Myc Down-regulation as a Mechanism to Activate the Rb Pathway in STAT5A-induced Senescence*S

Received for publication, August 23, 2007, and in revised form, October 2, 2007 Published, JBC Papers in Press, October 3, 2007, DOI 10.1074/jbc.M707074200

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Senescence is a general antiproliferative program that avoids the expansion of cells bearing oncogenic mutations. We found that constitutively active STAT5A (ca-STAT5A) can induce a p53- and Rb-dependent cellular senescence response. However, ca-STAT5A did not induce p21 and p16^{INK4a}, which are responsible for inhibiting cyclin-dependent protein kinases and engaging the Rb pathway during the senescence response to oncogenic ras. Intriguingly, ca-STAT5A led to a down-regulation of Myc and Myc targets, including CDK4, a negative regulator of Rb. The down-regulation of Myc was in part proteasome-dependent and correlated with its localization to promyelocytic leukemia bodies, which were found to be highly abundant during STAT5-induced senescence. Introduction of CDK4 or Myc bypassed STAT5A-induced senescence in cells in which p53 was also inactivated. These results uncover a novel mechanism to engage the Rb pathway in oncogene-induced senescence and indicate the existence of oncogene-specific pathways that regulate senescence.

The Rb (retinoblastoma) family controls cell proliferation by providing a barrier for cell cycle transitions (1). In molecular terms, this barrier consists of repression of the E2F family of transcription factors, which control the synthesis of genes required for cell cycle progression (2). Growth factors activate the cyclin-dependent protein kinase $(CDK)^2$ -cyclin complexes that phosphorylate Rb, inhibiting its binding to E2F factors (1). Gain-of-function mutations in genes that stimulate cell cycle progression can potentially disable the Rb barrier, leading to tumorigenesis. Hence, for efficient tumor suppression, normal cells must avoid Rb inactivation by oncogenes. Studies on the senescent cell cycle arrest in response to oncogenic *ras* have provided a general model of Rb activation by oncogenes in normal cells. Aberrant *ras* activity induces the expression of CDK inhibitors of the INK4 family such as $p15^{INK4b}$ (3) and $p16^{INK4a}$ (4). As a consequence, Rb accumulates in its active hypophosphorylated form during Ras-induced senescence. This pathway is critical for Ras-induced senescence because disabling either $p16^{INK4a}$ or the Rb family inhibits the process (4, 5).

It is not clear whether the INK4 proteins are universal mediators of Rb activation in response to oncogenic signaling or whether other molecular mechanisms can engage Rb to regulate senescence. To answer this question, we have developed a senescence model in normal human fibroblasts by expressing ca-STAT5A, a constitutively active allele of STAT5A (6). This allele was shown to be constitutively phosphorylated at tyrosine residues, localized to the nucleus, and transcriptionally active (7). Hence, ca-STAT5A provides a persistent and unregulated STAT5A signal similar to that observed in some human tumors (8, 9). We show here that ca-STAT5A engages the Rb pathway by down-regulating Myc instead of the p16^{INK4a}-mediated mechanism described in RasV12-induced senescence. As a consequence, cells expressing ca-STAT5 have a decrease in the expression of Myc targets such as CDK4 and an increase in the CDK inhibitor p15^{INK4b}. In agreement, ca-STAT5A-induced senescence is blocked when CDK4 or Myc is overexpressed in combination with dominant-negative p53 or the E6 protein of human papillomavirus. We conclude that the pathways regulating Rb by oncogenic signals are oncogene-specific and discuss the relevance of this concept for human cancers.

