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## *Review*

# Tumor suppression by p53: making cells senescent

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**Summary.** Cellular senescence is a permanent cell cycle arrest and a potent tumor suppression mechanism. The p53 tumor suppressor is a sequence-specific transcription factor and acts as a central hub sensing various stress signals and activating an array of target genes to induce cell cycle arrest, apoptosis, and senescence. Recent reports showed that restoration of p53 induces premature senescence and tumor regression in mice with hepatocarcinomas or sarcomas. Thus, p53-mediated senescence is capable of eliminating cancer cells *in vivo*. p63 and p73, two homologues of p53, have similar function in cell cycle arrest and apoptosis. However, the role of p63 and p73 in cellular senescence is elusive. In this review, we will discuss how p53 regulates senescence and future studies about p53 family members in senescence.

**Key words:** p53, p21, Cellular senescence, Tumor suppression

### Introduction

Cellular senescence is an irreversible cell cycle arrest in response to stress signals, including telomere dysfunction (Harley et al., 1990), oncogene activation (Serrano et al., 1997), and DNA damage (te Poele et al., 2002). Cellular senescence is a safeguard mechanism that may prevent aged cells or cells bearing mutations from expansion by inducing a permanent cell cycle arrest (Sherwood et al., 1988). Indeed, cellular senescence was shown to be activated at the initial steps of premalignant transformation (Braig et al., 2005; Campisi, 2005; Collado et al., 2005; Dimri, 2005). Conditional activation of K-rasV12 in mice leads to the development of both adenomas (premalignant) and adeno-carcinomas (malignant). Interestingly, senescent cells are only present in premalignant lesions, but not in malignant tumors. Thus, it was hypothesized that oncogene-induced senescence may help to restrict tumor progression and loss of senescence in malignant tumor cells may be partly due to loss of the p53 tumor suppressor. However, whether induction of senescence is sufficient to repress tumor *in vivo* is controversial. Recent reports showed that conditional restoration of p53 in mice with hepatocarcinomas, sarcoma, or lymphoma is able to promote tumor regression (Martins et al., 2006; Xue et al., 2007; Ventura et al., 2007). Although regression of lymphoma was associated with apoptosis, regression of hepatocarcinomas and sarcomas was associated with senescence. Thus, in addition to p53-mediated apoptosis, p53-mediated cellular senescence is a potent pathway to eliminate cancer cells *in vivo*.

The p53 tumor suppressor is the most commonly mutated gene in human cancers (Olivier et al., 2002). Loss of p53 in both mutant mice and Li-Fraumeni syndrome patients correlates with early onset of multiple tumors (Li and Fraumeni, 1969; Birch et al., 2001; Gasco et al., 2003). p53 functions as a sequence-specific transcription factor at the crossroads of cellular stress response pathways (el-Deiry et al., 1992). In response to various stress signals such as DNA damage, hypoxia, or activated oncogenes, the p53 protein is activated in a specific manner by posttranslational modifications and leads to DNA repair, cell cycle arrest, apoptosis, or cellular senescence (Ko and Prives, 1996; Prives and Hall, 1999; Hofseth et al., 2004). It has been shown that the level and/or activity of p53 were increased in senescent cells (Serrano et al., 1997) and overexpression of p53 was sufficient to induce premature senescence in p53-null cells (Sugrue et al., 1997; Wang et al., 1998). In contrast, inactivation of p53 using viral proteins (SV40 large T antigen or HPV-16 E6 protein) or gene targeting results in an extension of cell lifespan (Itahana et al., 2001). Similarly, loss of p53 heterozygosity or

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expression of dominant negative p53 also extends cell lifespan (Rovinski and Benchimol, 1988; Bond et al., 1994). Thus, p53 is a pivotal mediator of cellular senescence. However, although loss of p53 alone is sufficient for mouse cells to bypass senescence, additional inhibition of p16 is required for human cells to bypass senescence (Smogorzewska and de Lange, 2002). In addition, lack of p53 significantly diminishes but does not abrogate DNA damage-induced premature senescence (Schmitt, 2007). These suggest that senescence can occur through a p53-independent mechanism.

