The Regulation of Cellular Functions by the p53 Protein: Cellular Senescence

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Transformed cells have properties that allow them to survive and proliferate inappropriately. These characteristics often arise as a result of mutations caused by DNA damage. p53 suppresses transformation by removing the proliferative or survival capacity of cells with DNA damage or inappropriate cell-cycle progression. Cellular senescence, marked by morphological and gene expression changes, is a critical component of p53-mediated tumor suppression. In response to stress, p53 can facilitate an arrest and senescence program in cells exposed to stresses such as DNA damage and oncogene activation, preventing transformation. Senescent cells are evident in precancerous adenoma-type lesions, whereas proliferating, malignant tumors have bypassed senescence, either by p53 mutation or inactivation of the p53 pathway by other means. Tumors that have retained wild-type p53 often show a p53-mediated senescence response to chemotherapy. This response is actually detrimental in some tumor types, as senescent cells can drive relapse by persisting and producing cytokines and chemokines through an acquired secretory phenotype.

SENESCENCE: A PERMANENT CELL-CYCLE ARREST IN FIBROBLASTS MEDIATED BY p53

p53 is able to block cells with the potential to become a tumor from proliferating largely through its ability to act as a transcription factor. One of the best-characterized mechanisms of tumor suppression mediated by p53 is apoptotic cell death (Lowe et al. 1993; Symonds et al. 1994). It is intuitive that entirely removing a damaged cell from an organism will prevent that cell from ever becoming a tumor. More recently, accumulating evidence from biochemical studies, animal models, and clinical data has

revealed that cellular senescence plays a vital role in p53-mediated tumor suppression.

Cellular senescence is a permanent cell-cycle arrest caused by stress and is accompanied by altered gene expression and morphological changes that distinguish it from terminal differentiation (Campisi 1997). It was first described by Leonard Hayflick (1965) during serial cultivation of human diploid fibroblasts (HDFs) from the lung. These normal, p53 wild-type cells cease proliferation after a fixed number of population doublings in culture. Subsequent research has shown that these normal fibroblast cells undergo telomere shortening at each divi-

Editors: Guillermina Lozano and Arnold J. Levine

Additional Perspectives on The p53 Protein available at www.perspectivesinmedicine.org

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Cite this article as *Cold Spring Harb Perspect Med* 2017;7:a026112

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sion (Harley et al. 1990), with the telomeres reaching a critically short stage after a fixed number of passages (Bodnar et al. 1998). Short telomeres activate a DNA-damage response, resulting in cell-cycle arrest and replicative senescence (d'Adda di Fagagna et al. 2003; von Zglinicki et al. 2005). p53 is the critical mediator of this arrest (Itahana et al. 2001). Early experiments showed that introduction of SV40 large T antigen resulted in bypass of the senescence and arrest response to passaging in culture (Ide et al. 1983, 1984), and cells entered a state termed "crisis." In crisis, cells continue to divide, but at a rate that is matched by apoptosis (Neufeld et al. 1987; Radna et al. 1989). Eventually, immortal clonal populations will emerge that can maintain telomere length (Neufeld et al. 1987; Counter et al. 1992).

One of the functions of SV40 large T antigen is to inactivate p53, but it also has other activities that might obscure the precise role of p53 in replicative senescence. This role was more directly shown by gene targeting of p53 in human fibroblasts. Cells with one allele targeted will spontaneously lose the second allele, avoid senescent arrest, and continue through the cell cycle into crisis with eventual emergence of immortal cell populations (Wei et al. 2003).

In addition to mediating the onset of senescence, p53 is also important for maintaining the senescent phenotype. Introduction of a p53-inactivating peptide, large T antigen, or p53 siRNA by lentivirus into already senescent fibroblasts causes reentry into the cell cycle and loss of features of senescence (Beausejour et al. 2003). Interestingly, this occurred in cell lines with low levels of the cell-cycle inhibitor p16. In fibroblasts with relatively higher levels of p16 at senescence, inactivation of both p53 and Rb were required to reverse the phenotype (Beausejour et al. 2003). In sum, these studies show that p53-mediated senescence is a barrier to genomic instability that results from telomere erosion in fibroblasts during passage in culture, ultimately preventing acquired immortality.

