Review

Tipping the Balance: Cdk2 Enables Myc to Suppress Senescence

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Abstract

Intrinsic tumor-suppressor pathways protect healthy cells from transformation by activated oncogenes like MYC or RAS through induction of apoptosis or cellular senescence, respectively. However, when expressed together, MYC and RAS evade these barriers and initiate tumorigenesis. Although Ras suppresses Myc-induced apoptosis, the role of Myc in this cooperation has remained undefined. In a recent report, we showed that Myc represses Ras-induced senescence, thereby overcoming the second major barrier of tumorigenesis. Inhibition of Ras-induced senescence required specific phosphorylation of Myc by cyclin-dependent kinase 2 (Cdk2) and was abrogated by pharmacologic Cdk2 inhibitors, urging a reevaluation of this kinase as a therapeutic target in Myc- or Ras-driven tumors. Cancer Res; 70(17): OF1–5. ©2010 AACR.

Barriers to Malignant Transformation

Proto-oncogenes and tumor-suppressor genes act as positive and negative regulators of cell growth, respectively. When the balance between these factors is disturbed through mutations or epigenetic changes, cells enter a hyperproliferative, premalignant state. This condition may eventually lead to cancer as further lesions accumulate (1). However, oncogenic lesions also provoke intrinsic tumor-suppressive responses: apoptosis, an energy-dependent cell-death program; and cellular senescence, a state of irreversible growth arrest (1). In contrast to replicative senescence caused by telomere erosion, oncogene-induced senescence is a premature, stress-induced type of senescence. During recent years, cellular senescence has been observed in different types of premalignant human tumors, as well as in experimental mouse tumors in vivo, and has come to be recognized as an important tumor-suppressive mechanism (1–3). These failsafe mechanisms are enforced by two major guardians of aberrant cellular activity, the tumor-suppressor protein p53, which drives the expression of numerous targets involved in apoptosis and senescence, and the retinoblastoma protein pRB, which represses S-phase cell-cycle genes. A strong genetic and epigenetic selection exists to overcome these barriers during tumor development.

MYC and RAS are two classical oncogenes that both promote cell proliferation, but also typically induce tumor-suppressive failsafe mechanisms. Myc functions as a transcription factor that regulates a broad range of cellular processes affecting cell growth, whereas Ras, a small GTP-binding protein, transmits signals from growth factor receptors (1, 4, 5). Overactive Myc usually induces apoptosis by induction of Arf, which stabilizes p53 (6). In addition, Myc can induce apoptosis independent of p53 by suppressing anti-apoptotic genes such as BCL2 and BCLXL (5). In contrast, Ras induces senescence, also by activating Arf and p53 (7). In this case, activation of antiproliferative p53 targets, such as the cyclin-dependent kinase (Cdk) inhibitor p21Cip1, predominated. This protein inhibits the activity of Cdk1 and Cdk2, thereby arresting cells at the late G1 phase of the cell cycle. Oncogene-induced senescence through Ras also engages the pRb pathway by inducing the Cdk inhibitor p16ink4a, which inhibits the activity of Cdk4/6. p16 thereby ablates inhibitory phosphorylations of pRb carried out by Cdk4/6 (1). Further, hyperactive oncoproteins like Ras and Myc cause severe cellular stress leading to DNA damage, resulting in induced DNA damage responses that can lead to either apoptosis or oncogene-induced senescence.

Coexpression of Myc and Ras is sufficient for transformation of primary rodent cells as shown almost 30 years ago (5, 8). However, the mechanism behind this cooperativity has remained unsolved. Ras was later shown to suppress Myc-induced apoptosis through the phosphoinositide 3-kinase (PI3K)/Akt pathway. We hypothesized that Myc contributes to this partnership by suppressing senescence (Fig. 1A). In a recent report, we show that Myc, indeed, suppresses senescence and define the mechanisms behind this new Myc function (9).