**What is cellular senescence?**

In the early 1960s, Hayflick and Moorhead observed that normal human diploid cells have a limited number of passages *in vitro* after which they enter a metabolic active but non-proliferative phase defined as "replicative senescence" or "Haylick's limit" (Hayflick and Moorhead, 1961; Hayflick, 1965). Senescent cells are characterized by enlarged cell size, flattened morphology, inability to synthesize DNA, and expression of the biomarker, senescence-associated ß-galactosidase (SA-ß-gal) (Dimri et al., 1995). Senescent cells express a high level of lysosomal ß-gal which can be detected at suboptimal pH 6.0, whereas overexpression of lysosomal ß-gal itself is not able to initiate senescence (Le Gall et al., 1979; Kurz et al., 2000; Lee et al., 2006). Thus, the SA-ß-gal activity is the outcome of the highly elevated expression of lysosomal ß-gal. Years later, progressive telomere attrition during cell divisions was identified as the underlying mechanism for replicative senescence (Lundblad and Szostak, 1989; Harley et al., 1990). The telomeres are a sequence of repetitive bases, TTAGGG in humans, at the ends of linear chromosomes followed by a single-strand overhang (Blackburn, 1984). Binding of telomere-specific proteins to the single and double strand regions forms a nucleoprotein complex which caps the chromosome ends and protects chromosome termini from degradation, recombination, and end-fusion. Uncapping of the chromosome ends due to progressive telomere loss during each population doubling in somatic cells leads to cellular senescence by activating the DNA damage pathway (d'Adda di Fagagna et al., 2003). In addition, disruption of normal telomere status also leads to rapid induction of growth arrest (Karlseder et al., 2002). For example, TRF2, a key component of shelterin, the telomere-specific protein complex, binds to the duplex TTAGGG repeat array of mammalian chromosome ends (van Steensel et al., 1998). Loss of TRF2 or expression of mutant forms of TRF2 induces end-to-end chromosome fusions leading to a cell cycle arrest with the characteristics of cellular senescence (Karlseder et al., 2002)

In addition to stress signals originated from telomere dysfunction, aberrant oncogenic activities are also able to induce cellular senescence. Among oncogenes, Ras is the most frequently mutated gene in human cancers (Bos, 1989). The Ras oncogene family encodes small GTP-binding proteins that transduce mitogenic signals from G-protein coupled receptors in response to extracellular stimuli (Karnoub and Weinberg, 2008). Combined activation of Ras with a cooperating oncogene, such as Myc, E1A, and SV40 large T antigen (Land et al., 1983, Ruley, 1983), or with loss of a tumor suppressor, such as p53 and p16, leads to transformation of primary cells and tumor formation in animals (Kemp et al., 1993, Chintala et al., 1997). Interestingly, prolongd expression of an active Ras protein, H-RasV12, provokes an acute permanent cell cycle arrest, termed as "premature senescence", which is indistinguishable from replicative senescence (Serrano et al., 1997). There are several underlying mechanisms for oncogene-induced senescence (OIS). First, Ras-induced senescence partly depends on the activation of the Raf-MEK-ERK MAPK pathway. Since Ras induces cellular transformation via the same pathway, it is likely that the Ras-induced senescence is a fail-safe mechanism to limit the transformation potential of excessive Ras mitogenic signaling. Besides the Raf-MEK-ERK cascade, oncogenic Ras also activates the JNK-p38 MAPK pathway (Minden et al., 1994; Whitmarsh et al., 1997). Consistent with this, many but not all Ras effectors, including activated RAF, MEK, p38, and BRAF, were shown to induce senescence (Wang et al., 2002; Sun et al., 2007). Second, Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species (ROS) (Lee et al., 1999). It has been shown that expression of RasV12 results in an increase of intracellular and in particular, mitochondrial reactive oxygen species. In contrast, the extent of RasV12-induced senescence is shown to be decreased by repressing the production of ROS by culturing cells in a low oxygen environment (1% oxygen) compared to normoxia (20% oxygen). Third, OIS is an outcome of a DNA damage response (Di Micco et al., 2006). Cells undergoing senescence induced by oncogenic Ras contain clearly detectable DNA damage foci, whereas inactivation of DNA damage response pathway abrogates OIS and promotes cellular transformation. In addition, this robust DNA damage response in cells undergoing OIS is demonstrated to be triggered by oncogene-induced DNA hyper-replication.