Telomere shortening in the absence of p53 results in DNA damage and genomic instability, eventually leading to cellular immortality. Genetically engineered mice have shown that

p53 suppresses tumorigenesis in models with compromised telomeres. Mice engineered to lack telomerase activity are tumor prone, and this is accelerated by p53 loss (Chin et al. 1999; Rudolph et al. 1999). Further, p53-mediated senescence suppresses tumors in mice with deprotected telomeres (Cosme-Blanco et al. 2007; Feldser and Greider 2007). Interestingly, *Cdkn1a* (coding for p21) deletion can extend the life span of telomerase-deficient mice, without increasing the rate of cancer by improving viability in the stem cell compartment. In these mice, p21 loss impairs p53-mediated arrest and senescence, but leaves apoptosis intact (Choudhury et al. 2007). Thus, it is clear from multiple studies that the tumor suppressor p53 plays a critical role in vivo preventing genomic instability and transformation caused by short telomeres.

THE PATHWAY OF p53-MEDIATED SENESCENCE

The mechanism of how p53 mediates the senescence program has now been defined in numerous studies. As telomeres shorten, eventually a DNA damage signal is detected (d'Adda di Fagagna et al. 2003; von Zglinicki et al. 2005) and a classical DNA damage response is initiated (von Zglinicki et al. 2005). One step in this process is the activation of the ATM kinase (Wong et al. 2003), which phosphorylates both p53 and Mdm2 (Banin et al. 1998; Canman et al. 1998; Khanna et al. 1998; Maya et al. 2001), thus allowing the p53 protein to accumulate and initiate transcription of multiple target genes (Fig. 1) (Atadja et al. 1995; Bond et al. 1996).

Among the first p53 target genes discovered was the *CDKN1A* gene, which codes for the cyclin-dependent kinase (cdk) inhibitor p21. p21 was discovered simultaneously as an interacting partner and inhibitor of cdks (Xiong et al. 1993), as the primary transcript elevated in a p53-dependent fashion following DNA damage (el-Deiry et al. 1993), and as the primary transcript that induces senescence in HDFs (Noda et al. 1994). Each study complemented the other and produced a remarkably complete picture of p21 function and regula-

Figure 1. Mechanisms of cellular senescence. DNA damage is caused by multiple means, including oncogene activation, reactive oxygen species (ROS), cell division and replication, ultraviolet light (UV), and cancer therapies. These signals in turn can initiate p53 pathway signaling through increased ATM activity, causing Chk2-mediated phosphorylation of p53, which protects p53 from Mdm2-mediated degradation. Additionally, oncogene activation can induce ARF and p16 expression, which inhibit Mdm2 and cdk function, respectively. The resulting increased p21 and/or Rb activity can result in permanent cell-cycle arrest and senescence. Cellular senescence is marked by an enlarged, flattened morphology, increased lysosomal compartment, secretion of multiple cytokines and chemokines of the senescence-associated secretory phenotype (SASP), and sometimes formation of senescence-associated heterochromatin foci (SAHF).

tion. Subsequent studies have shown that p53 binds and transactivates the p21 promoter during replicative senescence of HDFs (Vaziri et al. 1997; Jackson and Pereira-Smith 2006a), and also during DNA damage induced by radiation (Jackson and Pereira-Smith 2006a). DNA damage of all sources is a potent activator of

p53 and can induce senescence in many cell types (Fig. 1).

p21 helps to execute the senescence program primarily by causing cell-cycle arrest. This is achieved through inhibition of cyclin/cdk complexes, which normally phosphorylate targets that promote cell-cycle progression (Sherr

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2000). One well-studied target of cdks is the RB family of proteins (Sherr and McCormick 2002). When p21 levels are elevated via p53-mediated transcription, cyclin/cdk complexes cannot phosphorylate and inhibit RB family members (Sherr 2000). This allows the RB family of proteins to recruit chromatin modifiers that silence expression of genes that drive cell-cycle progression (Ferreira et al. 2001; Narita et al. 2003).

