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Review

Tumor Cell Senescence in Cancer Treatment[1]

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Abstract

Cell senescence is broadly defined as the physiological program of terminal growth arrest, which can be triggered by alterations of telomeres or by different forms of stress. Neoplastic transformation involves events that inhibit the program of senescence, and tumor cells were believed until recently to have lost the ability to senesce. It has now become apparent, however, that tumor cells can be readily induced to undergo senescence by genetic manipulations or by treatment with chemotherapeutic drugs, radiation, or differentiating agents. Treatment-induced senescence, which has both similarities with, and differences from, replicative senescence of normal cells, was shown to be one of the key determinants of tumor response to therapy *in vitro* and *in vivo*. Although senescent cells do not proliferate, they remain metabolically active and produce secreted proteins with both tumor-suppressing and tumor-promoting activities. Expression of tumor-promoting factors by senescent cells is mediated, at least in part, by senescence-associated cyclin-dependent kinase inhibitors such as $p21^{Waf1/Cip1/Sdi1}$. Clinical and preclinical studies indicate that expression of different biological classes of senescence-associated growth-regulatory genes in tumor cells has significant prognostic implications. Elucidation of the genes and regulatory mechanisms that determine different aspects of tumor senescence makes it possible to design new therapeutic approaches to improving the efficacy and to decreasing the side effects of cancer therapy.

Senescence as an Anticarcinogenic Program of Normal Cells

Cell senescence, originally defined as proliferative arrest that occurs in normal cells after a limited number of cell divisions, has now become regarded more broadly as a general biological program of terminal growth arrest, a definition that is used throughout this review. Cells that underwent senescence cannot divide even if stimulated by mitogens, but they remain metabolically and synthetically active and show characteristic changes in morphology, such as enlarged and flattened cell shape and increased granularity (1). The most widely used surrogate marker with considerable (but not absolute) specificity to senescent cells is the SA-β-gal,[3] which is detectable by X-gal staining at pH 6.0 (2). SA-β-gal appears to reflect increased lysosomal mass of senescent cells (3). Senescent cells also produce many ECM components and secreted factors that affect the growth of their neighboring cells as well as tissue organization. In particular, paracrine factors produced by senescent cells have major effects on the growth and survival of tumor cells *in vitro* and *in vivo* (4, 5). Hence, senescence should not be viewed as merely an end point in a cell's life cycle but rather as a physiological state determined by the homeostatic programs of a multicellular organism.

Senescence ("growing old") was originally described in normal human cells explanted in culture; such cells undergo a finite number of divisions before permanent growth arrest (6). This gradual process of "replicative senescence" in human cells results primarily from the shortening and other structural changes of telomeres at the ends of the chromosomes (7). Telomeric changes in cells undergoing replicative senescence show similarities with DNA damage or may even directly involve such damage (8, 9). It is not surprising, therefore, that DNA damage was also found to induce rapid cell growth arrest, which was characterized as phenotypically indistinguishable from replicative senescence (7, 10). This "accelerated senescence," which does not involve telomere shortening, is also triggered in normal cells by the expression of mutant Ras or Raf (11, 12) and by some other forms of supraphysiological mitogenic signaling (1).

The key events in replicative and accelerated senescence of normal fibroblasts (the best-studied cellular system of senescence) are schematized in Fig. 1. Growth arrest of senescent cells is initiated with the activation of p53. In the case of replicative senescence, p53 protein is stabilized through the involvement of $p14^{ARF}$, a tumor suppressor that sequesters the Mdm2 protein, which promotes p53 degradation (13). Another protein that stimulates p53 under the conditions of replicative and RAS-induced accelerated senescence is promyelocytic leukemia (PML) tumor suppressor, which regulates p53 acetylation (14, 15). The activated p53 has multiple effects on gene expression, the most relevant of which in regard to senescence is transcriptional activation of $p21^{Waf1/Cip1/Sdi1}$, a pleiotropic inhibitor of different cyclin/CDK complexes (16). p21 induction causes cell cycle arrest in senescent cells (17, 18). The activation of p53 and p21 in senescent cells is only transient, and protein levels of p53 and p21 decrease after the establishment of growth arrest. Whereas p21 expression decreases, another CDK inhibitor, $p16^{Ink4A}$ becomes constitutively up-regulated, suggesting that p16 may be responsible for the maintenance of growth arrest in senescent cells (18, 19). Recent studies have implicated several positive and negative transcription regulatory factors (ETS, ID-1, BMI-1) in the transcriptional activation of p16 in senescent cells (20, 21). Other CDK inhibitors, $p27^{Kip1}$ (22, 23) and $p15^{Ink4b}$ (24), were also shown to play a role in fibroblast senescence. The best known (but by no means the only) mechanism for growth arrest induced by CDK inhibitors is the blockage of CDK-mediated inhibitory phosphorylation of tumor suppressor protein Rb. At the onset of senescence, Rb is converted to its active hypophosphorylated form, which sequesters and inhibits E2F transcription factors that are necessary for cell proliferation. The cellular levels of Rb decrease, however, after the onset of senescence in normal fibroblasts (19), suggesting that Rb may not play an active role in the maintenance of the senescent phenotype.

Both replicative and accelerated senescence are believed to be essential anticarcinogenic programs in normal cells. Replicative senescence imposes a limit on the total number of divisions a cell can undergo, and it should be expected, therefore, to interfere with tumor

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[3] The abbreviations used are: SA-β-gal, senescence-associated β-galactosidase (activity); ECM, extracellular matrix; CDK, cyclin-dependent kinase; IGF, insulin-like growth factor; IGFBP, IGF binding protein; MAPK, mitogen-activated protein kinase; FACS, fluorescence-activated cell sorting; TGF, transforming growth factor. RA, retinoic acid; NFκB, nuclear factor κB; EPLIN, epithelial protein lost in neoplasm; βAPP, β-amyloid precursor protein.

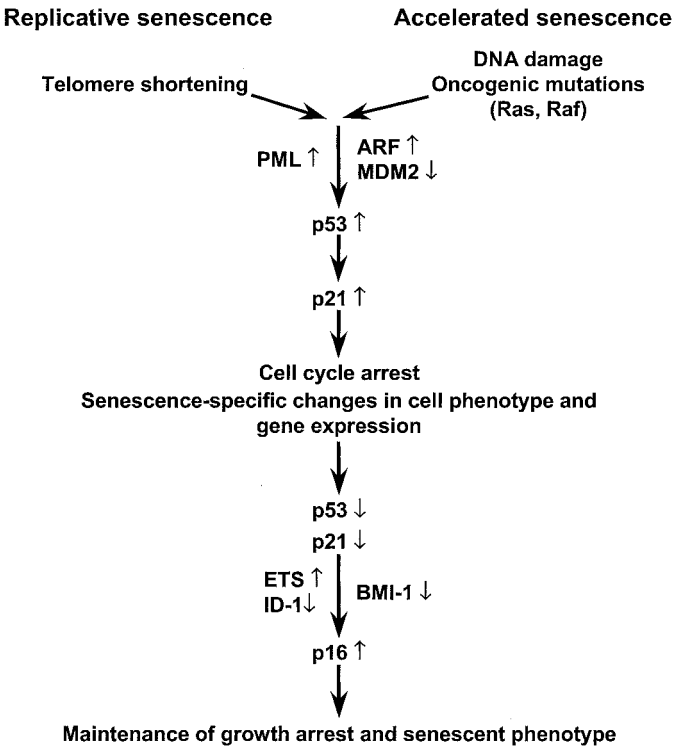

Fig. 1. Key events in replicative and accelerated senescence of normal fibroblasts. *PML*, promyelocytic leukemia.

growth. However, studies with mice deficient for the enzyme telomerase, which counteracts the shortening of telomeres, have yielded a more complicated picture. Telomere shortening, which occurs in telomerase-deficient mice after several generations, was found paradoxically to promote the rate of spontaneous carcinogenesis, most probably because telomeric aberrations destabilize the genome (7, 25). On the other hand, telomerase-deficient mice were more resistant to carcinogenesis under several conditions that increase the rate of tumor initiation, in agreement with the anticarcinogenic role of replicative senescence (7). The tumor-suppressive function may be more central to the program of accelerated senescence, which prevents the outgrowth of cells that have experienced oncogenic mutations (such as *RAS* or *RAF* mutations) or that underwent genome-destabilizing DNA damage.

