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ACCELERATED CELLULAR SENESCENCE IN SOLID TUMOR THERAPY

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Accelerated cellular senescence (ACS) is an emerging concept that implicates sustained, telomere-independent cell cycle arrest of neoplastic cells in response to chemotherapeutic agents, ionizing radiation, oxidative stress, or the presence of selective oncogenic stimuli. Recent evidence suggests that a subset of tumor cells induced in a state of reversible ACS can escape cell cycle arrest and resume proliferation accounting for cancer progression. The purpose of this review is to describe our current understanding of ACS including signaling pathways of senescence escape, role of senescence biomarkers, and rationale for senescence-based therapy. This article is part of a Special Issue entitled "Apoptosis: Four Decades Later".

***Key Words*: cellular senescence, cell cycle arrest, tumor cells, chemotherapeutic agents, senescence-based therapy.**

INTRODUCTION

Malignant solid tumors treated with chemotherapy and radiation typically exhibit disappointingly low response rates. The majority of advanced tumors are limited to only partial responses and delayed cancer progression is observed despite continued therapy. Conventional cancer therapeutics have been recognized to activate DNA damage signaling pathways that lead to apoptotic cell death. There is increasing evidence that apoptosis may not be the dominant pathway whereby tumor cells lose their proliferative capacity in response to cancer treatment. Cellular senescence, as first described by Hayflick [1] in 1961 while studying normal human fibroblasts, is defined as a quiescent state of proliferative arrest despite preservation of cell viability and maintained metabolic activity [2]. Replicative senescence has long been described for normal tissues grown under culture conditions and "aging"-associated physiological arrest has been shown to limit the replicative lifespan of normal cells in response to gradual erosion of the telomere. Senescent cells can be identified by characteristic morphologic features including enlarged and flattened cell shape with increased cytoplasmic granularity, nuclear polyploidy, and expression of the senescence marker, β-galactosidase (SA-β-gal) [3, 4]. Cellular senescence has also been observed in neoplastic cells and has been increasingly recognized as a tumor suppression mechanism accounting for the proliferative arrest observed in many benign tumors [5]. Malignant tumors are characterized by their ability to bypass replicative senescence, but can be induced into a state of cell cycle arrest following cancer treatment termed accelerated cellular senescence (ACS). Mounting evidence suggests that ACS is a prominent solid tumor response to therapy [6, 7] which most reasonably accounts for early treatment responses by prolonging cell cycle arrest. However, subsets of senescent cancer cells are capable of escaping senescence and resuming cell division leading to eventual tumor progression. The purpose of this overview is to describe our current understanding of ACS including signaling pathways of senescence escape, role of senescence biomarkers, and rationale for senescence-based therapy.

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Abbreviations used: ACS – accelerated cellular senescence; ATM/ATR – ataxia-telangiectasia-mutated/-Rad3; CAR – coxsackie-adenovirus receptor; CDKI – cyclin dependent kinase inhibitor; CTX – chemotherapy; DDR – DNA damage response; IAP – inhibitor of apoptotic protein; PAI-1 – plasminogen activator inhibitor-1; PET – positron emission tomography; SA-b-gal – senescence-associated b-galactosidase, 5-FU – 5-flourouracil.

SENESCENCE RESPONSE TO CANCER THERAPY AND REVERSIBILITY

Reversibility of ACS fundamentally distinguishes senescence from programmed cell death (apoptosis and autophagy) and mitotic catastrophe as cells enter a sustained period of replicative arrest with the possibility of cell cycle reentry. It follows that senescent cells are destined for either terminal cell death or eventual bypass of senescence (escape) to resume replication. Cell fate during ACS appears to be an important determinant of cancer treatment efficacy. Rare cancer cells following recovery from chemotherapy can escape senescence and resume proliferation which has been estimated to occur at a frequency of 1×10^6 cells [8]. Escape from therapy-induced senescence has been consistently demonstrated, but the mechanisms regulating cell cycle reentry of senescent cell remains poorly understood.