A Novel Nonredundant Function of Cdk2 Enables Myc to Suppress Oncogene-Induced Senescence

Using primary rodent cells exogenously expressing Myc and/or oncogenic H-Ras, we confirmed the ability of Ras to inhibit Myc-driven apoptosis. Importantly, whereas Ras
alone efficiently forced these cells into cellular senescence, coexpression with Myc interfered with this process (Fig. 1A). Suppression of senescence was also observed when coexpressing Myc with oncogenic Raf or Mek, indicating that Myc interferes with events downstream of Ras/Raf signaling (9). These findings are in good agreement with a recent report showing that Myc suppresses activated $\text{BRAF}$-induced senescence in primary melanocytes (10). Because the Myc phospho-acceptor site Thr 58 has been implicated in apoptosis signaling by Myc (5), we next investigated whether Myc's ability to repress senescence overlapped with its apoptosis-inducing functions. Phosphorylation of Thr 58 is known to require a priming phosphorylation at Ser 62, and, therefore, both Thr 58 (T58A) and Ser 62 (S62A) alanine mutants were examined. Interestingly, T58A had no effect, whereas the S62A mutant completely lost its capacity to repress Ras-induced senescence. Importantly, mutating this site to aspartic acid (to mimic constitutive phosphorylation) fully restored repression of senescence, showing that phosphorylation of Ser 62 is required for evasion of senescence in Myc/Ras cells. This finding shows that Ser 62 has an important regulatory function independent of Thr 58. The S62A mutant intriguingly induced a similar number of foci as the wild-type Myc together with Ras (although these foci contained numerous senescent cells; ref. 9); this may indicate that the phosphorylation does not affect Myc-induced proliferation per se, but rather the decision whether to exit the cell cycle permanently in response to senescence signals.

Several kinases have previously been suggested to phosphorylate Myc Ser 62, including mitogen-activated protein kinases (MAPK) and Cdk1 (5). To search for new potential Ser 62 kinases, we did kinase-inhibitor and/or knockdown experiments and uncovered cyclin E/Cdk2 as a major Ser 62 kinase (Fig. 1A), which was further confirmed by in vitro studies. Strikingly, selective pharmacologic inhibition of Cdk2 or enforced expression of the Cdk2 inhibitor p27Kip1

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**Figure 1.** Cdk2 controls Myc-mediated suppression of senescence. A, apoptosis and cellular senescence are two main barriers of tumor development. The oncogenes Myc and Ras induce apoptosis and senescence, respectively. Ras can inhibit Myc-induced apoptosis via PI3K. Our new finding shows that Myc suppresses Ras-induced senescence. This process requires Cdk2-mediated phosphorylation of Myc. Because Myc stimulates Cdk2 activity, Myc and Cdk2 establish an autostimulatory loop in suppression of senescence. Cdk2 also contributes in a nonredundant manner to suppression of senescence, independent of Myc phosphorylation. B, upon depletion or inhibition of Cdk2, Myc loses its capacity to suppress senescence and turns into an inducer of senescence. C, the proposed mechanism of suppression of oncogene-induced senescence by Myc comprises the following: (1) Myc enhances the activity of cyclin E/Cdk2. (2) Myc and cyclin E/Cdk2 physically interact, resulting in phosphorylation of Myc at Ser 62. (3) The latter enables Myc to regulate senescence-associated genes such as $p21$, $p16$, BMI-1, and hTERT, resulting in suppression of oncogene-induced senescence as part of cancer initiation and maintenance. Inhibition of either Cdk2 or Myc abrogates this mechanism, resulting in senescence and tumor suppression.
Cdk2 Enables Myc to Suppress Senescence

abrogated the ability of Myc to repress Ras-induced senescence (Fig. 1B), whereas inhibition of other kinases including Cdk1, Cdk9, and Mek1/2 had no effect. Because Cdk2 function in the cell cycle can be compensated by other Cdsks (11), our results suggest that Cdk2 plays a novel nonredundant function in mediating Myc-repressed senescence through Ser 62 phosphorylation (9). Importantly, reduced Ser 62 phosphorylation and induced senescence upon Cdk2 inhibition were also seen in established human p53-deficient, Myc-expressing leukemic tumor cells, suggesting that Cdk2-mediated suppression of senescence plays a role not only in initiation but also in the maintenance of tumors. Inhibition of Cdk2 activity and subsequent induction of senescence could be accomplished through small molecules selectively targeting Cdk2, by p27Kip1 overexpression, or by treatment with interferon (IFN)-γ, which is a physiologic inducer of p27. We next asked whether this Cdk2-mediated regulation might occur at Myc-target promoters involved in cellular senescence. Indeed, by chromatin immunoprecipitation we found interactions between Myc, Cdk2, and p27Kip1 directly at hTERT, BMI-1, p21Cip1, and p16INK4A promoters. Interestingly, inhibition of Cdk2 activity as a result of treatment with IFN-γ or pharmacologic substances led to decreased association of Myc with chromatin, correlating with reduced Ser 62 phosphorylation but increased association of p27Kip1. Further, analysis of the mRNA levels of these target genes showed increased expression of p21Cip1 and p16INK4A but decreased expression of BMI-1 and hTERT, consistent with a senescent phenotype (Fig. 1C; ref. 9).