As a p53 target, p21 plays a critical role in p53-dependent senescence. Similar to cells that lack p53, normal HDFs that lack p21 also fail to arrest after serial passage (Brown et al. 1997; Herbig et al. 2004), and inactivation of p21 by injection of specific antibodies results in reinitiation of S phase (Ma et al. 1999). Alternatively, forced expression of p21 can induce senescence when ectopically expressed in vitro (Fang et al. 1999; Chen et al. 2002). These studies define p21 as a potent mediator of p53-induced arrest and senescence in response to cell stress and DNA damage.

MARKERS OF SENESCENCE

As research expanded away from HDFs into other types of cultured cells and in vivo models, determining whether a cell was actually senescent, as opposed to temporarily arrested or differentiated, presented a challenge. “Permanent” cell-cycle arrest can be tested in vitro, but determining this in vivo is considerably more intractable as no established set of markers exists to clearly identify these cells. No single marker is universal to all senescent cells, and probably no feature other than eventual cell-cycle progression can exclude a cell from being classified as senescent (Collado and Serrano 2010; Sharpless and Sherr 2015). One can develop a degree of confidence, however, if several factors are examined concurrently to identify senescent cells (Fig. 1). These include lack of positive staining of proliferation markers (such as Ki67 or BrdU incorporation) combined with the presence of cell-cycle inhibitors (p21, p16, p15, or hypophosphorylated Rb family members). Additionally, senescent cells secrete many cytokines and chemokines, a feature known as the senes-

cence-associated secretory phenotype (SASP) (Acosta et al. 2008; Coppe et al. 2008; Kuilman et al. 2008; Wajapeyee et al. 2008). Many of these genes are up-regulated in cells made senescent by diverse stimuli and of all different origins, and thus can be used as markers of senescence.

The most commonly used marker for senescent cells is positive staining of β -galactosidase at pH 6 (senescence-associated β -galactosidase, or SA- β Gal) (Dimri et al. 1995), which takes advantage of the increased lysosomal compartment by senescent cells (Kurz et al. 2000; Lee et al. 2006). Senescent cells also form heterochromatic foci (SAHF), which mark areas of gene silencing, including those involved in cellular proliferation (Narita et al. 2003). Finally, senescent cells undergo a marked morphological change in vitro, becoming enlarged and flattened (Campisi 1997). Although there are obviously many known characteristics of senescent cells, one alone is not sufficient for positive identification. As an example of careful identification of senescence, in serial histological tissue sections of colon adenomas, senescent cells were identified by a lack of Ki67 staining combined with positive staining for p16 and the SASP factor interleukin (IL)-8, although proliferating, nonsenescent regions of the adenomas stained inversely: positive for Ki67 and negative for p16 and IL-8 (Kuilman et al. 2008). Thus, the use of multiple markers, such as those that identify proliferative arrest, expression of cdk inhibitors, and expression of SASP factors, are necessary to clearly define senescence in vivo.

OTHER STRESSES THAT CAUSE SENESCENCE MEDIATED BY p53: ONCOGENE ACTIVATION

The first cause of senescence described was a result of telomere erosion after serial passage in tissue culture (Hayflick 1965). Since then, however, many other stresses have been shown to induce senescence in normal cells. Perhaps the most relevant to tumor suppression is the induction of senescence in response to ectopically activated oncogenes (Fig. 1).

Oncogene-induced senescence (OIS) was first described by Serrano et al. in a landmark

study (Serrano et al. 1997). They showed that normal fibroblasts transduced with a retroviral vector-expressing oncogenic Ras undergo a short burst of proliferation, followed by the onset of a phenotype reminiscent of fibroblasts in replicative senescence. The cells exposed to activated Ras showed elevated expression of p21 and p16, hypophosphorylated Rb, cessation of BrdU incorporation, acquisition of morphological changes, and positive SA- β Gal staining (Serrano et al. 1997). Furthermore, it was discovered that OIS in mouse cells could be avoided through additional loss of the tumor suppressor p53. Mouse embryo fibroblasts with a genetic ablation of *p53* bypassed the senescence response to activated Ras, and instead increased proliferation rate. The viral oncogenes used to inactivate p53 required additional Rb inactivation to bypass OIS (Serrano et al. 1997).