In contrast, much is known about oncogene-induced senescence which has been proposed as a tumor suppressor mechanism in premalignant states such as dysplastic melanocytic nevus [9, 10], neurofibroma [11], and Barrett's esophagus [12]. In these premalignant cells, oncogenic stress appears to trigger premature senescence through components of the DNA damage response (DDR), the MEK/ERK, and the p14ARF pathways, whose signals converge

onto the p53/p21 or the p16/pRB replicative senescence pathway [13, 14]. Progression of premalignant lesions into invasive cancer necessitates additional loss of tumor suppressor functions within these pathways. Therapy-induced ACS invoked in malignant tumors following cancer treatment, therefore, almost always takes place in the absence of p53, p21, p16, and pRB function in epithelial solid tumors. These findings may explain the decade long observation that mutational status of these tumor suppressor genes often fail to reliably predict clinical outcome [15–17].

Despite these differences between oncogene- and therapy-induced ACS in premalignant and malignant tumors, components of ataxia telangiectasia-mutated/-Rad3 (ATM/ATR) and the DDR pathways also transduce DNA damage signals in response to chemotherapy [18–20]. Chk1 and Chk2 serine/threonine kinases and their downstream effectors, mediate signals caused by stalled replication forks, single and double stranded breaks, and telomere dysfunction resulting in activation of G_1-, S- and G_2/M-cell cycle checkpoints (Fig. 1) [18, 21]. The senescence program appears to be triggered by protracted checkpoint activation leading to terminal cell cycle arrest and eventual cell death by delayed apoptosis or autophagy. An emerging body of evidence now suggests that a subpopulation of therapy-induced senescent cells can reverse or escape ACS and evade cell death [22–24]. The viability of these escape cells must be maintained during senescence and they must acquire mechanisms to overcome barriers of cell cycle reentry. Therefore, a clear understanding of molecular determinants of senescence reversibility is crucial to reinforce terminal senescence response in cancer therapeutics. The following is a brief review of key components of therapy-induced senescence reversibility in the absence of p53 and p16 pathway functions (Fig. 1).

Cdk1

Activated cyclin B1/Cdk1 complex is the master switch for cell entry into mitosis. In response to DNA damage, Chk1/2 phosphorylates and inactivates Cdc25C phosphatase, which prevents dephosphorylation of cyclin B1/Cdk1 complex and is typically confined to an inactive state by inhibitory phosphorylation of Cdk1 at 14T and 15Y during G_2 [25]. This negative regulatory event is currently believed to be mediated by the Wee1/Mik1 family of protein kinases [26, 27]. Wee1 itself is also regulated by phosphorylation and can be phosphorylated by Chk1 *in vitro*. Additionally, the Kip/Cip family of cyclin-dependent kinase inhibitors p21 and p27 can directly bind the cyclin B1/Cdk1 complex and down-regulate Cdk1 kinase activity. The consequence of DNA damage for a vast majority of cancer cells with defective p53 function is a rapid cell cycle arrest at G_2. Recent evidence now suggests that down-regulation of Cdk1 protein level is required to maintain cell cycle dormancy during senescence (Fig. 1). This biphasic Cdk1 regulation has been observed in a variety of systems, including senescent fibroblasts [28] and several human cancer cell lines [8, 29–32]. Down-regulation of Cdk1 may be necessary in senescence as its activation has been implicated during apoptosis of YAC lymphoma cells in response to a lymphocyte granule protease [33] and inactivation of Cdk1 in this instance was shown to block apoptosis. A number of cellular proteins also appear to target Cdk1, which in turn suppresses senescence. These include the JNK activation kinase MKK7 and NIMA-related mitotic kinase Nek6 [29, 30]. Embryonic fibroblasts derived from MKK7$^{-/-}$ homozygous knockout mice spontaneously undergo G_2/M cell cycle arrest and premature senescence in conjunction with down-regulation of Cdk1. Nek6 was recently found to prevent reduction of cyclin B1/Cdk1 following chemotherapy treatment in H1299 and EJ carcinoma cells and thereby suppress the senescence response.

