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Review

Epigenetic regulation of p16^{Ink4a} and Arf by JDP2 in cellular senescence

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Abstract

In response to accumulating cellular stress, cells protect themselves from abnormal growth by entering the senescent stage. Senescence is controlled mainly by gene products from the p16^{Ink4a}/Arf locus. In mouse cells, the expression of p16^{Ink4a} and Arf increases continuously during proliferation in cell culture. Transcription from the locus is under complex control. p16^{Ink4a} and Arf respond independently to positive and negative signals, and the entire locus is epigenetically suppressed by histone methylation that depends on the Polycomb repressive complex-1 and -2 (PRC1 and PRC2). In fact, the PRCs associate with the p16^{Ink4a}/Arf locus in young proliferating cells and dissociate in aged senescent cells. Thus, it seems that chromatin-remodeling factors that regulate association and dissociation of PRCs might be important players in the senescence program. Here, we summarize the molecular mechanisms that mediate cellular aging and introduce the Jun dimerization protein 2 (JDP2) as a factor that regulates replicative senescence by mediating dissociation of PRCs from the p16^{Ink4a}/Arf locus.

Keywords: Arf; epigenetic; Jun dimerization protein 2 (JDP2); p16^{Ink4a}, senescence.

Introduction

Primary untransformed cells stop growing after several weeks of cell culture and enter an irreversible growth arrest stage called replicative senescence, which is related to 'cellular aging'. The senescence response can be induced by various cellular stresses, including the loss of telomeres, the accumulation of oxidative stress, and genotoxic stress by chemical agents. DNA damage generates responses that protect cells from aberrant growth. These responses include cell cycle arrest, DNA repair and apoptosis, as well as autophagy and senescence. Autophagy is a cellular process by which cellular components are degraded by lysosomes. It acts as a tumor suppressor by scavenging damaged organelles (1). Cells that fail to recover from damage through DNA repair and autophagy undergo apoptosis and senescence. Senescence protects normal cells from abnormal growth signals and oncogenic transformation, by stopping progression of the cell cycle. It can be induced by various signals, including activation of the MAP kinase pathway by oncogenic Ras. Senescence increases the activities of two well-known tumor suppressors, pRb and p53. Interestingly, acute inactivation of these proteins by shRNA or CRE-loxP dependent conditional knockdown allows mouse embryonic cells to re-enter the cell cycle from the senescent state, suggesting that these proteins are also required for maintaining the senescent phenotype (2, 3). pRb and p53 expression is regulated by two distinct proteins, p16^{Ink4a} and Arf, respectively, that are encoded by the *cdkn2a* locus (4) (Figure 1). The expression of p16^{Ink4a} increases dramatically with increasing numbers of cell division of primary fibroblast in culture (5–7), as well as in rodent and human models *in vivo* (8, 9). Recently, the level of expression of p16^{Ink4a} has begun to be considered to be a 'biomarker of aging' and the regulation of its aging dependent expression has become a matter of considerable interest. In this review, we will summarize the roles of p16^{Ink4a} and Arf in the cell cycle, and describe established and novel mechanisms for the regulation of their expression.

p16^{Ink4a} and the Rb pathway

A number of genes that are essential for cell cycle progression, such as cyclin E1 (Figure 2), are transcribed at the beginning of the G1 phase by the E2F family of transcription factors. E2F is controlled by the Rb family of proteins, pRb, p107 and p130 (10, 11). Early in G1, unphosphorylated Rb proteins bind to the E2F family of proteins and inactivate their function (12, 13). During G1, the Rb proteins are inactivated by phosphorylation by the Cdk4/6-cyclinD complexes, thereby allowing transcription of E2F-dependent genes, including cyclin E1. Upregulated cyclin E1 forms a complex with cdk2, which mediates hyperphosphorylation of the Rb proteins, an essential requirement for the G1/S transition. p16^{Ink4a} is an allosteric inhibitor of cdk4/6 (14). Binding of p16^{Ink4a} changes the conformation of cdk4/6, which prevents

