Sin3B Expression Is Required for Cellular Senescence and Is Up-regulated upon Oncogenic Stress

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Abstract

Serial passage of primary mammalian cells or strong mitogenic signals induce a permanent exit from the cell cycle called senescence. A characteristic of senescent cells is the heterochromatinization of loci encoding pro-proliferative genes, leading to their transcriptional silencing. Senescence is thought to represent a defense mechanism against uncontrolled proliferation and cancer. Consequently, genetic alterations that allow senescence bypass are associated with susceptibility to oncogenic transformation. We show that fibroblasts genetically inactivated for Sin3B are refractory to replicative and oncogene-induced senescence. Conversely, overexpression of Sin3B triggers senescence and the formation of senescence-associated heterochromatic foci. Although Sin3B is strongly up-regulated upon oncogenic stress, decrease in expression of Sin3B is associated with tumor progression in vivo, suggesting that expression of Sin3B may represent a barrier against transformation. Together, these results underscore the contribution of senescence in tumor suppression and suggest that expression of chromatin modifiers is modulated at specific stages of cellular transformation. Consequently, these findings suggest that modulation of Sin3B-associated activities may represent new therapeutic opportunities for treatment of cancers. [Cancer Res 2009;69(16):6430–7]

Introduction

Various cellular stresses have been shown to trigger senescence in cultured cells, including telomere attrition, accumulation of DNA damage, and increased expression of the products of the INK4a/ARF locus (1). However, the biological significance of cellular senescence remains controversial. Recent reports have suggested that it may serve as a barrier against tumorigenesis in vivo (2–4), as well as a driving force in organismal aging (5–7). In mouse cells, cellular senescence is driven primarily by p16ARF, which serves as a sensor for oncogenic signals to activate the p53 response (8). Consistent with the notion that p16ARF, and not p16INK4a, mediates replicative and oncogene-induced senescence in mouse cells, murine fibroblasts genetically inactivated for p16ARF failed to arrest on serial passaging or forced expression of activated Ras, whereas p16INK4a-null primary fibroblasts are not immortal and remain susceptible to oncogene-induced senescence (9–11). Disruption of E2F-mediated transcription using a dominant-negative form of E2F1 prevents p19ARF-, p53-, or activated Ras–mediated senescence (12). Reinforcing the importance of the E2F proteins and their target genes in senescence is the established role for Rb and Rb-like proteins in senescence (13, 14). Upon undergoing senescence, E2F target loci are embedded in nuclear foci, termed senescence-associated heterochromatic foci (SAHF; ref. 15), which are characterized by the presence of constitutive heterochromatin-specific marks, including H3K9me3 and HP1 proteins, and by the absence of acetylated histones. The enzymes responsible for the chromatin modifications observed at E2F target loci during senescence are not completely understood.

Importantly, loss-of-function experiments have indicated that bypass of Ras-induced senescence correlates with oncogenic transformation. For example, p53- and p16ARF-null fibroblasts are transformed upon activated Ras overexpression (9, 16), leading to the notion that immortalization correlates with susceptibility to cellular transformation on oncogenic activation. Consistently, activation of a senescence program has been evidenced in a variety of preneoplastic lesions in human, including melanocytic nevi and prostatic adenomas (3, 4), whereas further progression of the tumor requires inactivation of the senescence pathway.

To further examine the interplay between chromatin modifications, senescence, and oncogenic transformation, we investigated the role of one potential effector protein, Sin3B. The Sin3 complex is a large multiprotein complex that is recruited by several sequence-specific transcription factors. It is characterized by the presence of the Sin3A or the highly related Sin3B protein, which serve as scaffold proteins between transcription factors and the repressor activities of the complex (17). Among these repressive activities, the histone deacetylase activity carried out by Sin3-associated HDAC1 and HDAC2 is essential for the transcriptional repression mediated by the Sin3 complex. Several lines of evidence suggest the involvement of the mammalian Sin3 complex in establishing the senescent phenotype. First, a Sin3-containing complex has been shown to be integral in the formation of pericentric heterochromatin (18), heterochromatic regions molecularly similar to SAHFs. Furthermore, genetic inactivation studies in the mouse showed that Sin3B, although dispensable for cellular growth, is necessary to mediate the onset of quiescence by transcriptional repression of E2F-responsive genes (19, 20). Therefore, we investigated the ability of Sin3B-null fibroblasts to undergo replicative or oncogene-induced senescence. We show here that Sin3B is required for heterochromatinization and silencing of E2F target genes. Although primary cells devoid of Sin3B are resistant to senescence, they are not transformed upon expression of activated Ras, thus uncoupling immortalization from susceptibility to oncogenic transformation. Importantly, expression...
of Sin3B increases upon oncogenic stress, both in vitro and in vivo, correlating with the induction of the senescence program, but is strongly decreased as tumors progress, making it a potential therapeutic target in cancer.