Given the importance of DNA damage response in cell cycle arrest and senescence, it is not surprising that DNA damage agents, such as radiation, chemotherapeutic drugs, and oxidative stresses, induce premature senescence. Typically, stress-induced senescence (SIS) and OIS do not lead to significant telomere shortening (Wei and Sedivy, 1999, Gorbunova et al., 2002). For example, hydrogen peroxide treatment ceases DNA replication and induces a permanent cell cycle arrest in human diploid fibroblasts (Chen and Ames, 1994; Bladier et al., 1997). In addition, oxidative stress originated from UVB irradiation promotes premature senescence in human skin diploid fibroblasts

(Borlon et al., 2007). Moreover, appropriate levels of doxorubicin or etoposide, inhibitors of topoisomerase II that can induce DNA double-strand breaks (Nelson and Kastan, 1994), induce a permanent cell cycle arrest in normal proliferating fibroblasts (Robles et al., 1999). Contrary to normal somatic cells, most cancer cells have extended or infinite life spans. Thus, tumor cells were thought to have escaped from senescence. However, recent studies showed that cancer chemotherapeutic drugs, including doxorubicin, camptothecin, and cisplatin, are able to arrest tumor cells by initiating premature senescence *in vitro* and *in vivo* (Chang et al., 1999b, Elmore et al., 2002, Han et al., 2002, te Poele et al., 2002, Roninson, 2003, Farrell et al., 2004). Camptothecin is an inhibitor of topoisomerase I and can induce DNA double strand breaks (Liu et al., 2000). Cisplatin creates DNA lesions by binding to DNA and in particular the formation of intrastrand cross-links between adjacent guanines (Reedijk and Lohman, 1985). In a summary, cellular senescence is a common stress response in response to genomic instability originated from telomere dysfunction, oncogene activation, and DNA damage.

Here, we would like to note that cellular senescence is defined as an irreversible cell cycle arrest, but is not necessarily irreversible depending on the expression of pRb and p53. Inactivation of p53 was found to stimulate the re-entrance into the cell cycle of senescent cells with low levels of p16, but not those with high levels of p16 (Beausejour et al., 2003). In addition, several reports showed that upon inactivation of pRb, the pRb-induced senescent cells resumed to synthesize DNA, but most died in the process rather than proliferated (Xu et al., 1997; Tiemann and Hinds, 1998; Alexander et al., 2003). Moreover, a small number of p16-induced senescent cells appeared to enter S phase upon removal of p16-expression (Dai and Enders, 2000).

## p53 is an effector of stress signals leading to senescence

### *The role of p53 in replicative senescence*

Several studies have revealed that DNA damage foci appear at the telomeres of senescent cells (d'Adda di Fagagna et al., 2003; Takai et al., 2003). These foci contain multiple DNA damage-response proteins, such as γ-H2AX, 53BP1, MDC1, NBS1, MRE11, and RAD17. Moreover, senescent cells express activated forms of Ataxia-telangiectasia mutated (ATM) and its downstream target, Chk2. Phosphorylation of p53 at Ser-15 by ATM and at Ser-20 by Chk2 inhibits p53 degradation by MDM2, a RING finger E3 ligase which ubiquitinates and degrades p53 through ubiquitin-mediated proteolysis. However, loss of ATM does not attenuate senescence as expected. It has been shown that human diploid fibroblasts from AT patients with mutation in the ATM gene are associated with accelerated telomere shortening and undergo senescence (Takai et al., 2003). Indeed, other than functioning as a DNA damage response mediator for telomere attrition, ATM also plays a role in maintaining the integrity of telomeres (Pandita, 2002). In addition, localization of ATM- and Rad3-related (ATR) and ATR-interacting protein (ATRIP) to telomeres was observed in the $ATM^{-/-}$ cells (Herbig et al., 2004). ATR activates its substrate ChK1 and then phosphorylates p53 at Ser 15 and regulates p53 activity. It has been shown that ATM plays a major role transmitting the telomere DNA damage signal in normal cells but ATR becomes more prominent in $ATM^{-/-}$ cells (Herbig et al., 2004). Moreover, ectopic activation of ATR is sufficient to trigger a cell cycle arrest in $ATM^{-/-}$ cells suggesting that ATR has complementary role for ATM deficiency (Herbig et al., 2004).