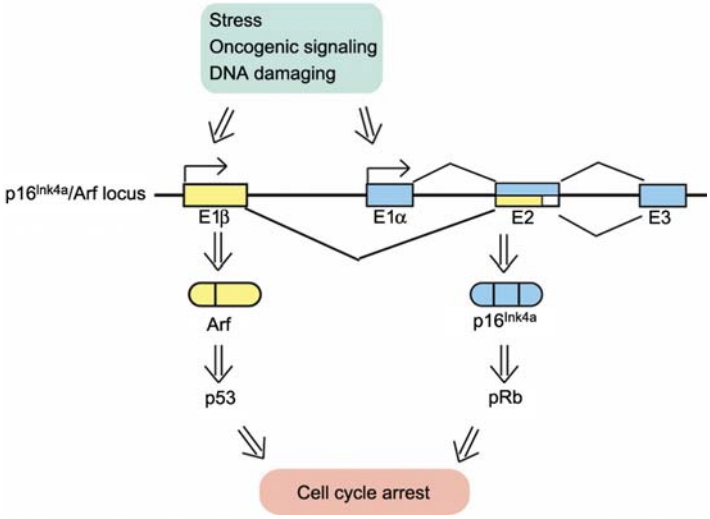

Figure 1 Schematic structure of the $p16^{Ink4a}$/Arf locus.
Signaling from stress, oncogenic activation, and DNA damage activate transcription from Exon 1β and Exon 1α of Arf and $p16^{Ink4a}$, respectively. As a result of splicing, Arf (yellow) and $p16^{Ink4a}$ (blue) share common Exon 2 and Exon 3 sequences but are translated from alternative open reading frames that code for different amino acid sequences. Arf and $p16^{Ink4a}$ activate the p53 and pRb axes, respectively.

their interaction with cyclin D (15). Therefore, $p16^{Ink4a}$ acts as an inhibitor of the cell cycle in G1 by modulation of the Rb pathway. $p16^{INK4A}$ is often lost in a variety of human malignancies, such as glioblastoma, melanoma, and pancreatic adenocarcinoma (16). By contrast, $p16^{INK4A}$ upregulation induces cell cycle arrest and senescence.

Arf and the p53 pathway

p53 is one of the most studied tumor suppressors. It mediates cell cycle arrest in G1 and G2, as well as apoptosis (Figure 3). A number of downstream targets of p53 are involved in these processes, including $p21^{Cip/waf1}$ for G1 arrest (17), 14-3-3 sigma and GADD45 for G2 arrest (18, 19) and $p21^{Bax}$, PUMA, Fas/Apo1 and Killer/DR5 for apoptosis (20–24). p53 is regulated at the levels of protein stability and activity, and to some extent transcription and translation (25, 26). In non-stressed cells, p53 protein levels are very low because of degradation mediated by the E3 ubiquitin ligase activity of MDM2, which targets p53 for ubiquitin-dependent proteolysis (27). MDM2 is a transcriptional target of p53, thus p53 directly activates the expression of its own negative regulator, producing a potent negative feedback regulatory loop (28). There are several stress-responsive kinases, which, by phosphorylating p53, inhibit its degradation by MDM2 and increase its transcriptional activity (29–31). DNA damage rapidly activates ataxia telangiectasia mutated (Atm) and ataxia telangiectasia related (Atr), which phosphorylate the checkpoint kinases Chk1 and Chk2, which in turn propagate the signal to downstream effectors such as p53 (32, 33). Chk1 and Chk2 phosphorylate p53 on Ser20, which prevents efficient recruitment of MDM2. Thus, p53 is stabilized and its expression level is increased in response to stress signaling.

MDM2 is regulated by Arf. Arf is predominantly localized in nucleoli and stabilized by binding to nucleophosmin. In response to stress signaling, Arf is released from nucleophosmin and translocates to the nucleoplasm, where it interacts with MDM2, inhibits its E3 ubiquitin ligase activity and blocks nucleocytoplasmic shuttling of the MDM2-p53 complex. The consequence of activation of Arf is stabilization and activation of p53 (34, 35).