Materials and Methods

Cellular analysis. IMR-90 human fibroblasts were grown in DMEM plus 10% FCS and penicillin/streptomycin and used before reaching P20. Mouse embryonic fibroblasts (MEF) were generated from E13.5 embryos. Retroviral infection, growth curves, bromodeoxyuridine (BrdUrd) incorporation, senescence-associated β-galactosidase (SA-β-gal) staining, and protein analysis were conducted as described in refs. 16, 19, 21. P values were calculated using Student’s t test.

Quantitative reverse transcription-PCR. Reverse transcription was done by using Moloney murine leukemia virus polymerase and oligo(dT) primers. Real-time PCR analyses were done using the SYBR Green method (primer sequences available on request). Results were reported as relative to the abundance of β2-microglobulin transcripts.

Chromatin immunoprecipitation. Immunoprecipitations were done essentially as described by Boyd and colleagues (22) using Sin3B (Santa Cruz Biotechnology), H3 (Abcam), H3K9me3 (Abcam), HDAC1 (Millipore), and HP1γ (Millipore) antibodies, followed by analysis of recovered chromatin using real-time PCR (primer sequences and detailed protocols available on request).

Protein analysis. Paraformaldehyde-fixed cells were incubated with antibodies against H3K9me3 (Abcam), H3K4me3 (Abcam), and PML (Santa Cruz Biotechnology). Secondary antibodies were obtained from Molecular Probes. Nuclei were counterstained using either propidium iodide or 4,6-diamidino-2-phenylindole (DAPI) as indicated and analyzed on a Zeiss LSM 510 confocal microscope. For Western blot, antibodies used were Sin3B (Santa Cruz Biotechnology), Ras (Santa Cruz Biotechnology), Sin3A (Santa Cruz Biotechnology), p19 (Santa Cruz Biotechnology), Flag M2, and tubulin (Sigma). For immunohistochemistry, Sin3B (A-20, Santa Cruz Biotechnology) and MCL-1 (S-19, Santa Cruz Biotechnology) antibodies were used.

Results

Genetic inactivation of Sin3B prevents entry into replicative senescence. To establish immortalized cell lines, Sin3B+/+ and Sin3B−/− MEFs were generated and cultured using a standard 3T3 protocol. As expected, Sin3B+/+ MEFs proliferated for 8 to 10 passages before losing proliferative capacity, at which point, immortalized cultures ultimately emerged. Surprisingly, Sin3B−/− cells proliferated steadily for up to 26 passages (Fig. 1A), suggesting that Sin3B genetic inactivation promotes spontaneous immortalization. In mouse cells, accumulation of genotoxic damage resulting from oxidative stress is believed to account for replicative senescence (23). To assess the contribution of oxidative stress to the growth differences observed upon Sin3B inactivation, primary MEFs were cultured in low-oxygen conditions (3% O2). By contrast to what we observed in high-oxygen conditions, the growth rates of Sin3B+/+ and Sin3B−/− primary MEFs were comparable over 18 passages, consistent with a specific function of Sin3B in replicative senescence (Supplementary Fig. S14). The percentage of Sin3B−/− MEFs that were positive for SA-β-gal staining was significantly lower than that of their wild-type counterparts at passage 6 (Fig. 1B). This observation corroborates the noticeable differences in cell morphology after six to eight passages between Sin3B−/− and Sin3B+/+ MEFs, which appeared significantly more flat and enlarged, irrespective of the cell density (Fig. 1C). Of note, p53 levels were greatly increased upon UV irradiation in both Sin3B+/+ and Sin3B−/− fibroblasts at passage 6 (Supplementary Fig. S18). This suggested that the p53 pathway remained intact in Sin3B−/− cells despite their immortal growth and that loss of Sin3B alone is sufficient to immortalize primary fibroblasts. Altogether, these results strongly argue that the absence of Sin3B delays replicative senescence in the absence of alteration of p53 status.