Taken together, our findings strongly suggest that an important function of Myc in oncogenic transformation is to overcome oncogene-induced senescence during both tumor initiation and maintenance. Further, our results show that Cdk2 plays a crucial, nonredundant function in this process by phosphorylating Myc at Ser 62 and by acting as a transcriptional cofactor in the regulation of genes essential for initiation and maintenance of oncogene-induced senescence (Fig. 1).

Mechanistic Roles of Myc Ser 62 and Cdk2, and Their Potential as Targets for Future Cancer Treatment

As described, Cdk2 modulates the capacity of Myc to suppress senescence by phosphorylating Myc at Ser 62. However, it is still unclear exactly what role phospho-Ser 62 plays in this process. Physiologic or pharmacologic inhibition of Cdk2 leads to a substantial loss of Myc from binding sites at senescence-associated genes, indicating that phosphorylation of Ser 62 is necessary for stable association of Myc with certain promoters. These results are consistent with previous data from Benassi and colleagues, which suggested that Erk-mediated Ser 62 phosphorylation is required for the recruitment of Myc to the gamma–glutamyl-cysteine synthetase (γ-GCS) promoter in response to oxidative stress (12). Interestingly, inhibition of other Ser 62 kinases had no effect in our study, suggesting that phosphorylation of Ser 62 is required but not sufficient for Myc/Cdk2-mediated suppression of senescence. It is, therefore, likely that Cdk2 targets additional substrates in a nonredundant manner. It will be important to shed light on this regulation in future studies.

In a recent report by Campaner and colleagues (13), in collaboration with the laboratory of Bruno Amati (European Institute of Oncology, Milan, Italy), we found that activation of Myc (in the absence of other oncogenes) induced cellular senescence in primary mouse embryonic fibroblasts (MEF) with homozygous deletion of Cdk2, but not in wild-type MEFs (Fig. 1B). Similar observations were made previously in cells lacking the Werner syndrome protein (WRN), a helicase implicated in DNA repair (14). Myc-induced senescence was shown to require intact Arf/p53/p21 and p16/Rb pathways (13), as previously reported for Ras (7). Further, activated Myc has been reported to induce DNA damage and DNA damage response through increased replication stress and generation of reactive oxygen species signaling (5), also similar to Ras. Because DNA damage response is a major trigger of oncogene-induced senescence, it is not surprising that overactive Myc is potentially able to provoke premature senescence. This finding was supported by our observation that Myc-induced senescence in Cdk2-depleted cells was inhibited by antioxidants and also by caffeine, which interferes with DNA damage response signaling (13). Apparently Cdk2 and WRN are able to rescue cells from Myc-induced senescence. The presence of Cdk2 also overcomes senescence induced by oxidative stress (13).

An intriguing question raised in a minireview by van Riggelen and Felscher (15), commenting on the report by Campaner and colleagues, is how Myc can act both as an inducer (Fig. 1B; refs. 13, 14) and a repressor (Fig. 1A and C; ref. 9) of senescence. The latter view is further supported by the observations that depletion of Myc in BrafV600E-expressing melanoma cells induces senescence, and that senescence is a frequent outcome of turning off Myc in several Myc-regulatable tumor models in mice (10, 15, 16). One observation that may help to resolve this question is that Myc stimulates both WRN expression and Cdk2 activity (4, 14, 15), indicating that Myc initiates an auto-protective loop by which Myc could suppress its own senescence-promoting activity (Fig. 1A). Interestingly, both Cdk2 and WRN have been suggested to have functions in DNA repair (14, 17). A plausible hypothesis is, therefore, that inhibiting and/or depleting Cdk2 or WRN in Myc-driven tumors leads to excessive DNA damage, triggering senescence (Fig. 1B). However, Cdk2 status did not affect senescence induced by Ras alone (13). Therefore, suppression of Ras-induced senescence requires activated Myc, along with Cdk2 (9), as described above. It is unclear at present if the function of Cdk2 in suppressing Myc-induced senescence is distinct or analogous to its Myc-dependent role in suppressing Ras-induced senescence. For instance, it remains to be determined whether Sen 62 phosphorylation has any role in the proposed auto-protective loop discussed above.