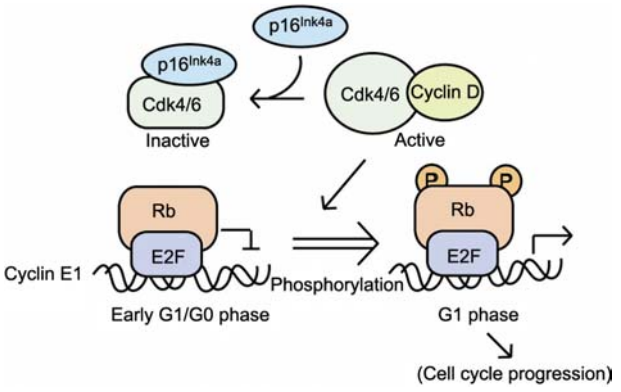

Figure 2 The $p16^{Ink4a}$-pRb pathway.
In the G0/early G1 phases of the cell cycle, unphosphorylated pRb (Rb in the figure) forms a complex with the E2F family of transcription factors, preventing E2F-dependent transcription. Phosphorylation of pRb by the Cdk4/6-cyclinD complex leads to transcriptional activation and expression of genes, such as cyclin E1, that are essential for cell cycle progression. $p16^{Ink4a}$ binds to Cdk4/6, inhibits the interaction with cyclin D, and thereby blocks Cdk4/6 kinase activity and phosphorylation of pRb. Overall, the expression of $p16^{Ink4a}$ induces cell cycle arrest by inhibiting pRb phosphorylation.

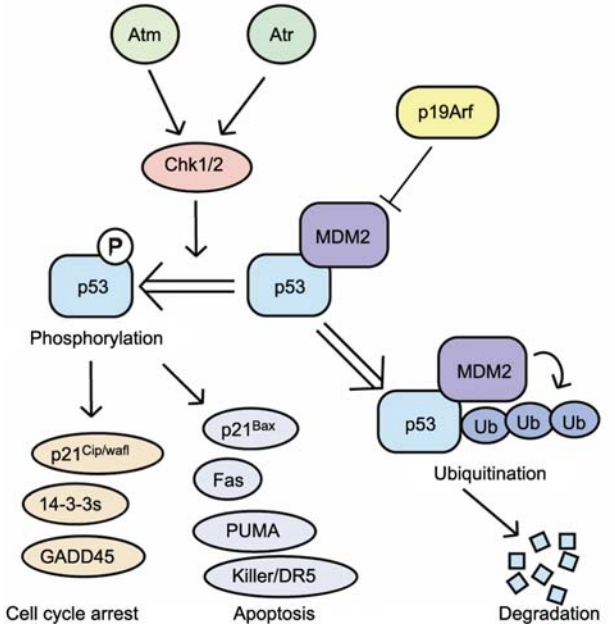

Figure 3 The Arf-p53 pathway.
In non-stressed cells, p53 protein levels are very low because of ubiquitin-dependent degradation mediated by MDM2. p53 is stabilized by different mechanisms. DNA damage activates the Atm and Atr kinases that phosphorylate Chk1/2. Chk1/2 phosphorylate p53 on ser20, which inhibits MDM2 binding and thereby stabilizes p53. By contrast, Arf stabilizes p53 by binding to MDM2 and inhibiting its activity. Upregulation of p53 leads to cell cycle arrest and apoptosis.

Transcription regulation of p16^{Ink4a}

Transcriptional regulation of the p16^{Ink4a} gene is an important event in cellular senescence (Figure 4). p16^{Ink4a} expression is increased during replicative senescence as well as premature senescence induced by oncogenic activation (5). Its expression is regulated by transcription activators such as Ets1/2 and the basic helix-loop-helix (b-HLH) protein E47, as well as transcription inhibitors that include the Id-1 HLH protein (36–39). In addition, the p16^{Ink4a} locus is epigenetically repressed by the Polycomb Repressive Complexes-1 and -2 (PRC1 and PRC2), which methylate lysine 27 of histone H3 (H3K27) (40). In transformed cells, in which the cell cycle is not arrested by a senescence program, the CpG islands on the p16^{Ink4a} promoter and exon 1 are methylated and the p16^{Ink4a} gene is silenced (41, 42). Understanding the role of the different factors that regulate the p16^{Ink4a} gene is important to elucidate the molecular mechanism of the cellular aging program. Here, we will describe some of these factors, including transcription activators, inhibitors, and epigenetic regulators.