We investigated the possibility that Sin3B might be essential not only for the establishment but also for the maintenance of a senescent phenotype in mouse cells as previously reported for the Rb protein (25). Primary MEFs harboring one floxed Sin3B allele and either one wild-type Sin3B allele (Sin3B+/+) or one null Sin3B allele (Sin3B−/−) were generated. These MEFs were serially propagated using a 3T3 protocol, and at passage 8, virtually 100% of the cells were senescent as revealed by SA-β-gal staining of a subset of the cells (Supplementary Fig. S24). Senescent cells were then infected with adenovirus expressing Cre recombinase, resulting in efficient and rapid excision of the floxed allele (Supplementary Fig. S2C). As shown in Supplementary Fig. S2B, acute deletion of Sin3B was not sufficient to restore cellular proliferation in senescent cells. Together, these results show that although Sin3B is required for the induction of senescence, it is, by contrast to Rb (25), dispensable for the maintenance of the senescent state.

Genetic inactivation of Sin3B prevents oncogene-induced senescence but is not sufficient for Ras-induced transformation. Given the requirement for Sin3B during replicative senescence (Fig. 1), we investigated whether a Sin3B-dependent pathway is equally engaged in oncogene-induced senescence. RasV12 was
retrovirally transduced into early-passage primary MEFs, leading to high-level expression of activated Ras as well as a reproducible up-regulation of endogenous Sin3B (Fig. 2A). As previously reported (16), expression of activated Ras in wild-type MEFs induced senescence, as evidenced by the strong increase in the proportion of cells staining positive for SA-β-Gal (Fig. 2B, left) and the significant reduction in BrdUrd incorporation (Fig. 2B, right). By contrast, the proportion of senescent cells was significantly reduced and BrdUrd incorporation was only marginally affected by RasV12 expression in Sin3B−/− cells (Fig. 2B). Therefore, we investigated the possibility that RasV12-dependent activation of the p19ARF/p53 pathway is impaired in Sin3B−/− fibroblasts. As previously reported, expression of p19ARF was induced upon transduction of activated Ras into wild-type cells (Fig. 2C; ref. 26). Surprisingly, in Sin3B−/− cells overexpressing activated Ras, the level of p19ARF protein was comparable with that detected in Sin3B+/+ cells. However, the amount of p19ARF detected in the absence of oncogenic stress was significantly higher in Sin3B−/− cells compared with Sin3B+/+ cells (Fig. 2C). This result is consistent with the previous observation that p19ARF is an E2F target (27) and that E2F-driven repression is impaired in the absence of Sin3B (19). Furthermore, our observation that Sin3B−/− fibroblasts do not senesce upon RasV12 expression, in spite of high levels of p19ARF, strongly suggests that Sin3B functions downstream of p19ARF in oncogene-induced senescence. These results show that, although Sin3B participates in the transcriptional repression of p19ARF under normal culture conditions, it is required for the induction of senescence upon activation of the p19ARF/p53 pathway.

Recent work has suggested that the DNA damage response pathway may be a driver of oncogene-induced senescence (28, 29). To confirm that differential activation of the DNA damage response does not account for the changes in onset of senescence between Sin3B-wild-type and Sin3B-null cells upon induction of oncogene-induced stress, we examined the levels of γH2AX after infection with activated Ras. As shown in Supplementary Fig. S3A, no difference in the amount of γH2AX-positive cells was observed in Sin3B-wild-type or Sin3B-null cells following RasV12 infection. Along with the normal p53 accumulation upon UV treatment in Sin3B−/− cells (Supplementary Fig. S3B), these results suggest that Sin3B acts downstream of the DNA damage response pathway to mediate senescence.

