Review Article

Role of Jun dimerization protein 2 (JDP2) in cellular senescence

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Stable cell-cycle arrest is known as “cellular senescence” and is triggered by various stresses. Senescent cells show a series of alterations, including a flat and enlarged morphology, increased in non-specific acidic β -galactosidase activity, chromatin condensation, and changes in gene expression patterns. The onset and maintenance of senescence are regulated by two tumor suppressor proteins, p53 and Rb. The expression of p53 and Rb is regulated by two distinct proteins, Arf and p16^{Ink4a}, respectively, which are encoded by *cdkn2a*. Jun dimerization protein (JDP2) is a histone chaperone, which has activities in the inhibition of histone acetyltransferase and in nucleosome assembly/disassembly. Therefore, JDP2 plays key roles in cell growth, cell differentiation, and senescence by regulating the expression of genes. JDP2 inhibits the recruitment of polycomb repressive complexes (PRC-1 and PRC-2) to the promoter of the gene encoding p16^{Ink4a} and inhibits the methylation of lysine 27 of histone H3 (H3K27). In fact, the PRCs associate with the *p16^{Ink4a}/Arf* locus in young proliferating cells and dissociate from it in aged senescent cells. Therefore, it seems that chromatin-remodeling factors that regulate the association and dissociation of PRC and are controlled by JDP2 might be important players in the senescence program. The molecular mechanisms that underline the action of JDP2 in cellular aging and replicative senescence by mediating the dissociation of PRCs from the *p16^{Ink4a}/Arf* locus are discussed.

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Introduction

The structure of chromatin changes to allow greater accessibility to the DNA by transcription factors during the activation of a gene¹⁻³). It has been suggested that the structural change to a more accessible state not only involves the modification of histones and nucleosomal arrays, but also results from changes in nucleosome integrity caused by the displacement of the histones⁴). It has been demonstrated that histone chaperones play a critical role in these processes⁵⁻⁸). Therefore, it is tempting to speculate that histone chaperones are important in the compaction of the chromatin. Consequently, it is important to determine whether certain corepressors of transcription influence the deposition and assembly of nucleosomes via the regulation of histone chaperone activity.

Primary cultures of untransformed cells stop growing after several weeks and undergo senescence, a phenomenon that is related to so-called “cellular aging”. Senescence protects normal cells from abnormal growth signals and oncogenic transformation, and impairs their reprogramming to pluripotent stem cells⁹) by interrupting the cell cycle. Senescence is induced not only by cellular aging but also by the forced activation of the mitogen-activated protein (MAP) kinase pathway and by genotoxic stressors, such as hydrogen peroxide and certain DNA-damaging compounds. Senescence increases the activities of two well-known tumor suppressors, Rb and p53. The expression of Rb and p53 is regulated by two distinct proteins, p16^{Ink4a} and Arf, respectively, which are encoded by the *cdkn2a* locus (Fig.1, ref. 10). The expression of p16^{Ink4a} increases dramatically with increasing numbers of cell divisions in primary fibroblasts in culture, and in rodent and human models *in vivo*. In this review, we summarize the roles of p16^{Ink4a} and Arf in the cell cycle, and describe a novel mechanism for the regulation of their expression.

p16^{Ink4a} and the Rb pathway

E2F is controlled by the Rb family of proteins, pRb, p107, and p130^{11,12}). Early in G1, unphosphorylated Rb proteins bind to the E2F family of proteins and inactivate their function^{13,14}). During G1, the Rb proteins are inactivated by phosphorylation by Cdk4/6-cyclinD complexes, thereby allowing the transcription of E2F-dependent genes, including cyclin E. Upregulated cyclin E forms a complex with cdk2, that mediates the hyperphosphorylation of the Rb proteins, an essential requirement for the G1/S transition. p16^{Ink4a} is an allosteric inhibitor of cdk4/6. Binding to p16^{Ink4a} changes the conformations of cdk4/6, which prevents its interaction with cyclin D^{15,16}). Therefore, p16^{Ink4a} acts as an inhibitor of the cell cycle at G1 by modulating the Rb

pathway. p16^{Ink4a} is often lost in a variety of human malignancies, including glioblastoma, melanoma, and pancreatic adenocarcinoma¹⁷). In contrast, the upregulation of p16^{Ink4a} induces the cell-cycle arrest and senescence^{16,17}).