In agreement with a role of senescence in cancer prevention, the process of carcinogenesis almost inevitably involves one or more events that inhibit senescence. Tumor cells avoid replicative senescence through the up-regulation of telomerase, or (less frequently) by using alternative mechanisms of telomere maintenance (ALT; Ref. 7). Telomerase expression does not prevent accelerated senescence induced by DNA damage (26), but both replicative and accelerated forms of senescence are inhibited by the inactivation of p53 or p16, two of the most commonly disabled tumor suppressors in different types of cancer. As a result, most tumor cells have both senescence-promoting changes (short telomeres, RAS mutations) and senescence-inhibiting adaptations (activation of telomerase, inactivation of p53 and/or p16). Until a few years ago, it had been a common assumption that neoplastically transformed cells are no longer capable of senescence. Today we know, however, that tumor cells can undergo senescence and can be forced into this process by various genetic manipulations and by epigenetic factors, including conventional anticancer drugs, radiation, and differentiating agents.

Inducing Senescence in Tumor Cells by Genetic Modifications

The earliest means used to induce senescence in "immortal" tumor cell lines was somatic cell fusion with normal cells or with other tumor cell lines. These studies (reviewed in Ref. 27) have demonstrated that senescence is dominant over immortality, and they have identified four senescence-determining complementation groups. A senescence-determining gene of one complementation group has been isolated from chromosome 4 and termed *MORF4*. Transfection of *MORF4*, which encodes a transcription factor-like protein, induces cessation of proliferation and the senescent phenotype after 18–35 population doublings in different tumor cell lines that belong to the same complementation group (28).

In the past 5 years, the transfection of many growth-inhibitory genes into tumor cell lines was shown to produce stable growth arrest, followed by the appearance of senescence-associated phenotypic changes and SA-β-gal expression. Growth-inhibitory genes that induce senescence-like growth arrest in tumor cells are listed in Table 1; most of these genes are known to play a role in the program of senescence in normal cells. These genes include *RB*, which induces senescence through a pathway that appears to depend on the induction of p27 (29), p53, and two p53-related proteins (p63 and p73), several CDK inhibitors (p21, p16, $p57^{Kip2}$, and $p15^{Ink4b}$), and IGFBP-rP1, a member of the IGF binding protein family, which is often up-regulated in normal senescent cells. Constitutively active mutants of two genes stimulating the MAPK pathway, including *RAF-1* and MAPK kinase *MKK6* (which specifically activates p38HOG), also induced tumor cell senescence.

Is the senescence-like growth arrest induced in tumor cells by the overexpression of growth-inhibitory genes irreversible? The ability of tumor cells to recover once the expression of a growth-inhibitory gene has been turned off has been investigated for *p53*, *p21*, and *p16* tumor suppressors, which were expressed in tumor cells from regulated

Table 1 *Genes that induce senescence-like growth arrest in tumor cells*

Gene	Tumor cell line	Reference
p53	EJ bladder ca[a]; NSCLC H358 lung ca	(30, 104)
p63	EJ bladder ca	(105)
p73	EJ bladder ca	(105)
RB	MDA-MB-468 breast ca; 5637 bladder ca; Saos-2 osteosarcoma	(106)
$p21^{Waf1/Cip1/Sdi1}$	HT1080 fibrosarcoma; EJ bladder carcinoma; H1299 lung ca	(31, 32, 107, 108)
$p16^{Ink4A}$	U-1242 MG glioma; U251 glioma; U2-OS osteosarcoma	(33, 109, 110)
$P57^{Kip2}$	U343 MG-A, U87 MG, and U373 MG astrocytoma	(111)
$p15^{Ink4B}$	U251 glioma	(110)
E2 papillomavirus protein (inhibitor of E6 and E7)	HeLa, HT-3, and CaSki cervical ca	(37) (38)
IGFBP-rP1	M12 prostate ca; MCF-7 breast ca	(100, 112)
Activated RAF-1	LNCaP prostate ca	(75)
Activated MKK6	U2-OS osteosarcoma	(43)

[a] ca, carcinoma; NSCLC, non-small cell lung cancer.

promoters (30–33). In all of these cases, the ability of the cells to grow and form colonies after the promoter shutoff was inversely related to the duration of expression of the tumor suppressor, with very few cells recovering after prolonged induction (4–5 days). The failure to recover after the release from p21-induced growth inhibition was also shown to depend on the level to which p21 expression was induced (34). The latter study, in which p21 was expressed from an inducible promoter in HT1080 fibrosarcoma cells, has also addressed the mechanism of the failure of the cells to recover after the shutoff of p21. All of the cells, despite their senescent phenotype and regardless of the duration or the magnitude of p21 induction, were found to reenter the cycle and replicate their DNA. On entering mitosis, however, most of the cells that were released after prolonged arrest developed grossly abnormal mitotic figures and either died through mitotic catastrophe or underwent senescence-like growth arrest in a subsequent cell cycle (34). It remains to be determined whether the failure to recover from senescence-like growth arrest induced by any other growth-inhibitory genes is attributable to a genuinely permanent cell cycle arrest that can be maintained without the inducing protein.

Another type of genetic manipulation that induces senescence in tumor cells is based on inhibiting the tumor proteins that counteract senescence. Somewhat surprisingly, the inhibition of telomerase by a dominant-negative mutant was found to induce cell death rather than senescence in tumor cell lines (35). This result can be understood in light of the antiapoptotic function of telomerase, which may be independent of its effect on telomeres (36), and which may also reflect the induction of mitotic catastrophe by abnormal telomeric structures. In contrast to the outcome of telomerase inhibition, senescence was readily induced in cervical carcinoma cells by inhibiting papillomavirus oncoproteins E6 and E7, which inhibit *p53* and *Rb* tumor suppressors, respectively. Introduction of bovine papillomavirus protein E2, a negative regulator of both E6 and E7, into several human cervical carcinoma cell lines induced accelerated senescence in almost 100% of tumor cells (37, 38). The effect of E2 was not accompanied by telomere shortening (37), and it was not prevented by constitutive overexpression of telomerase (39). Induction of senescence by E2 was associated with p53 stabilization and with strong induction of p21, and it was prevented by using p21-inhibiting antisense oligonucleotides or by increasing the expression of E6 or E7 (38). These results demonstrate that tumor cells are "primed" to undergo accelerated senescence once senescence-restraining mechanisms that inhibit the p53 and Rb pathways are removed. Enhancement of the extant program of accelerated senescence in tumor cells can be viewed, therefore, as a biologically justified approach to cancer therapy.

