Suppression of tumorigenesis by the p53 target PUMA

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Communicated by Bruce W. Stillman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, May 10, 2004 (received for review April 4, 2004)

The p53 tumor suppressor regulates diverse antiproliferative processes such that cells acquiring *p53* mutations have impaired cell-cycle checkpoints, senescence, apoptosis, and genomic stability. Here, we use stable RNA interference to examine the role of PUMA, a p53 target gene and proapoptotic member of the Bcl2 family, in p53-mediated tumor suppression. *PUMA* short hairpin RNAs (shRNAs) efficiently suppressed *PUMA* expression and p53-dependent apoptosis but did not impair nonapoptotic functions of p53. Like *p53* shRNAs, *PUMA* shRNAs promoted oncogenic transformation of primary murine fibroblasts by the *E1A*/*ras* oncogene combination and dramatically accelerated *myc*-induced lymphomagenesis without disrupting p53-dependent cell-cycle arrest. However, the ability of PUMA to execute p53 tumor suppressor functions was variable because, in contrast to *p53* shRNAs, *PUMA* shRNAs were unable to cooperate with oncogenic *ras* in transformation. These results demonstrate that the p53 effector functions involved in tumor suppression are context dependent and, in some settings, depend heavily on the expression of a single proapoptotic effector. Additionally, they demonstrate the utility of RNA interference for evaluating putative tumor suppressor genes *in vivo*.

The p53 tumor suppressor is a transcription factor that controls diverse cellular processes such as DNA repair, cell-cycle checkpoints, senescence, apoptosis, and angiogenesis (1). In principle, disruption of each of these activities alone or in combination could explain the potent impact of *p53* mutations on tumorigenesis. Attempts to identify relevant p53 activities have used mouse models or cells derived from these animals to determine whether disruption of individual p53 effectors can mimic *p53* loss during tumorigenesis. To date, the biological consequences of inactivating these effectors have not been as severe as those obtained by inactivating *p53* itself. For example, disruption of either *bax* (an apoptotic regulator) or *p21* (a proliferation inhibitor) does not recapitulate *p53* loss in promoting transformation or tumorigenesis (2–4). Although these observations suggest that disruption of multiple p53 functions is required to support tumorigenesis, it is also clear that p53 coordinates each activity through multiple effectors whose single inactivation is not sufficient to completely disable each activity (5).

Other studies have taken a more global approach to specifically target p53 activities, albeit with apparently contradictory results. For example, during *myc*-induced B cell lymphomagenesis, coexpression of *bcl2*, which completely disables apoptosis downstream of p53, mimics *p53* mutations in producing aggressive malignancies that retain p53-dependent cell-cycle checkpoints (6). Although these results suggest that apoptosis is the primary p53 activity responsible for tumor suppression in this model, p53 deficiency, but not expression of *bcl2*, is efficient at promoting T cell lymphomagenesis (7–9). Furthermore, mice harboring p53 point mutants that are incapable of transactivating p53 proapoptotic targets fail to develop the T cell lymphomas characteristic of p53-null mice (7, 10). In the latter setting, genomic instability, and not apoptosis, was proposed to explain the advantage of *p53* mutations during tumorigenesis.

PUMA (p53 up-regulated modulator of apoptosis) is a "BH3-only" member of the Bcl2 family that was initially identified from differential gene expression studies as a p53 target gene and a potent inducer of apoptosis (11, 12). PUMA acts by modulating Bax activity to facilitate cytochrome *c* release from the mitochondria, thereby triggering the apoptotic cascade (11). *PUMA*-deficient colon carcinoma cells and MEFs derived from *PUMA*-deficient mice are resistant to several apoptotic stimuli, including those acting through p53 (13, 14). In fact, the similar phenotypes of $PUMA^{-/-}$ and $p53^{-/-}$ cells suggest that PUMA is an essential p53 effector during apoptosis under some conditions. However, $PUMA^{-/-}$ mice are not overtly tumor-prone, again suggesting that simultaneous inactivation of multiple p53 effector functions is critical for tumorigenesis (15).