It has been shown that genetic deletion of p53 or of the Ink4a locus renders cells both resistant to senescence and susceptible to transformation upon forced expression of activated Ras, enforcing the hypothesis that bypassing senescence is a critical step in transformation (30). However, by contrast to p53 or p19ARF, deletion of Sin3B was not sufficient to allow soft-agar growth upon activated Ras overexpression (Fig. 2D, left). Yet, forced expression of SV40 large T antigen and activated Ras in Sin3B−/− primary MEFs led to the formation of transformed foci, indicating that Sin3B−/− fibroblasts are not inherently refractory to cellular transformation (Fig. 2D, right). Interestingly, expression of large T antigen and activated Ras in Sin3B−/− cells resulted in a significantly higher number of transformed colonies than that in Sin3B+/+ cells. The basis for the increased susceptibility of Sin3B−/− primary MEFs to transformation by large T antigen/Ras remains elusive, but it is possible that disruption of the E2F repression pathway sensitizes primary fibroblasts to transformation. Altogether, these experiments strongly suggest that Sin3B is required for oncogene-induced senescence. However, in the absence of additional genetic lesions, its inactivation is not sufficient to promote cellular transformation upon oncogene activation.
Sin3B is recruited to the promoters of E2F target genes upon oncogenic stress and its presence correlates with their heterochromatinization and silencing. It has been previously observed that transcriptional repression of E2F targets correlates with the onset of either replicative or oncogene-induced senescence (12). Therefore, we investigated whether inactivation of Sin3B impairs the transcriptional repression program triggered by oncogenic stress in primary fibroblasts. We first observed that the amount of transcripts corresponding to every E2F target gene we looked at was significantly increased upon Sin3B deletion, irrespective of the presence of RasV12, consistent with our previous observations (Fig. 3A and B, compare Sin3B^+/− to Sin3B^−/−; ref. 19). We then confirmed the down-regulation of several E2F targets, including cycA2 and ccne1, in wild-type primary fibroblasts upon RasV12 overexpression (Supplementary Fig. S4; Fig. 3A). By contrast to their wild-type counterparts, Sin3B^−/− MEFs did not exhibit transcriptional repression of any of these E2F target genes upon RasV12 overexpression (Supplementary Fig. S4, left; Fig. 3A and B). A second group of E2F target genes, including cdk2a and mcm5, were not affected by RasV12 in wild-type cells but were significantly up-regulated in Sin3B^−/− cells (Fig. 3B). This observation is in agreement with the recent demonstration of heterogeneous transcriptional responses among E2F target genes (31) and correlates with the finding that forced expression of oncogenes in primary fibroblasts induces senescence through uncontrolled transition from G1 to S phase (28, 32). Altogether, these experiments indicate that the impairment of oncogene-induced senescence in Sin3B^−/− MEFs closely correlates with altered E2F target transcription. 

To show the direct role of Sin3B in mediating E2F transcriptional control, we performed chromatin immunoprecipitation experiments. We found that Sin3B is strongly and specifically enriched at E2F target promoters upon RasV12 overexpression, but not at nonrelevent promoters such as oct4 (Supplementary Fig. S4, left; Fig. 3C). In control experiments done in Sin3B-null cells, we observed no increase in the amount of Sin3B-associated chromatin on senescence induction. We confirmed that expression of activated Ras induces recruitment of heterochromatin marks, including H3K9me3 and HP1γ at E2F target promoters (Supplementary Fig. S4 and B, right; Fig. 3D; ref. 15). Importantly, we showed that this enrichment for heterochromatin marks at E2F target promoters upon oncogenic stress requires the presence of Sin3B (Supplementary Fig. S4; Fig. 3D). Of note, total levels of H3K9me3 were not affected by the absence of Sin3B (Supplementary Fig. S5). Consistent with the notion that HP1γ is a marker rather than an inducer of senescence (33), we observed an increase in global levels of HP1γ upon RasV12 overexpression only in the presence of Sin3B (Supplementary Fig. S5). Altogether, these observations suggest that the recruitment of Sin3B and the subsequent modifications to the promoters of E2F target genes are required for accurate transcriptional repression of these pro-proliferative genes and the resultant onset of oncogene-induced senescence.