Induction of Senescence in Tumor Cell Lines by Chemotherapy, Radiation, and Retinoids

The propensity of tumor cells to undergo senescence in response to damage was demonstrated by the analysis of the effects of chemotherapeutic drugs and radiation on cell lines derived from different types of human solid tumors (40). A wide variety of anticancer agents induced senescence-like morphological changes and SA-β-gal expression in tumor cells. When equitoxic (ID_{85}) doses of different agents were applied to HT1080 fibrosarcoma cells, the strongest induction of the senescent phenotype was observed with DNA-interactive agents doxorubicin, aphidicolin, and cisplatin; a somewhat weaker response was seen with ionizing radiation, cytarabine, and etoposide; and the weakest effect was seen with microtubule-targeting drugs (Taxol and vincristine). Induction of senescence by the drugs was dose-dependent, and it was detectable even at the lowest drug doses that had a measurable growth-inhibitory effect. Moderate doses of doxorubicin induced the senescent phenotype in 11 of 14 cell lines derived from different types of human solid tumors (40). Other investigators have demonstrated the induction of the senescent phenotype in different tumor cell lines treated with cisplatin (41), hydroxyurea (42, 43), doxorubicin (44, 45), camptothecin (46), or bromodeoxyuridine (47, 48). Drug-induced senescent phenotype in tumor cells was not associated with telomere shortening and was not prevented by the overexpression of telomerase (45). Notably, in some of the cell lines, the senescent phenotype was observed in 10–20% of the cells even without drug treatment (40), suggesting that tumor cell senescence could develop spontaneously, possibly in response to subtle changes in the cell environment.

Drug-induced senescent phenotype was specifically associated with the tumor cells that underwent terminal growth arrest in response to treatment (31, 40, 49). The most conclusive evidence for this came from the analysis of growth-arrested and proliferating cells that were separated after release from the drug. This FACS-based separation procedure involves labeling cells with a lipophilic fluorophore PKH2, which stably incorporates into the plasma membrane and distributes evenly between daughter cells, resulting in gradual decrease in PKH2 fluorescence with increasing numbers of cell divisions (50). In the experiment shown in Fig. 2 (from Ref. 49), HCT116 cells were exposed to 200 μM doxorubicin for 24 h and then were labeled with PKH2. Changes in PKH2 fluorescence were monitored on subsequent days. Drug-treated HCT116 cells remained growth arrested ($PKH2^{hi}$) for 2–3 days after the removal of doxorubicin, but a proliferating cell population ($PKH2^{lo}$) emerged starting from day 4. A large fraction of cells, however, remained $PKH2^{hi}$ and did not change their fluores-

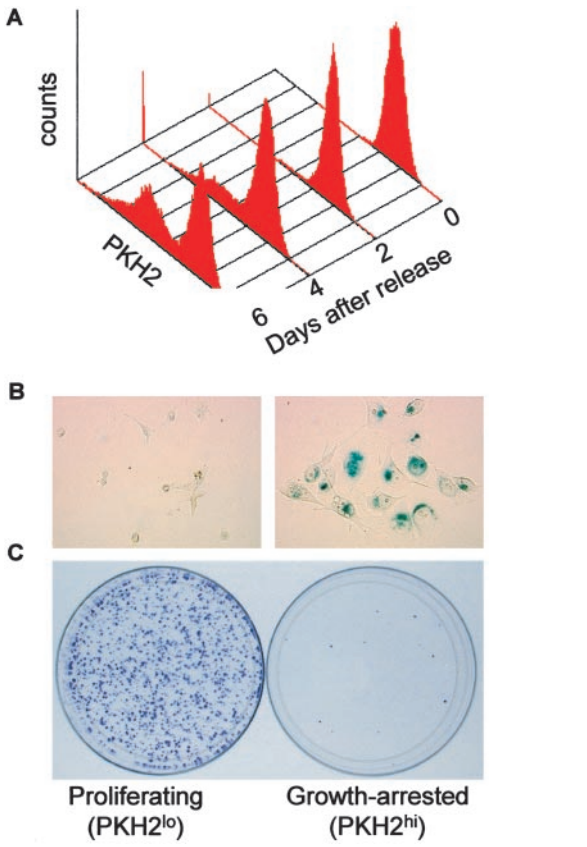

Fig. 2. Doxorubicin-induced senescence in HCT116 colon carcinoma cells (from Ref. 49). *A*, FACS profiles of PKH2 fluorescence of HCT116 cells on the indicated days after release from doxorubicin. *B*, SA-β-gal staining of $PKH2^{hi}$ (*right*) and $PKH2^{lo}$ (*left*) populations, separated 6 days after release from the drug, photographed at ×200. *C*, colony formation by $PKH2^{hi}$ and $PKH2^{lo}$ populations, separated 9 days after drug treatment and plated at 10,000 live cells per 10-cm plate.

cence throughout the experiment, indicating that these cells did not divide even after release from the drug (Fig. 2*A*). Six to 9 days after treatment, the cells were separated by FACS into $PKH2^{hi}$ (growth-arrested) and $PKH2^{lo}$ (proliferating) fractions. The $PKH2^{hi}$ cells were large, flat, and SA-β-gal+, but $PKH2^{lo}$ cells did not express the markers of senescence and were otherwise indistinguishable from the untreated cells (Fig. 2*B*). The $PKH2^{lo}$ (proliferating) but not the $PKH2^{hi}$ (growth-arrested) cells gave rise to colonies (Fig. 2*C*), indicating that the senescent phenotype of doxorubicin-treated HCT116 cells is associated with terminal growth arrest (49). On the other hand, when doxorubicin-treated HT1080 fibrosarcoma cells were analyzed by a similar procedure, most of the cells in the $PKH2^{hi}$ population divided once or twice after release from doxorubicin before undergoing terminal growth arrest, indicating that drug-induced senescent phenotype can be associated with both an immediate and a delayed terminal growth arrest (40).

The role of senescence as a determinant of treatment response was also indicated by two other *in vitro* studies. One of these studies found that expression of the MDR1 P-glycoprotein, which acts both as a drug efflux pump and as an inhibitor of apoptosis, protects a HeLa derivative and NIH 3T3 cells from radiation-induced apoptosis, but it does not increase their clonogenic survival. This apparent paradox was resolved by finding that a decrease in the fraction of apoptotic cells was accompanied by a commensurate increase in the fraction of cells undergoing either senescence or mitotic catastrophe (the principal nonapoptotic form of cell death), indicating that the latter responses, without apoptosis, are sufficient to stop the proliferation of tumor cells (51). The second study identified a novel phenotype of resistance to multiple DNA-interactive drugs, and showed that this form of resistance was associated with a decreased senescence response (52).