We have proposed that not all p53 effector functions contribute to tumor suppression, and, instead, that loss of specific p53 activities can play crucial roles that are context-dependent (6, 16). For example, whereas apoptosis appears to be the primary p53 activity limiting *myc*-induced lymphomagenesis, both apoptosis and cellular senescence contribute to chemotherapy responsiveness (6, 17). Because PUMA can be a specific and essential mediator of p53-dependent apoptosis, the impact of *PUMA* loss on malignant phenotypes should reveal the relative contribution of apoptosis to p53-mediated tumor suppression in different contexts. Therefore, we used stable RNA interference (RNAi) to acutely suppress *PUMA* expression in settings where p53 has established tumor-suppressor activity. In contrast to studies with traditional knockout mice, studies with RNAi exploit short hairpin RNAs (shRNAs) to acutely and stably suppress gene expression, providing an extremely rapid approach to study loss-of-function effects *in vitro* and *in vivo* (16, 18). Also, RNAi can produce hypomorphic expression states that may more closely mimic basal expression states or the activity of a mutated gene than a null allele. Here, we use this approach to identify PUMA as a potential tumor suppressor and highlight important features of the p53 tumor-suppressor network.

Materials and Methods

Cells and Gene Transfer. Primary murine embryonic fibroblasts were derived from WT and $p53^{-/-}$ day 13.5 embryos and maintained as described in refs. 19 and 20. Retroviruses encoding shRNAs expressed from the U6 promoter were generated by PCR with a pGEM U6 promoter template (16). The shRNA sequences encoded inverted repeats of 29 nt separated by an 8-nt spacer. The inverted repeats corresponded to nucleotides 772–802 (shPU-2) or 500–528 (shPU-3) of the mouse *PUMA* cDNA (NM_133234) and had >3-nt differences compared with any other murine genes as determined by BLAST. The shp53 sequence used was the same as the "p53.2" published in ref. 16. The resulting PCR products were cloned directly into the *Hpa*I site of the murine stem cell virus (MSCV) phosphoglycerate kinase (PGK)-Puro-internal ribosome entry site (IRES)-GFP vector

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Abbreviations: HSC, hematopoietic stem cell; IRES, internal ribosome entry site; MEF, mouse embryo fibroblast; MSCV, murine stem cell virus; PGK, phosphoglycerate kinase; RNAi, RNA interference; shRNA, short hairpin RNA.

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(16) or, for the *p53* shRNA vector used *in vitro*, pQCXIX PGK-Puro-IRES-GFP (modified from Clontech's pQCXIX). The exact primer sequences and cloning strategies are available from the authors upon request. All animal protocols were approved by Cold Spring Harbor Laboratory in accordance with National Institutes of Health guidelines.

Retroviral-mediated gene transfer was performed by using Phoenix packaging cells (G. Nolan, Stanford University, Stanford, CA) as described in ref. 20. Oncogenic *ras* (*H-RasV12*) (20) and *E1A* were expressed by using WZL-Hygro-based retroviral vectors (Ariad Pharmaceuticals, Cambridge, MA). *E1A/H-RasV12* was expressed by using a modified pBabe H-RasV12 retroviral vector (20). Infected cell populations were selected by culture in puromycin (2 μg/ml, 3 days) or hygromycin (100 μg/ml, 3 days) to eliminate uninfected cells.

PUMA Suppression and Functional Assays. Cells were extracted in RIPA buffer (50 mM Tris, pH 7.4/150 mM NaCl/1% Triton X-100, 0.1% SDS/1% sodium deoxycholate) supplemented with Complete Mini protease inhibitors (Roche Diagnostics). We assessed protein expression by immunoblotting as described in ref. 21, with primary antibodies directed against PUMA (1:300) from ProSci (San Diego), p53 (1:500) from NovoCastra (Newcastle, U.K.), or α-tubulin (1:4,000) from Sigma. Mouse embryo fibroblasts (MEFs) used in *E1A/ras* death assays were plated into 12-well plates (10^5 cells per well) in medium containing either 10% or 0.1% FBS and incubated for $\approx$36 h. Cell viability was analyzed by trypan blue exclusion, and at least 200 cells were scored for each sample.

For BrdUrd incorporation assays, 1×10^5 $p53^{-/-}$ MEFs or WT MEFs infected with MSCV control, shPU-3, or shp53 viruses were plated in triplicate on sterile coverslips in six-well dishes. Twelve hours after plating, MEFs were exposed to 6-Gy γ-radiation. Fourteen hours later, cells were pulsed with BrdUrd for 4 h. BrdUrd incorporation and colony formation assays were performed as described in ref. 16. For lymphoma DNA damage checkpoint analysis, tumor-bearing mice were either irradiated at 6 Gy or left untreated. Thirty-six hours later, lymphoma cells were harvested from irradiated and control mice, fixed in 70% ethanol, and stained with propidium iodide for cell-cycle analysis (6).