Subsequent to this study, p53 and/or Rb family-dependent OIS has been shown in numerous cell types exposed to diverse oncogenic signals (Gorgoulis and Halazonetis 2010). In addition to Ras activity, senescence can be induced in vitro in various cell types by activation of signals downstream from Ras, including B-RAF (Zhu et al. 1998; Michaloglou et al. 2005), MEK (Lin et al. 1998), AKT (Astle et al. 2012), and PIK3CA (Kim et al. 2007; Astle et al. 2012). Upstream of Ras, activation of fibroblast growth factor receptors (FGFRs) (induces p53-dependent senescence) (Ota et al. 2009) and ERBB2 (Her2/neu, p53 dependence was not examined) can induce senescence (Trost et al. 2005). Additionally, loss of negative regulators of Ras and PI3 kinase, such as neurofibromatosis type 1 (NF1) (Courtois-Cox et al. 2006) and PTEN (Chen et al. 2005; Kim et al. 2007), result in increased signaling and senescence. Other oncogenic signals such as those from c-Mos and the Bcr-Abl fusion protein can induce senescence, although p53 dependence was not examined in these studies (Bartkova et al. 2006; Wajapeyee et al. 2010).

These experiments clearly show the induction of senescence in normal cells in response to oncogene activation and the p53 and Rb mechanisms that often drive the response, thus creating a well-supported hypothesis that this program is tumor suppressive in vivo.

IN VIVO ROLE OF p53-MEDIATED ONCOGENE-INDUCED SENESCENCE

Evidence of the in vivo role of p53-mediated senescence in tumor suppression has come from multiple studies. Cells in mice that carry a hypomorphic *p53* allele greatly favor transactivation of *Cdkn1a* and arrest/senescence programs, and fail to activate apoptotic genes (Liu et al. 2004). This hypomorphic p53 protein still suppresses spontaneous tumor formation by a *Cdkn1a*-dependent mechanism (Liu et al. 2004; Barboza et al. 2006) and can also suppress Myc-driven lymphoma (Post et al. 2010) and pancreatic cancer (Morton et al. 2010), extending life span in all cases when compared with *p53* null or mutant alleles.

These studies showed a p53–p21-senescence pathway suppresses tumor formation. However, some questions were unaddressed, including when, during the transformation from normal cell to malignant cell, does p53-mediated senescence act to prevent cancer and extend life span?

Subsequent studies in mouse models and clinical specimens addressed this question, showing that cells driven to senescence by p53 are prevalent in arrested, premalignant adenomas, whereas the proliferating carcinoma regions of tumors lack markers of senescence (Collado and Serrano 2010). This relationship has been observed in the prostate with *Pten* deletion, and in the lung and mammary gland with Ras pathway activation.

Mice with *p53* deletion in the prostate have no tumor phenotype, whereas mice with *Pten* deletion develop nonlethal prostatic intraepithelial neoplasias (PIN) that are positive for SA- β Gal, p19Arf, p53, and p21 (Chen et al. 2005). Remarkably, mice with deletion of both *Pten* and *p53* in the prostate, however, develop invasive carcinoma that is lethal by 7 months of age. Deletion of *p53* allowed bypass of the senescence in response to *Pten* loss in the prostate, resulting in rapid tumor progression and mortality (Chen et al. 2005).

Ductal epithelial cells in the mammary gland are also sensitive to ectopic Ras signals. Sarkisian et al. used an inducible model system

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in which the degree of Ras activation in the mammary could be controlled (Sarkisian et al. 2007). Potent inducible activation of H-RasG12V resulted in reduced mammary duct elongation in puberty followed by induction of senescence, as detected by p16, PML, and SA- β Gal staining (Sarkisian et al. 2007). Weaker induction of Ras caused hyperplasias in a p53 heterozygous background that could occasionally progress to tumors that spontaneously up-regulated Ras signaling. These tumors also bypassed the p53 senescence response that was observed acutely following potent Ras activation by undergoing loss of the wild-type p53 allele (Sarkisian et al. 2007).