Aside from cytotoxic drugs and radiation, tumor cell senescence was also found to be induced by TGF-β (53) and by compounds that are usually referred to as "differentiating agents," including sodium butyrate (54) and retinoids. The induction of senescence has been analyzed in most detail for retinoids, natural and synthetic derivatives of vitamin A, which regulate cell growth and differentiation through their effects on gene expression. The latter effects are mediated by the binding of retinoids to retinoid receptors that act as regulators of transcription. Retinoid-induced growth arrest of tumor cells is commonly assumed to result from the induction of differentiation, and, in some cases, this assumption has been corroborated by the appearance of differentiation-specific markers in retinoid-treated cells. It has now become apparent, however, that retinoid treatment can also induce senescence rather than differentiation (55). In particular, exposure of MCF-7 breast carcinoma cells to a low noncytotoxic dose of *all-trans* RA induced growth arrest and the senescent phenotype (40). cDNA microarray analysis showed that this response involves the up-regulation of several senescence-associated genes but not of any markers of epithelial differentiation (56). In another example (57), two sublines of the same neuroblastoma cell line SK-N-SH showed different morphological responses to RA. In one subline, RA induced neuronal differentiation, as defined by morphological and antigenic markers. In the other subline, RA treatment produced morphological features of senescence and SA-β gal expression. Significantly, RA treatment increased the levels of p21 in the differentiating SK-N-SH cells, but it *decreased* p21 expression in the subline undergoing senescence, suggesting that p21 could act as a switch between differentiation and senescence in retinoid-treated cells (57). RA was also shown to decrease p21 levels in MCF-7 cells (58). In contrast to RA-induced senescence, p21 levels are increased under the conditions of senescence induced by DNA-damaging chemotherapeutic drugs (31, 49). As discussed in a later section, these differences in p21 expression are paralleled by major differences in the spectra of genes that are up-regulated in senescent tumor cells.

Senescence as a Determinant of *in Vivo* Treatment Response

The induction of tumor senescence by anticancer agents is not limited to cell culture. SA-β-gal staining showed that the senescent phenotype develops in human tumor xenografts grown in nude mice and treated *in vivo* with a retinoid (fenretinide; Ref. 40) or with doxorubicin (59). Recently te Poele *et al.* (44) became the first to investigate the correlation between the senescence response and chemotherapeutic treatment in clinical cancer. This study used newly sectioned material from frozen archival breast tumors of patients who had or had not received neoadjuvant chemotherapy (the CAF regimen: cyclophosphamide, doxorubicin, and 5-fluorouracil). The senescent phenotype was detected by SA-β-gal activity, and the tumors were also stained for p53 and p16. Although SA-β-gal enzymatic activity is unstable even in freshly frozen tissue samples, te Poele *et al.* have succeeded in demonstrating SA-β-gal in 15 (41%) of 36 treated tumors. Remarkably, SA-β-gal staining was confined to tumor cells, whereas normal tissue was completely negative, suggesting that chemotherapy-induced senescence is a specific response of tumor cells. Tumor sections of patients who had not received chemotherapy showed SA-β-gal staining of isolated tumor cells in 2 of 20 cases, suggesting that spontaneous senescence also occurs in clinical cancer. SA-β-gal staining in breast cancer was associated with low p53 staining, indicative of the lack of mutant p53, and with high staining for p16 (44).

In another recent study (60), both senescence and apoptosis were shown to determine an *in vivo* response to chemotherapy in Eμ-myc lymphoma, a transgenic mouse model of B-cell lymphoma. When the program of apoptosis in Eμ-myc lymphoma was inhibited by transduction with a retrovirus that expresses the anti-apoptotic gene *BCL-2*, senescence became the principal tumor response to cyclophosphamide, as indicated by apparently complete cessation of DNA replication and mitosis and by drastic induction of the SA-β-gal marker. The senescence response (along with apoptosis) was also observed in the absence of *BCL-2*. Treatment-induced senescence became undetectable on the knockout of either p53 (which also abolished the apoptotic response) or p16 (which had no effect on apoptosis). Inhibition of either apoptosis or senescence in Eμ-myc lymphoma made these tumors significantly more sensitive to chemotherapy, indicating that both of these physiological programs contribute to treatment success (60).

It should be noted, however, that senescence is not the only anti-proliferative response that determines treatment response in the absence of apoptosis. Another principal effect of anticancer agents is mitotic catastrophe, cell death resulting from abnormal mitosis, which usually ends in the formation of large cells with multiple micronuclei and uncondensed chromatin (reviewed in Ref. 59). Etoposide-induced mitotic catastrophe in HeLa cells was greatly increased when apoptosis was inhibited by BCL-2 (61), and, as mentioned above, both mitotic catastrophe and senescence were augmented in irradiated tumor cells with MDR1-suppressed apoptotic response (51). There are as yet no *in vivo* studies in which all three responses have been analyzed at the same time. Such analysis should elucidate the relative contribution of apoptosis, senescence, and mitotic catastrophe to the overall outcome of cancer treatment.

p53, p21, and *p16* in Tumor Cell Senescence

Some observations indicate that *p53*, *p21*, and *p16*, which regulate replicative and accelerated senescence in normal cells (see

Fig. 1), also play a role in treatment-induced senescence of tumor cells. Treatment-induced senescence in murine Eμ-myc lymphoma required wild-type *p53* and *p16* (60), and p16 expression correlated with SA-β-gal staining in treated breast cancers (44). As mentioned above, *p53* and *p16* are frequently inactivated in cancers. If these genes were required for treatment-induced senescence, one could expect that the majority of tumors, which are deficient in one or both of these genes, would be unable to undergo senescence. This, however, is not the case. Chemotherapeutic drugs readily induced senescence *in vitro* and *in vivo* in *p16*-deficient tumor cell lines, such as HT1080 and HCT116 (40). Furthermore, moderate doses of doxorubicin induced the senescent phenotype in *p53*-null Saos-2 cell line, in SW480 and U251 cells carrying mutant *p53*, and in HeLa and Hep-2 cell lines, in which *p53* function has been inhibited by papillomavirus protein E6 (40). In the breast cancer study of te Poele *et al.* (44), 20% of the SA-β-gal+ tumors showed high *p53* staining (suggestive of *p53* mutations) and 13% of the SA-β-gal+ tumors did not stain for *p16*, indicating that wild-type *p53* and *p16* induction are not necessary for senescence in clinical breast cancer.

The role of p53 and p21 in treatment-induced senescence was analyzed in HT1080 fibrosarcoma cells in which p53 function and p21 expression were blocked by a p53-derived genetic suppressor element and in HCT116 colon carcinoma cells with homozygous knockout of p53 or p21. In both cell lines, the inhibition or knockout of p53 or p21 strongly decreased but did not abolish drug- or radiation-induced senescence, as determined by PKH2 analysis of cell division and by SA-β-gal staining (31). Hence, p53 and p21 act as positive regulators of accelerated senescence in tumor cells, but they are not absolutely required for this response. In addition, as described above, retinoid-induced senescence involves a decrease rather than an increase in p21 expression. These observations suggest that some genes other than *p53*, *p21*, or *p16* are likely to play a role in accelerated senescence of tumor cells.

