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Novel ARF/p53-independent senescence pathways in cancer repression

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Abstract

Cellular senescence, which can be induced by various stimuli, is a stress response that manifests as irreversible cell cycle arrest. Recent studies have revealed that cellular senescence can serve as a critical barrier for cancer development. Induction of cellular senescence by oncogenic insults, such as Ras over-expression or by inactivation of PTEN tumor suppressor, triggers an ARF/p53-dependent tumor-suppressive effect which can significantly restrict cancer progression. Given the important role of the ARF/p53 pathway in cellular senescence and tumor suppression, drugs that stabilize p53 expression have been developed and tested in clinical trials. However, a major hurdle for p53 targeting in cancer treatment arises from the frequent deficiency or mutation of ARF or p53 in human cancers, which, in turn, profoundly compromises their tumor-suppressive ability. Recent discoveries of novel regulators involved in ARF/p53-independent cellular senescence not only reveal novel paradigms for cellular senescence but also provide alternative approaches for cancer therapy.

Keywords

Skp2; p53; Cellular senescence; Cancer therapy

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Introduction

Cellular senescence was originally defined by Hayflick and Moorhead in 1961 based on their in vitro observations that primary fibroblasts ceased to replicate after about 50 cell divisions [1, 2]. Later, this phenotype was characterized by numerous studies, and it is now applied in general to the irreversible cell growth arrest caused by various stress stimuli [3–7]. In addition to cultivation-induced proliferation exhaustion, a myriad of other stress stimuli, including telomere dysfunction, DNA damage accumulation, and genotoxic stress, have been shown to initiate “replicative senescence” [3–7]. Senescent cells display enlarged and flattened cell morphology, sustained metabolic activity, and elevated expression of senescence-associated β-galactosidase (SA-β-gal), the best-characterized marker for senescence [8–10]. Moreover, senescent cells can also secrete interleukins or insulin-like growth factor-binding protein 7 (IGFBP7) to reinforce senescence responses [11–13]. Importantly, senescence serves as a protective mechanism by which aged or mutated cells are eliminated through induction of permanent cell cycle arrest. Several lines of evidence suggest that senescence occurs not only in the in vitro cell culture system but also in various tissues in vivo [14–18] and serves as a critical barrier for cancer development [19–22].

In addition to replicative senescence, cellular senescence can also be elicited by several oncogenes, a phenomenon termed oncogene-induced senescence (OIS). In 1997, Serrano et al. first demonstrated that primary human or rodent cells undergo cellular senescence in vitro upon ectopic expression of an activated Ras mutant ($HRas^{V12}$) [5]. This phenomenon can be also elicited by other oncogenes, such as Raf, Mek, and BRaf [19, 23, 24].

The p53 transcription factor is a central player in activation of a series of signal transduction cascades to induce cell cycle arrest, apoptosis, and senescence in response to various stress signals. In the presence of stress stimuli, p53 is induced and elicits senescence both in vitro and in vivo [5, 22, 25]. Conversely, p53 targeting or inactivation by viral proteins, such as SV40 large T antigen or HPV-16 E6 protein, prolongs cellular life span [26–29]. Moreover, genetic depletion of p53 abolishes the senescence response driven by oncogenic $HRas^{G12V}$ or $BRaf^{V600E}$, in turn promoting cancer progression [5, 20, 30–32]. These observations underscore the importance of p53 in cellular senescence and suggest that activating the p53-dependent senescence pathway can be a potential strategy for cancer therapy.

Although senescence response mostly depends on the ARF (alternative reading frame) or p53 pathway, recent studies reveal that several novel senescence responses can occur independently of ARF or p53 activation. In this review, we will summarize recent advances in identifying molecular mechanisms underlying senescent phenotypes. In particular, we will focus on the regulation of ARF/p53-independent senescence pathways and consider the possible roles of these novel senescence regulators in the context of potential therapeutic avenues.