Transcription activators

Ets1 and Ets2 activate the *p16^{INK4A}* gene in response to activation of the Ras/MEK/MAP kinase pathway by directly binding to the ets consensus sites on the promoter (39). In human fibroblasts, hyperactivation of Ets2 by overexpression of oncogenic Ras induces G1 arrest, premature senescence, and increased expression of p16^{INK4A}. Ets2 seems to be the main regulator of p16^{INK4A} expression in the case of oncogenic premature senescence, whereas Ets1 plays a role in replicative senescence (43, 44). The b-HLH protein, E47, binds to DNA and proteins through its basic and HLH domains, respectively. The E47 homodimer binds specifically to the E-box (CANNTG) on the p16^{INK4A} promoter (38). E47 overexpression inhibits proliferation of some tumor cell lines by inducing p16^{INK4A} expression. Inhibition of E47 by RNA interference significantly reduces the expression of p16^{INK4A} and delays the onset of senescence (36). Similarly, heterodimerization of E47 with ectopically expressed Tal1 inhibits the expression of p16^{Ink4a} (45).

Transcription inhibitors

Id-1 is a negative transcription regulator of p16^{Ink4a}. Id-1 expression correlates negatively with p16^{Ink4a} expression during the process of senescence (36, 39). The expression of p16^{Ink4a} in mouse embryonic fibroblasts (MEFs) is higher in Id-1-deficient compared with wild type (wt) MEFs (37).

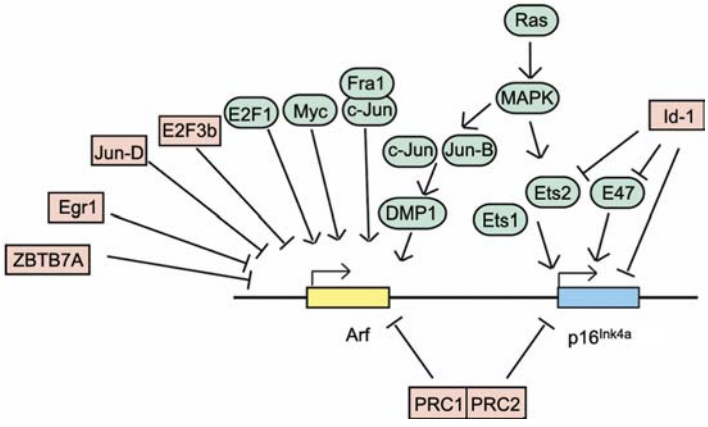

Figure 4 Regulation of the p16^{Ink4a} and Arf transcription.
Green circles and red squares indicate activators and repressors, respectively, of p16^{Ink4a} and/or Arf transcription.

Overexpression of Id-1 delays replicative senescence by inhibiting $p16^{INK4A}$ in human keratinocytes and endothelial cells (46, 47). Id-1 does not have a basic DNA-binding domain, unlike the b-HLH protein E47. Instead, Id-1 inhibits transcription of $p16^{Ink4a}$ by heterodimerization with Ets2 (39). Id-1 also heterodimerizes with E47 and inhibits its transcriptional activity (48).