### *The role of p53 in OIS*

While the adenovirus E1A and c-Myc activate p53 to promote apoptosis, oncogenic Ras induces p53 to initiate premature senescence. It has been shown that oncogenic Ras arrests cells at G1 along with a significant increase in the abundance of p53 (Serrano et al., 1997). Upregulation of p53 in oncogene-induced senescence results from two major signaling pathways. First, activation of ARF via the MAPK pathway has been shown to be required for oncogenes signaling to p53. Indeed, it has been shown that ARF interacts with MDM2 and prevents MDM2-mediated degradation of p53 (Zhang and Xiong, 2001). Mice lacking ARF alone are highly prone to spontaneous tumor formation, which is similar to p53-deficient mice (Donehower et al., 1992; Kamijo et al., 1997). In addition, ectopic expression of ARF induces cell cycle arrest in cells containing wild-type p53 but not mutant p53, whereas ARF-null MEFs do not undergo replicative senescence and can be transformed by oncogenic H-Ras alone (Kamijo et al., 1997). Moreover, in the absence of ARF, coexpression of oncogenic Ras and p53 was unable to promote premature senescence in MEFs (Ferbeyre et al., 2002). However, ARF is not required for OIS in human cells (Wei et al., 2001). These data suggest that ARF may function as a mediator of Ras signaling to p53 in mouse cells. Second, DNA damage response is required for the activation of p53 in response to oncogenes. It has been reported that OIS is accompanied by DNA replicative stress, including prematurely terminated DNA replication forks and DNA double-strand breaks caused by hyper-DNA replication (Bartkova et al., 2006; Di Micco et al., 2006). Consistent with this, oncogenic Ras-induced senescence is accompanied with activation of DNA damage response effectors, such as ATM/ATR and Chk2/Chk1, and inactivation of these DNA damage response effectors by RNA interference attenuates OIS (Mallette et al., 2007; Mallette and Ferbeyre, 2007; Halazonetis et al., 2008; Toledo et al., 2008). Moreover, increased production of reactive oxygen species by oncogenic Ras also acts through the p53 pathway to

induce senescence (Itahana et al., 2003).

*The role of p53 in SIS*

In response to DNA damage stress signals, including oxidative stress, ionizing radiation, and chemotherapies, the ATM/ATR-p53 pathway is activated leading to premature senescence in both normal and tumor cells (Schmitt, 2007). Upon treatment with doxorubicin, camptothecin, and cisplatin, SA-ß-gal was present in multiple human cancer cells lines carrying wild-type p53, including MCF7, HCT116, LS174T, HCA-7, A2780, and HT1080, but not in DLD1, MDA-MB-231, and PC3 cells with mutant p53 (Chang et al., 1999b). In addition, knockdown of p53 in MCF7 and HT1080 cells, or knockout of p53 in HCT116 cells significantly decreases, but not abolishes doxorubicin-induced premature senescence (Chang et al., 1999a; Qian et al., 2008). Moreover, in response to doxorubicin treatment, SA-ß-gal positive cells were still observed in p53 null Saos-2 cells, in SW480 and U251 cells carrying mutant p53, and in Hela and Hep-2 cells in which p53 is inactivated by E6 protein (Chang et al., 1999b). These data suggest that p53 plays an important role in SIS, but some other genes may also play a role in SIS in cancer cells. Interestingly, cell cycle arrest is the initial step of cellular senescence, and it has been well established that DNA damage also activates p53 to induce cell cycle arrest and cell death (Caelles et al., 1994; Sabbatini et al., 1995). The outcome of p53 activation may depend on the cell type, amount of DNA damage, levels of p53 induced, different p53 post-translational modifications, recruitment of different co-factors to p53, and transcriptional activation of different sets of p53 target genes (Hansen and Oren, 1997; Wahl and Carr, 2001). Some cell cycle regulatory genes, such as CIP/KIP family of cyclin-dependent kinase inhibitors (p21/p27/p57) (Brown et al., 1997; Nijjar et al., 1999; Collado et al., 2000; Alexander and Hinds, 2001; Herbig et al., 2004) and Rb family of pocket proteins (pRb/p130/p107) (Kiyono et al., 1998; Braig et al., 2005; Kapic et al., 2006; Lehmann et al., 2008), are shown to play a role in cellular senescence. Therefore, it is likely that cells make a decision between undergoing premature senescence and apoptosis in response to DNA damage via a similar mechanism to that by which cells make a decision between cell cycle arrest and apoptosis. However, this needs to be further explored.