Stimuli of cellular senescence

Senescence represents a fail-safe mechanism that serves as a critical barrier for cell growth and tumor progression. In many cases, cells undergo senescence when cellular growth is critically deregulated. Senescence can be initiated by various extrinsic or intrinsic stress stimuli (Fig. 1). Replicative senescence results from telomere erosion generated by cell passaging [33, 34]. Dysfunctional (shortened/damaged) telomeres trigger a DNA damage response (DDR) that detects damaged DNA needing repair and subsequently initiates long-term cell cycle arrest [35, 36]. The DDR-activated signal transduction cascades involve the activation of ATM (ataxia telangiectasia mutation), ATR (ATM and Rad3 related), CHK1 (checkpoint kinase 1), and CHK2 (checkpoint kinase 2), which in turn induce a p53-dependent senescence response [36–38]. In studies to validate the role of telomere erosions

in senescence in vivo, Greider's and Chang's groups independently demonstrated that telomere shortening in mice elicited p53-dependent cellular senescence and restricted tumor formation [39, 40]. Apart from telomere-induced senescence, other telomere-independent insults, such as oxidative response, ultraviolet radiation, and ionizing radiation, can also contribute to senescence [6, 7, 41–43].

Among all senescence stimuli, OIS draws the most attention because of its role in tumor suppression. The first identified oncogene to induce senescence was Ras. Expressing high levels of oncogenic Ras in primary fibroblasts causes cells to suffer morphological and molecular changes indistinguishable from senescence [5]. In addition to the Ras oncogene, the Raf/Mek pathway downstream of Ras has also been shown to induce cellular senescence in vitro [23, 24], suggesting that Ras may activate the Raf/Mek pathway to induce senescence. Along with these in vitro observations, overexpressing oncogenic Ras or Raf induces senescence in vivo, further underscoring the importance of the Ras/Raf axis in senescence [20, 30–32]. In line with this evidence, recent studies showed that distal effectors of the Ras pathway, such as the E2F family of transcription factors, also trigger senescent phenotypes in certain circumstances. For example, enforcing the expression of E2F1 in normal fibroblasts can elicit the senescent phenotype [44]. Additionally, induction of *E2f3* transgene expression in mice initially promotes hyperproliferation in tissues, but subsequently thwarts cellular division and provokes OIS to restrict tumor formation [45]. It will be interesting to determine in the future whether any of these E2F family proteins contributes to the Ras-mediated senescence response. However, it should be noted that E2F target genes, such as S-phase promoting genes, are silenced during cellular senescence and may account for the stability of the senescence state [46]. Thus, hyperactivation or inactivation of E2F family transcription factors may contribute to cellular senescence.

In addition to the Ras/Raf/Mek cascade, oncogenic PI3K/Akt signaling and the PTEN tumor suppressor are also involved in cellular senescence [21, 47]. Complete loss of *Pten* in mouse prostate leads to cellular senescence, which significantly limits the ability of *Pten* loss to induce invasive prostate cancer [21]. Consistently, acute *Pten* loss or overexpression of active Akt1 in mouse embryonic fibroblasts (MEFs) triggers p53/p21-dependent senescence [21]. Moreover, Akt1 hyperactivation can also induce p27-dependent cellular senescence in vivo, which profoundly limits the ability of Akt1 to induce invasive prostate cancer [48]. Conversely, compared with wild-type cells, *Akt1/2*-deficient cells exhibit fewer senescent cells upon Ras overexpression [49]. Although hyperactivation of PI3K/Akt signaling causes senescence, disruption of this pathway seems to also contribute to senescence. Cichowski's group found that abrogation of PI3K signaling promoted senescence induced by aberrant activation of the Ras/Raf pathway [50], although the mechanism by which the inhibition of PI3K signaling impedes the senescence response remains to be determined. These studies suggest that PI3K/Akt signaling displays distinct functions in senescence response, which may be caused by distinct cell types. Nevertheless, these studies highlight the importance of the PI3K/Akt pathway in cellular senescence.

As Akt activation is known to antagonize p53 and p27 expression and activity, how Akt hyperactivation induces p53 and p27 expression is still a conundrum. One recent study provides a partial explanation for this phenomenon. Pandolfi and colleagues revealed that acute *Pten* inactivation leads to mTOR hyperactivation, which in turn enhances p53 protein translation and cellular senescence [51]. This finding provides a direct link between mTOR and p53 induction in cellular senescence in the presence of acute *Pten* inactivation. However, another study suggested that p27 induction due to *Pten* loss or Akt hyperactivation is independent of mTOR activation [48]. More studies need to be done to further unravel the mechanism by which Akt regulates p27 expression during cellular senescence.