Epigenetic regulators

The $p16^{Ink4a}$ locus is also regulated epigenetically. The locus is transcriptionally silenced by trimethylation of lysine 27 of histone H3 (H3K27) in young proliferating primary cells. By contrast, $p16^{Ink4a}$ expression increases in aged and senesced cells as a result of the loss of H3K27 trimethylation (49). The methylation of H3K27 and the silencing of the $p16^{Ink4a}$ locus is mediated by PRC2 and PRC1. PRC1 contains several subunits, including polycomb (CBX2, 4, 6, 7, or 8 in humans), polyhomeotic (PH1 or PH2 in humans), Bmi1, Ring1B and other subunits (50), whereas PRC2 is composed of Ezh2, Suz12, and Eed (40, 51). In PRC2, Ezh is the catalytic subunit that methylates H3K27 (40); whereas, the other components are indispensable for the function of the complex. Suz12 is essential for complex formation and the di- and trimethylation of H3K27 *in vivo* (52, 53). By contrast, Eed is required for global H3K27 methylation, including monomethylation (54). In PRC1, the CBX subunit recognizes and binds to trimethylated H3K27 (55, 56). Ring1B has E3 ligase activity for ubiquitylation of histone H2A, whereas Bmi1 acts as a cofactor (57, 58). Ubiquitylation of H2A by PRC1 prevents elongation of RNA polymerase II (59). PRC1 has been shown, by electron microscopy, to contribute to compaction of chromatin (60). Therefore, a possible molecular mechanism of PRC-mediated gene silencing might be that Ezh2 and other subunits of PRC2 trimethylate histone H3K27, which acts as a binding site for PRC1 that ubiquitylates H2A and compacts chromatin, lead to inhibition of elongation of RNA polymerase. In addition, PRCs can inhibit earlier steps of transcription, as PRC1 interacts with components of the basal transcription machinery, the TAFs (TATA box-binding protein associated factors) (61, 62). This inhibition does not appear to involve blocking access of RNA polymerase to the promoter, as the PRCs and transcription factors bind to target genes at the same time (59, 62–65). In summary, $p16^{Ink4a}$ transcription is inhibited by PRC2 that methylates histone H3K27, which in turn recruits PRC1. PRC1 represses $p16^{Ink4a}$ expression in young proliferating cells. In aged and stressed cells, H3K27 trimethylation marks are lost and PRC1 dissociates from the $p16^{Ink4a}$ locus, resulting in transcriptional activation by Ets1 and/or Ets2, and cell entry into senescence.

An important aim is to understand how the H3K27 trimethylation mark is lost in senescing cells. The various possibilities include the presence of (i) as yet unidentified histone demethylases and/or inhibitors of methyl transferases, and (ii) histone chaperones that recruit histone variants to chromatin. A recent study reported that the H3K27 specific demethylase, JMJD3, is induced by Ras-Raf signaling as well as environmental stresses. JMJD3 is recruited to the $p16^{Ink4a}$ locus and contributes to transcriptional activation of $p16^{Ink4a}$ (66, 67). We propose that senescence is regulated by the Jun dimerization protein 2 (JDP2), which has histone binding and chaperon activity. JDP2 is a member of the AP1 family of transcription factors that activates transcription of $p16^{Ink4a}$, as described below (68).

Regulation of Arf

The contribution of Arf to senescence is still controversial. In general, it seems that $p16^{INK4A}$ plays a central role in senescence and tumor suppression in human cells, whereas Arf has a relatively more prominent role in mouse cells. In humans, mutations are found specifically in $p16^{INK4A}$, rather than ARF. $p16^{INK4A}$ mutations are frequent in primary cancers, and occur during the establishment of immortal cell lines (16, 69). In addition, signaling by the Ras oncogene and telomere shortening induce p53- and ARF-independent growth arrest (70, 71). By contrast, in MEFs, Arf expression correlates with the onset of senescence, and cells lacking Arf do not senesce in culture (7, 72). Mice strains with targeted deletions of $p16^{Ink4a}$ or Arf are tumor prone, whereas animals lacking both $p16^{Ink4a}$ and Arf have a more severe phenotype (42, 72–75). Signaling from oncogenic Ras activates transcription of the DMP1 (cyclin D-binding Myb-like protein 1) gene via the MAP kinase pathway and AP1 transcription factors such as c-Jun and Jun-B. DMP1 binds to and activates transcription of the Arf promoter (76). This pathway is important, as oncogenic Ras fails to activate Arf in MEFs from *Dmp1*-null mice (77).

Curiously, factors that activate Arf expression can have different phenotypic effects: Ras induces senescence whereas Myc induces apoptosis. Overexpression of Myc in B-lymphocytes augments cell proliferation, which is counteracted by the ARF-p53-MDM2 pathway. Suppression of the ARF-p53-MDM2 pathway inhibits Myc-induced apoptosis and facilitates B cell lymphoma formation (78). However, another report indicates that induction of Arf requires high and continuous Myc activity, and physiological levels of Myc are not enough to stimulate the Arf promoter (79).

E2F transcription factors activate the Arf promoter. E2F1 stimulates the expression of ARF and activates the ARF-p53-$p21^{WAF1}$ axis, which blocks cell proliferation. This block is removed by loss of function of the ARF-MDM2-p53 pathway, resulting in E2F1-induced S-phase entry (80). E2F family members bind directly to the Arf promoter, as has been shown by the ChIP assay (81–83). Ectopic expression of E2F1, E2F2 and E2F3 activate the ARF promoter in human cells (82). By contrast, an isoform of E2F3, E2F3b, represses the Arf promoter in MEFs (83).