EXPERIMENTAL PROCEDURES

Cells and Retroviruses—Normal human diploid fibroblasts (HDFs), BJ cells (obtained from Dr. S. W. Lowe), and IMR90 cells (American Type Culture Collection) were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone, Logan, Utah) and 1% penicillin G/streptomycin sulfate (Invitrogen). Primary human mammary epithelial cells were cultured in MEGM® (Clonetics) containing 10 ng/ml human epidermal growth factor, 5 μ g/ml insulin, 0.5 μ g/ml hydrocortisone, 50 μ g/ml gentamicin, 50 ng/ml amphotericin B, and 52 μ g/ml bovine pituitary extract at 37 °C with 5% CO₂.

Retroviral vectors are described under supplemental "Experimental Procedures." Retrovirus-mediated gene transfer, cell proliferation analysis (growth curves and bromodeoxyuridine and [³H]thymidine incorporation assays), cell cycle analysis, and senescence assays were done as described (6, 10).

Protein Expression Analysis—To prepare total cellular protein, cells were collected by trypsinization, washed with phosphate-buffered saline, lysed in 100 μ l of SDS sample buffer (60 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, and 5% 2-mer-

^{*} This work was supported in part by the Canadian Institutes of Health Research (to G. F.) and a fellowship from the Fonds de Recherche en Santé du Québec (to F. A. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

^(S) The on-line version of this article (available at http://www.jbc.org) contains supplemental "Experimental Procedures," Figs. 1 and 2, and references.

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² The abbreviations used are: CDK, cyclin-dependent protein kinase; ca-STAT5A, constitutively active STAT5A; HDFs, human diploid fibroblasts; PML, promyelocytic leukemia; PIPES, 1,4-piperazinediethanesulfonic acid.



FIGURE 1. STAT5A activation induces senescence in human cells. A, bromodeoxyuridine (BrdU) incorporation of IMR90 cells with a control vector (V), oncogenic ras (R), or STAT5A1*6 (5A). Senescence-associated β -galactosidase (SABGal) staining results (percent of positive cells) are listed at the bottom. B, cell cycle analysis of IMR90 cells infected with a control vector or STAT5A1*6. C, Western blot analysis of STAT5 and phospho-STAT5 in IMR90 cells expressing a control vector or its derivatives expressing STAT5A1*6 or STAT5B1*6 (5B) and in K562 cells. pY-STAT5, tyrosine-phosphorylated STAT5. D and E, senescence-associated β -galactosidase staining of IMR90 cells and human mammary epithelial cells, respectively, infected with the indicated control and retroviral vectors. The percent S.D. of senescence-associated β -galactosidase-positive cells is indicated in each panel. Data represent three independent experiments done with cells collected 6 days post-selection. STAT5wt, wild-type STAT5.

captoethanol), and boiled for 5 min. For Western blotting, 20 μ g of total cellular protein was separated by SDS-PAGE and transferred to Immobilon-P membranes (Millipore Corp.). The primary antibodies used are described under supplemental "Experimental Procedures." Signals were revealed after incubation with anti-mouse or anti-rabbit secondary antibody coupled to peroxidase (Amersham Biosciences) by enhanced chemiluminescence (ECL, Amersham Biosciences) or Lumi-Light^{PLUS} (Roche Applied Science). Fluorescence microscopy was done as described previously (11) and is also detailed under supplemental "Experimental Procedures." To visualize Myc in promyelocytic leukemia (PML) bodies, the protocol was modified by extracting the cells with cytoskeletal buffer (10 mM PIPES (pH 6.8), 100 mM NaCl, 300 mM sucrose, and 3 mM MgCl₂) containing 0.5% Triton X-100. Cells were then fixed and treated for immunofluorescence as described (11).

Luciferase Assays—H1299 cells were seeded at 2.5 \times 10⁵ cells/well in 6-well plates. Cells were transfected using Lipofectamine 2000 (Invitrogen) with 2 μ g of firefly luciferase reporter plasmid under the control of the CDK4 (provided by

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C. V. Dang, The Johns Hopkins University, Baltimore), p21, or MDM2 promoter; 0.5 µg of Renilla luciferase reporter plasmid under the control of the β -globin promoter; 250 ng of Myc- or p53-expressing plasmid; and increasing amounts (0, 0.1, or 1 μ g) of pBabeSTAT5A1*6 (ca-STAT5A). The total quantity of plasmid was kept constant by adding the empty vector pBabe. Cells were harvested 24 h after transfection and processed for Dual-Luciferase assay (Promega) according to the manufacturer's instructions.