Other proto-oncogenes engaged in OIS are β-catenin and Myc. Although β-catenin has been linked to tumor progression, aberrant β-catenin expression induces growth arrest with features of senescence in vitro, and its transgenic expression in lymphocytes triggers the DDR and cellular senescence in vivo [52, 53]. In Emu-myc transgenic mouse models, constitutive Myc expression elicits cellular senescence in vivo by directly activating the p53-dependent pathway and indirectly increasing TGF-β secretion from macrophages [54, 55]. Although Myc overexpression triggers cellular senescence, its deficiency also causes cellular senescence in MEFs [56, 57], suggesting that the senescence program can be elicited by deregulated Myc expression.

Oxidative stress and the DDR, in addition to being the two major causes of replicative senescence, are also downstream events of OIS. For instance, an increase in reactive oxygen species (ROS) levels triggered by activated oncogenes, such as Ras and Myc, ultimately contributes to a senescence response in vitro [58, 59]. Moreover, the DDR is found to be induced in cells with Ras-induced senescence, and inactivation of the DDR abolishes OIS and promotes cellular transformation in vitro [60, 61]. It will be interesting to examine whether the DDR and oxidative stress indeed contribute to OIS in vivo.

A series of recent studies suggest that the occurrence of OIS is contingent upon culture conditions, cell types, and oncogenic protein levels. For example, when MEFs are cultured in serum-free medium, they become resistant to Ras-induced senescence [62], suggesting that mitogenic stimulation is critical for OIS. Restoration of p53 in *p53*-deficient mice was shown to trigger senescence in liver carcinoma and sarcoma, but not in lymphoma [22, 63]. However, a recent study using a different mouse tumor model showed that p53 restoration also elicits senescence in lymphoma [55]. Thus, distinct genetic alterations may affect the ability of p53 to trigger senescence. Moreover, overexpression of various Ras mutants, including $NRas^{G12D}$ and $KRas^{G12D}$, has been shown to induce senescence in various cancer types, including premalignant lung adenomas [20, 64], but this phenomenon is not consistently observed in lung lesions of KRas-mutant knock-in mice. One study showed that there was no senescence in lung lesions of $KRas^{G12D}$ knock-in mice, but another study revealed that senescence occurs in lung adenomas of $KRas^{G12V}$ knock-in mice [65, 66]. Thus, deepening our understanding of the molecular basis and tissue specificity of OIS will not only provide novel insights into how cellular senescence is regulated but also aid in developing therapeutic strategies and assessments for cancer treatments.

ARF/p53-dependent senescence pathway

Extensive studies from the past decade have revealed that p53 is a major senescence effector that responds to various external and internal stress signals by activating a set of important target genes. As previously stated, the DDR is initiated during telomere erosion and contributes to cellular senescence. DDR proteins, such as ATM, Chk2, and ATR, are activated upon telomere shortening and are actively involved in the senescence process [36–38]. Activated ATM or ATR then phosphorylates p53 and protects it from ubiquitination and degradation by MDM2, in turn eliciting senescence programs. Likewise, DNA damage agents, such as doxorubicin, can also trigger the DDR, leading to p53-dependent cellular senescence. The supporting evidence comes from the observation that SA-β-gal expression and cellular senescence are only induced in cells carrying wild-type p53, but not in cells harboring mutated or inactivated p53, when treated with chemotherapy agents [67]. In addition, p53 not only is critical for replicative senescence but also contributes to OIS [5, 21, 30, 32]. Moreover, it is shown that OIS is partly mediated through oncogene-driven DDR [68]. Similarly, recent studies using genetically engineered mice in which p53 expression can be switched on or off have supported the notion that tumor development due to *p53* deficiency can be inhibited after *p53* expression is restored [69], and in certain cancer types,

tumor regression can be triggered by cellular senescence [22, 63]. Conversely, p53 ablation eliminates senescence and promotes tumor progression in $BRaf^{V600E}$-induced lung tumors and $HRas^{G12V}$-induced mammary tumors [30, 32].