Among the AP1 family of transcription factors, the c-Jun and Fra1 (Fos-related antigen-1) heterodimer is an activator of Arf transcription in both human and mouse cells (summarized in Figure 4). Knockdown of Fra1 in human cells or deficiency of *c-Jun* in MEFs results in reduced expression

of Arf (84). In contrast, Jun-D seems to be a repressor of Arf, as MEFs lacking Jun-D express elevated levels of Arf, growth arrest in a p53-dependent manner and senesce prematurely (85). Arf is also transcriptionally repressed by EGR1 and POKEMON (ZBTB7A). Egr1-null MEFs express increased levels of Arf, but escape replicative senescence with decreased expression of p53, $p21^{Cip1/Waf1}$ and other p53 downstream proteins (86). POKEMON binds to the consensus sequence [5′-(G/A)(C/A)GACCCCCCCCC-3′] both on the mouse Arf and human ARF promoters and inhibits their expression. POKEMON-null MEFs senesce prematurely as a result of upregulation of Arf, as senescence can be overcome by mutation of Arf (87).

Senescence and aging in humans and mouse

Cellular senescence appears to be related to organismal ageing. Cellular senescence involves processes that include telomere shortening, the accumulation of DNA damage and activation of the Ink4a/Arf locus. Their contributions to senescence seem to be different in humans and mice. Cultured mouse fibroblasts undergo senescence, even though they have long telomeres and high telomerase activity. Senescence is abrogated by the loss of the Ink4a/Arf locus (73). In human cell cultures, ectopic expression of telomerase is sufficient to overcome senescence by maintaining the length of telomeres (88). In mice, telomere length maintenance is important, as telomerase-deficiency shortens their lifespan and leads to premature aging (89–91). In human kidney and skin (92, 93), as well as in the majority of mouse tissues (7, 8), age-dependent accumulation of INK4A has been observed. In the case of oncogene induced senescence, there is *in vivo* evidence that Arf is an important factor for activation of p53 tumor suppression (94, 95). However, another study showed that components of the DNA-damage signaling cascade, including ATM and CHK2, are critical for activation of p53 in response to oncogenic signals (96–98). The differences between humans and mice could be due to species specificity and/or experimental conditions. Cellular senescence appears to be related to organismal ageing because the same processes appear to be involved. Genetic variants of the INK4A/ARF locus are linked to age-associated disorders, such as general frailty, heart failure, and type 2 diabetes (99–104). Mutations of telomerase or proteins that affect telomerase activity are linked to human premature aging syndromes, including dyskeratosis congenital and aplastic anemia, (105). There are increases in DNA mutation, DNA oxidation, and chromosome losses during organismal aging. It seems reasonable to assume that all three factors, activation of Ink4a/Arf locus, telomere shortening, and the accumulation of DNA damage, could have cooperative effects on aging in physiological situations. Understanding the mechanisms of cellular senescence is currently of wide interest and it is important in identifying new components, such as JDP2.

Jun dimerization protein 2 (JDP2)