Arf and the p53 pathway

p53 is known to mediate cell-cycle arrest in G1 and G2, and apoptosis (Fig.2). A number of downstream targets of p53 are involved in these processes, including p21^{Cip/waf1} in G1 arrest¹⁸), 14-3-3 sigma and GADD45 in G2 arrest^{19,20}), and p21, Bax, PUMA, Fas/Apo1, and Killer/DR5 in apoptosis²¹⁻²⁵). p53 is regulated at the levels of protein stability and activity, and to some extent at transcription and translation^{26,27}). In unstressed cells, p53 protein levels are very low because its degradation is mediated by the E3 ubiquitin ligase activity of MDM2, which targets p53 for ubiquitin-dependent proteolysis²⁸). MDM2 is a transcriptional target of p53, so p53 directly activates the expression of its own negative regulator, producing a potent negative feedback regulatory loop²⁹). There are several stress-responsive kinases, which, by phosphorylating p53, inhibit its degradation by MDM2 and increase its transcriptional activity³⁰⁻³²). DNA damage rapidly activates the ataxia telangiectasia mutated (Atm) and ataxia telangiectasia related (Atr) proteins, which phosphorylate the checkpoint kinases Chk1 and Chk2, which in turn propagate the signal to downstream effectors such as p53^{33,34}). Both Chk1 and Chk2 phosphorylate p53 at Ser 20, which prevents the efficient recruitment of MDM2. Thus, p53 is stabilized and its expression level is increased in response to stress signaling. Arf is predominantly localized in the nucleoli and is 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 the nucleocytoplasmic shuttling of the MDM2-p53 complex. Therefore, the consequences of the activation of Arf are the stabilization and activation of p53^{35,36}).

Transcriptional regulation of p16^{Ink4a}

The transcriptional regulation of the *p16^{Ink4a}* gene is an important event in cellular senescence (Fig.3). The expression of p16^{Ink4a} is increased during replicative senescence and in the premature senescence induced by oncogenic activation. Its expression is regulated by transcriptional activators such as Ets1/2 and the basic helix-loop-helix (b-HLH) protein E47, as well as by transcriptional inhibitors including the Id-1 HLH protein³⁷⁻⁴⁰). The *p16^{Ink4a}* locus is also epigenetically repressed by the polycomb repressive complexes-1 and -2 (PRC1 and PRC2, res-

Fig.1 Schematic representation of the *p16^{Ink4a}/Arf* locus

Signaling from stress, oncogenic activation, and DNA damage activates the transcription from exon 1 β and exon 1 α of Arf and p16^{Ink4a}, respectively. As the 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 encode different amino acid sequences. p16^{Ink4a} and Arf activate the Rb and p53 axes, respectively.

Fig.2 Schematic representation of the Arf-p53 pathway

In unstressed cells, p53 protein levels are very low because of its ubiquitin-dependent degradation, mediated by MDM2. DNA damage activates the Atm and Atr kinases, which phosphorylate Chk1 and Chk2. Both of Chk1 and Chk2 then phosphorylate p53 at Ser20, which inhibits MDM2 binding and thereby stabilizes the p53 protein. In contrast, Arf stabilizes p53 by binding to MDM2 and inhibiting its activity. The upregulation of p53 leads to cell-cycle arrest and apoptosis.

Fig.3 Regulation of the transcription of the *p16^{Ink4a}/Arf* locus

Blue circles and orange squares indicate the activators and repressors, respectively, of p16^{Ink4a} and/or Arf transcription.

pectively), which methylate lysine 27 of histone H3 (H3K27)⁴¹. In transformed cells, in which the cell cycle is not arrested by a senescence program, the CpG islands in the *p16^{Ink4a}* promoter and exon 1 are methylated and the *p16^{Ink4a}* gene is silenced^{42,43}.

Understanding the role of all the factors that regulate the expression of *p16^{Ink4a}* is important in clarifying the molecular mechanism of cellular aging. We describe here some of these factors, including transcriptional activators and inhibitors, and epigenetic regulators.