p53 is a transcription factor that is known to turn on target genes involved in various cellular responses. Among them, p21 is an important downstream effector for p53-mediated cellular senescence. Notably, p21 is found to be overexpressed in senescent cells, and forced expression of p21 induces premature senescence even in *p53*-null cells [70]. Moreover, inactivation of *p21* expression in human fibroblasts bypasses senescence and extends the cellular life span [71]. However, *p21*-deficient MEFs undergo senescence and are resistant to Ras-induced transformation [72], suggesting that the role of p21 in cellular senescence may vary among species. Since numerous p53 target genes other than p21 have also been identified, it will be interesting to determine whether there are additional target genes involved in p53-dependent senescence pathways. If so, do they work redundantly or distinctly to effect p53-dependent senescence processes?

p63 and p73 are also characterized as members of the p53 family and, like p53, are involved in cellular senescence [73–76]. Because of their high sequence homology in DNA binding and activation domains, p53, p63, and p73 are likely to share similar downstream transcription targets and overlapping biological functions [77]. Consistent with this notion, ectopic expression of p63 and p73 triggers cell cycle arrest and apoptosis in a manner similar to that of p53. Like p53, p73 has been shown to repress transcription of human telomerase reverse transcriptase (hTERT), which maintains telomere length and regulates senescence response. This similar function implies that p73 can also trigger telomere erosion and in turn activate replicative senescence [73, 78]. These findings suggest that upregulation of p73 and p53 favors a senescence response.

The role of p63 in cellular senescence appears to be inconsistent. One study showed that p63 overexpression in cells that lack functional p53 can elicit replicative senescence [76]. In contrast, another study demonstrated that *p63* deficiency in embryos or primary keratinocytes dramatically induces expression of two senescence markers, SA-β-gal and PML, accompanied by cell cycle arrest and cellular senescence [79].

p63 is expressed in two isoforms, ΔNp63 (N-terminally deleted p63) and TAp63 (transcriptionally active p63), with two distinct promoters. A recent study utilizing the *TAp63*-specific conditional mouse model demonstrated that the TAp63 isoform is required for Ras-mediated cellular senescence in MEFs and that overexpression of TAp63 triggers cellular senescence through p21 and pRB, but not the ARF/p53 pathway [80]. On the contrary, another study revealed that *TAp63* deficiency in skin epithelial cells can initiate cellular senescence [81]. These results suggest that the TAp63 isoform plays a distinct role in the regulation of cellular senescence in a tissue-specific manner. With regard to ΔNp63, its downregulation reduced cell proliferation and induced cellular senescence in human keratinocytes [82]. Given that ΔNp63 and TAp63 regulate transcription of genes with distinct biological functions in cancer and development [83], it is important to investigate specific downstream targets responsible for ΔNp63- and TAp63-triggered senescence responses.

ARF is an upstream regulator of p53. It activates and induces p53 by preventing MDM2-mediated p53 ubiquitination and degradation. Comparable to the role of p53 in cellular senescence, *Arf* deficiency in MEFs inhibits cellular senescence and eventually leads to cell immortalization [84], whereas induction of *Arf* expression activates p53-dependent senescence in fibroblasts [85]. Moreover, *Arf*-deficient mice are highly prone to spontaneous development of tumors, such as lymphoma and sarcoma, a phenotype that was also observed

in *p53*-null mice [86, 87]. Interestingly, the *CDKN2A* locus, which encodes ARF and INK4A (p16), is de-repressed, leading to induction of ARF and p16 transcription when senescence stimuli are encountered [88–90]. Oncogenes, such as Ras and E2F, induce the expression of ARF tumor suppressor, leading to p53-dependent cellular senescence [91, 92]. Although the ARF/p53 pathway is responsible for oncogene-induced cellular senescence, oncogene-induced DNA damage resulting from replication stress or ROS triggers an ARF-independent DDR, leading to p53 activation and senescence [59–61, 93].

Although ARF has been shown to modulate many biological functions by regulating p53 activation, one recent report showed that ARF may function independently of p53 to regulate cellular senescence in certain cell types [94]. The authors demonstrated that *Pten* loss in prostate epithelial cells drives p53-dependent senescence, but concomitant inactivation of *Arf* and *Pten* surprisingly does not suppress p53 activation and cellular senescence. However, *Arf* deficiency in MEFs abolishes cellular senescence upon complete *Pten* inactivation [94], indicating that p53-triggered senescence is regulated variably in different cell types.