Inhibition of Cell Cycle Progression Genes and Induction of Intracellular and Secreted Growth Inhibitors in Senescent Tumor Cells

Additional determinants of drug-induced senescence in tumor cells were identified by gene expression profiling of doxorubicin-induced senescence in HCT116 colon carcinoma cells, which are p16-deficient and wild-type for p53 (49). The proliferating and senescent fractions of HCT116 cells were separated by PKH2 labeling and flow sorting 6–9 days after 1-day doxorubicin treatment (Fig. 2) and were used for RNA extraction. cDNA microarray hybridization followed by reverse transcription-PCR analysis of individual genes revealed major biological clusters of genes that were either down-regulated or up-regulated in senescent cells. More than one-half of all of the genes that were strongly inhibited in senescent cells are known to play a role in cell cycle progression, with the largest groups of genes involved in mitosis and DNA replication. The inhibition of these genes became apparent 1–2 days after doxorubicin treatment and was likely to contribute to the maintenance of drug-induced growth arrest. Analysis of HCT116 cell lines with homozygous disruption of either *p53* or *p21* demonstrated that doxorubicin-induced inhibition of cell cycle progression genes was fully dependent on *p21* (49). Furthermore, ectopic expression of *p21* in HT1080 fibrosarcoma cells was sufficient to inhibit the transcription of the same set of genes that are down-regulated in doxorubicin-treated cells (4). This effect of *p21* is exerted at the level of transcription, and it is mediated at least in part by negative regulatory elements in the corresponding promoters, such as CDE/CHR (cell cycle dependence element/cell cycle gene homology region; Ref. 62).

In addition to the down-regulation of genes required for cell cycle progression, senescent HCT116 cells were also found to up-regulate multiple genes with growth-inhibitory activities (Table 2). Concerted and sustained induction of such genes explains the growth arrest of senescent cells despite the lack of p16 and suggests that this arrest is maintained by many apparently redundant mechanisms. Several of these genes are known or putative tumor suppressors that are silenced in the course of neoplastic transformation but become reactivated with the onset of senescence. The up-regulated growth inhibitors include *p21*, tumor suppressor *BTG1*, a related gene *BTG2*, and candidate tumor suppressor *EPLIN*. Of special interest, senescent HCT116 cells also overexpress several secreted growth inhibitors, including serine protease inhibitor Maspin, a tumor suppressor shown to inhibit the invasion and angiogenesis of breast and prostate cancers, as well as MIC-1/pTGF-β (a member of the TGF-β family), IGF-binding protein 6 (*IGFBP-6*), and amphiregulin, an epidermal growth factor (EGF)-related factor that inhibits the growth of several carcinoma cell lines although promoting the growth of normal epithelial cells (49). Exposure to chemotherapeutic drugs and radiation was previously shown to induce a similar set of secreted tumor-suppressing factors, and paracrine growth-inhibitory activities of the damaged cells have been documented by conditioned media and coculture assays (63). The finding that the same factors are stably overexpressed by senescent cells suggests that such cells may provide a reservoir of tumor-suppressing factors that may contribute to the long-term success of chemotherapy.

Induction of secreted tumor-suppressing factors was previously found to be mediated by *p53* (63). Analysis of *p53*-deficient HCT116 cells showed, however, that induction of senescence-associated growth inhibitors (intracellular or secreted) showed either no dependence on *p53* (*BTG1*, *IGFBP-6*) or limited *p53* dependence (*BTG2*, *EPLIN*, *Maspin*, *MIC-1*, *amphiregulin*). The latter genes were still induced in the absence of *p53*, albeit their induction was delayed or diminished relative to the cells with the wild-type *p53* (49). These results help to explain why *p53* deficiency diminishes but does not abolish drug-induced senescence (31). *p21* knockout in HCT116 cells had no effect on the induction of senescence-associated growth inhibitors (except for *EPLIN*; Ref. 49), explaining why *p21*-deficient cells can still undergo senescence (31). On the other hand, the reduction in the senescence response of *p21*-deficient cells can be readily explained by a failure to inhibit the transcription of cell cycle progression genes and by the lack of *p21* itself.

Concerted induction of several growth-inhibitory genes was also observed in retinoid-induced senescence of MCF-7 breast carcinoma cells (56). cDNA microarray hybridization and reverse transcription-PCR analysis showed that RA-induced senescent phenotype of MCF-7 cells is associated with the strong induction of four genes with growth-inhibitory activity. These genes (Table 2) encode intracellular inhibitors EPLIN and FAT10 (a ubiquitin family member), as well as a secreted growth-inhibitor IGFBP-3, which is related to IGFBP-6 up-regulated in senescent HCT116 cells), and a tumorigenicity-suppressing cell adhesion protein βIG-H3. Interestingly, a survey of the published genes that are inducible by retinoids in different types of tumor cells showed that retinoids induce many other intracellular and secreted growth inhibitors, most of which are also known to be up-regulated in senescent cells (55). βIG-H3, IGFBP-3, and a related inhibitor IGFBP-4 were also up-regulated in HeLa cells by bromodeoxyuridine treatment, under the conditions that induce senescence in this cell line (48). Induction of multiple growth-inhibitory proteins

Table 2 *Tumor-inhibiting and tumor-promoting genes induced in senescent tumor cells*

Gene	Senescent cells/inducer	Reference
Intracellular growth inhibitors		
$p21^{Waf1/Cip1/Sdi1}$ (pleiotropic CDK inhibitor)	Different tumor cell lines/various drugs and radiation	(31, 42, 48, 49)
$p16^{Ink4A}$ (CDK4/6 inhibitor; tumor suppressor)	K562 leukemia/hydroxyurea; murine Eμ-myc lymphoma/cyclophosphamide	(42, 60)
$p27^{Kip1}$ (CDK2 inhibitor)	K562 leukemia/hydroxyurea	(42)
BTG1 (tumor suppressor)	HCT116 colon ca[a]/doxorubicin	(49)
BTG2 (BTG1-related)	HCT116 colon ca/doxorubicin	(49)
EPLIN (actin-binding protein; putative tumor suppressor)	HCT116 colon ca/doxorubicin MCF-7 breast ca/retinoids	(49, 56)
FAT10 (ubiquitin family)	MCF-7 breast ca/retinoids	(56)
Secreted and cell-surface-associated tumor-inhibiting proteins		
IGFBP-3 (IGF-binding protein; cytostatic; apoptogenic)	HeLa cervical ca/bromodeoxyuridine; MCF-7 breast ca/retinoids	(48, 56)
IGFBP-6 (IGF-binding protein; cytostatic)	HCT116 colon ca/doxorubicin	(49)
IGFBP-4 (IGF-binding protein; cytostatic)	HeLa cervical ca/bromodeoxyuridine	(48)
Maspin (serine protease inhibitor; inhibits invasion and angiogenesis; tumor suppressor)	HCT116 colon ca/doxorubicin	(49)
MIC-1 (TGF-β family)	HCT116 colon ca/doxorubicin	(49)
βig-H3 (ECM protein; inhibits tumorigenesis)	HeLa cervical ca/bromodeoxyuridine; MCF-7 breast ca/retinoids	(48, 56)
Secreted and cell-surface-associated tumor-promoting proteins		
TGFα (mitogen; EGFR ligand)	HCT116 colon ca/doxorubicin	(49)
Cyr61 (angiogenic; mitogenic)	HCT116 colon ca/doxorubicin	(49)
CTGF (Cyr61-related; angiogenic; mitogenic)	HT1080 fibrosarcoma/p21	(4)
Epithelin/granulin (mitogenic)	HT1080 fibrosarcoma/p21	(4)
Galectin-3 (antiapoptotic)	HT1080 fibrosarcoma/p21	(4)
Prosaposin (antiapoptotic)	HT1080 fibrosarcoma/p21 HCT116 colon ca/doxorubicin	(4, 49)
βAPP (Alzheimer's β amyloid precursor; mitogenic)	HT1080 fibrosarcoma/p21 HCT116 colon ca/doxorubicin	(4, 49)
Cathepsin B (serine protease; promotes invasion)	HT1080 fibrosarcoma/p21	(4)
Plasminogen activator inhibitor-1 (serine protease inhibitor; promotes angiogenesis and invasion)	HeLa cervical ca/bromodeoxyuridine; HT1080 fibrosarcoma/p21	(4, 48)
Dual-function growth regulators		
Amphiregulin (EGF family; mitogenic in normal cells; antimitogenic in tumor cells)	HCT116 colon ca/doxorubicin	(49)
Activin A (TGF-β family)	HT1080 fibrosarcoma/p21	(4)
CD44 (cell adhesion molecule; pleiotropic growth modulator)	HCT116 colon ca/doxorubicin	(49)
Jagged-1 (Notch receptor; stem cell growth regulator; angiogenic)	HCT116 colon ca/doxorubicin	(49)