Transcriptional activators:

Both Ets1 and Ets2 activate *p16^{Ink4a}* expression through the activation of the Ras-MEK-MAP kinase pathway by directly binding to the ETS consensus sites in its promoter⁴⁰. In human fibroblasts, the hyper-activation of Ets2 by the overexpression of the Ras oncogene induces G1 arrest, premature senescence, and the increased expression of *p16^{Ink4a}*. Ets2 seems to be the main regulator of *p16^{Ink4a}* expression during oncogenic premature senescence, whereas Ets1 plays a role in replicative senescence^{44,45}. 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) in the *p16^{Ink4a}* promoter³⁹. The overexpression of E47 inhibits the proliferation of some tumor cell lines by inducing the expression of *p16^{Ink4a}*. The inhibition of E47 by RNA interference significantly reduced the expression of *p16^{Ink4a}* and delayed the onset of senescence³⁷. Similarly, the hetero-dimerization of E47 with ectopically expressed, Tal1 inhibited the expression of *p16^{Ink4a}*⁴⁶.

Transcriptional inhibitors:

The expression of Id-1 correlates negatively with *p16^{Ink4a}* expression during the process of senescence^{37,40}. The expression of *p16^{Ink4a}* in mouse embryonic fibroblasts (MEFs) was higher in those from Id-1-deficient mice than in wild-type MEFs³⁸. The overexpression of Id-1 delayed replicative senescence by inhibiting *p16^{Ink4a}* in human keratinocytes and endothelial cells^{47,48}. Id-1 does not have a basic DNA-binding domain, unlike the b-HLH protein E47. Instead, Id-1 inhibits the transcription of *p16^{Ink4a}* by heterodimerization with Ets2⁴⁰. Id-1 also hetero-dimerizes with E47, and inhibits its transcriptional activity⁴⁹.

Epigenetic regulators:

The *p16^{Ink4a}* locus is also regulated epigenetically. The locus was transcriptionally silenced by the trimethylation of lysine 27 of histone H3 (H3K27) in young proliferating primary cells. In contrast, the expression of *p16^{Ink4a}* increases in aged and senescent cells with the loss of H3K27 trimethylation⁵⁰. The methylation of H3K27 and the silencing of the *p16^{Ink4a}* locus are mediated by PRC1 and PRC2. PRC1 contains a number of subunits,

including polycomb (CBX2,4,6,7, or 8 in humans), polyhomeotic (PH1 or PH2 in humans), Bmi1, Ring1B, and other subunits⁵¹, whereas PRC2 is composed of Ezh2, Suz12, and Eed subunits^{41,52}. In PRC2, Ezh2 is the catalytic subunit, which methylates H3K27⁴¹, 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*^{53,54}. In contrast, Eed is required for global H3K27 methylation, including its monomethylation⁵⁵. In PRC1, the CBX subunit recognizes and binds to trimethylated H3K27^{56,57}. Ring1B has E3 ligase activity for the ubiquitylation of histone H2A, whereas Bmi1 acts as a cofactor^{58,59}. The ubiquitylation of H2A by PRC1 prevents transcript elongation by RNA polymerase II⁶⁰.

PRC1 has been shown, by electron microscopy, to contribute to the compaction of the chromatin⁶¹. Therefore, a possible molecular mechanism of PRC-mediated gene silencing might involve the trimethylation of histone H3K27 by Ezh2 and the other subunits of PRC2. This then acts as a binding site for PRC1, which ubiquitylates H2A and compacts the chromatin, leading to the inhibition of transcript elongation by RNA polymerase.

PRCs might also inhibit earlier steps in transcription, because PRC1 interacts with components of the basal transcription machinery, the TATA-box-binding protein-associated factors (TAFs)^{62,63}. This inhibition does not appear to involve blocking the access of RNA polymerase to the promoter, because the PRCs and transcription factors bind to the target genes at the same time^{60,63-66}. In summary, *p16^{Ink4a}* transcription is inhibited by PRC2, which methylates histone H3K27, which in turn recruits PRC1. PRC1 represses *p16^{Ink4a}* expression in young proliferating cells. In aged and stressed cells, the H3K27 trimethylation marks of PRC1 are lost and PRC1 dissociates from the *p16^{Ink4a}* locus, resulting in the transcriptional activation of *p16^{Ink4a}* by Ets1 and/or Ets2, and the entry of the cell into senescence.