ARF/p53-independent senescence pathways

Although most stress stimuli elicit cellular senescence primarily by activating the ARF/p53 pathway, recent reports unravel novel mediators whose inactivation induces cellular senescence independently of that pathway (Fig. 2). *VHL* (von Hippel-Lindau) is a tumor suppressor that is frequently mutated in many human cancers [95]. It is well-established that VHL displays tumor-suppressive activity by promoting ubiquitination and degradation of oncogenic hypoxia-inducible factor (HIF). Surprisingly, acute inactivation of the *VHL* tumor suppressor causes cellular senescence in vitro and in vivo. The senescence response is due to neither HIF accumulation nor p53 activation but instead depends on Rb activation and p400 reduction [96]. Interestingly, *HIF1α* deficiency in MEFs also induces senescence by regulating macrophage migration inhibitory factor (MIF) expression [97]. Another report, however, showed that loss of *VHL* in MEFs causes HIF-dependent growth arrest in vitro, although it is unclear whether senescence is induced in that scenario [98].

Interestingly, *VHL* inactivation also decreases *Skp2* mRNA levels and increases p27 expression [96]. Consistent with this notion, overexpression of the human T-lymphotropic virus type 1 (HTLV-1) Tax protein also downregulates Skp2 expression, accompanied by cellular senescence [99]. These results suggest that Skp2 reduction or p27 induction may have a direct role in cellular senescence.

While VHL is ubiquitously expressed in somatic tissues, the tumor spectrum linked to VHL loss is only limited to certain tissues. The fact that acute *VHL* inactivation induces senescence responses provides a possible explanation as to why *VHL* loss in various somatic tissues only leads to development of certain types of cancers. We speculate that only those *VHL*-deficient cells with additional mutations or molecular changes that overcome senescence responses may eventually develop tumors.

Skp2, which belongs to the family of F-box proteins, exhibits E3 ligase activity by forming the Skp2-SCF (Skp1-Cul1-Rbx1-F-box) complex. Earlier studies have shown that Skp2 regulates cell cycle progression and proliferation by targeting ubiquitination and degradation of its substrates, such as cell cycle inhibitor p27 [100, 101]. Subsequent studies of human cancer samples revealed that Skp2 is overexpressed in a variety of human cancers and is correlated inversely with p27 levels, suggesting that Skp2 overexpression may play an important role in human cancer development [102, 103]. Other studies using xenograft mouse tumor models have supported the oncogenic role of Skp2 in cancer development [104–106]. Moreover, recent work using the *Skp2*-deficient mouse model has revealed that

Skp2 is required for cancer development in multiple tumor conditions, including *Pten*, *Arf*, and *pRB* inactivation [107, 108].

The direct role of Skp2 in cellular senescence is supported by our recent work, which reveals that genetic *Skp2* inactivation evokes cellular senescence in vitro and in vivo in the context of *Pten* or *Arf* loss, although loss of *Skp2* alone is not sufficient to elicit senescence [107]. Strikingly, the senescence response driven by *Skp2* inactivation along with *Pten* inactivation or *Arf* loss neither activates the p53 pathway nor elicits the DDR (Fig. 3). Instead, cell cycle inhibitors p27 and p21 and endoplasmic reticulum stress protein Atf4 are induced and synergistically contribute to this senescence response [107].