**p53 target genes involved in senescence**

The tumor suppression functions of p53 are manifested through the activation of its downstream genes resulting in cell cycle arrest, senescence, and apoptosis (Harms et al., 2004). Although many target genes have been identified, those involved in p53-dependent cellular senescence are still poorly understood (Levine et al., 2006). Several molecular markers, such as p21, PML, PAI-1, and DEC1, are shown to be sufficient to mediate senescence downstream of p53.

p21, a pleiotropic inhibitor of cyclin/cyclin-dependent kinases, is a classic p53 target (el-Deiry et al., 1992). p21 initiates growth arrest by preventing pRb phosphorylation by cyclin-dependent kinases (el-Deiry et al., 1993), or by binding to and inactivating E2F (Afshari et al., 1996) and PCNA (Li et al., 1994; Waga et al., 1994). Thus, p21 plays a major role in inducing p53-dependent G1 arrest following DNA damage (el-Deiry et al., 1992, 1994). In addition, in senescent cells, increased p53 activity is accompanied by increased binding of p53 to the p21 promoter (Jackson and Pereira-Smith, 2006). Thus, it has been hypothesized that p21 is an important effector of p53-dependent cellular senescence. p21 was first identified as an overexpressed gene in senescent cells (Noda et al., 1994) and later was found to be capable of inducing premature senescence in p53-null H1299 cells (Wang et al., 1999). However, disruption of p21 in human diploid fibroblasts results in extended cellular lifespan but not immortalization (Brown et al., 1997). In agreement with this, MEFs derived from p21-null mice eventually enter senescence (Pantoja and Serrano, 1999). Moreover, lack of p21 diminishes but does not abrogate DNA damage-induced premature senescence in tumor cells (Schmitt, 2007). Furthermore, p53 and p21 double-null cells undergo senescence induced by coexpression of oncogenic Ras and p53 (Castro et al., 2004). These data suggest that p21 is not essential for p53-mediated senescence, although is sufficient to trigger senescence in the absence of p53.

Promyelocytic leukemia PML, a RING finger nuclear phosphoprotein, is a tumor suppressor and originally identified in acute promyelocytic leukemia (APL) patients (Reymond et al., 2001). PML is an essential component of the PML nuclear bodies (PML-NBs), which function as a docking area recruiting regulatory proteins including p53 to organize nuclear processes (Borden, 2002). The level of PML was found to be increased in both Ras-induced premature senescence and replicative senescence (Ferbeyre et al., 2000; Pearson et al., 2000). Overexpression of PML is capable of inducing premature senescence by stabilizing p53 via promoting p53 acetylation on Lys-382 and phosphorylation on Ser-15 and -46 (Ferbeyre et al., 2000; Pearson et al., 2000; Bischof et al., 2002). In contrast, deacetylation of p53 antagonizes PML-induced premature senescence (Langley et al., 2002). PML is expressed as seven isoforms. However, only PML-IV is able to regulate p53 activity and cause senescence (Bischof et al., 2002). Moreover, PML is capable of enhancing p53 stability through sequestering MDM2 (Bernardi et al., 2004) or inhibiting MDM2-mediated p53 degradation via prolonging the phosphorylation of p53 on ser-20 by Chk2 (Louria-Hayon et al., 2003). Interestingly, it has been shown that PML is a direct p53 target and functions as an effector for p53 activities (de Stanchina et al., 2004). Thus, in response to oncogene activation and DNA damage, induction of PML is p53-dependent and knockdown of PML attenuates p53-

induced apoptosis and senescence. Together, PML and p53 form a positive regulatory feedback loop during cellular senescence.

PAI-1, plasminogen activator inhibitor-1, is upregulated in aging fibroblasts *in vivo* and *in vitro* and is considered a marker of replicative senescence (Mu and Higgins, 1995). PAI-1 inhibits cell proliferation by physically association with uPA and inhibits its activity. uPA, a secreted protease, promotes G1/S transition through activating a mitogenic signaling cascade by increasing the bioavailability of growth factors (Andreasen et al., 2000). PAI-1 is transcriptionally regulated by p53 (Kunz et al., 1995; Zhao et al., 2000). Inhibition of PAI-1 by RNA interference leads to escape from replicative senescence and ectopic expression of PAI-1 induces premature senescence in proliferating p53-deficient mouse or human fibroblasts (Kortlever et al., 2006). Therefore, PAI-1 is a critical downstream target of p53 in the senescence response of both mouse and human diploid fibroblasts.

