003; Green et al. 2001). Additionally, HIF1α suppresses the activation of CSC25A phosphatase (Goda et al. 2003). Under extreme conditions of hypoxia (≤0,8mm Hg) or even in anoxia, cell proliferation arrest was achieved in an HIF1α-independent manner at different checkpoints (Freiberg et al. 2006). HIF1α-induced expression of downstream targets is accomplished at varying conditions, for example, expression of classical target genes of HIF1α such as VEGF, GLUT1, PGK1, etc., mainly relies on HIF-1α's binding to HRE within the specific promoter site. In contrast, expression of p21[CIP1] has been linked to a noncanonical way engaging the HIF-1alpha PAS domains binding and dislocation of Myc from p21[CIP1] promoter, which functions as a silencer (Koshiji and Huang 2004). Undoubtedly, the mechanisms involved in HIF1α's targeting effect on different genes may vary depending on the cell type or context. In previous reports, HIF-1α was shown to induce activation of TWIST in human mesenchymal stem cells (MSCs), which decreased the expression of p21[CIP1] by transposition of

E2A from the p21^{CIP1} promoter, thereby allowing MSCs to bypass senescence in hypoxia (Tsai et al. 2011).

HIF-1α and HIF-2α may also have distinct effects on regulation of the cell cycle and cell proliferation as exemplified in clear cell renal carcinoma, where HIF-1α is involved in limitation of cell growth but HIF-2α initiation of tumor growth (Gordan et al. 2007, 2008). Thus, in hypoxia, various strategies are employed to regulate the gene expression that mainly differs with the level of oxygen, or with the nature of the involved transcription factor, or the target gene or the cell type. Although it is not yet completely understood, these differences may certainly impact on overall regulation of senescence in primary cells.

P53 is the central transcription factor activated upon various stresses such as DNA damage to activate the failsafe mechanisms such as cell cycle arrest, DNA repair, induction of apoptosis, or senescence. Hypoxia is among those stress factors inducing p53 activation (Chen 2016). This is despite the fact that the level of hypoxia desired to activate p53 is rather severe (almost anoxia) than that required for HIF1α. Previous reports indicate a direct interaction between HIF1α and p53 that likely occurs to enhance p53 stabilization and HIF1α degradation (Chen et al. 2003; Blagosklonny et al. 1998; Hammond et al. 2002; Hansson et al. 2002; Ravi et al. 2000). Presumably, retaining p53 stabilization by HIF1α would promote induction of senescence; however, secondary targets seem to take the action to cross-regulate p53 or HIF1α. In the case of p53, its target, PML, has been shown to repress the translation of HIF1α via the inhibition of mTOR (de Stanchina et al. 2004). Conversely, p53 and the interconnected responses including senescence are inhibited by the direct binding of MIF, which is a downstream target of HIF1α (de Stanchina et al. 2004; Ferbeyre et al. 2000; Jung et al. 2008; Petrenko et al. 2003). Collectively, these data substantiate that HIF1α and p53 can exert opposing activities in various contexts and also secondary effectors can be in charge.

5.4.2   Impact of Hypoxia in Metabolism

In hypoxia, cells display various adaptations, including the metabolic change from aerobic to anaerobic metabolism, so-called Warburg effect, to promote tumor survival under inappropriate conditions (Warburg et al. 1927). HIF-1α plays a central role in inducing the transcriptional activation of the vast majority of genes involved in the glycolysis such as the glucose transporters GLUT1 and GLUT3, or glycolytic enzymes PGI, PFK1, aldolase, TPI, GAPDH, PGK, PGM, enolase, PK, PFKFB1–4, and PDK1 and MXI1 implicated in obstruction of the mitochondrial functions (Semenza 2007; Denko 2008).

Glycolysis serves as a source of ATP under hypoxic conditions and reduces the level of oxidative stress that results in increased life span. Evidently, overexpression of the glycolytic enzyme phosphoglucose isomerase (PGI) and phosphoglycerate mutase (PGM) genes has been associated with bypass of senescence (Denko 2008; Kondoh et al. 2005). Overall, these data suggest that hypoxia and HIF-1α-induced

activation of glycolysis and concomitant reduction of oxidative stress also contributes bypass of senescence.