Tumorigenicity Assays. For tumorigenicity assays, 1×10^6 cells per 0.25 ml of PBS were injected s.c. into NIH Swiss athymic nude mice (Taconic Farms) and monitored as described in ref. 22. MEFs from two different embryo preparations were infected with retroviruses containing shRNAs targeting *PUMA* or *p53* in the presence of MSCV vector, *ras*, or *E1A/ras*. Isolation, infection, and transplantation of hematopoietic stem cells derived from embryonic day 15 WT or *Eμ-myc* was performed as described in ref. 6. Reconstituted animals were monitored for illness by lymph node palpation, by monitoring overall morbidity, and, in some cases, by whole-body fluorescence imaging (8). Overall survival was defined as the time from stem cell reconstitution until the animal reached a terminal stage and was killed. In all cases, terminal animals harbored large tumor burdens. Statistical analysis was performed with a one-way ANOVA test by using PRISM (Version 3.0, GraphPad, San Diego).

Immunophenotyping was performed on three shPUMA and three shp53-induced lymphomas. Briefly, 1×10^6 freshly harvested lymphoma cells were washed two times in PBS with 2% FBS. Cells were then incubated for 1 h in 200 μl of PBS with 10% FBS containing 1:100 dilutions of phycoerythrin-conjugated B220, Thy1, and IgM (Pharmingen). After incubation with surface antibodies, cells were washed two times in PBS with 2% FBS. Flow cytometry analysis was performed on a Becton Dickinson LSRII cell analyzer equipped with FACSVANTAGE DIVA software.

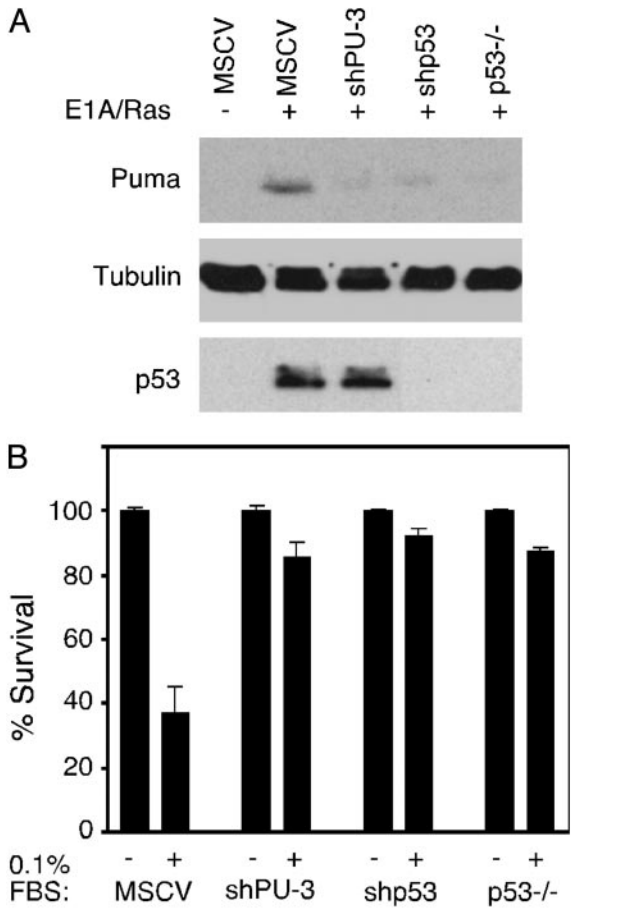

Fig. 1. Analysis of *PUMA* shRNA function *in vitro*. (*A*) Western blot analysis showing p53 and PUMA levels in *E1A/ras* MEFs stably expressing an MSCV control vector or *PUMA* and *p53* shRNAs. Tubulin is shown as a loading control. (*B*) *E1A/ras* MEFs transduced with an MSCV control vector or *p53* and *PUMA* shRNAs were incubated in high/low serum for 36 h after which viability was assessed by trypan blue exclusion.