RNA Analysis—For real-time reverse transcription-PCR or semiquantitative reverse transcription-PCR, 2–5 μ g of total RNA was reverse-transcribed with a RevertAidTM H minus first strand synthesis kit (Fermentas Life Sciences) using oligo(dT) primers, and the resulting first strand cDNA was used as a template for PCR. Specific primers are described under supplemental "Experimental Procedures."

RESULTS

ca-STAT5A Induces Senescence in Human Cells-We reported that the DNA damage response (DDR) is required to induce p53 during STAT5-induced senescence (6). However, the mechanism of activation of the Rb pathway in response to ca-STAT5A remained unknown. То investigate how STAT5A

engages the Rb pathway, we used a retrovirus that directs the expression of ca-STAT5A in HDFs. As a control, we infected cells with a virus expressing RasV12, which is known to activate Rb by inducing p16^{INK4a}. RasV12- or ca-STAT5A-expressing HDFs proliferated normally during the first 4 days after retroviral infection, indicating that the immediate effect of the introduction of these oncogenes does not inhibit cell growth. However, both RasV12- and ca-STAT5A-expressing cells dramatically decreased their DNA synthesis 8 days after retroviral infection (6 days post-selection) (Fig. 1A) and remained in a non-dividing state for at least 20 days. Cells expressing ca-STAT5A arrested their proliferation with a prominent G₁ DNA content (Fig. 1B) and displayed the morphology of flat cells that stained positive for senescence-associated B-galactosidase (Fig. 1A and supplemental Fig. 1A). Notably, the levels of phospho-STAT5A displayed by ca-STAT5A-infected fibroblasts were comparable with those found in the leukemia cell line K562 (Fig. 1*C*).

Next, we used a wild-type allele of STAT5A to investigate if the ability to induce senescence requires tyrosine phosphoryl-

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FIGURE 2. ca-STAT5A engages the Rb pathway by down-regulation of CDK4. A, Western blot analysis of the Rb pathway in IMR90 cells bearing a control vector (V), RasV12 (R), or STAT5A1*6 (5A). B, quantitative real-time PCR for CDK4 in IMR90 cells infected with a control vector or STAT5A1*6. C, CDK4 cooperates with E6 to bypass STAT5A-induced senescence. Shown is the senescence-associated β -galactosidase staining of IMR90 cells bearing a control vector or its derivative expressing STAT5A1*6 infected with empty vectors or with vectors expressing CDK4R24, E6, or both CDK4R24 and E6. The percent S.D. of senescence-associated β -galactosidasepositive cells is indicated in each panel. Data represent three independent experiments done with cells collected 6 days post-selection.

ation of STAT5. We found that STAT5A-induced senescence was observed only with the allele STAT5A1*6 and not with wildtype STAT5A (Fig. 1D). Also, ca-STAT5B (known as STAT5B1*6), which has the same activating mutations as STAT5A1*6 but is not constitutively phosphorylated to the same extent (Fig. 1*C*), was not able to induce senescence in a significant proportion of the cells (data not shown). Hence, as reported for oncogenic ras (12), the senescence phenotype depends on the strength of signaling.