DEC1, a basic helix-loop-helix transcription factor, was firstly identified as a gene expressed in differentiated embryo-chrondrocyte and induced by retinoic acid in mouse (Boudjelal et al., 1997; Shen et al., 1997). DEC1 functions as a transcription repressor by directly binding to class B E-boxes (St-Pierre et al., 2002; Li et al., 2003), by interacting with components of the basal transcription machinery (Boudjelal et al., 1997; Shen et al., 2002; Zawel et al., 2002), or by recruiting an HDAC co-repressor complex (Sun and Taneja, 2000). DEC1 is implicated in cell cycle regulation, differentiation, circadian rhythm, and apoptosis in response to various extracellular stimuli (Boudjelal et al., 1997; Yoon et al., 2001; Honma et al., 2002; Li et al., 2002; Miyazaki et al., 2002). In addition, DEC1 is a novel senescence marker which is upregulated in oncogene K-rasV12-induced senescence (Collado et al., 2005). We recently showed that DEC1 is a target gene of the p53 family members and mediates p53-dependent cellular senescence. In addition, overexpression of DEC1 alone is sufficient to initiate G1 arrest and cellular senescence, whereas knockdown of DEC1 attenuates DNA damage-induced senescence in MCF7 cells (Qian et al., 2008). Moreover, DEC1 inhibits the expression of ID1 (Qian and Chen, 2008), a member of the ID (inhibitor of differentiation or DNA binding) subfamily of the bHLH transcription factors (Norton, 2000). ID1 plays an important role in senescence. It has been shown that ID1 is an oncogene and down-regulated in arrested or senescent cells (Hara et al., 1994). In contrast, ectopic expression of ID1 extends the life-span of human keratinocytes (Alani et al., 1999, Nickoloff et al., 2000). We found that ID1 expression is repressed upon DNA damage, whereas knockdown of p53 or DEC1 releases this inhibition. In addition, DEC1 represses ID1 expression through binding to the E-box elements in the proximal promoter region of the ID1 gene. Thus, we hypothesized that p53 trans-repressional activity on ID1 expression is mediated by its own target DEC1. However, knockdown of DEC1 or overexpression of ID1 partially attenuates DNA damage-induced senescence, which indicates the involvement of other unknown targets.

Over 100 target genes of p53 have been identified (Agarwal et al., 1998, Nakamura, 2004, Harms et al., 2004, Harris and Levine, 2005). For example, Bax, Puma, Noxa, Fas and Killer/DR5 are involved in apoptosis. However, genetic studies have shown that p53 is still able to induce apoptosis in the absence of each individual one. This suggests that p53 activates a redundant set of genes to fulfill its functions: some genes yet to be found may play an indispensable role in response to a specific senescence signal, or different combinations may be required in different tissues. Given the importance of the p53 transcriptional activities in regulating cellular responses, it is likely that other target genes are involved in p53-dependent cellular senescence.

## p53 family members, p63 and p73, in cellular senescence

p53 was believed to be expressed predominantly as a single isoform. Recently, it was found that multiple isoforms of p53 are produced by using alternative promoters and undergoing alternative splicing (Bourdon et al., 2005). One of the isoforms, named ΔNp53 in human and p44 in mouse, is generated from an alternative translation start site located in exon 4 at codon 40 in human and condon 41 in mouse. In the absence of the N-terminal transactivation domain, ΔNp53 is not able to transactivate p21 and MDM2 in p53-null background (Courtois et al., 2002). Consistent with this, ΔNp53 is tumorigenic in cells deficient in p53 (Mowat et al., 1985), but is growth suppressive in cells with wild-type p53 (Rovinski et al., 1987). Moreover, p44-knockin mice exhibit a phenotype of growth suppression and premature aging, which may be due to the altered expression ratio between p53 and p44 (Maier et al., 2004). Therefore, the ratio of expression levels of p53 isoforms is important for p53 activity in growth suppression and senescence.