[a] ca, carcinoma; EGF, epidermal growth factor; EGFR, EGF receptor.

appears, therefore, to be a general phenomenon in treatment-induced senescence of tumor cells.

Tumor Senescence Is Associated with the Induction of Tumor-Promoting Genes

Inhibition of cell proliferation, however, is not the only aspect of tumor senescence with potential clinical implications. Replicative senescence of normal fibroblasts is characterized by changes in the expression of multiple proteins (1). Some of the proteins that are highly expressed in senescent cells have long-range pathogenic effects, including βAPP (64), as well as degradative enzymes, inflammatory cytokines, and growth factors, which may contribute to carcinogenesis and tumor progression (1). Indeed, coculture and conditioned media experiments showed that normal human fibroblasts undergoing either replicative or accelerated senescence stimulate the growth of transformed epithelial cells *in vitro* and *in vivo* (5). These paracrine effects of senescent fibroblasts closely resemble the cancer-promoting activities of tumor-associated stromal fibroblasts (65). The procarcinogenic function of normal senescent cells *in vivo* is also supported by the findings that SA-β-gal expression in normal human hepatocytes is strongly correlated with the presence of hepatocellular carcinoma in the surrounding liver (66), and that prostate enlargement correlates with SA-β-gal expression in prostate epithelial cells (67).

In agreement with these observations in normal senescent cells, doxorubicin-treated senescent HCT116 carcinoma cells also showed increased expression of genes for many proteins with diverse paracrine activities. In fact, secreted factors, ECM components, ECM receptors and other integral membrane proteins make up 33 of 68 genes with known functions that are strongly induced in senescent HCT116 cells (in contrast, only 2 of 64 known genes that are down-regulated in such cells belong to this category). The secreted proteins up-regulated in senescent HCT116 cells include not only the above-described growth inhibitors but also several proteins with mitogenic, antiapoptotic, and angiogenic activities (Table 2; Ref. 49). Some of these proteins are an ECM component Cyr61 with mitogenic and angiogenic functions, an antiapoptotic and mitogenic ECM factor prosaposin, transforming growth factor TGFα, and several proteases that may potentially contribute to metastatic growth. Several other genes induced in senescent cells encode cell adhesion and cell-cell contact proteins and ECM receptors, including several integrins and syndecan-4, involved in angiogenesis. Other transmembrane proteins induced in senescent cells are βAPP, which has mitogenic activity (68), another amyloid precursor, BRI, associated with an Alzheimer's-like disease, and growth-regulatory proteins CD44 and Jagged-1 (49). Thus, senescence-associated changes in gene expression involve the induction of both tumor-suppressive and tumor-promoting proteins, as well as proteins involved in age-related diseases other than cancer. Relative expression of different biological classes of senescence-associated genes is likely, therefore, to determine whether tumor senescence would have a mostly positive or a mostly negative effect on the outcome of treatment.

Role of CDK Inhibitors in Senescence-associated Changes in Gene Expression: Implications for Tumor-Promoting Stromal Fibroblasts

About one-third of senescence-associated genes that were induced by doxorubicin in HCT116 cells showed decreased or delayed induction in a p21−/− derivative of this cell line, indicating that p21 plays

a role not only in the inhibition but also in the induction of gene expression in senescent cells. Some of the genes that showed p21 dependence encode secreted mitogenic/antiapoptotic proteins, such as prosaposin, TGFα, and βAPP (49). These findings were in accord with the results of cDNA microarray analysis of the effects of p21 on gene expression in HT1080 fibrosarcoma cells (4). p21 induction in the latter cells produces growth arrest and the senescent phenotype, inhibits transcription of multiple genes, most of which are involved in cell cycle progression, and also leads to the induction of a set of genes with important paracrine activities. Altogether, 40% of p21-inducible genes encode secreted proteins, ECM components, or ECM receptors. Most of the genes induced by p21 in HT1080 cells were also induced in WI-38 normal human fibroblasts infected with a p21-expressing adenoviral vector[4] and in a human melanoma cell line treated with a polyamine-depleting regimen that induces strong p21 expression (69). Furthermore, the effects of p21 on the induction of gene expression in HT1080 cells can be largely reproduced by another senescence-associated CDK inhibitor, p16.[5]

Many p21-induced genes are known to be up-regulated during replicative senescence (4). Furthermore, products of many genes that are induced by p21 have been linked to age-related diseases, including Alzheimer's disease, amyloidosis, atherosclerosis, and arthritis. Some examples are βAPP, serum amyloid A, tissue transglutaminase, connective tissue growth factor (CTGF), and $p66^{Shc}$, a positive mediator of oxidative stress, knockout of which increases toxin resistance and the life span in mice (70). Another group of p21-induced genes encode secreted proteins with known mitogenic, antiapoptotic, or angiogenic activities (Table 2), such as prosaposin, epithelin/granulin, galectin-3, CTGF, or VEGF-C. The induction of such genes produces paracrine growth-promoting activities, as demonstrated by the fact that conditioned media from p21-arrested HT1080 cells has mitogenic and antiapoptotic effects (4). These paracrine effects of p21 induction mimic the tumor-promoting activities that were demonstrated in different types of senescent fibroblasts (5) and in tumor-associated stromal fibroblasts (65). As discussed elsewhere (71), all of the treatments that are known to induce the tumor-promoting functions of stromal fibroblasts also result in p21 induction, suggesting that p21 or related proteins could be responsible for the paracrine tumor-promoting functions of stromal fibroblasts.