An important aim is to understand how the H3K27 trimethylation signal is lost in senescing cells. The possible explanations are as follows: (1) the action of as yet unidentified histone demethylases and/or inhibitors of the methyl transferases involved in H3K27 trimethylation; (2) histone chaperones recruit histone variants to the chromatin to alter gene expression. A recent report has shown that the H3K27-specific demethylase JMJD3 is induced by Ras-Raf signaling, as well as by the environmental stresses. Jumonjic domain-containing protein 3 (JMJD3) is recruited to the *p16^{Ink4a}* locus and contributes to the transcriptional activation of *p16^{Ink4a}*^{67,68}. We propose that senescence is regulated by Jun dimerization protein 2 (JDP2), which has histone-binding and chaperone activities. JDP2 is a member of the

AP1 family of transcription factors, and activates the transcription of $p16^{\text{Ink4a}}$, as described below⁶⁹.

Regulation of Arf

The contribution of Arf to senescence is still controversial. In general, it seems that $p16^{\text{Ink4a}}$ plays a central role in senescence and tumor suppression in human cells, whereas Arf has a relatively more prominent role in mouse cells. In human cells, mutations are specifically found in $p16^{\text{Ink4a}}$, rather than in ARF. Mutations of $p16^{\text{Ink4a}}$ are frequently observed in primary cancers, and occur during the establishment of immortal cell lines^{17,70}. Signaling by the Ras oncogene and telomere shortening also induce p53- and ARF-independent growth arrest^{71,72}. In contrast, in MEFs, the expression of Arf correlates with the onset of senescence and cells lacking Arf do not senesce in culture^{73,74}. Mice strains with targeted deletions in $p16^{\text{Ink4a}}$ or Arf are tumor prone, whereas animals lacking both $p16^{\text{Ink4a}}$ and Arf have a more severe phenotype^{46,74-77}. Signaling from oncogenic Ras activates the transcription of the cyclin D-binding Myb-like protein 1 gene (*Dmp1*) via the MAP kinase pathway and AP1 transcription factors, such as c-Jun and Jun-B. DMP1 binds to and activates the transcription of the Arf promoter⁷⁸. This pathway is important, because oncogenic Ras fails to activate Arf in MEFs from *Dmp1*-null mice⁷⁹.

Curiously, factors that activate Arf expression can have different phenotypic effects: Ras induces senescence, whereas Myc induces apoptosis. The overexpression of Myc in B lymphocytes augments cell proliferation, which is counteracted by the ARF-p53-MDM2 pathway. The suppression of the ARF-p53-MDM2 pathway inhibits Myc-induced apoptosis and facilitates the formation of B-cell lymphoma⁸⁰. However, another study has been shown that the induction of Arf requires high and continuous Myc activity and that physiological levels of Myc are insufficient to stimulate the Arf promoter⁸¹.

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 the loss of function of the ARF-MDM2-p53 pathway, resulting in E2F1-induced S-phase entry⁸².

E2F family members bind directly to the Arf promoter, as has been shown with a chromatin immunoprecipitation (ChIP) assay⁸³⁻⁸⁵. The ectopic expression of E2F1, E2F2, and E2F3 activated the ARF promoter in human cells⁸³. In contrast, an isoform of E2F3, E2F3b, repressed the Arf promoter in MEFs⁸⁴. Among

the AP1 family of transcription factors, the c-Jun and Fos-related antigen-1 (Fra1) heterodimer is an activator of Arf transcription in both human and mouse cells (Fig.3). The knock-down of FRA1 in human cells or a deficiency of c-Jun in MEFs results in the reduced expression of Arf⁸⁶. In contrast, JunD seems to be a repressor of Arf, because MEFs lacking JunD express elevated levels of Arf, and display p53-dependent growth arrest and premature senescence⁸⁷. Arf is also repressed transcriptionally by EGR1 and ZBTB7A (pokeron). Egr1-null MEFs express increased levels of Arf, but escape replicative senescence with reduced expression of p53, p21^{Cip1/Waf1} and other p53 downstream proteins⁸⁸. Zbtb7a-null MEFs senesce prematurely because of the up-regulation of Arf expression since senescence can be overcome by the mutation of Arf⁸⁹.