Since Skp2 regulates activity of Cdk (cyclin-dependent kinase) family proteins by targeting p21 and p27 ubiquitination and degradation, it is conceivable that Cdk inhibition may also induce senescence. Like Skp2 inhibition, inhibiting the activity of Cdks (Cdk2, Cdk4, and Cdk6) can also trigger cellular senescence [109, 110]. Owing to the functional overlap among Cdks, determining which Cdk affects senescence is of relevance in cancer research. A recent report indicates that Cdk2 plays a role in restricting cellular senescence. Loss or inactivation of Cdk2 causes sensitization to Myc-triggered senescence, in turn preventing lymphoma development [110, 111]. Interestingly, the senescence response driven by *Cdk2* deficiency is likely independent of p53 activity but may depend on p21 and p16 induction [110]. Moreover, a recent study provides genetic and pharmacological evidence demonstrating that ablation of Cdk4 provokes senescence responses to attenuate KRas-driven lung adenocarcinoma [112]. In addition, constitutive Cdk4 activation bypasses cellular senescence in vitro and promotes in vivo tumorigenesis driven by carcinogens TPA and DMBA, known to induce Ras mutation [113]. It should be noted that *Cdk4* inactivation induces an immediate senescence response only in the lungs and not in other tissues, providing an explanation for the limited efficacy of a selective Cdk4 inhibitor in treating leukemia and breast tumors [112]. The similarity between the role of Skp2 and Cdks in the regulation of cell proliferation and cellular senescence suggests that Skp2 and Cdks may act through common signaling cascades for regulating cell cycle progression and senescence.

Apart from the protein regulators described above, micro-RNAs (miRNAs) are also implicated in cellular senescence. miRNAs are small noncoding RNAs that can negatively regulate gene expression by either inhibiting translation or promoting RNA degradation [114]. Recent studies have shown that some classes of miRNAs display oncogenic activity to promote cancer progression and metastasis, while other classes of miRNAs are tumor suppressors that can negatively regulate these processes by triggering cellular senescence or apoptosis [115, 116]. For instance, miR-34a expression was found to be induced under BRaf overexpression and was correlated with a cellular senescence response [117]. Interestingly, although p53 was also induced upon BRaf overexpression and contributed to BRaf-mediated cellular senescence, it was not responsible for miR-34a induction in this case. Subsequent experiments revealed that c-Myc is targeted by miR-34a during senescence [117]. These results suggest that in addition to p53, miR-34a upregulation may contribute to the senescence response upon BRaf overexpression by repressing c-Myc expression. Since p53 is shown to mediate the DDR and regulate a subset of miRNAs, such as miR-192 and miR-215 [118, 119], it may be relevant to investigate whether these miRNAs are also involved in cellular senescence regulation and subsequent tumor development.

Senescence in tumor suppression and tumor targeting

As mentioned above, a senescence response arrests cell growth and acts as a brake for cancer progression. In response to aberrant oncogenic insults, the p53 tumor suppressor plays a crucial role in promoting the senescence process, suggesting that targeting p53 may

be a potentially useful strategy for treating human cancers. Indeed, *p53*-deficient tumors regress when the p53 level is rescued in vivo [22, 63]. Recently, a p53-stabilizing small molecule has been developed and shown to display senescence-inducing effects on cancer cells [120]. Moreover, mice bearing tumors with intact senescence programs show better response to chemotherapy agents than those harboring tumors with senescence defects [121].

Because Myc overexpression is present in numerous human cancers and transgenic mice with Myc overexpression develop spontaneous cancers, targeting Myc may be a potential approach for cancer treatment [122]. In support of this notion, inactivation of Myc by an artificial dimerization partner known as Omomyc triggers tumor regression along with apoptosis and senescence responses [123].

Since p53 is the most commonly mutated gene in human cancers, applying a p53-dependent cellular senescence strategy may not be applicable for tumors with *p53* loss or inactivation. In this scenario, targeting *p53*-independent cellular senescence pathways may be the key for ensuring success of human cancer treatments. Since inactivation of *Skp2* elicits p53-independent cellular senescence, targeting oncogenic Skp2 may be an ideal approach for treating advanced human cancers with *p53* inactivation. In support of this notion, a small molecule (MLN4924) indirectly targeting the Skp2-SCF complex has been shown to trigger p53-independent senescence and repress prostate tumors with p53 inactivation [107]. Thus, developing specific Skp2 small molecule inhibitors and testing their in vivo efficacy are of importance and may be beneficial for the treatment of human cancers.

Since Cdk2 inactivation also elicits p53-independent cellular senescence, Cdk2 may be another ideal target for treating cancers with *p53* inactivation. This idea can be tested immediately, as several small molecule inhibitors of Cdk2 have already been developed. In fact, two small molecule inhibitors of Cdk2 have been shown to trigger senescence in Myc-overexpressed leukemia cancer cells with ablated p53 function [111], although their efficacy on tumor growth in vivo remains to be determined.