JDP2 was identified as a binding partner of c-Jun in a yeast two-hybrid screen (106). JDP2 forms a heterodimer with c-Jun and inhibits AP-1-mediated activation of transcription (106). Similarly, JDP2 was isolated in a yeast two-hybrid screen using ATF-2 as the 'bait' (107). JDP2 homodimers or heterodimers with ATF-2 and other members of the Jun family proteins bind to several DNA consensus elements, including AP-1 sites, cyclic AMP response elements (CREs), and TPA response elements (TREs) (106, 107). JDP2 stimulates the formation of DNA supercoils in assays using relaxed DNA and core histones, indicating that JDP2 has histone chaperon activity (108). Moreover, JDP2 is able to bind histones and inhibit their acetylation. Cellular functions of JDP2 involve transcriptional repression or activation, depending on the cell type (107–112). JDP2 represses UV-dependent apoptosis by inhibiting p53 gene transcription in NIH3T3 cells (110), whereas others have not observed any significant effect of JDP2 overexpression on p53 in MEFs (111). JDP2 inhibits transcription of cyclin D1 (113) in myoblast (C2C12) and rhabdomyosarcoma cells. In contrast, it activates the cyclin D1 promoter by collaborating with CHOP10 in NIH3T3 cells (114). In MEFs, JDP2 acts as a repressor of stress-induced transcription of ATF3 (115). JDP2 is implicated in various biological processes, such as proliferation, differentiation, and apoptosis (106, 109–111, 116, 117). For example, JDP2 overexpression inhibits retinoic acid-dependent differentiation of embryonic carcinoma F9 cells (112). Recently, we reported that MEFs from Jdp2 knockout mice ($Jdp2^{-/-}$ MEFs) are susceptible to adipocyte differentiation, indicating that JDP2 is involved in differentiation (118). Subsequently, we found that $Jdp2^{-/-}$ MEFs could proliferate for longer periods of time than wt MEFs (68).

JDP2 and replicative senescence

We analyzed aging-dependent proliferation of MEFs from $Jdp2^{-/-}$ transgenic mice in the presence of environmental (20%) or low (3%) oxygen. $Jdp2^{-/-}$ MEFs continued to divide even after 6 weeks, whereas wt MEFs almost stopped proliferating and entered senescence in environmental oxygen. By contrast, neither wt MEFs nor $Jdp2^{-/-}$ MEFs succumbed to replicative senescence at lower oxidative stress. These results demonstrate that MEFs lacking Jdp2 can escape from irreversible growth arrest caused by environmental oxygen (summarized in Figure 5). The expression of $p16^{Ink4a}$ and Arf were repressed in aged $Jdp2^{-/-}$ MEFs (40 days) compared to wt MEFs. In 3% oxygen, at the equivalent time (40 days), wt MEFs expressed lower levels of $p16^{Ink4a}$ and Arf compared with 20% oxygen, whereas $Jdp2^{-/-}$ MEFs maintained low-level expression of $p16^{Ink4a}$ and Arf. These observations indicate that the aging-associated expression of $p16^{Ink4a}$ and Arf are dependent on oxygen stress and that JDP2 controls the expression of both $p16^{Ink4a}$ and Arf. We did not find extreme downregulation of the upstream repressors of $p16^{Ink4a}$/Arf, Bmi1, and Ezh2, in the absence of JDP2, sug-

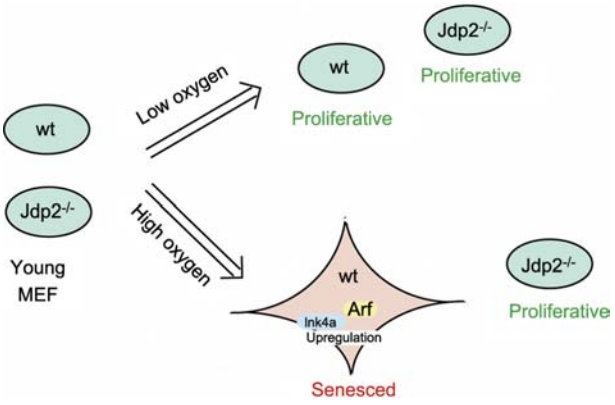

Figure 5 JDP2-deficient mouse embryo fibroblasts (MEFs) escape replicative senescence.
In high environmental oxygen (20%), wild type MEFs become senesced after several weeks in culture, whereas Jdp2$^{-/-}$ MEFs remain proliferative. p16^{Ink4a} and Arf expression are upregulated in wild type MEFs, but not in Jdp2$^{-/-}$ MEFs. In low oxygen (3%), neither wild type nor Jdp2$^{-/-}$ MEFs senesce.

gesting that JDP2 is not a regulator of their expression. Interestingly, JDP2 expression in wt MEFs increased in the presence of 20% oxygen, but not in 3% oxygen, suggesting that its expression depends on oxygenic stress, and that accumulated JDP2 could play a role in transcription activation of p16^{Ink4a}/Arf. Studies using chromatin immunoprecipitation demonstrated that methylation of H3K27 on the p16^{Ink4a}/Arf locus was higher in Jdp2$^{-/-}$ MEFs compared with wt MEFs, and binding of PRC1 and PRC2 to the p16^{Ink4a} and Arf promoter were more efficient in Jdp2$^{-/-}$ MEFs than in wt MEFs. These observations suggest that, in the absence of JDP2, H3K27 is methylated by PRC2 and the p16^{Ink4a}/Arf locus is silenced by PRC1, whereas increased expression of JDP2 helps to release PRC1 and PRC2 from the p16^{Ink4a}/Arf locus and thereby decreases H3K27 methylation.