Senescence and aging in human and mouse

Cellular senescence appears to be related to organismal aging. Cellular senescence involves processes that include telomere shortening, the accumulation of DNA damage, and the activation of the $p16^{\text{Ink4a}}$ /Arf locus. The contributions of these factors to senescence seem to differ in humans and mice. Cultured mouse fibroblasts undergo senescence even when they have long telomeres and high telomerase activity. Senescence is abrogated by the loss of the $p16^{\text{Ink4a}}$ /Arf locus⁷⁵. In human cell cultures, the ectopic expression of telomerase is sufficient to overcome senescence by maintaining the length of the telomeres⁹⁰. In mice, the maintenance of telomere length is important because telomerase deficiency shortens their lifespan and leads to premature aging⁹¹⁻⁹³. The age-dependent accumulation of INK4A has been observed in the human kidney and skin^{94,95}, as well as in the majority of mouse tissues^{93,96}. In oncogene-induced senescence, there is *in vivo* evidence that Arf is the important factor in the activation of p53 tumor suppression^{96,97}. However, another study has shown that components of the DNA-damage signaling cascade, including Atm and Chk2, are critical for the activation of p53 in response to oncogenic signals⁹⁸⁻¹⁰⁰. These differences between humans and mice could be attributable to species specificity and/or experimental conditions. Cellular senescence appears to be related to organismal aging because the same processes appear to be involved. Genetic variants of the $p16^{\text{Ink4a}}$ /ARF locus are linked to age-associated disorders, such as general frailty, heart failure, and type 2 diabetes¹⁰¹⁻¹⁰⁶. Mutations in telomerase or in proteins that affect telomerase activity are linked to premature human aging syndromes, including congenital dyskeratosis and aplastic anemia¹⁰⁷. There are increases in DNA

mutations, DNA oxidation, and chromosome loss during organismal aging. It seems reasonable to assume that all three factors, the activation of the *p16^{Ink4a}/Arf* locus, telomere shortening, and the accumulation of DNA damage, have cooperative effects on aging in physiological situations. Understanding the mechanisms of cellular senescence is currently of wide interest and it is important that we identify new components of this process, such as JDP2.

JDP2 regulates the AP-1 mediated activation of transcription

JDP2 has been identified as a binding partner of c-Jun in yeast two-hybrid screening experiments, based on the recruitment of the SOS system¹⁰⁸. JDP2 forms heterodimers with c-Jun and represses the AP-1-mediated activation of transcription¹⁰⁸. Similarly, Jdp2 was isolated by yeast two-hybrid screening with ATF-2 as the “bait”¹⁰⁹. JDP2 was also shown to associate with both the CAAT/enhancer-binding protein- γ ¹¹⁰ and the progesterone receptor¹¹¹. JDP2 is constitutively expressed in many cell lines and represses the transcriptional activity of AP-1¹¹². Moreover, JDP2 is rapidly phosphorylated at threonine residue 148 when cells are exposed to UV irradiation, oxidative stress, or inhibitor-induced depressed levels of translation by JNK¹¹³. Although a novel JNK-docking domain is necessary for the p38-mediated phosphorylation of JDP2 at threonine residue 148, this domain is not sufficient for this process¹¹⁴. JDP2 binds to both cAMP- and TRE-response elements on DNA as a homodimer and as a heterodimer with ATF-2 and members of the Jun family, respectively^{108,109}. JDP2 inhibits UV-induced apoptosis by suppressing the transcription of the p53 gene¹¹⁴. Given the roles of AP-1 in cellular transformation and the reported repression of Jun- and ATF-2-mediated transcription by JDP2, we have demonstrated that JDP2 inhibits the oncogenic transformation of chicken embryonic fibroblasts¹¹⁵. JDP2 also modulates the expression of cyclin D1 and p21, which have opposing effects on cell-cycle progression. JDP2 interferes with the progression of the cell cycle by reducing the levels of cyclin D1 and at the same time, increases the expression of p21^{116,117}. The forced expression of JDP2 promotes the myogenic differentiation of C2C12 cells, which is accompanied by the formation of C2 myotubes and the strong expression of major myogenic markers. Moreover, the ectopic expression of JDP2 in rhabdomyosarcoma cells induces incomplete myogenesis and the incomplete formation of myotubes. These cells become committed to differentiation via the p38-MAPK pathway¹¹⁸. A similar enhancement of cell differentiation was reported during the induction of osteoclast formation