The senescence response to STAT5A was also documented in BJ cells, another strain of normal human fibroblasts (supplemental Fig. 1*B*), and in primary mammary epithelial cells (Fig. 1E). Hence, STAT5A-induced senescence is not restricted to IMR90 fibroblasts and occurs in mammary epithelial cells, a cell type in which STAT5 plays a role in both normal physiology (13-15) and breast cancer (15-17). We concluded that ca-STAT5A induced senescence in normal cells when activated to the same extent as found in human cancers, providing a new model to investigate the mechanisms of Rb activation during oncogene-induced senescence.

ca-STAT5A Induces the Down-regulation of CDK4 and the Activation of the Rb Pathway in Human Cells-The activation of the Rb pathway in response to oncogenes in normal cells is known to involve induction of CDK inhibitors such as p21, p16^{INK4a}, and p15^{INK4b} (18). To better characterize the mechanism of Rb activation during ca-STAT5A-induced senescence, we measured the levels of the upstream regulators and downstream effectors of the Rb pathway (Fig. 2A). To begin with, senescence induced by ca-STAT5A was characterized by Rb hypophosphorylation, down-regulation of the E2F-dependent genes cyclin A and MCM6, and up-regulation of ezrin. The latter was shown to be induced by Rb, mediating the characteristic flat morphology of senescent cells (19). Intriguingly and in CDK4 down-regulation was also observed at the mRNA level (Fig. 2B). In consequence, oncogenic STAT5A engages the Rb pathway by a mechanism different from the one reported during Ras-induced senescence. Next, we investigated whether the observed reduction in CDK4 levels is important for STAT5-in-

contrast to Ras-induced senes

cence, ca-STAT5A did not induce

p16^{INK4a} or p21 but led to a moderate down-regulation of CDK4.

duced senescence. To do the experiment, we first prepared IMR90 cells expressing human papillomavirus 16 E6 to inactivate the p53 pathway. This was necessary because in IMR90 fibroblasts it is not possible to bypass Ras- or STAT5-induced senescence by isolated inactivation of the p53 or Rb pathway (supplemental Fig. 2). As expected, expression of E6 (which

blocks the p53 pathway) or CDK4 (which blocks the Rb pathway) did not rescue ca-STAT5A-induced senescence. However, E6 and CDK4 together efficiently blocked senescence (Fig. 2C).

Mechanism of CDK4 Down-regulation during STAT5-induced Senescence-The decrease in CDK4 protein and mRNA levels during STAT5-induced senescence suggests either a decrease in CDK4 gene expression or an increase in mRNA turnover. We first investigated the effects of STAT5A on the CDK4 promoter and its main known regulator, c-Myc (20). The CDK4 promoter contains Myc-binding sites, and as reported previously (20), it is stimulated by Myc in transient transfection assays (data not shown). Transfection of ca-STAT5A together with Myc and the CDK4 promoter-reporter blocked Myc activity in H1299 human lung tumor cells (Fig. 3A). However, ca-STAST5A did not affect the ability of p53 to stimulate the p21 and MDM2 promoters (Fig. 3A). Hence, ca-STAT5A can specifically inhibit Myc activity. Because there are no known STAT5A-binding sites in the CDK4 promoter, these results suggest that ca-STAT5A can block general Myc activity.

To confirm this hypothesis, we measured other Myc target genes during STAT5-induced senescence. We found a downregulation of classic Myc targets such as Cdc7 and Cdc25c (Fig. (3B) (21). The magnitude of the decrease was similar to the decrease of the E2F target thymidine kinase-1. We also observed an increase in the Myc-repressed gene p15^{INK4b} (22). This CDK inhibitor may further activate Rb by inhibiting CDK6 and the remaining levels of CDK4 in cells expressing ca-STAT5A. Taken together, our data indicate that a downregulation of Myc can engage the Rb pathway via two mechanisms: down-regulation of CDK4 and release of $p15^{INK4b}$ from repression.



FIGURE 3. **STAT5A antagonizes Myc activity.** *A*, Dual-Luciferase assay in H1299 cells transiently transfected with the *CDK4*, p21, or *MDM2* promoter-firefly luciferase plasmid; the β -globin promoter-*Renilla* luciferase plasmid; and the indicated vectors. *, significant decrease in luciferase activity (Student's *t* test, *p* < 0.001) compared with control cells. *B* and *C*, quantitative real-time reverse transcription-PCR of different Myc target genes in IMR90 cells infected with a control vector (*V*) or STAT5A1*6 (*5A*). RNA samples were collected 6 days post-selection.