**Sin3B up-regulation is sufficient to induce senescence.** We initially observed an increase in the level of Sin3B protein upon Ras induction (Fig. 2A). To document the specificity of this increase, the levels of Sin3B and of the related Sin3A protein were investigated. As shown in Fig. 4A (left), whereas Sin3B protein levels increased 3- to 

![Figure 3](chart.png)

**Figure 3.** Sin3B directly regulates the chromatin modifications and transcription of E2F target genes in primary MEFs infected with RasV12. **A and B,** relative amount of transcripts as determined by quantitative RT-PCR corresponding to the indicated E2F target genes in early-passage Sin3B+/+ (n = 2; black columns) or Sin3B^−/− (n = 2; white columns) MEFs infected with vector or RasV12. Shown is the average of two independent experiments. **C,** chromatin immunoprecipitations (ChIP) on promoters of the indicated genes with an anti-Sin3B antibody in early-passage Sin3B^+/+ (n = 2; black columns) or Sin3B^−/− (n = 2; white columns) MEFs infected with vector or RasV12. Shown is the average of two independent experiments. **D,** chromatin immunoprecipitation of the dihydrofolate reductase (dhfr) promoter with the indicated antibodies in early-passage Sin3B^+/+ (n = 2; black columns) or Sin3B^−/− (n = 2; white columns) MEFs infected with vector or RasV12. Shown is the average of two independent experiments. Bars, SE. *P < 0.05.
4-fold upon expression of activated Ras, the levels of Sin3A remained unchanged, emphasizing the specificity of Sin3B in the cellular response to oncogenic stress. As shown by quantitative reverse transcription-PCR (RT-PCR) analysis, the increase in Sin3B levels upon activated Ras expression occurs at the transcriptional level (Fig. 4A, right).

Having established that Sin3B is required for cellular senescence and that Sin3B levels are up-regulated upon oncogenic stress, we investigated whether Sin3B overexpression in primary cells was sufficient to engage the senescence program. Early-passage wild-type MEFs were infected with retroviruses encoding either full-length Sin3A or full-length Sin3B (Fig. 4B). Although Sin3B overexpression was sufficient to significantly increase the proportion of cells positive for SA-β-gal compared with the control, overexpression of Sin3A had no effect (Fig. 4C). This result indicated that overexpression of Sin3B, but not Sin3A, is sufficient to engage a senescence program.

Because the abundance of pericentriolar heterochromatin present in mouse cells makes visualization of SAHF structures difficult, we likewise infected human primary diploid fibroblasts (IMR90) with retroviruses encoding full-length mouse Sin3B or activated Ras. As expected, overexpression of activated Ras in IMR90 triggered the onset of senescence as detected by SA-β-gal staining (Fig. 5A). By contrast, <20% of same-passage IMR90 cells infected with the corresponding empty retroviral vector were positive for SA-β-gal staining (Fig. 5A). Strikingly, upon Sin3B overexpression, ~40% of the cells were positive for SA-β-gal staining, a significant increase in the amount of senescent cells compared with empty vector–infected cells (Fig. 5A).

Consistent with the ability of Sin3B to induce SA-β-gal positivity in primary human fibroblasts, Sin3B-overexpressing IMR90 displayed nuclear foci that were brightly stained for H3K9me3 and DAPI, comparable with those observed on overexpression of activated Ras (Supplementary Fig. S6A; Fig. 5B). Furthermore, these DNA dense foci excluded histone marks that correspond to transcriptionally active chromatin, such as H3K4me3 (Supplementary Fig. S6B). We also noted that the well-characterized subnuclear structures called PML bodies were not disrupted on Sin3B overexpression (Supplementary Fig. S6B), suggesting that the effect of Sin3B on the formation of SAHF is specific and does not result from an overall alteration of nuclear organization. The action of Sin3B downstream of Ras in the establishment of senescence was further substantiated by the observation that overexpression of RasV12, but not of Sin3B, leads to relocalization of the propidium iodide staining within the nucleolus (Supplementary Fig. S6B; Fig. 5B). Overall, these results show that, upon overexpression, Sin3B is able to coordinate the histone modifications characteristic of heterochromatin and contributes to the formation of SAHF.