by the receptor activator of the NF κ B-ligand (RANKL)¹¹⁹. Unlike other members of the AP-1 family, the levels of JDP2 remain constant in response to a large variety of stimuli, such as UV, irradiation, and retinoic acid (RA), which affect the levels of other factors involved in cell-cycle control. The induction of JDP2 expression was only observed during the differentiation of F9 cells to muscle cells and osteoclasts. Therefore, JDP2 may provide a threshold for exit from the cell cycle and a commitment to differentiation. Further studies of the regulation of the cell cycle and the differentiation of cells induced by JDP2 should be very instructive. It is also interesting that JDP2 is one of the candidate oncoproteins that collaborate in the oncogenesis associated with the loss of p27 as the result of insertional mutations¹²⁰. Recent studies of tumor cells have demonstrated that JDP2 is a tumor suppressor¹²¹. We have also found that JDP2 is a repressor of the activation of transcription via AP-1, and a negative regulator of the RA-induced differentiation of mouse embryonic F9 cells^{122,123}.

JDP2 inhibits histone acetyltransferase (HAT) activity

We have reported previously that Jdp2 represses the transactivation mediated by p300¹²³. Both p300 and ATF-2 have HAT activity^{124,125}. It was recently shown that p300 acetylates ATF-2 protein *in vitro* at lysine residues 374 and 357 and that ATF-2 is essential for the acetylation of histones H4 and H2B *in vivo*^{126,127}. We found that acetylation by p300 is inhibited in a dose-dependent manner by JDP2, when added exogenously. We also found that JDP2 was not acetylated by p300 under our experimental conditions. The inhibitory effect of JDP2 on histone acetylation is induced by p300, CBP, PCAF, and Gcn5. The overexpression of JDP2 apparently represses the RA-induced acetylation of lysines 8 and 16 of histones H4 and H3.

JDP2 has intrinsic nucleosome-assembly activity *in vitro*

The TAF-I β protein, which is a component of the INHAT complex identified by Seo et al.^{128,129}, is a histone chaperone that binds directly to core histones and facilitates the assembly of nucleosomes *in vitro*^{126,127}. JDP2 interacted directly with all the core histones tested and inhibited the p300-mediated acetylation of those histones. To our surprise, JDP2 also introduced supercoils into circular DNA in the presence of core histones, to levels similar to those observed for yCia1p and CIA1. Therefore, JDP2 appears to have significant histone chaperone activity *in vitro*¹²³. We have also shown that the HAT-inhibitory activity of

Fig.4 JDP2-deficient mouse embryonic fibroblasts (*Jdp2*^{-/-} MEFs) escape replicative senescence

In high environmental oxygen (20%), wild-type MEFs become senescent after several weeks in culture, whereas *Jdp2*^{-/-} MEFs remain proliferative. The expression of p16^{Ink4a} and Arf is upregulated in wild-type MEFs, but not in *Jdp2*^{-/-} MEFs. In low oxygen (3%), neither wild-type nor *Jdp2*^{-/-} MEFs become senescent.

Fig.5 Model for the 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.

Fig.6 Model for the epigenetic regulation by JDP2

During the exposure of cells with oxidative stress, retinoic acid (RA), RANKL, TPA and adipocyte inducing hormones, the histone H3, H4K8 and H4K16 as well as H3K27 were masked by JDP2 proteins and prevent the attack of histone modification enzymes like HAT (p300/CBP, pCAF, GCN5, MOF etc) and HMT (Ezh2 etc) in the cellular senescence⁶⁹ and cell differentiation¹³¹. This is a novel mechanism of JDP2 to inhibit the histone modification. The gene locus is either the *p16*^{Ink4a}/*Arf* locus or C/EBP δ locus.

JDP2 is involved, to some extent, in the repression of transcription by JDP2, whereas the maximal capacity of JDP2 to suppress the RA-mediated activation of the c-Jun promoter requires the recruitment of histone deacetylases (HDACs).