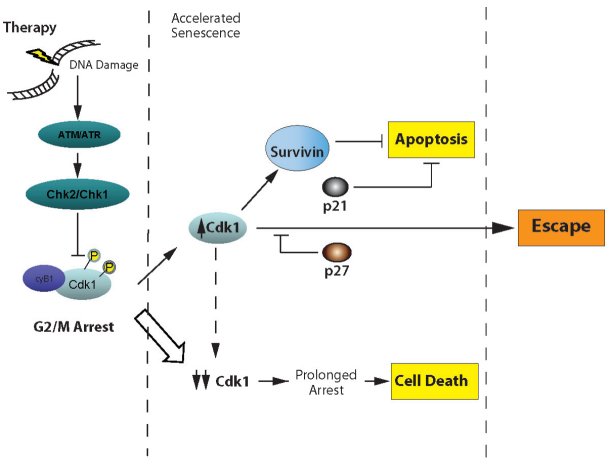

Fig. 1. Pathways of Therapy-induced ACS in p53-null cancer cells. The signals induced by therapy related DNA damage are transduced by the components of the DDR pathway involving ATM/ATR, Chk1/Chk2, and Cdc25C (not depicted) resulting in inactivation of the cyclin B1-Cdk1 complex and rapid cell cycle arrest in G_2. As cells enter ACS, further down-regulation of Cdk1 level reinforces the dormancy. Based on marker studies [63], the overwhelming majority of senescent cells transition from a state of potential cell cycle reversibility to irreversibility. The reversible cells, however, maintain a relatively high Cdk1 expression and kinase activity which is essential for senescence viability and escape. Survivin, whose function depends on Cdk1 phosphorylation, inhibits apoptosis. p21 also blocks apoptosis but through a mechanism that unrelated to Cdk1 kinase activity. Another Cip/Kip protein p27 also bind to Cdk1 and directly inhibit Cdk1 kinase and Cdk1 mediated function in senescence. Reversible senescent cells reenter cell cycle while the irreversible senescent cells eventually die by delayed apoptosis, autophagy or other mechanisms

Despite the requirement of low Cdk1 levels in ACS, Cdk1 activity is required to maintain viability of senescent cells during therapy-induced senescence. Work in our laboratory has demonstrated that abrupt disruption of Cdk1 kinase activity by pharmacological inhibitors or through genetic modulation predictably elicits apoptosis of camptothecin-induced senescent H1299 cells [8]. On the other hand, aberrantly high Cdk1 levels are typically found in senescence escape cells and ectopic expression of a constitutively activated version of Cdk1 in senescent cells facilitates escape. Altogether, these findings suggest that a subpopulation of senescent cells that manages to escape cell cycle arrest may be inherently different in their biological makeup, perhaps by over-expressing anti-apoptosis proteins that protect cells from the pro-apoptotic effects of high Cdk1 activ-

ity. One such protein is the inhibitor of apoptotic protein (IAP) survivin, which is further discussed below. Notably Cdk1 has been shown to promote immortalization of normal human foreskin fibroblasts [34]. When Cdk1, or cyclin A, is transduced into these cells in primary culture, spontaneous immortalized colonies emerge.

Interestingly these immortalized cells have consistently lost alleles of p53 or p21, raising the possibility that Cdk1 level may represent a critical senescence barrier in the p53-defective background of most cancer cells.

Survivin

Survivin, a 16.5 kDal nuclear protein, is the smallest member of the human IAP family [35, 36]. Survivin is expressed in a cell cycle-dependent manner with a marked rise during mitosis and functions to regulate cell division [37–39]. The protein is phosphorylated at the threonine-34 (T34) residue by Cdk1, which stabilizes survivin and appears necessary for interaction with the mitotic spindle and inhibition of caspase-9 apoptotic activity [39]. In HeLa cells, the microtubule inhibitor, taxol, activates a putative survival checkpoint through the up-regulation of Cdc2/Cdk1 kinase activity which leads to the phosphorylation and accumulation of survivin. Suppression of survivin phosphorylation by the Cdc2/Cdk1 kinase inhibitor, flavopiridol, was shown to enhance adriamycin-induced apoptosis [40].