**Sin3B levels are modulated during oncogenic progression in vivo.** Previous reports have shown that the bypass of senescence plays an important role in oncogenic progression in animal models (2, 34) and that reactivation of the senescence program may be sufficient to lead to tumor clearance (35). Although we have observed that Sin3B expression is induced upon oncogenic stress in vitro (Fig. 4A), we sought to investigate the relevance of this result as it relates to tumor progression in vivo. We used a well-characterized mouse model that exhibits multistage pancreatic ductal adenocarcinoma progression. In this model, pancreas-specific expression of activated K-Ras, driven by its own promoter, leads to the generation of local premalignant lesions, namely, metaplastic acini, which have been shown to stain positive for senescence markers (3). These metaplastic acinar structures are believed to be precursors for pancreatic intraepithelial neoplasias (PanIN lesions), which subsequently progress to pancreatic ductal adenocarcinoma in association with homozygous loss of Ink4a/Arf or p53 (36–38). We investigated here the levels of expression of Sin3B in K-Ras–activated, p53–heterozygous mouse pancreas at different stages of tumor progression. Sin3B expression is strongly up-regulated in metastatic acini and to a lesser extent in PanINs compared with adjacent normal pancreas or pancreas from wild-type animals.
Quantification of the Sin3B signal in sections from at least eight independent animals per type of lesion revealed a significant up-regulation of Sin3B in preneoplastic lesions compared with normal pancreas (Fig. 6B). This result is consistent with our in vitro observations that Sin3B expression is up-regulated upon oncogenic stress (Fig. 4A). Consistent with a previous report showing the presence of markers of senescence in preneoplastic lesions in a Ras-induced mouse model of pancreatic cancer (3), we also observed positivity for markers of senescence in metaplastic lesions (Fig. 6C). Importantly, in pancreatic ductal adenocarcinoma, Sin3B expression decreases dramatically and is comparable with the levels detected in normal pancreas (Fig. 6Aa–d). Altogether, these

![Figure 5. Sin3B overexpression induces senescence in primary human fibroblasts. A, percentage of SA-β-Gal-positive, early-passage IMR90 cells 5 d after infection with the indicated retroviruses and puromycin selection (>200 cells per point). Columns, average of two independent experiments done in duplicate; bars, SE. Differences between pBABE and RasV12 and between pBABE and Sin3B are significant (P < 0.05). B, immunofluorescence of IMR90 cells 5 d after infection with the indicated constructs and puromycin selection using antibody to H3K9me3 (green) and counterstained with propidium iodide (PI; red).](#)

![Figure 6. Expression of Sin3B throughout tumorigenesis. A, a and a', Sin3B immunostaining on wild-type mouse pancreas. a', the inset shown in a (dashed lines). b and b', Sin3B immunostaining on mouse pancreas (Pdx-Cre'; Lox-Stop-Lox-K-Ras; p53−/−) with acinar-ductal metaplasia. Note the strong nuclear staining in metaplasia. b’, the inset shown in b (dashed lines). c and c’, Sin3B immunostaining on mouse pancreas (Pdx-Cre'; LSL-K-Ras; p53−/−) with acinar-ductal metaplasia (white arrow) and PanIN lesions (black arrow). c’, the inset shown in c (dashed lines). d and d’, Sin3B immunostaining on mouse pancreas (Pdx-Cre'; LSL-K-Ras; p53−/−) with ductal adenocarcinoma. d’, the inset shown in d (dashed lines). Pancreas sections from at least eight independent animals were observed for each type of lesion. Shown are representative sections of more than eight independent slides analyzed. B, quantification of the Sin3B signal in normal pancreas (N), metaplastic lesions (M), or pancreatic adenocarcinoma (A). At least eight samples were scored for each type of lesion, and quantification ranges from 0 (no signal) to 4 (strong signal). Columns, mean; bars, SD. C, MCL-1 immunostaining on metaplastic lesions (left) or adenocarcinoma (right) in the same mouse model. Shown are representative sections from multiple independent animals.](#)
observations suggest that Sin3B is up-regulated in early premalignant lesions but becomes strongly down-regulated on full oncogenic transformation. Consistent with the results obtained in the mouse model, the Oncomine online database revealed that Sin3B levels are significantly ($P < 10^{-5}$) reduced in multiple cancer types compared with the corresponding normal tissues (Supplementary Fig. S7). Along with the results of this study, the demonstration that chromatin modifications associated with cellular senescence represent a barrier against tumorigenesis in vivo (2) strongly suggests that the modulation of Sin3B-associated activities act as a nonclassic tumor suppression mechanism and could serve as a potential therapeutic approach in preventing oncogenic transformation.