Senescence has also been detected in premalignant lesions in the lung in a K-RasV12 lung cancer model. Lung adenomas were positive for the senescence markers p16, p15, and new markers discovered in the same study, Dec1 and Dcr2 (Collado et al. 2005). Rare lesions that progressed to adenocarcinoma had bypassed senescence (Collado et al. 2005). The role of p53 (and requirement of p53 inactivation to bypass senescence) was not examined; however, other Ras models in the lung have shown (similar to *Pten* in prostate discussed above) that p53 null alleles greatly accelerate tumor formation and progression to invasive lung cancer (Jackson et al. 2005), consistent with bypass of senescence.

In premalignant ductal lesions of murine pancreata driven by Ras activation, p53-induced senescence was detected by positive staining for SA- β Gal, p21, and IGFBP7 (SASP factor) (Morton et al. 2010). Interestingly, senescence was induced by a single wild-type allele in the presence of a mutant p53 allele (*p53R172H*). In mice heterozygous for mutant p53 that progress to frank malignancy, or mice homozygous for mutant p53, p53 activity was not evident and senescence had been bypassed (Morton et al. 2010).

Similar results were found for B-Raf activation in mouse lung (Dankort et al. 2007; Shai et al. 2015): OIS occurs in early lesions, but subsequent deletion of p53 results in acceleration and progression of tumor growth. Human melanocytic nevi with activation of the Ras

pathway, via *BRAF* mutation, were also positive for senescent markers, such as SA- β Gal and p16, and lack of Ki67 staining, in contrast to keratinocytes in normal skin (Michaloglou et al. 2005). Similar results were observed in a mouse model of B-Raf activation in melanocytes (Dhomen et al. 2009). The role of p53 in inducing arrest and senescence was not directly addressed in these studies. However, in contrast to other models, such as lung and prostate, it does not appear that loss of p53 is required to bypass senescence and progress to malignancy in melanoma, as these tumors are predominantly p53 wild-type. It is possible that the p53 pathway is inactivated by other means.

In a study that expanded our understanding of senescence and tumor suppression, Kang et al. showed that activation of oncogenic Ras in murine liver cells caused senescence, as shown by positive staining for p21, p16, and SA- β Gal. Interestingly, these senescent cells are cleared by the immune system, and failure to do so led to development of hepatocellular carcinoma (Kang et al. 2011).

THE COST OF TUMOR SUPPRESSION BY SENESCENCE

Although the immediate consequence of p53-mediated cellular senescence is tumor suppression, it is hypothesized that the accumulation of senescent cells over the course of the lifetime of an individual can result in aging phenotypes (Campisi 2005; Rodier et al. 2007). Although this theory has not been strictly proven, evidence exists suggesting the possibility it could indeed be true. Cells positive for senescence markers, such as SA- β Gal or p16, accumulate in aged individuals (Dimri et al. 1995; Hornsby 2002). Unfortunately, reducing or eliminating activity of p53 to examine the effect on aging is not possible, as an organism with compromised p53 succumbs to tumors early in life. However, p53 loss has been shown to rescue some models of progeria and senescence.

Mice with a mutation in the *Zmpste24* gene recapitulate Hutchinson–Gilford progeria and show numerous premature aging phenotypes, including cellular senescence, as detected by

elevated expression of p21 in the heart and liver and SA- β Gal staining in the kidney and other organs (Varela et al. 2005). Deletion of p53 resulted in partial rescue of the aging phenotypes. Mice null for both *Zmpste24* and *p53* no longer stained positive for SA- β Gal in the kidney and had a longer life span than p53 wild-type, *Zmpste24* null mice (Varela et al. 2005).

p53 can also mediate senescence in developing embryos with compromised genomic maintenance. Stress caused by homozygous loss or mutation of the DNA repair gene *Brca1* causes late gestation embryonic lethality in mice because of widespread senescence, as detected by extensive SA- β Gal staining throughout the embryo as well as elevated p21 expression (Cao et al. 2003). When crossed into a p53 heterozygous null background, the developmental defects are completely rescued, no senescence is observed in the embryo, and mice can live 6 to 12 months (Cao et al. 2003). The mice do show aging phenotypes later in life, possibly because of the activity of the remaining wild-type p53 allele.