In addition to p53, p63 and p73 were identified to be members of the p53 family (Kaghad et al., 1997; Schmale and Bamberger, 1997). p63 and p73 share similar structure with p53, especially in the DNA binding and activation domains (Courtois et al., 2004). Both p63 and p73 are expressed as multiple isoforms due to utilization of two separate promoters, the upstream P1 promoter and the P2 promoter in intron 3 (Harms et al., 2004; Prives and Manfredi, 2005). The P1 promoter produces transcriptional active (TA) isoforms, whereas the P2 promoter produces N-terminally deleted (ΔN) isoforms. Both TA and ΔN p63 and p73 transcripts can undergo C-terminal splicing resulting in three p63 isoforms ($\alpha$-$\gamma$) and seven p73 isoforms ($\alpha$-$\eta$). In addition, several p73 isoforms are produced through alternative N-terminal splicings. The presence of these

different isoforms indicates the functional diversity of p63 and p73.

The sequence homology among p53, p63 and p73 indicates the possibility of similar functions for this gene family. Since p53 is a crucial tumor suppressor, it is not unreasonable to suspect that p63 and p73 may be tumor suppressors. Surprisingly, inactivating mutations of p63 and p73 are rarely observed in human cancers (Moll and Slade, 2004). In addition, p63 and p73 knockout mice exhibit severe developmental abnormalities but not increased cancer susceptibility (Mills et al., 1999; Yang et al., 1999, 2000). p63-/- mice are defected in limb and skin formation and lack ectodermal derivatives, including mammary glands, teeth, and hair (Mills et al., 1999; Yang et al., 1999). p73-/- mice are born with severe neurological defects, including hippocampal dysgenesis and hydrocephalus, chronic infection and inflammation, and abnormalities in pheromone sensory pathways (Yang et al., 2000). These suggest that p63 and p73 play a key role in development. However, p63-/- mice are born alive, but they die hours later due to dehydration. p73-/- mice live up to 4 to 6 weeks and die due to chronic infections. These put an obstacle to observing the role of p63 and p73 in cancer development. To overcome this, mouse models with knockout of specific isoforms of p63 and p73 were generated, and suggested that p63 and p73 are also involved in tumor formation and regulating cellular senescence. It has been shown that tissue-specific p63 conditional knockout mice have a shortened lifespan and display features of accelerated aging correlated with induction of cellular senescence by conditional p63 ablation in skin (Keyes et al., 2005). Similar early-aging associated phenotypes were observed in mice with a deletion mutation of the first six exons in the p53 gene (Tyner et al., 2002). In addition, p63+/-;p73+/- mice develop spontaneous tumors and further loss of p63 or p73 in p53+/- mice exhibit higher tumor burden and metastasis compared to p53+/- mice (Flores et al., 2005). Moreover, mice deficient in TAp73 are tumor prone and show accelerated aging (Tomasini et al., 2008). These observations suggest that as a member of the p53 family, p63 and p73 play a role in tumorigenesis and aging.

Because of highly conserved sequence identity in the DNA binding domains among the p53 family, both p63 and p73 are capable of binding to the consensus p53 response element and activate some p53 targets, including p21, DEC1, and MDM2 (Zhu et al., 1998; Harms et al., 2004; Qian et al., 2008), and transient expression of p63 and p73 induces cell cycle arrest and apoptosis (Zhu et al., 1998; Dohn et al., 2001). In addition, p63 and p73 are found to participate in DNA damage-induced apoptosis (Yang et al., 1998; Stiewe and Putzer, 2000). Interestingly, it has been shown that p63 and p73 are required for p53-dependent apoptosis in MEFs upon DNA damage (Flores et al., 2002), but not in mature T cells upon treatment with radiation or glucocorticoid (Senoo et al., 2004). Thus, the role of p63 and p73 in the p53-pathway may vary under different conditions. However, whether p63 and p73 are also capable of initiating senescence is not clear. It has been shown that like p53, p73 can regulate the expression of human telomerase reverse transcriptase (hTERT), which adds TTAGGG repeats to chromosome ends (Racek et al., 2005; Beitzinger et al., 2006). Telomere elongation by enforced expression of hTERT extends the lifespan of normal cells (Bodnar et al., 1998). Interestingly, p53 is able to repress hTERT mRNA expression via blocking the accessibility of Sp1 to the Sp1 sites within the hTERT core promoter (Xu et al., 2000, Kanaya et al., 2000) or indirectly through p21 by forming a repressive pRb/E2F complex on an atypical E2F site of the hTERT promoter (Shats et al., 2004). Like p53, TAp73 isoforms can also inhibit the hTERT gene expression via Sp1 binding sites within the hTERT core promoter (Racek et al., 2005; Beitzinger et al., 2006). Although ectopic expression of TAp73 isoforms ($\alpha$,ß,$\gamma$,$\delta$) was found to downregulate the hTERT promoter activity in H1299 cells (Racek et al., 2005), other report showed that ectopic expression of TAp73ß does not lead to suppression of hTERT transcription in the same cell system (Toh et al., 2005). Moreover, both TAp73$\alpha$ and TAp73ß can release p53-mediated suppression on the hTERT promoter by reducing p53 level through upregulation of MDM2 (Toh et al., 2005). Furthermore, $\Delta$Np73 antagonizes p53 and TAp73-mediated inhibition of hTERT via interfering with the activity of p53, TAp73