JDP2 and replicative senescence

We analyzed the aging-dependent proliferation of MEFs from Jdp2^{-/-} transgenic mice in the presence of environmental (20%) or low (3%) oxygen. The Jdp2^{-/-} MEFs continued to divide, even after six weeks, whereas the wild type MEFs almost stopped proliferating and entered senescence under environmental oxygen. Conversely, neither wild-type MEFs nor Jdp2^{-/-} MEFs succumbed to replicative senescence at lower oxidative stress. These results demonstrate that MEFs lacking Jdp2 can escape from the irreversible growth arrest caused by environmental oxygen (Fig.4). The expression of p16^{Ink4a} and Arf were repressed in aged Jdp2^{-/-} MEFs (40 days) compared with their levels in wild-type MEFs. In 3% oxygen, at the equivalent time (40 days), wild-type MEFs expressed lower levels of p16^{Ink4a} and Arf compared with those in 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 is dependent on oxygen stress and that JDP2 controls the expression of both p16^{Ink4a} and Arf. We found no dramatic down-regulation of the upstream repressors of p16^{Ink4a}/Arf, Bmi1 and Ezh2, in the absence of JDP2, suggesting that JDP2 does not regulate their expression. Interestingly, JDP2 expression in wild-type MEFs increased in the presence of 20% oxygen, but not in the presence of 3% oxygen, suggesting that its expression depends on oxygenic stress, and that accumulated JDP2 may play a role in the transcriptional activation of p16^{Ink4a}/Arf. Studies based on ChIP have demonstrated that the methylation of H3K27 at the p16^{Ink4a}/Arf locus was higher in Jdp2^{-/-} MEFs than in wild-type MEFs, and that the binding of PRC1 and PRC2 to the p16^{Ink4a} and Arf promoters was more efficient in Jdp2^{-/-} MEFs than in wild-type 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 the increased expression of JDP2 helps to release PRC1 and PRC2 from the p16^{Ink4a}/Arf locus, thereby reducing H3K27 methylation. Our data demonstrate that JDP2 is an important factor regulating cellular senescence. The loss of JDP2 allows MEFs to escape senescence and conversely, the overexpression of JDP2 induces cell-cycle arrest. The absence of JDP2 reduces the expression of both p16^{Ink4a} and Arf, which inhibit cell-cycle progression. We propose a model that takes into account these results. The accumulation of oxidative stress

and/or other environmental stimuli during aging upregulates JDP2 expression in primary untransformed cells. Increased JDP2 helps to remove PRC1 and PRC2, which are responsible for the methylation of histone H3, from the p16^{Ink4a}/Arf locus, leading to increased p16^{Ink4a} and Arf expression and entry into the senescence (Fig.5). There is some evidence that Jdp2 acts as a tumor suppressor: Jdp2 inhibits the Ras-dependent transformation of NIH3T3 cells¹³⁰ and Jdp2 gene disruptions are often found in the lymphomas induced by insertional mutagenesis caused by the Moloney murine leukemia virus in MYC/Runx2 transgenic mice¹³¹. Here, we suggest that Jdp2 not only inhibits the transformation of cells but also plays a role in the induction of cell senescence. Both functions of JDP2 might be important for its role in inhibiting tumor formation. Our findings also provide new insights into the molecular mechanisms by which senescence is induced in the context of the epigenetic regulation of the p16^{Ink4a}/Arf locus.

Concluding remarks

Like differentiation and tumorigenesis, senescence is associated with dynamic changes in gene expression, which are regulated by chromatin remodeling. Here, we have shown that the expression of p16^{Ink4a} and Arf is up-regulated in response to accumulating environmental stresses, oncogenic signaling, and DNA-damaging signals, and that they in turn induce irreversible cell-cycle arrest by activating the Rb and p53 pathways, respectively. The expression of p16^{Ink4a} and Arf is epigenetically regulated by PRC1 and PRC2, which associate with these locus, methylate histone H3 in young cells, and dissociate in aged and senescent cells. Several factors that upregulate or downregulate the expression of the p16^{Ink4a}/Arf locus, have been reported. An important question, that must be addressed, is how these different factors regulate senescence. Do they affect only euchromatin or do they also regulate heterochromatin? Do they modify the chromatin structure by recruiting HDACs, HATs, histone methyltransferases, histone chaperones, and/or other molecules (see Fig.6)?

Addressing these precise functions in the context of epigenesis should help us to understand how senescence and, in a broader context, aging are regulated.

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