Conclusion and perspective

Cellular senescence was initially regarded to be an artifact induced by cell culture stress but now has been well characterized as an intrinsic cell-protective mechanism against stress signals that can help eliminate damaged or arrested cells. In particular, the “tumor-blunt” effect triggered by a senescence response has drawn the most attention and sheds light on a novel therapeutic strategy for cancer treatment. Traditional cancer treatment strategies aim at executing cell death and apoptosis, which are regarded as prerequisites for preventing malignant cell growth. However, recent studies in multiple mouse tumor models demonstrate that cellular senescence can occur in vivo and can provide a critical barrier for cancer development [19–22, 30–32, 45, 107], suggesting that “pro-senescence” therapy may provide an efficient alternative strategy for cancer prevention and treatment.

Although enormous progress has been made in the previous decade in characterizing cellular senescence in tumor suppression, several important areas of inquiry still remain to be addressed. First, since senescence response can be reversed in vitro by inactivating both p16/Rb signaling and p53 [124], does the same phenomenon occur in vivo and if so, does it contribute to tumor relapse? Second, the senescence regulators described above are involved not only in cellular senescence but also in the regulation of cell apoptosis. For instance, modulating activity of p53, TAp63, Cdk2, or Skp2 triggers the apoptosis program, which can potentially contribute to tumor regression. Thus, it is important to examine how these two important cell-protective mechanisms interact. Does cell senescence occur

independently of apoptosis? What determines whether cells undergo either apoptosis or a senescence response? Third, although accumulating evidence has supported the important role of cellular senescence in tumor suppression, some studies suggest that cellular senescence may also have a tumor-promoting effect. For instance, it was shown that senescent cells may acquire a senescence-associated secretory phenotype, which empowers senescent cells to secrete proinflammatory interleukins, growth factors, and protein/extracellular components, which may stimulate the malignant phenotype of neighboring cells [125, 126]. Hence, understanding how the senescence response operates with regard to its tumor suppression and tumor promotion processes is required before this concept can be applied to cancer treatment. Nevertheless, the advances in identifying p53-independent senescence regulators not only shed new light on cellular senescence programs but also provide an important step toward developing a “pro-senescence” therapy for clinical application.

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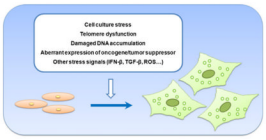

Fig. 1.

Cellular senescence induced by various stimuli. Telo-mere shortening, damaged DNA accumulation, oncogene/tumor suppressor dysregulation, or other stress signals (e.g., cell culture shock, interferon-β, transforming growth factor-β, or oxidative stress) will trigger cells to acquire a senescent phenotype that includes cell growth arrest, enlarged and flattened morphology, and sustained metabolic activity

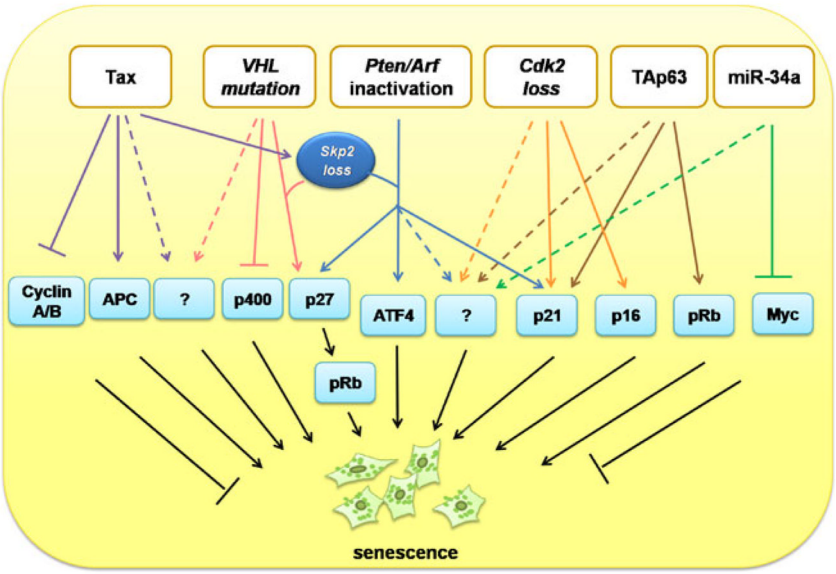

Fig. 2.