WILD-TYPE p53 TUMORS: CAN SENESENCE AFFECT RESPONSE AND OUTCOME?

Cancer is a disease marked by uncontrolled proliferation that is commonly paired with decreased DNA repair capabilities. In the hope of exploiting these characteristics, chemotherapies that cause DNA damage are commonly used in the clinic (Gianni et al. 2009). Ideally, a rapidly proliferating cancer cell will be overwhelmed with DNA damage it cannot repair efficiently and die, whereas normal somatic cells will be able to recover. Although effective in many circumstances, especially in blood-borne cancers (Weller 1998; Zenz et al. 2008), this is not always the case. For many solid tumors, only a partial response is observed following chemotherapy, leaving significant residual disease (Weller 1998; Symmans et al. 2007). Recent studies have shown that apoptosis is not always the primary p53-mediated response to DNA damage from chemotherapy. In many cases, p53 drives arrest and senescence, resulting in incomplete eradication of the tumor.

Senescence as a response to chemotherapy can potentially be a desirable outcome. Intuitively, it makes sense that permanent cell-cycle arrest would halt tumor progression and prevent relapse. Additionally, senescent cells can be cleared from tissue by the immune system much like cells that have undergone apoptosis, leading to tumor regression (Xue et al. 2007). However, secretion of multiple cytokines and growth factors by the SASP can act via paracrine and autocrine signaling and can have protumorigenic effects on nearby cells (Velarde et al. 2013; Perez-Mancera et al. 2014). To circumvent unwanted outcomes, these competing facets of therapy-induced senescence (TIS) must be considered when designing cancer therapy strategies.

DNA-DAMAGING CHEMOTHERAPY INDUCES p53-DEPENDENT CELLULAR SENESENCE IN VITRO

The role of p53 in inducing cellular senescence has been well documented in cancer cells as a response to chemotherapy and DNA damage. This was first described in a study in which doxorubicin, a DNA-damaging chemotherapeutic drug, was administered to multiple cancer cell lines. All cell lines with wild-type p53 showed increased SA- β gal staining, indicative of senescence, whereas cell lines that did not become senescent were mutant for p53 and instead underwent mostly apoptosis or mitotic death (Chang et al. 1999). In subsequent studies, DNA-damaging drugs were also found to induce senescence in p53 wild-type colon, ovarian, and breast adenocarcinoma cell lines, as detected by SA- β gal staining, increased p53, p21, p16, and hypophosphorylated Rb (te Poele et al. 2002). Additionally, this induced senescence was shown to be p53-dependent, as HCT116 colon carcinoma cell lines null for either p53 or p21 did not increase SA- β gal staining after chemotherapy treatment (te Poele et al. 2002). MCF-7 breast cancer cells, which are wild-type for p53, also undergo senescence in response to doxorubicin (Elmore et al. 2002; Jackson and Pereira-Smith 2006b). This response is altered when p53 activity is compromised by expression of the HPV-16 E6 oncogene (Elmore et al. 2002) or by siRNA

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silencing (Jackson et al. 2012), resulting in a cell death response instead of a senescent one.

It is apparent from these in vitro studies that presence of wild-type p53 in many cancer cell lines can direct a senescent response after chemotherapy in vitro (Roninson 2002; Ewald et al. 2010). Conversely, those tumors lacking wild-type p53 activity do not senesce and instead undergo cell death, which could potentially give a more favorable initial response to chemotherapy.

THERAPY-INDUCED SENESCENCE AND p53 IN VIVO

Evidence for TIS in vivo was described by te Poele et al. (2002) in breast cancer and expanded on in other studies using more tractable mouse models. Breast cancer is rarely cured by chemotherapy, and residual disease is usually present at surgery following neoadjuvant chemotherapy (Symmans et al. 2007). Senescence was examined in samples taken from breast cancer patients that had undergone a preoperative DNA-damaging chemotherapy regimen. When compared with tumors from patients who had not received any previous treatment, there was a higher occurrence of senescence as detected by SA- β gal and p16 staining in tumors judged to be p53 wild-type because of a lack of stabilized p53 protein (an indicator of p53 mutation) (te Poele et al. 2002).