We have found that survivin is consistently up-regulated in cancer cells that have managed to escape therapy-induced senescence [41] and survivin appears to account for Cdk1-mediated survival function. Virally transduced survivin expression in senescent cells, for example, both reduces apoptosis and promotes senescence escape. A short peptide derived from the Cdk1 phosphorylation domain on survivin has been shown to efficiently block survivin phosphorylation and induces rapid apoptosis in senescence escape cells. Consistent with our findings, F14512 is a novel epipodophylootoxin derivative that preferentially induces ACS while promoting both survivin and phosphor-survivin expression in HBL melanoma cells [42]. The knockdown of survivin using SiRNA converts the predominately F14512 senescence response to apoptosis. More recently, survivin over-expression has been shown to reverse spontaneous senescence in stem cell marker ABCG-negative IRG37 melanoma cells [43]. Survivin expression occurs with high frequencies in many types of human cancers including 85–96% of lung cancer specimens [44], 100% of colon adenocarcinoma [45], 71% of prostate adenocarcinomas [46], 80% of glioblastomas [47] and nearly 100% of laryngeal carcinomas [48]. Survivin expression has been associated with unfavorable clinical prognosis in cancers of the breast, esophagus, stomach, pancreas, and colon [44, 49, 50]; and has been shown to correlate with therapy resistance in a variety of clinical settings. For example, in one analysis of 60 advanced ovarian cancers treated with Taxol, complete pathological response was produced in 100% of survivin-negative tumors but only 43% of survivin-expressing tumors [51]. These findings support speculation that survivin is an important determinant of cell cycle reversibility for cells in ACS.

Cyclin-dependent Kinase Inhibitors: p16, p21 and p27

CDKIs p16, p21 and p27 interact with multiple cyclin and cyclin-dependent complexes during cell cycle regulation and therapy-induced DDR, and likely provide cytoprotective functions [52, 53]. p16 binds to CDK4/6 and prevents phosphorylation of Rb. The p16/RB axis has been linked to both physiological and ACS, where a complex of genetic and epigenetic controls regulate p16 expression [54]. p21 and p27 are well known for their roles as regulators of the G_1 cell cycle progression [55]. p21 mediates an anti-apoptotic effect through its known interaction with stress activated protein kinases, apoptosis signal-regulating kinase 1 in the cytoplasm [56], procaspase-3 in the mitochondria [57], or by its release from Cdk2 in the nucleus [58]. During mitosis, the presence of p27 has been proposed to prevent premature entry into S-phase during the mitotic cell cycle, whereas p21 appears to suppress Cdk-1 mediated apoptosis leading to tolerance of genotoxic stress [59].

It is clear that the presence or the absence of functional p53, single or combination of CDKIs and the type of stressor can each dramatically alter the function of intact CDKI during senescence. Using single cell analysis to characterize senescent cells derived from human ataxia telangiectasia and Li-Fraumeni syndrome, the expression of either p16 or p21 was shown to correlate with senescence induced by ionizing radiation depending on the presence or the absence of p53 [60]. We have demonstrated in the p53-null, p16-silenced H1299 cells, both p21 and p27 appear to interact with the cyclin B1/Cdk1 complex; however, only p27 modulates the Cdk1 kinase activity following DNA damage (Fig. 1). While the knockdown of p27 suppresses ACS, knockdown of p21 results in massive apoptosis. This suggests that both of these Kip/Cip family members may serve distinct pro- and anti-apoptotic functions during senescence. Therefore, the determinants of therapy-induced ACS in cancer cells may be highly variable dependent upon distinct senescence pathways.