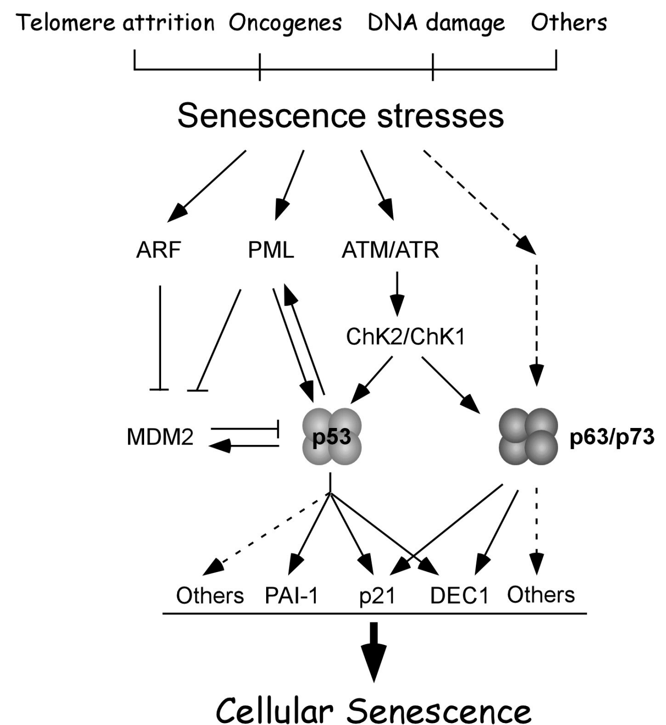


**Fig. 1.** p53 pathways in cellular senescence. Solid lines, known pathways. Dashed lines, undefined pathways.

and, in addition, induces hTERT expression by interfering with E2F-RB-mediated repression of the hTERT core promoter (Beitzinger et al., 2006). These data indicate that p73 regulates hTERT through p53-like or unique mechanisms. However, whether regulation of hTERT, a regulator of replicative senescence, is a mechanism by which p73 modulates cellular senescence remain to be characterized. In addition, the common targets of p53 and p73 or the unique p73 targets involved in cellular senescence need to be identified in the future.

For p63, induced ablation of p63 in primary keratinocytes causes an arrest with enhanced expression of senescence markers, SA-ß-gal and PML (Keyes et al., 2005). Reports showed that both p53 and p16 pathways are involved in senescence induced by p63 deficiency (Keyes and Mills, 2006). In addition, several regulators of cell cycle and senescence, such as PML, p21, 14-3-3δ, and IGFBP3, are shown to be positively or negatively regulated by TAp63/ΔNp63 (Keyes and Mills, 2006). However, how p63 fits into the senescence-regulatory pathway needs to be further explored. Taken together, the story of p53 family members in cellular senescence is far more complicated than we expected. Development of efficient antibodies specifically against each isoform and generation of cell lines or mice models with single isoform expression, knockdown, or knockout are needed to further elucidate the role of p53 family proteins in cellular senescence.

## Conclusions

It is clear that cellular senescence is an important anti-cancer barrier and the p53 tumor suppressor plays a pivotal role in this process. How p53 family members are involved in the senescence pathway was summarized in Fig.1. However, the complexity of the p53 family proteins makes this story even more complicated. For example, what is the function of each individual p53 isoform in senescence? How p63 and p73 are implicated in cellular senescence? Since p63 and p73 are rarely mutated in tumors, what is the significance of p63 and p73 in mediating senescence? Do p53 family proteins affect each other in this process? Therefore, future studies uncovering these questions will further our understanding of the integrated functions of the p53 family members in normal tissue development, cancer formation, and aging. This will fulfill our knowledge about the p53 family members in tumorigenesis and may provide valuable clues to solve the current clinical problems.

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