FIGURE 4. Decrease in Myc during STAT5A-induced senescence and Myc localization to PML bodies. *A*, Westem blot analysis of Myc during STAT5A-induced senescence in IMR90 cells. The same probe was reprobed with an anti-tubulin antibody as a loading control. The expression of the transgene was evaluated by probing for phospho-STAT5. *B*, indirect immunofluorescence of Myc in STAT5A-induced senescence. IIMR90 cells infected with STAT5A1*6 were treated with either Me₂SO (*DMSO*) or the proteasome inhibitor MG132 at 5 µg/ml for 4 h. Every cell showed a similar increase in Myc levels; three representative nuclei are shown. *BF*, bright-field. *C*, PML immunofluorescence of IMR90 cells with a control vector (*V*) or STAT5A1*6 (*SA*). *DAPI*, 4',6-diamidino-2-phenylindole. *D*, co-localization of Myc in a subset of PML bodies. during STAT5-induced senescence using confocal microscopy. *Arrows* indicate co-localization of Myc to PML bodies.

protein levels (Fig. 4A). Treatment with the proteasome inhibitor MG132 rescued Myc levels in the nucleus of every cell as assessed by immunofluorescence (Fig. 4B). This result suggested that proteasomedependent degradation was part of the mechanism of Myc down-regulation. Next, we looked for STAT5 target genes that could influence Downloaded from www.jbc.org at BIBLIO DE LA SANTE on January 8, 2008 Myc degradation by the proteasome. We first focused on PML, which is known to be regulated by STAT transcription factors and to contain STAT5-binding sites in its promoter (23). Also, PML interacts with Myc and induces its degradation (24-26). As expected, we found that ca-STAT5A induced the formation of PML bodies when expressed in normal human fibroblasts (Fig. 4C). Also, using confocal microscopy, we revealed that a fraction of these PML bodies co-localized with Myc during STAT5-induced senescence (Fig. 4D). Although the extent of co-localization was moderate, it was similar to previous reports of Myc localization to PML bodies (24, 25). Together, the data are consistent with a model of dynamic localization of Myc to PML bodies, where Myc may undergo degradation and/or modifications that ultimately target it to the proteasome. These results contrast with previous data showing that STAT5A is required to induce Myc in lymphoid cells treated with interleukin-2 (27). We believe that the down-regulation of Myc we observed in STAT5A-induced senescence is not an immediate and direct consequence of normal STAT5 signaling but the result of a

cellular response to sustained and

unregulated STAT5 signaling.

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Mechanism of Myc Down-reg-

ulation during STAT5A-induced

Senescence-Next, we investigated

Myc levels and activity in the con-

text of ca-STAT5A-induced senes-

cence. STAT5-induced senescence

caused a dramatic reduction of Myc

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FIGURE 5. **Myc cooperates with E6 to bypass STAT5A-induced senescence.** *A*, senescence-associated β -galactosidase staining of IMR90 cells bearing a control vector (*V*) or its derivative expressing ca-STAT5A infected with empty vectors or with vectors expressing wild-type Myc (*wtMyc*), Myc(T58A), and E6 as indicated. LxSn is the empty control vector for E6. *B*, Western blot analysis of CDK4 expression in STAT5-infected cells expressing Myc or Myc(T58A). *C*, Western blot analysis of Myc expression in STAT5infected cells expressing wild-type Myc (*wtMyc*) or Myc(T58A). The loading control (*LC*) is a nonspecific band. *pSTAT5*, phospho-STAT5.