Our data demonstrate that JDP2 is one of the important factors that regulate cellular senescence. Loss of JDP2 helps MEFs to escape from senescence and, inversely, overexpression of JDP2 induces cell cycle arrest. The absence of JDP2 decreases the expression of both p16^{Ink4a} and Arf, whose gene products inhibit cell cycle progression. Taken together, we propose a model that takes into account these results. The accumulation of oxidative stress and/or other environmental stimuli during aging upregulate JDP2 in primary untransformed cells. Increased JDP2 helps to remove PRC1 and PRC2, which are responsible for methylation of histone H3 on the p16^{Ink4a}/Arf locus, leading to increased p16^{Ink4a} and Arf expression and entry into the senescent stage (Figure 6). There is some evidence that Jdp2 acts as a tumor suppressor: Jdp2 inhibits Ras-dependent transformation of NIH3T3 (117), and Jdp2 gene disruptions are often found in lymphomas induced by insertional mutagenesis with Moloney murine leukemia virus in MYC/Runx2 transgenic mice (119). Here, we suggest that Jdp2 not only inhibits transformation of cells but also plays a role in induction of cell senescence. Both functions of JDP2 might be important for its role in inhibiting tumor formation. Moreover, our findings give new insights into understanding the molecular mechanisms by which senescence is induced in the context of epigenetic regulation of the p16^{Ink4a}/Arf locus.

Concluding remarks

Similar to differentiation and tumorgenesis, senescence is associated with dynamic changes of gene expression, which are regulated by chromatin remodeling. Here, we have seen that p16^{Ink4a} and Arf are upregulated in response to accumulating environmental stresses, oncogenic signaling and DNA damaging signal, and that they in turn induce irreversible cell cycle arrest by activating the pRb and p53 pathways, respectively. The expression of p16^{Ink4a} and Arf are epigenetically regulated by PRC1 and PRC2, which associate with these loci and methylate histone H3 in young cells and dissociate in aged and senesced cells. Several factors that upregulate or downregulate the p16^{Ink4a}/Arf locus have been reported. An important question, that needs to be addressed, is how do these different factors regulate senescence: do

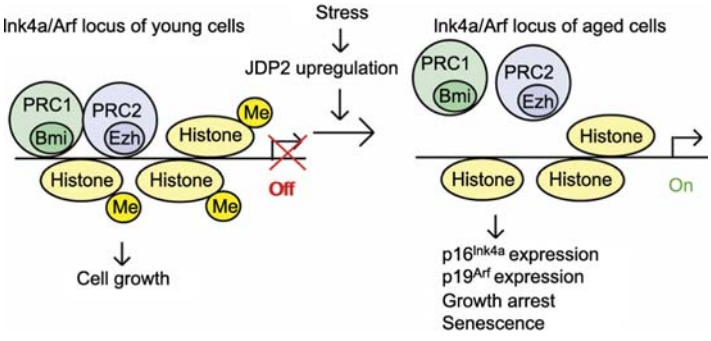

Figure 6 Model for epigenetic regulation of the p16^{Ink4a}/Arf locus by JDP2.
Young primary cells exposed to oxidative stress accumulate JDP2. In the presence of JDP2, PRC1 and PRC2 dissociate from the p16^{Ink4a}/Arf locus and histone H3 on the promoter is demethylated. Finally, p16^{Ink4a} and Arf are expressed and the aged cells senesce.

they affect only euchromatin or do they also regulate heterochromatin, do they modify chromatin structure by recruiting histone deacetylases, histone acetylases, histone methyltransferases, histone chaperones, and/or others. Addressing their precise functions in the context of epigenesis will help us to understand how senescence and, in a broader context, aging are regulated.

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