Induction of gene expression by p21 occurs at the level of transcription, because p21 stimulated the activity of all six of the tested promoters of p21-inducible genes.[6] Although p21 is best known as an inhibitor of cyclin/CDK complexes, it also interacts with many transcription factors and cofactors and regulators of signal transduction (16), which can account for its pleiotropic effects on gene expression. One of the effects of p21 is the augmentation of transcription factor NFκB (72). This effect is mediated through the activation of transcription cofactors/histone deacetylases p300 and CBP, which augment not only NFκB but also many other inducible transcription factors (72). The stimulation of p300 and CBP by p21 is mediated through a repressor domain of p300/CBP termed CRD1 (73). Recently, the ability of p21 to stimulate p300-mediated transactivation of different genes was investigated in U-2 OS osteosarcoma cells cotransfected with p21, p300 fused to yeast Gal4 DNA-binding protein domain, and reporter constructs containing core promoters of different genes linked to Gal4 DNA-binding sites (74). p21 enhanced the effect of p300 on the core promoters of five genes that are known to be induced by p21 and on three strong promoters of viral origin, but p21 did not stimulate the transactivation of four core promoters from genes that are not induced by p21. The effect of p21 on promoter transactivation by p300 appears, therefore, to be an important determinant of the selectivity of the induction of gene expression by p21. The ability of a core promoter to respond to p21 was found to be determined primarily by the sequences flanking the TATA box (74). The effect of p21 in this system does not appear to be mediated by cyclin/CDK binding, because two p21 mutants deficient in such binding were as active in stimulating the effect of p300 as was the wild-type p21 (74). On the other hand, the same mutants showed little or no effect on the transcription of p21-inducible genes or complete promoters of such genes in HT1080 cells,[6] suggesting that there may be cell type-specific differences in the induction of disease-associated genes by p21.

The involvement of p21 and other CDK inhibitors in the expression of genes associated with the undesirable effects of senescence suggests that treatments that induce senescence without activating p21 or p16 may be more beneficial in the long term. In agreement with this hypothesis, none of the genes that were found by cDNA microarray analysis to be up-regulated in retinoid-induced senescence of MCF-7 breast carcinoma cells encode secreted factors with tumor-promoting activities, or other disease-promoting factors (such as amyloid proteins; Ref. 56). This result is likely to reflect the lack of p21 or p16 induction in RA-induced senescence of MCF-7 cells. Thus, the positive effects of tumor senescence (permanent growth arrest of tumor cells and secretion of tumor-suppressing factors) can be separated from the disease-promoting activities of senescent cells.

Prognostic Implications of Tumor Senescence: Examples from Prostate Cancer

Senescent cells appear within the tumor either as a consequence of treatment or, spontaneously, as a result of environmental stress or sporadic inactivation of senescence-restraining mechanisms in an individual cell. Senescent cells are generally resistant to apoptosis, and senescent fibroblasts in culture are known to survive for more than a year. The persistence of senescent cells in the tumor is a double-edged sword. On one hand, the senescent tumor cells do not proliferate and, furthermore, serve as a reservoir of secreted factors that inhibit tumor growth. On the other hand, the same cells can also produce secreted factors with mitogenic, antiapoptotic, and angiogenic activities. These tumor-promoting functions of senescent cells are determined to a large extent by the expression of p21 and p16. The presence of senescent cells in the tumor and the relative abundance of different proteins produced by the senescent cells are important biological factors that should have significant prognostic implications for the disease outcome. One would expect that more aggressive tumors might contain few or no senescent cells. Alternatively, such tumors may have a substantial fraction of senescent cells that express CDK inhibitors (p16 or p21), as well as senescence-associated tumor-promoting factors that are up-regulated by CDK inhibitors. On the other hand, tumors containing senescent cells that express high levels of secreted growth inhibitors but little p16 or p21 should have a more favorable prognosis. This concept is illustrated in Fig. 3.

Although the breast carcinoma study of te Poele *et al.* (44) is the only one thus far that has directly addressed the development of tumor senescence in clinical cancer, there is abundant evidence in the literature in support of various aspects of the model shown in Fig. 3. This can be illustrated especially well by the results of clinical and biological studies in prostate cancer as follows (similar examples can be found for many other tumor types):

(*a*) a prostate carcinoma cell line LNCaP growing in culture contains 10–15% SA-β-gal+ cells, suggesting that prostate cancer cells can undergo spontaneous senescence. The senescent fraction in this

[4] M. Swift, B. D. Chang, E. V. Broude, and I. B. Roninson, unpublished observations.
[5] B. D. Chang, M. Shen, and I. B. Roninson, unpublished observations.
[6] J. Poole, B. D. Chang, and I. B. Roninson, unpublished observations.

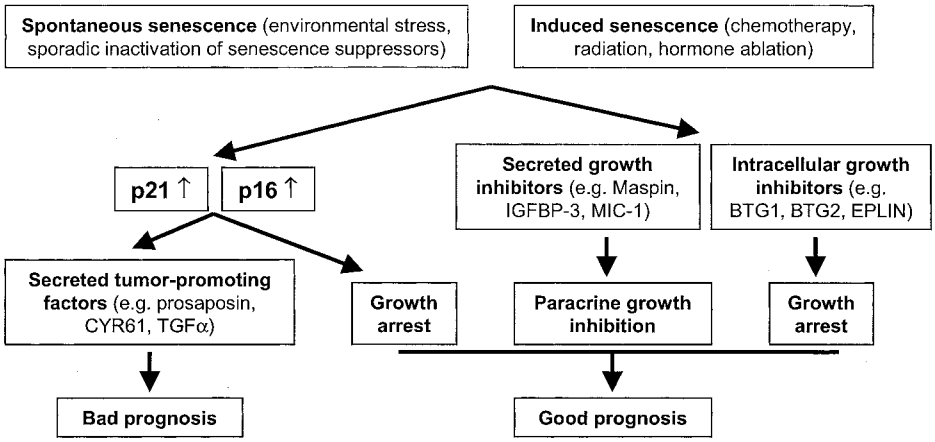

Fig. 3. Tumor senescence and its consequences.

cell line is increased by doxorubicin (40) or by transfection with an activatable form of c-Raf (75), thus demonstrating the susceptibility of prostate cancer cells to accelerated senescence;

(*b*) indirect observations in radiation therapy of prostate cancer suggest that the induction of senescence may be a primary mode of treatment response. In particular, complete regression of prostate cancers was reported in some patients to take more than a year after radiation treatment (76). This slow course of tumor disappearance seems most consistent with radiation-induced senescence. In an example from another tumor type, regression of desmoid tumors took up to 2 years after radiation treatment (77);

(*c*) p16, the CDK inhibitor primarily associated with senescence, is a tumor suppressor, which is infrequently mutated in prostate cancer (78). p16 expression was not detectable by immunohistochemistry in the normal prostate (79), but it is observed in close to one-half of prostate carcinomas (79). In all of these studies, p16 was found to be an unfavorable prognostic marker for prostate cancer (80, 81). In particular, p16 expression was an independent indicator of early relapse after radical prostatectomy, and p16 was elevated in cancers relative to benign prostatic hyperplasia (BPH). These paradoxical adverse correlations of the tumor suppressor p16 are readily explained by the ability of p16 to up-regulate secreted tumor-promoting factors. Notably, higher p16 levels in tumor cells have also been associated with the history of androgen ablation treatment (79), a result that probably reflects the induction of prostate cancer senescence by this treatment. Strong adverse correlations for p16 expression have also been reported in breast cancer (82–84);

(*d*) similar unfavorable prognostic correlations were found for another CDK inhibitor, p21, in the majority of studies that analyzed this protein in prostate cancer (85–90). p21 expression in prostate cancer showed no correlation with p53 status, suggesting that p21 is induced in this tumor primarily by p53-independent mechanisms (85). Like p16, p21 was found to be an independent marker of early relapse after prostatectomy, and it was associated with high pathological grade and high Ki-67 index. p21 was also shown to be a highly significant marker of progression from androgen-dependent to androgen-independent cancer (90). The adverse prognostic role of p21 is disputed in some reports (91, 92), but a potential explanation for such discrepancies is suggested by the findings of Sarkar *et al.* (88). The latter study noted a dependence of p21 correlations on the racial background (p21 is a strong independent marker of negative prognosis in Caucasians but not in African Americans) and suggested that some as yet unknown genetic factors may affect the role of p21 in prostate cancer. Studies of p21 expression in other tumor types produced both favorable prognostic correlations (reflecting the role of p21 as a marker of wild-type p53 function) and unfavorable correlations, similar to those in prostate cancer (71);