causal to the induction of senescence via the Rb pathway, we prepared HDFs in which the p53 pathway was inactivated using E6. In this way, we can study the role of the Myc/CDK4/Rb pathway in isolation. Introduction of ca-STAT5A in E6-expressing HDFs induced senescence as described above (Fig. 2C). However, coexpression of ca-STAT5A with Myc and E6 dramatically reduced the numbers of senescent cells (Fig. 5A). An allele of Myc with increased stability due to the mutation T58A (28) was even more potent in blocking STAT5A-induced senescence. Both Myc and Myc(T58A) were also able to rescue CDK4 expression in STAT5A-expressing cells (Fig. 5B). Of note, although the degradation mechanism triggered by ca-STAT5A also affected the stability of the Myc alleles expressed from a retroviral vector, the final levels of Myc were much higher than in control cells (Fig. 5C). These results indicate that exogenous Myc is able to overcome the Myc inhibitory effects of cells expressing ca-STAT5A.

DISCUSSION

We report here a novel mechanism of activation of the Rb pathway in response to oncogenic signals. An allele of STAT5A that mimics the constitutive activation of STAT5 seen in many human tumors activates the Rb pathway by suppressing Myc expression and activity. During STAT5A-induced senescence, at least two direct Myc targets were identified as mediators of Rb activation in response to low Myc activity. First, the levels of the CDK4 protein kinase were moderately but consistently low. Second, the levels of the Myc-repressed gene p15^{INK4b}, a CDK inhibitor, were high. These mechanisms can cooperate to keep Rb hypophosphorylated, preventing E2F activity, which is required for cell cycle progression. This reduced Myc activity is important for STAT5A senescence because the process was



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FIGURE 6. Model for STAT5A-induced senescence indicating independent connections of the Rb and p53 pathways with the senescence program. A DNA damage signal connects ca-STAT5A to p53 via ATM, but there is no evidence so far that DNA damage is also responsible for the activation of the Rb pathway. An important feature of this model is the proposed positive feedback loop between Myc and CDK4 via E2F. This loop explains how CDK4 can rescue cells from the low Myc state we have discovered in STAT5-induced senescence. DDR, DNA damage response.

rescued by enforced expression of Myc or CDK4 in cells in which p53 was inactivated by E6. In agreement with our model, it has been reported that enforcing inhibition of *myc* expression can lead to cellular senescence in both normal and tumor cells (29, 30). Significantly, our work establishes an endogenous pathway of *myc* repression that counteracts the oncogenic stress provided by constitutive activation of STAT5A.

Our model to explain STAT5A-induced senescence presents two tumor suppressor pathways that can act independently to mediate the process. ca-STAT5A can induce senescence in cells in which either the Rb or p53 pathway is intact but not in cells in which both pathways are disabled (Fig. 6). These independent abilities of the Rb and p53 pathways to regulate senescence ensure robustness in tumor suppression pathways, preventing cell transformation when p53 or Rb is accidentally inactivated. It is not clear yet if the Rb and p53 pathways are connected to oncogenes through entirely different mechanisms. Recent results have established that p53 is activated in response to a variety of oncogenes via the DNA damage response (6, 31, 32). During STAT5A-induced senescence, ablation of ATM expression using RNA interference does not prevent Rb-dependent senescence (6), suggesting that the ATM branch of DNA damage signaling does not connect oncogenes to the Rb pathway. However, STAT5-induced DNA damage could still signal to the Rb pathway via ATM-independent mechanisms that could eventually end in regulating Myc levels (Fig. 6).