SENESCENCE MARKERS OF TREATMENT RESPONSE

Standard chemotherapy regimens have been shown to exert their effects by forcing cancer cells to enter a state of dormancy and absent proliferation despite the preservation of metabolic activity. These senescent cancer cells are phenotypically characterized by features of enlarged and flattened shape with increased cytoplasmic granularity, nuclear polyploidy, and expression of pH-restricted senescence-associated b-galactosidase (SA-b-gal). Evidence of *in vivo* ACS is accumulating and has been reported for several types of cancer. A retrospective study of archival tumor samples obtained from patients with breast carcinoma following cyclophosphamide, doxorubicin, and 5-FU therapy found SA-b-gal expression in 41% of patients treated with prior chemotherapy com-

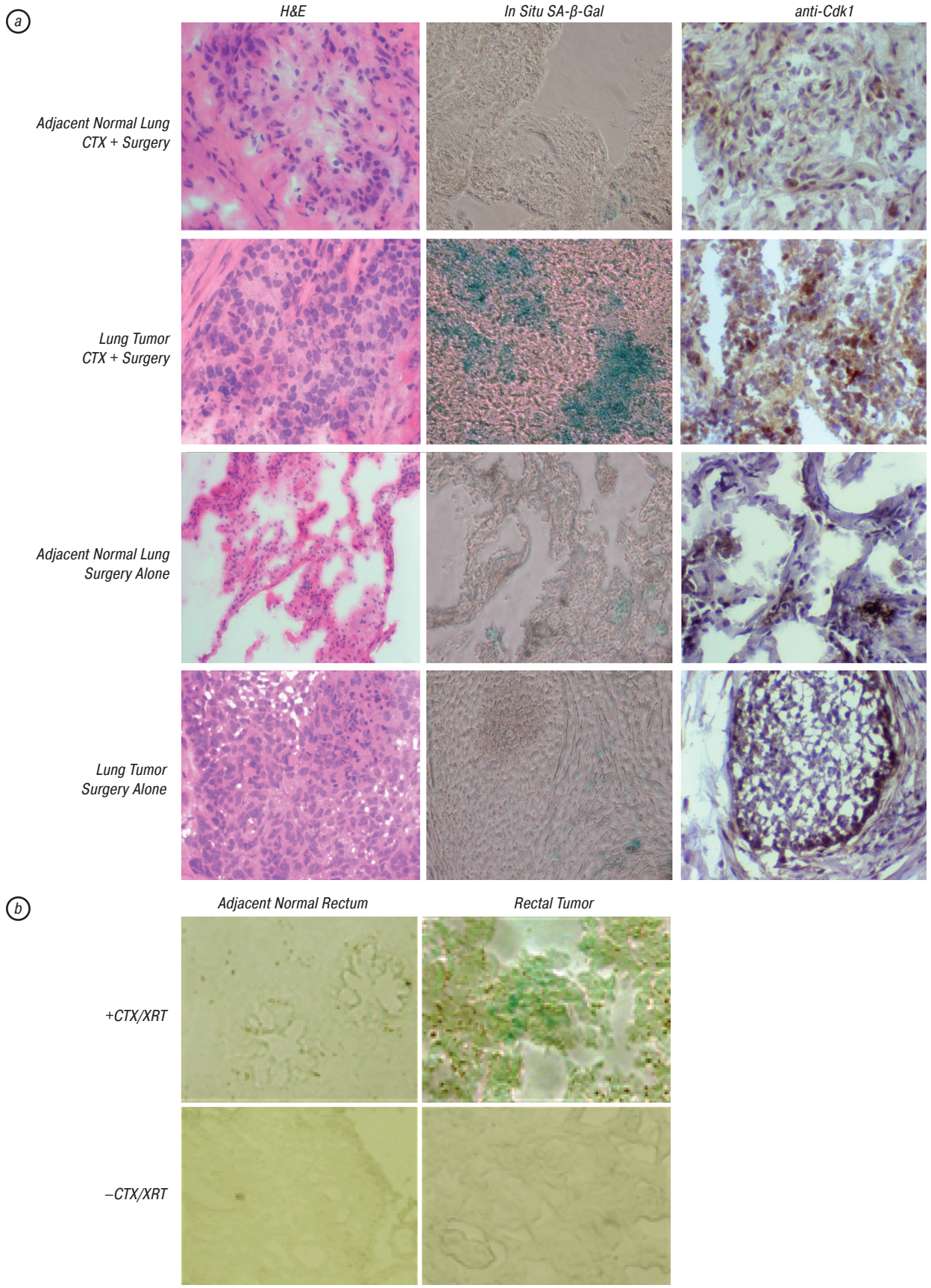

Fig. 2. ACS in human cancers following treatment. *a*: Hemotoxylin-Eosin, *in situ* SA-β-galactosidase and anti-Cdk1 IHC staining of surgery specimens derived from 2 patients with intermediate stage non-small cell lung carcinoma. One patient received neoadjuvant chemotherapy (CTX) prior to surgery (top panel) and the other underwent surgery alone (lower). Tumor or adjacent normal lung specimens were analyzed. Senescence response is clearly demonstrated in the tumor sample of the patient who received chemotherapy but not in the adjacent normal lung or tumor specimen from the patient who did not receive chemotherapy. *b: In situ* SA-b-galactosidse staining of freshly frozen specimens derived from 2 patients with rectal cancer, one of whom received neoadjuvant chemotherapy (CTX) and radiation (XRT). Evidence of therapy-induced ACS is clearly shown

pared to only 10% of specimens from patients who underwent surgery alone [61]. SA-b-gal expression correlated with high expression levels of p16 but inversely with the p53 expression indicative of p53 mutations. Interestingly 20% of tumor samples among the p53 overexpressing samples were positive for ACS which suggests that while p53-dependent mechanisms promote ACS, p53-independent mechanisms likely mediate ACS response to therapy. In transgenic murine models using Bcl-2 over-expressing lymphomas, tumor response to cyclophosphamide was shown to correlate with senescence response which was attenuated by the accumulation of either p53 or p16 mutations [62].