5.4.3 Impact of Hypoxia in OIS

Given the significant effect of hypoxia on replicative and drug-induced senescence, explicating the role of hypoxic environment in the regulation of oncogene-induced senescence becomes extremely interesting as OIS constitutes the fundamental part of tumor suppression (Collado and Serrano 2006; Di Micco et al. 2021; Collado and Serrano 2005). OIS is identified as a genetically programmed failsafe mechanism withstanding as an essential initial block against malignant transformation by its tumor preventive act (Collado and Serrano 2006; Di Micco et al. 2021; Collado and Serrano 2005). Activation of p53-p21^{CIP1} and/or p16^{INK4A}–pRb signaling is essential for OIS (Collado and Serrano 2006; 2005). Undoubtedly, in murine cells when p53 or its upstream partner p14/p19^{ARF} is inactivated, H-RasV12-induced senescence is abrogated (Serrano et al. 1997). Conversely, in human cells inactivation of p16^{INK4A} appears more crucial compared to p53 as the accomplishment of OIS entirely depends on activation p16^{INK4A} (Brookes et al. 2002). In experimental models of OIS, in normoxic conditions, ectopic expression of oncogenic RAS (HRASV12) in primary fibroblasts induced senescence that is accompanied by increased intracellular in particular mitochondrial reactive oxygen species (Lee et al. 1999). However, under low oxygen conditions RASV12-expressing senescent cells can be rescued due to the decreased production of reactive oxygen species (Welford et al. 2006; Lee et al. 1999). Moreover, in hypoxic conditions (1% O_2), RasV12 is incapable of triggering the increase in the expression of p21^{CIP1} and thus induction of senescence activation (Lee et al. 1999). Intriguingly, in a different study utilizing MEFs, it was shown that intracellular levels of ROS are increased in hypoxia and essentially required for hypoxic activation of HIF-1α, which in turn leads to ultimate extension of replicative life span (Welford et al. 2006). These conflicting data on ROS may be due to the differences in hypoxic conditions or cell types, but its involvement in the modulation of cellular senescence or the regulation of hypoxia cannot be underestimated. Of note, ROS has been reported to play a dual role by contributing simultaneously to two signaling pathways that have opposite functions in tumorigenesis, namely, RAS-RAF-MEK1/2-ERK1/2 and the p38 mitogen-activated protein kinases (MAPK) pathways (Hutter et al. 2002). RAS-RAF-MEK1/2-ERK1/2 signaling is extensively linked to oncogenesis, whereas the p38 MAPK pathway promotes cancer suppression implicating oncogene-induced senescence, inflammation-induced senescence, replicative senescence, contact inhibition, and DDR (Ogrunc et al. 2014; Hutter et al. 2002). Thus, these studies suggest that ROS may not be considered as an absolute tumor-promoting or -suppressing factor.

In line with others, our group has also shown that hypoxia prevents Ras-provoked senescence *(RASV12)* in human diploid fibroblasts (HDFs) involving HIF1α governed p53 and p21^{CIP1} downregulation and reduced DDR (Kilic Eren and

Tabor 2014). As previously shown, HIF1α and p53 proteins may have direct interactions, mostly allowing HIF-1α-dependent p53 stabilization or p53-dependent HIF-1α degradation (Kilic Eren and Tabor 2014; Blagosklonny et al. 1998). Accordingly, downstream effectors of p53 and HIF-1α may also regulate each other reciprocally (Goda et al. 2003; Hammer et al. 2007). As confirmed in replicative senescence, MIF as a target of HIF-1α directly binds and inhibits p53 and p21^{CIP1} (Fig. 5.3) (Welford et al. 2006).

In studies conducted under normoxic conditions, p16^{INK4a} is widely accepted as an essential modulator of oncogene-induced senescence and found to be upregulated predominantly through its implication of the RB pathway (Collado and Serrano 2006; Collado et al. 2005; Collado and Serrano 2005). In contrast, studies accomplished in hypoxia presented intriguing data showing the expression of p16^{INK4A} is downregulated in decreased oxygen levels (Kilic Eren and Tabor 2014). Our group reported that p16^{INK4A} is downregulated in RASV12-expressing HDFs in an HIF1-α-independent manner in hypoxic conditions (Fig. 5.3) (Kilic Eren and Tabor 2014). Additionally, in another study hypoxia/anoxia (0.1% O_2) downregulated the expression of p16^{INK4A}, depending on constitutive activation of PI3K/Akt but not HIF1α (Box and Demetrick 2004). Thus, inactivation of the p16^{INK4A}-Rb and p53-p21 tumor suppressor pathways is essential for overcoming OIS. Of note, previous studies substantiated p16^{INK4A} and p53 are mutated, and/or inactivated that in most human cancers either in the initial stage or during the progression of tumorigenesis (Sarkar et al. 2000; Rivlin et al. 2011). For example, senescence can be induced in noninvasive neoplastic papillary bladder cells via activation of the p16^{INK4A}-Rb pathway in vitro, but this is not possible in aggressive bladder carcinoma cells due to the inactivation of p16^{INK4A}-Rb and p53 signaling while cells developing into aggressive carcinoma (Sarkar et al. 2000; Romagosa et al. 2011).