Taken together these results indicate that Myc and Cdk2 constitute a senescence switch; Myc cooperates with activated Cdk2 to suppress Ras-induced senescence and to
dampen the consequences of its own oncogenic stress, but in the absence of Cdk2 activity, the senescence signaling of Myc through the Arf/p53/p21 and p16/Rb pathways dominates, and it becomes an inducer of senescence (Fig. 1A and B).

The results from the reports by Hydbring and colleagues and Campaner and colleagues (9, 13) suggest that Cdk2 inhibition could potentially become a new therapeutic principle for treatment of Myc/Ras- and Myc-driven tumors (Fig. 1B and C). In support of this notion, depletion of Cdk2 delayed Myc-induced B-cell lymphoma in a p33-dependent manner, correlating with increased cellular senescence (13). These findings were further corroborated by a recent report showing that an apoptosis-deficient mutant of p53 still suppressed Myc-driven lymphomagenesis through increased senescence (18). Further, our own unpublished results indicate that systemic administration of a Cdk2 inhibitor delays leukemogenesis in a Myc/BclxL-driven mouse model. Moreover, Cdk2 inhibition was recently shown to efficiently inhibit growth of MYCN-amplified neuroblastoma cells (19), emphasizing that Cdk2 inhibition could be a successful treatment option in several Myc-driven tumors. Previously, its relevance as a therapeutic target has been questioned because many cancer cell lines were not growth inhibited after Cdk2 inhibition (20). However, most of this work has focused on colon cancer cell lines with defective p16ink4a expression, which presumably would weaken induction of senescence. Later, Cdk2 inhibition was indeed shown to have strong antiproliferative effects in BRCA1-deficient breast cancer and melanoma cell lines (17, 21), although senescence was not measured in these studies. Because Ras is a stronger inducer of senescence than Myc, we envisage that Cdk2 inhibition will have greater potential to cure tumors harboring both deregulated Myc and Ras, or functionally similar oncopgenes. Other tumor types of therapeutic interest are certainly those with connections to Cdk2 activity, such as tumors with decreased p27 expression and/or elevated expression of cyclin E or Skp2, an E3 ligase that targets p27 and Myc for degradation, but promotes Myc function in transcription (5). Interestingly, deletion of Skp2 was recently shown to suppress tumorigenesis through increased senescence (22).

Is reestablishment of intrinsic senescence mechanisms in tumors by Cdk2 or Myc inhibition (Fig. 1C), or by other means, a promising strategy for cancer treatment? In fact, many current cancer treatments cause senescence or apoptosis depending on the context, as a result of DNA damage (5). It has been debated whether the risk of tumor relapse using "senescence therapy" is higher than with treatments that cause apoptosis; the argument presented is that dead tumor cells are better than living, but senescent, tumor cells. Accepting this argument, senescence induction is an alternative treatment option for tumors that display defective apoptotic signaling. However, in both strategies, a major cause of relapse is likely to be tumor-initiating cells that are resistant to treatment. Finding the optimal treatment strategy to combat tumor-initiating cells in various tumor types is still an open question. Another concern with senescence induction as a therapeutic principle is the secretion of inflammatory cytokines and growth factors from metabolically active senescent cells (senescence-associated secretory phenotype or SASP), which could affect both tumor cells and surrounding normal cells in the tissue and potentially stimulate tumor growth (2, 3). However, some of these cytokines, such as interleukin (IL)-6 and IL-8, can reinforce the senescent state and also contribute to recruitment of cells from the innate immune system, leading to clearance of senescent cells (2). Therefore, further studies are warranted for a better understanding of the benefits and drawbacks of senescence therapy in combating cancer.

In addition to Cdk2, pharmacologic inhibition of its essential sibling Cdk1 also led to prolonged survival of mice with Myc-driven lymphoma or hepatoblastoma, in this case because of increased cancer-cell apoptosis (23). This finding suggests that Myc-driven tumors could be combated either through apoptosis or senescence induction by these two different Cdk inhibitors, either alone or in combination, depending on the type of tumor targeted. A very recent report suggests that these antitumorigenic mechanisms can enforce each other through crosstalk with the tumor environment (24). In future cancer therapy, such treatment decisions will hopefully be based on the molecular diagnosis of individual tumors with respect to the genetic and epigenetic status of such intrinsic tumor-suppressor systems.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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