Demonstration of senescence in human tumor samples raises the possibility that senescence markers may have prognostic value for cancer treatment. Our group has reported *in vivo* evidence of chemotherapy-induced senescence in patients treated for advanced lung and colorectal cancer [8, 63] (Fig. 2). We conducted a clinicopathological study to determine whether senescence response correlates with clinical outcome in patients with locally advanced non-small cell lung cancer (stages II and IIIA, AJCC 6th edition) who underwent neoadjuvant (preoperative) therapy prior to surgery. A total of eighteen lung cancer patients were included with a median follow-up time of 27 months. ACS was detected in 78% (14/18) of patients according to tumor-specific SA-b-gal expression relative to adjacent normal lung tissues (Fig. 2 *a*). Viable tumor cells were confirmed on pathology in all 14 SA-b-gal expressing specimens. A Kaplan — Meier survival analysis was performed to compare the outcome of these two subgroups (Fig. 3) and demonstrated decreased overall survival in patients with tumors that over-expressing SA-b-gal compared to patients without detectable senescence marker expression. Despite the limited number of patients in this small pilot study, it nonetheless reached statistical significance with p=0.04 on a two-tailed Kaplan — Meier analysis. Within the limitations of this preliminary observation, we propose that senescence response may predict disease recurrence and adverse treatment outcome. Most recently the negative prognostic effect of senescence response was shown in patients who underwent neoadjuvant chemotherapy for malignant pleural mesothelioma [64]. This study demonstrated that elevated expression of plasminogen activator inhibitor-1 (PAI-1), a surrogate marker for senescence response, was also associated with statistically inferior survival. These findings collectively suggest that ACS leading to terminal growth arrest is a physiological mechanism of DDR during cancer therapy and could be used to predict clinical outcome.