(*e*) prostate cancers also express senescence-associated growth inhibitors with good prognostic correlations. One of the most commonly used markers in prostate cancer is IGFBP-3, a secreted protein that induces growth arrest and apoptosis. Low IGFBP-3 levels in the plasma, alone or in combination with high IGF-1, have been associated with the presence of advanced prostate cancer (93, 94), and an increase in serum IGFBP-3 has been used as an indicator of treatment response (95). IGFBP-3 is induced at senescence in different types of normal and tumor cells (Table 2; Ref. 55). In particular, IGFBP-3 is consistently up-regulated in senescent prostate epithelial cells and silenced in prostate cancer cell lines and tumors (96). This senescence-associated growth inhibitor is induced in prostate cancer cells by many antiproliferative agents (97, 98). Another IGFBP family member, IGFBP-rP1 increases during senescence of normal prostate epithelial cells and is down-regulated in prostate cancer (99). Overexpression of IGFBP-rP1 induces growth arrest and the senescent phenotype in the M12 prostate cancer cell line (100); and

(*f*) the strongest correlations with good prognosis in prostate cancer have been reported thus far for another senescence-associated tumor suppressor, serine protease inhibitor Maspin. Maspin is induced to a very high level by DNA damage in several tumor cell lines, including LNCaP prostate carcinoma (101). The absence or low levels of Maspin in prostate cancer samples have been correlated to higher

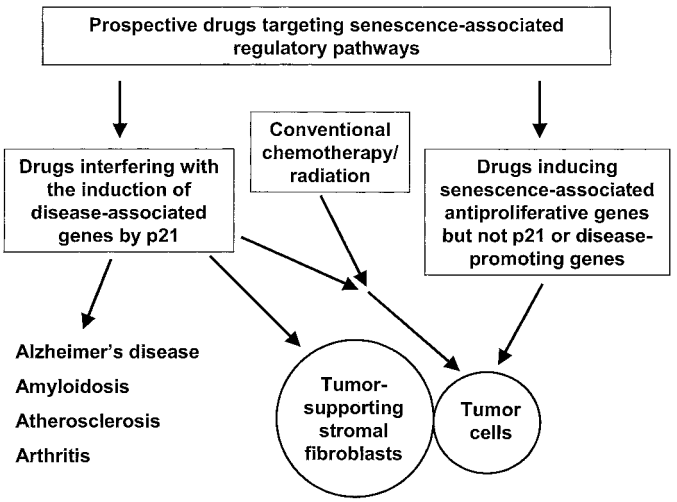

Fig. 4. Strategies for senescence-based therapy in cancer treatment.

tumor stages, histological dedifferentiation and early relapse (102). Conversely, Maspin expression was strongly elevated in the cancers of patients treated with neoadjuvant androgen ablation therapy, and treatment-induced Maspin was specifically associated with tumor cells that showed morphological effects of the treatment (103).

The above observations in prostate cancer seem to be in excellent agreement with the model depicted in Fig. 3. As predicted by the model, markers of senescence are observed in untreated tumors, but their expression is elevated after treatment. Furthermore, senescence-associated growth inhibitors (Maspin and IGFBP-3) correlate with good prognosis, whereas p16 and p21 correlate with bad prognosis. With the identification of multiple senescence-associated growth regulators (Table 2), it should now be possible to investigate their expression and coexpression in tumor tissues by conventional immunocytochemical techniques. Such analyses should allow us to determine the relationship between the expression of these proteins, tumor staging, and treatment outcome. It should also be possible to determine whether radiation or chemotherapy-induced tumor senescence leads to sustained secretion of senescence-associated growth inhibitors into bodily fluids. Production of such proteins could be an important factor in preventing the tumor growth. New diagnostic approaches that will arise from understanding the biology of tumor senescence may be of considerable benefit in the management of cancer patients.

Potential for Developing Senescence-based Anticancer Drugs

Activating the program of senescence in tumor cells seems an attractive approach to cancer treatment. This response to chemotherapy is induced by a wide variety of anticancer agents, even under the conditions of minimal cytotoxicity. Even if not all of the tumor cells are rendered senescent as a result of treatment, such cells may provide a reservoir of secreted tumor-suppressing factors that will inhibit the growth of nonsenescent cells. On the other hand, senescent cells can overexpress secreted tumor-promoting factors, as well as proteins associated with various pathological conditions. The side effects of senescence, which are mediated at least in part by CDK inhibitors, may have potential adversarial effects in the short term (growth stimulation of nonsenescent tumor cells) and in the long term (increased likelihood of *de novo* carcinogenesis and the development of age-related diseases). On the basis of these considerations, senescence-oriented therapeutic strategies may include two general strategies. The first direction is to develop the agents that will interfere with the induction of disease-promoting genes by CDK inhibitors. The second strategy is to develop drugs that will induce tumor cell senescence without up-regulating p21 (which, unlike p16, is almost never inactivated in tumors) or p21-inducible disease-promoting genes. These approaches are schematized in Fig. 4.

Elucidation of the mechanisms that mediate the induction of transcription by p21 or by other CDK inhibitors should provide the essential information for developing compounds that will prevent such induction. Such agents may include inhibitors of p21-stimulated transcription factors and cofactors, such as NFκB or p300. Alternatively, these compounds may be identified by *de novo* screening of chemical libraries, based on their effect on the expression of p21-inducible genes or promoter-reporter constructs. Agents that prevent the induction of gene expression by p21 are likely to interfere with the tumor-promoting paracrine activities of senescent cells that arise spontaneously or as a result of conventional chemotherapy or radiation therapy. These compounds may also block the tumor-promoting activities of stromal fibroblasts and may potentially be useful in the chemoprevention of age-related diseases, such as Alzheimer's disease or atherosclerosis (Fig. 4).

The second strategy in Fig. 4 is based on the development of agents that would induce tumor cell senescence without its associated side effects. The feasibility of this strategy is suggested by the finding that retinoid-induced senescence of breast carcinoma cells involves the induction of the tumor-suppressing but not of the disease-promoting genes. The therapeutic use of retinoids is limited by the fact that these drugs act though retinoid receptors, which are readily lost in tumor cells. Senescence-associated growth-inhibitory genes, however, contain no discernible retinoid receptor-binding sites in their promoters, and they appear to be induced by retinoids through an indirect mechanism (55). Furthermore, the same proteins are induced by retinoids and by nonretinoid drugs, such as doxorubicin or bromodeoxyuridine (Table 2). It seems likely, therefore, that the mechanisms that produce concerted up-regulation of retinoid-inducible growth inhibitors may also be stimulated by other inducers of senescence. Identification of senescence-associated growth-inhibitory genes makes it possible to develop high-throughput screening systems for agents that induce such genes. Thus, the elucidation of the biological aspects of tumor cell senescence offers plausible approaches to the development of novel therapeutic strategies to stop the growth of tumor cells.

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