DDR via ATM/ATR kinases is a key mediator of all types of senescence including OIS (Di Micco et al. 2011), However, whether or not it is implicated in the prevention of senescence in hypoxic conditions is still not completely elucidated. In general, particularly low levels of hypoxia (0.1% O_2) were shown to persuade DDR, in which both ATR and ATM kinases interceded signaling activated to mediate subsequent induction of p53-dependent apoptosis (Hammond et al. 2002; Hammond and Giaccia 2004). Several studies reported the effect of hypoxia on DDR and its relationship with cell cycle arrest or senescence (Kilic Eren and Tabor 2014; Hammond and Giaccia 2004). Our group previously reported that hypoxia decreased the phosphorylation levels of key mediators of DDR, including ATM, ATR, and Chk1 and Chk2 kinases in RASV12-expressing HDFs, suggesting a role in bypassing OIS under hypoxic conditions (Kilic Eren and Tabor 2014). Thus, the DDR barrier prevailing the induction of senescence upon oncogenic stress emerges as another mechanism that must have been successfully inactivated in cells that have fully undergone malignant transformation. Indeed, the defects in the DDR barrier, such as ATM and p53 inactivating mutations, have been frequently shown in human cancers (Bartek 2011; Bartek et al. 2007a, b). Remarkably, overexpression of HIF-1α has been demonstrated in premalignant, malignant, and metastatic lesions in the most common types of cancer (Zhong et al. 1999; Lu and Kang 2010;

Semenza 2000). In particular, the expression of HIF1α in premalignant tissues in the early stages of cancer formation even before the onset of histological initiation of angiogenesis or invasion is exceptionally intriguing (Zhong et al. 1999). From this point of view, it is plausible that the expression of HIF1α in premalignant tissues may create a selective pressure in cells and may emerge as an antagonistic mechanism contributing to bypass senescence to further promote progression into the full malignancy.

SASP is one of the hallmarks of senescence comprising the secretion of various inflammatory and immune-modulatory cytokines, growth factors, and cell surface molecules as mentioned earlier. It is evident that SASP is per se a multifaceted process employed in the promotion of complex processes, including malign transformation and paracrine and autocrine responses of senescence (Acosta et al. 2013). In previous studies, the SASP factors such as chemokine receptor CXCR2 and its ligands IL-8 and GROα have been identified as key mediators of senescence and knockdown of these factors leads to a bypass of senescence (Lee et al. 1999). Additionally, inflammatory cytokines IL-6 and IL-8 are found to be critical for oncogenic BRAF-induced senescence (Hutter et al. 2002). Interestingly, among those SASP factors IL-6, IL-8, CXCR-2 PAI1, and GROα are regulated by hypoxia as targets of HIF1α in different settings (Box and Demetrick 2004; Sarkar et al. 2000; Rivlin et al. 2011; Romagosa et al. 2011). In addition, recently, hypoxia has been shown to downregulate the detrimental pro-inflammatory SASP factors in cultured cells and tissues through AMPK-mediated mTOR suppression (Romagosa et al. 2011). Thus, hypoxia may regulate several senescent-promoting factors or pathways through HIF-1α in a context-dependent manner.

Loss of VHL, the primary regulator of HIF-α in aerobic condition, has been also associated with induction of cell cycle arrest and senescence involving pRB through HIF1α, p400, and p27[CIP1] in mouse fibroblasts (Ohh et al. 2000; Young et al. 2008), which can be reversed by the increased oxygen levels (Welford et al. 2010). Likewise, in renal epithelial cells where moderate hypoxic condition is present (~10-50mm Hg) in vivo, loss of VHL was found to be inadequate to induce senescence, whereas addition of oxidative stress to existing conditions was sufficient (Young et al. 2008; Welford et al. 2010). These data suggest that in vitro in atmospheric oxygen conditions the mechanisms that are sufficient to induce senescence may not be sufficient under low oxygen tension in vivo.

In conclusion, previous studies have shown that cells cultured under hypoxic conditions are capable of bypassing oncogene-induced senescence through the modulation of main factors such as HIF1α, p53, p21[CIP1], and p16[INK4A] despite the fact that the modulation may depend on the severity of the hypoxia, or the cell type (Welford and Giaccia 2011; Kilic Eren and Tabor 2014; Welford et al. 2006). Given that oncogene-induced-senescence is activated as an intrinsic tumor suppressor barrier in the cell, it is of great importance to a comprehensive understanding of the molecular mechanisms and identifies the mediators of the hypoxia or HIF-1α primed bypass of oncogene-induced senescence.

5.5 Conclusions and Future Perspectives

Mammalian tissues are heterogeneous in their oxygen-delivering capacity and internal oxygen levels, which can be further influenced by the intrinsic and extrinsic factors. Consequently, an evolutionarily conserved adaptation mechanism has been developed to adjust the lack of oxygen in the tissues.

Senescence is an essential part of life and emerges as crucial for both physiological and pathophysiological conditions. The contemporary senescence knowledge mainly relies on the experiments achieved through atmospheric oxygen levels. Obviously, varying levels of hypoxia exist in different tissues in living organisms and activation of HIF1 pathway can exert preventive effects on instigation of senescence triggered by various stimuli including oncogenic activation. In particular, in the context of OIS, it is to be expected that hypoxic areas will become more vulnerable to malignant transformation. Also, considering the well-known role of hypoxia on aggressive tumor behavior of and resistance to cancer therapy, modulation of tumor hypoxia or HIF1 may be beneficial to restore the tumor-suppressive function of senescence as well as to obtain better outcome from conventional treatment approaches. Further investigations directed to better understand the hypoxia-related mechanisms and factors involved in bypassing oncogene-induced senescence will certainly contribute to the development of novel and effective cancer treatment strategies..

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