Previous studies have demonstrated that non-cancerous cells in replicative senescence are less prone to adenoviral infection as a result of reduced surface coxsackie-adenovirus receptor (CAR) expression [65, 66]. CAR has also been shown to mediate adenoviral-mediated gene transfer in a variety of human malignancies including glioma, melanoma, lung and pancreatic cancer [67–69]. We observed that tumor cells treated with chemotherapeutic agents and induced to a state of ACS could be differentiated by both their susceptibility to adenoviral transfection and levels of surface CAR expression [63]. Using both surface CAR expression and marker adenovirus transduction as a surrogate determinant for CAR, morphologically identical senescent tumor cell populations can be functionally distinguished by their ability to escape senescence. The subpopulation of senescent cells with increased surface CAR expression, retain the ability to escape cell cycle arrest. Conversely, low CAR expressing senescent cells appear confined to a prolonged senescent state destined for eventual cell death. The ability to characterize transitional senescent states based upon adenoviral marker transduction efficiency and CAR expression provides unique insights into the biological properties of ACS.

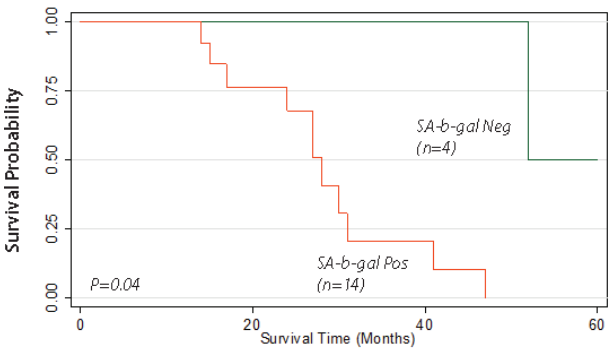

Fig. 3. Senescence response as a prognosticator of treatment outcome in 18 patients with intermediate stage (IIB, and IIIA) non-small cell lung cancer. These patients were enrolled in a institutional review board-approved protocol and received either chemotherapy or combined chemotherapy and radiation per recommendation of the multidisciplinary tumor board. At the time of surgery, tumor and adjacent normal lung specimens were collected and assayed for SA-b-gal (see example in Fig. 2). Patients were followed for a median time of 34 months. Evidence of ACS was detected in 78% (14/18) based on the SA-b-gal expression of the tumor relative to that of adjacent normal lung tissues. Viable tumor cells were found on pathology in all 14 SA-b-gal expressing specimens. Conversely, no viable tumor cells could be identified in the four cases which showed no evidence of senescence response. A two tailed Kaplan — Meier survival analysis was performed to compare the outcome of these 2 subgroups and shows a significantly decreased overall survival in patients with tumors that over-expressed SA-b-gal than patients whose surgery specimens showed no detectable senescence marker expression (p=0.04)

Tumor samples obtained from rectal cancer patients treated with preoperative chemoradiation prior to surgical resection were immunostained with CAR antibody and compared to patients undergoing resection without prior treatment [63]. Decreased CAR staining was observed in tumors treated with preoperative chemoradiotherapy compared to untreated tumors. Meanwhile CAR expression appeared unchanged in the surrounding normal colonic mucosa suggesting that down-regulation of CAR expression in response to preoperative chemoradiation is a tumor-specific property. This provocative finding in rectal cancer patients suggests that CAR expression and adenoviral transduction efficiency may be convenient methodologies to study fundamental regulatory events in ACS and

further studies are warranted to examine the role of CAR as a candidate therapy-induced senescence biomarker.

Additional senescence markers have been proposed with limited or unproven clinical efficacy. IGFBP-3, a serum protein shown to induce growth arrest and apoptosis, has been used as a treatment response marker in animal models of prostate cancer and found to be upregulated in senescent prostate cancer cells [70, 71]. However, clinical studies of IGF markers in cancer patients have shown limited effectiveness with disappointingly low sensitivity and specificity [72]. Other markers, such as the senescence associated heterochromatin foci [73], heterochromatin protein 1g [74,75], and PAI-1 [64, 74], have been applied in both *in vitro* and *in vivo* situations. Each of these suffers from the lacking of systematic studies to assess their value in clinical situations. There remains an important need to identify robust prognostic markers of senescence which can be studied in prospective clinical trials.