### Discussion

In this study, we investigated the ability of Sin3B-null fibroblasts to undergo replicative and oncogene-induced senescence. We showed that Sin3B is present at promoters undergoing heterochromatinization and is required for the induction of senescence. Notably, although primary cells devoid of Sin3B were resistant to senescence, they were not transformed upon expression of activated Ras, thus uncoupling immortalization from susceptibility to oncogenic transformation. Finally, we found that expression of Sin3B increased on oncogenic stress, consistent with the induction of the senescence program, but strongly decreased as tumors progress, suggesting that it may represent a barrier to oncogenic transformation.

Our results suggest that Sin3B coordinates the recruitment of chromatin modifiers to the promoters of E2F target genes, leading to their heterochromatinization. Although the identity of these modifiers is still under investigation, we hypothesize that the tethering of Sin3B leads to the coordinated removal of active chromatin marks and the addition of inactive marks. Supporting this hypothesis, it has recently been shown that Sin3B physically interacts with the H3K4 methyltransferase HP1γ at E2F promoters. One alternative explanation is that Sin3B recruitment at E2F target promoters upon oncogenic stress represents the first step in the silencing of these genes, as previously shown at the induction of quiescence (19). This initial step would then be necessary for the subsequent recruitment of specific chromatin modifiers, leading to the permanent silencing of these loci.

Interestingly, genetic ablation of Sin3B uncouples immortalization and sensitivity to oncogenic transformation. Indeed, Sin3B-null fibroblasts are immortal and do not exit the cell cycle on oncogenic stress, but expression of activated Ras is not sufficient to lead to a fully transformed state. This is reminiscent of what has been observed on simultaneous genetic ablation of Rb and p107 (40). Loss of Sin3B expression sensitizes to oncogenic transformation by large T antigen and Ras, and decrease in Sin3B expression is observed in several tumors in vivo, suggesting that Sin3B may act as a nonclassic tumor suppressor. One hypothesis is that, although Sin3B acts downstream of p19ARF to signal senescence, disruption of the senescence pathway is not sufficient to lead to transformation but allows the accumulation of deleterious mutations leading to the oncogenic phenotype. This is consistent with our observation that Sin3B-null cells infected with RasV12 do not exit the cell cycle, allowing them to accumulate additional mutations (Fig. 2B, right). Importantly, loss of Survivin/1/2, a family of chromatin modifiers that contribute to the formation of SAHF, was recently shown to accelerate tumorigenesis in a mouse model of B-cell lymphoma (2). In this regard, it will be informative to investigate the contribution of Sin3B to the prevention of cancer in vivo. Of note, multiple clinical trials are aimed at investigating the efficacy of inhibitors of histone deacetylases as therapeutic agents in human cancer (41). Based on our findings, inhibiting Sin3B-associated HDAC activity may promote transformation, rather than prevent it. Furthermore, modulation of Sin3B expression via stimulation of Sin3B transcriptional activity may provide a potential therapeutic approach to restore the senescence program in tumors. Our results suggest that understanding the precise contribution of discrete chromatin modifiers in oncogenesis may lead to a better targeted therapeutic approach modulating the function of specific chromatin modifiers at precise loci.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Acknowledgments

Received 2/12/09; revised 5/22/09; accepted 6/9/09; published OnlineFirst 8/4/09.

**Grant Support:** Rett Syndrome Research Foundation (G. David), March of Dimes (G. David), the American Federation for Aging Research (G. David), American Cancer Society (G. David and K.B. Grandinetti), NIH grant CA112226 (S.K. Logan), and NIH training grant CA009161 (K.B. Grandinetti).

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We thank Lawrence Gardner, Eva Hernando, and Iman Osman for helpful discussions, and Chris Van Oevelen and Brian Dynlacht for their help with E2F target analyses.

### References


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*Cancer Res* Published OnlineFirst August 4, 2009.

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