It is significant that in normal human fibroblasts, the expression of Myc acts genetically like that of E7 and CDK4, which are negative regulators of the Rb pathway. Consistent with this notion, enforced expression of Myc in human primary prostate epithelial cells inactivates the Rb pathway, leading to cellular immortalization (33). Also, Myc inactivation in diverse tumor types induces tumor regression via cellular senescence. However, inactivation of the Rb pathway prevents this tumor regression (30). It has been proposed that Myc acts directly on Rb to regulate cell proliferation (34). However, it is now well established that the Rb barrier to cell cycle progression is controlled by the concerted action of the CDKs, which phosphorylate Rb and release E2F transcription factors. Therefore, the effects of Myc to inhibit Rb during senescence are better explained by its ability to induce CDK4 and/or to repress the CDK inhibitor p15^{INK4b}. The inhibition of senescence by CDK4 does not mean that CDK4 is the only Myc target required to prevent activation of the Rb pathway in response to ca-STAT5A. In fact, a genetic screen for genes able to rescue the growth defect of myc null rat fibroblasts identified only c-myc and N-myc (35). This experiment suggests that there are no Myc targets that can substitute for Myc functions. To explain this apparent discrepancy, we propose that expression of CDK4 could initiate a positive feedback loop leading to sustained activation of endogenous Myc, via E2F1 (Fig. 6), which is a positive regulator of the *myc* gene (36). In this way, CDK4 can rescue the Myc defect in the context of STAT5-induced senescence.

Our data also imply that factors regulating Myc stability in response to oncogenes play important tumor suppressor roles. One of those factors is the PML protein, which has been shown to regulate Myc degradation (25, 26) and can be directly regulated by STAT5A or p53 (23, 37). PML forms nuclear bodies in which components of the proteasome have been detected (38). Hence, Myc can be degraded in PML nuclear bodies during STAT5-induced senescence. The evidence for a role of PML in Myc degradation remains correlative and will require further studies. PML is also induced by oncogenic ras via p53 (10, 37), but we did not find dramatic CDK4 or Myc down-regulation during Ras-induced senescence. It is known that Ras can induce Myc stability (39), explaining why we did not observe Myc and CDK4 down-regulation during Ras-induced senescence. Alternatively, other STAT5-regulated genes play a critical role in Myc degradation.

The enforced expression of Myc was sufficient to overcome the mechanisms of Myc down-regulation triggered by ca-STAT5A. Exogenous Myc was not completely degraded in cells expressing ca-STAT5A. This result suggests that the Myc degradation pathway can be saturated or inhibited by high levels of Myc, explaining why the amplification of the *c-myc* gene is a powerful oncogenic event (40). On the other hand, Myc is regulated at multiple levels, and it is possible that ca-STAT5A may engage other mechanisms that limit Myc activity, translation, or RNA levels (41–45). STAT5-induced senescence provides a model system to study the interplay among all Myccontrolling pathways.

Our work has important implications for tumors that are characterized by constitutive activation of STAT5. These include many frequent neoplasias such as several leukemias (46-49), Hodgkin lymphoma (50), breast cancer (51, 52), prostate cancer (8), and head and neck cancer (53). We anticipate that these tumors must have genetic or epigenetic changes inactivating the signaling pathways from STAT5A to senescence, including overexpression of Myc and loss of p53 target

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genes associated with senescence. Evidence for such genetic interactions can be found in the erythroleukemia cell line K562, which we showed has levels of endogenous phospho-STAT5 similar to those obtained with ca-STAT5A. This cell line has a mutated p53 gene (54) and deletions of p15^{INK4b} and p16^{INK4a}, which control the Rb pathway (55). These genetic alterations may prevent STAT5A-induced senescence in these cells.

Intriguingly, T cells from elderly humans have a higher basal level of phospho-STAT5 compared with those from young controls. In addition, in old but not young subjects, phospho-STAT5 is increased by stimulation with interleukin-6, a cytokine that increases with aging (56). These data suggest that the mechanisms of STAT5-induced senescence described here could be relevant to understanding the immunosenescence associated with human aging.

Acknowledgments—We thank V. Bourdeau, L. Chatel-Chaix, B. Mao, A. Montmarquette, and members of the Ferbeyre laboratory for comments and technical assistance and C. V. Dang, D. Galloway, T. Kitamura, S. W. Lowe, and S. Mader for cells and plasmids.

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