Senescent features were also observed in human breast cancer xenografts from mice that were administered chemotherapy. Tumors wild-type for p53 had increased senescent markers after treatment, whereas those mutant for p53 did not, and instead underwent mitotic catastrophe (Varna et al. 2009), although no data on outcome were collected. Senescence was also detected following chemotherapy treatment in lymphomas driven by transgenic Myc. These tumors (and others of myeloid origin) naturally undergo apoptosis in response to chemotherapy. However, when apoptosis was blocked by ectopic Bcl2 expression, senescence was induced (Schmitt et al. 2002). These studies show that chemotherapy is capable of inducing p53 and senescence within tumors, but how

this affects tumor response and relapse was not addressed.

THERAPY-INDUCED SENESCENCE CAN POSITIVELY OR NEGATIVELY AFFECT OUTCOME AND SURVIVAL

If senescent cells are permanently arrested following chemotherapy treatment, then theoretically senescence should be as effective a response as apoptosis. Accumulating evidence, however, suggests that senescence is actually a significantly inferior response, and senescent cells can actually drive relapse.

In a study using the MMTV-*Wnt1* transgenic mouse model of mammary tumors, p53 wild-type tumors had poor response to doxorubicin administration and relapsed sooner than tumors that were mutant for p53 (Jackson et al. 2012). The p53 wild-type tumors were positive for many markers of senescence, including positive staining for SA- β gal, negative staining for proliferation markers such as Ki67, and mRNA expression was increased for many cytokines and chemokines of the SASP (Jackson et al. 2012).

Similar results were found in another study using human-in-mouse xenografted breast tumor transplants. Triple-negative human tumors, which are often mutant for p53 and highly aggressive, were engrafted into the mammary fat pad of immune compromised mice (Ma et al. 2012). Tumors wild-type for p53 arrest in response to chemotherapy, resulting in a quicker relapse than mutant p53 tumors. Knockdown of p53 in the wild-type line enhanced drug sensitivity (Ma et al. 2012). A role for induction of senescence was not examined in this study, although induction of senescence in the p53 wild-type tumors would be consistent with observations in other studies (te Poele et al. 2002; Jackson et al. 2012).

A limited number of studies have examined p53 status, TIS, and outcome in human patients. In one study, senescent markers including SA- β gal and SASP gene PAI-1 were induced following DNA-damaging chemotherapy in some human tumor samples of malignant pleural mesothelioma (Sidi et al. 2011). Those pa-

tients with tumors that underwent senescence had less tumor regression and worse overall survival compared with those that did not undergo senescence, although p53 status was not examined (Sidi et al. 2011).

In other studies, poor response and reduced overall survival were linked with presence of wild-type p53 in both ovarian carcinoma and advanced gastric cancer (Bataille et al. 2003; Moreno et al. 2007; Wong et al. 2013). Choi et al. also showed that wild-type p53 status predicted poor response to chemotherapy in muscle-invasive bladder cancer (Choi et al. 2014). Interestingly, of the mutant p53 tumors that also showed a modest response to chemotherapy with reduced apoptosis, many had a gene signature consistent with wild-type p53 activation (Choi et al. 2014). These studies show in multiple contexts that the best responses to chemotherapy are often observed in patients with mutant p53, and that retention of wild-type p53 can result in a worse outcome, consistent with induction of arrest and senescence.

In breast cancer, how p53 affects outcome is less clear, and is dependent on what endpoint is used in the study. Approximately 30% of breast cancers carry a *TP53* mutation (TCGA-Network 2012). In the neoadjuvant setting, in which chemotherapy treatment precedes surgery, the probability of achieving a complete pathological response, with no residual disease present following treatment, is much higher in *TP53* mutant tumors (Bertheau et al. 2002, 2007; Chen et al. 2012; Esserman et al. 2012; Wang et al. 2015).