FUTURE DEVELOPMENT OF SENESCENCE-BASED CANCER THERAPY

Manipulating senescence response in tumors presents a novel approach to cancer treatment. For the majority of solid tumors, induction chemotherapy alone results in modest disease response rates of 20–40% and rarely results in complete tumor eradication [76]. Initial tumor responses with reduction in tumor size and volume are often followed by tumor growth and progression despite continued therapy. It is assumed that senescent tumor cells induced by anticancer agents are able to escape cell cycle arrest and resume proliferation accounting for cancer progression. Therapeutic strategies to enforce therapy-induced senescence and bypass escape pathways have been proposed. These include pharmacologic agents such as CDK inhibitors intended to block reversible senescence. One of the first pharmacologic CDK inhibitors to enter clinical trials is flavopiridol which has been shown to have antitumor effects in a wide range of solid tumors including renal, colon, and prostate cancer patients [77, 78]. Recent evidence in dose-escalation and dose-sequencing trials suggests that flavopiridol can potentiate the effects of standard chemotherapy agents [78]. It remains to be established whether CDK inhibitors can be effectively used with chemotherapy to modulate senescence response.

Statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) commonly used for the treatment of dyslipidemia have also been shown to decrease farnesylation and geranylgeranylation of several proteins essential for cellular proliferation and survival [79]. Statin agents have been reported to demonstrate a broad spectrum of anti-tumor activities and shown to modulate chemotherapeutic effects *in vitro* [80]. For example, Atorvastatin and lovastatin was shown to potentiate the effect of chemotherapeutic agents in lung cancer cell lines [81]. Statins therefore have been explored as potential preventive and therapeutic agents for human cancers [82, 83]. A retrospective study of from the VA Health Care System found evidence that statin use reduces the risk of lung cancer in the Veterans population [84]. This study showed that statin use for greater than 6 months was associated with an unexpected 55% risk reduction independent of race, age and tobacco use ($p<0.01$). Currently, only a few clinical trials have been performed to study statin treatment for human cancers. In a study of hepatocellular carcinoma patients, Pravastatin and 5-FU conferred a statistically significant survival advantage when compared with 5-FU alone [85]. The effect of statin was examined in patients with colorectal cancer treated with neoadjuvant chemoradiotherapy. Lovastatin used concurrently with neoadjuvant chemoradiation resulted in a higher complete pathological response rate compared to those who did not receive a statin drug (30% vs. 17%; $p=0.10$). Interestingly, statins have been shown to down-regulate several key targets of the Cdk1 pathway, including Cdk1 itself, cyclin B1, survivin, and up-regulate CDKI p27. We have demonstrated that statin drugs can block escape and reinforce senescence in colorectal cell lines previously exposed to chemotherapy (unpublished data). Paradoxically, statins administered alongside chemotherapy were found to promote senescence escape suggesting that statins may exhibit both agonistic and antagonistic effects on therapy-induced senescence. These early observations suggest that statin use in cancer therapy may require stringent scrutiny both in terms of dose intensity and administration schedule in relationship to chemotherapy to establish clinical efficacy.

CONCLUSION

Cellular senescence plays an important role alongside apoptosis in determining tumor responses to the stresses imposed by cancer treatment. There is accumulating evidence that conventional therapies including chemotherapy and radiation induce senescence-like phenotypes classified as ACS. Further studies of the mechanisms that influence transitional states in ACS are crucial for a better understanding of the signaling pathways that ultimately lead to either cancer death or progression. The identification and validation of robust senescence markers is needed to detect *in vivo* senescence and could be combined with tumor imaging modalities such as PET to provide a real-time measure of tumor response that would enable treatment modifications and lead to more personalized cancer therapies. Lastly, clinical trials incorporating pharmacologic agents designed to target senescence pathways are encouraged to investigate whether manipulation of senescence response can improve the clinical efficacy of anti-cancer agents and improve patient survival.

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