Novel regulators involved in p53-independent senescence pathways. Skp2 inactivation, concomitant with inactivation of PTEN, ARF, or VHL, activates downstream effectors, including p27, p21, ATF4, and p400, to mediate senescence responses. Skp2 down-regulation alone induced by HTLV Tax1 protein is also linked to cellular senescence. Inactivation of cell cycle regulators other than Skp2 (for instance, CDK2) is also engaged in senescence partially through activating p21 and p16. TAp63, which belongs to the p53 family, induces expression of p21 or pRb and subsequently contributes to the p53-independent senescent phenotype. Beyond these regulators, the senescence response is triggered by noncoding RNA. For instance, p53-independent miR-34a upregulation is involved in Braf-induced senescence by repressing Myc expression. *Solid lines* indicate known pathways. *Dashed lines* indicate undefined pathways

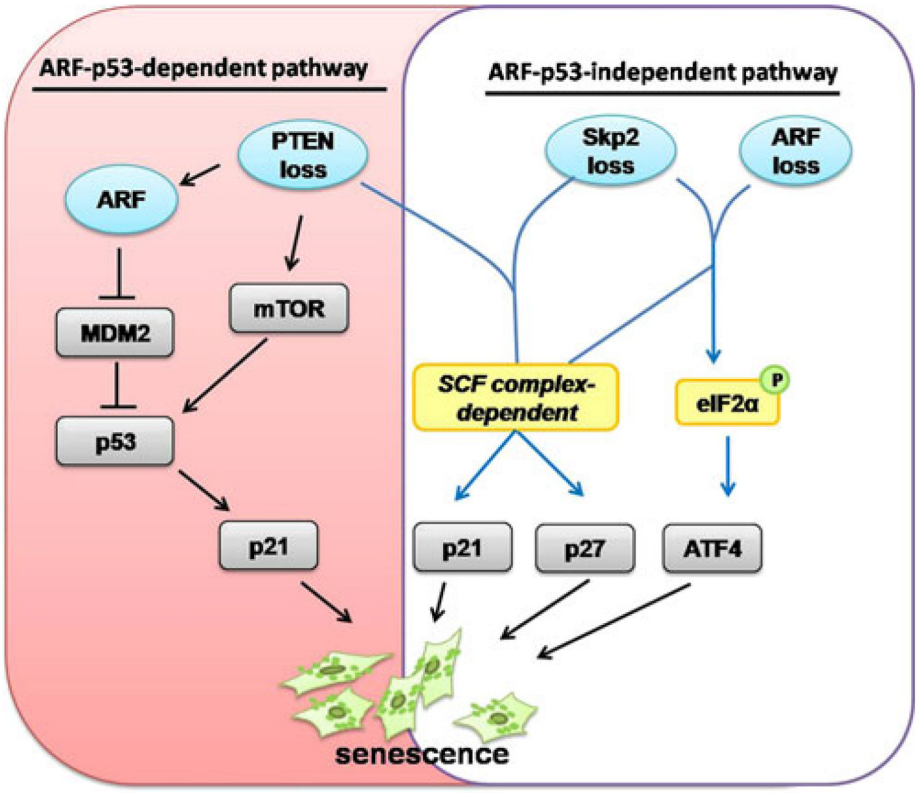

Fig. 3.

Crosstalk between ARF, PTEN, and the Skp2-SCF complex in ARF/p53-dependent and ARF/p53-independent pathways. In response to stress signals, ARF activation and PTEN loss could trigger cellular senescence through pathways both dependent on and independent of ARF/p53. Stress stimuli induce ARF to sequester MDM2, which then activates p53 and, downstream, p21, resulting in a p53-dependent cellular senescence response. Additionally, in the absence of PTEN, stress signals will trigger p53-dependent cellular senescence through the activation of ARF or mTOR. In the context of PTEN or ARF inactivation, Skp2 deficiency will increase expression of p21, p27, and ATF4, which eventually leads to an ARF/p53-independent senescence response. *Black lines* indicate known pathways. *Blue lines* demonstrate newly characterized ARF/p53-independent pathways