Although a better pathological response to chemotherapy normally predicts better overall survival, this correlation is muddled in breast cancer. The superior initial response of p53 mutant tumors to chemotherapy does not correlate with an improved outcome in the p53 mutant tumor patient population, which has either reduced or has made no difference in survival (Olivier et al. 2006; Esserman et al. 2012; Silwal-Pandit et al. 2014; Wang et al. 2015).

How a more robust response to chemotherapy fails to affect overall survival rates is confusing, but it is possible that averaging data from large patient pools of all responses is obscuring

the actual story. This possibility is suggested by a study that examined p53 status, patient responses, and outcome. For breast cancer patients given neoadjuvant therapy, those with *TP53* mutant tumors had a higher probability of a complete response (Bertheau et al. 2007), as was observed in the above-mentioned studies. In this study, however, patients that had a complete response were analyzed as a separate group. These patients had the best overall survival rates in the study, even compared with those with wild-type p53 tumors (Bertheau et al. 2007). Interestingly, the mutant p53 tumors with an incomplete response had a shorter overall survival than both the complete responders and wild-type p53 groups (Bertheau et al. 2007). Thus, p53 mutant tumors have both the best and the worst responses to chemotherapy based on patient survival. For breast cancer patient survival, wild-type p53-mediated TIS in a tumor is less favorable than a complete response but more favorable than an incomplete response in p53 mutant tumors. Other factors such as tumor heterogeneity will need to be explored to understand this conundrum.

WHY ARE SENESCENT CELLS DETRIMENTAL TO RESPONSE?

It is clear that in multiple cancer types the presence of senescent, p53 wild-type cells following treatment is correlated with a poor response. Although this is partly because of a lack of cell death and clearance of cells in the tumor, this is not the entire picture. Senescent cells with acquired SASP persist within the tumor and can impose protumorigenic properties on neighboring cells. It has now been thoroughly shown that many types of tumor cells made senescent by chemotherapy treatment produce cytokines and chemokines through the SASP (Coppe et al. 2008; Novakova et al. 2010; Jackson et al. 2012). Many of these cytokines and chemokines induce tumorigenic properties such as proliferation, survival, angiogenesis (Takamori et al. 2000; Dhawan and Richmond 2002; Karnoub and Weinberg 2006; Yang et al. 2006; Begley et al. 2008), and an increase in the cancer stem cell population (Achuthan et al. 2011; Cahu

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et al. 2012; Canino et al. 2012). Krtolica et al. (2001) showed that injection of tumor cells mixed with senescent fibroblasts cells greatly increased tumor growth in mice when compared with injections of tumor cells alone. However, another study showed that the presence of TIS cells did not increase tumor growth in prostate cancer (Ewald et al. 2008). Thus, while clearly the production of cytokines and chemokines by senescent cells fuels tumor growth, factors such as the number of senescent cells, the origin of these cells, and the quantity of the chemokines they produce will influence tumor growth.

CONCLUDING REMARKS

p53-mediated senescence convincingly contributes to both tumor suppression and response to therapy. Faced with a choice between OIS and progression during tumorigenesis or TIS and primary resistance in drug treatment, senescence is indeed a favorable outcome. However, the nature of senescent cells is to persist, and recent studies have shown that the secretory phenotype acquired by senescent cells may have detrimental effects, such as driving aging phenotypes or proliferation and survival of transformed cells. Future research investigating the choice between p53-mediated apoptosis and senescence and the fate of senescent cells in organisms (Jackson et al. 2011) will be needed to deepen our understanding of tumor suppression and drug response.

ACKNOWLEDGMENTS

The authors apologize to the many laboratories whose contributions were unable to be cited because of space limitations. The authors acknowledge funding from the Department of Defense W81XWH-14-1-0216 (J.G.J.) and the National Institutes of Health (NIH) CA82577 (G.L.).

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The Regulation of Cellular Functions by the p53 Protein: Cellular Senescence

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Cold Spring Harb Perspect Med 2017; doi: 10.1101/cshperspect.a026112 originally published online November 23, 2016

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