Results

***PUMA* shRNAs Can Suppress PUMA Levels and Activity.** MEFs expressing *E1A* or *myc* become sensitized to apoptosis after DNA damage or serum depletion (5). In contrast, oncogene-expressing MEFs derived from both $p53^{-/-}$ and $PUMA^{-/-}$ mice are resistant to apoptosis (14–15, 23), suggesting that PUMA is essential for p53-mediated cell death in this context. To determine whether suppression of *PUMA* by using RNAi could be effective, we generated *PUMA* shRNAs against distinct sequences in the *PUMA* gene (designated shPU-2 and shPU-3), cloned them into a retroviral expression vector, and tested their activity after introduction into MEFs. *PUMA* shRNAs were capable of efficiently suppressing PUMA expression, even in *E1A/ras*-expressing cells that harbor stabilized p53 (Fig. 1*A*) (data not shown). Furthermore, like a p53 shRNA, *PUMA* shRNAs protected *E1A/ras*-expressing MEFs against apoptosis after serum depletion (Fig. 1*B*). Therefore, *PUMA* shRNAs are capable of modulating *PUMA* expression and activity.

PUMA Suppression Does Not Affect p53-Dependent Cell-Cycle Arrest. p53 is essential for cell-cycle arrest after DNA damage (24). To rule out the possibility that PUMA affects p53 arrest functions, we examined the ability of MEFs expressing PUMA or p53 shRNAs to undergo cell-cycle arrest after γ-irradiation. Both vector control and shPUMA-expressing MEFs retained an intact

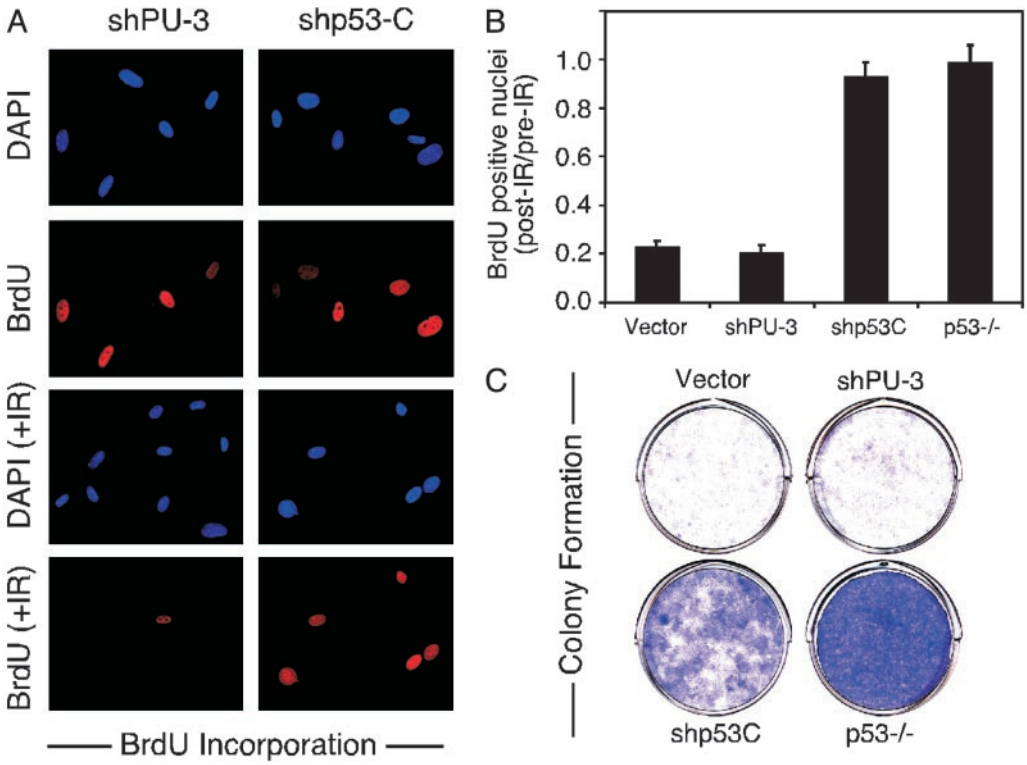

Fig. 2. *PUMA* suppression does not impair p53-mediated cell-cycle arrest. (*A*) Representative fields of a BrdUrd-incorporation assay showing the response of *p53* and *PUMA* shRNA-expressing MEFs to ionizing radiation. (*B*) Quantitation of the data in *A*, including MSCV control-infected and $p53^{-/-}$ MEFs. The relative ratios of BrdUrd incorporation for irradiated vs. nonirradiated cells are shown. (*C*) A colony-formation assay performed on $p53^{-/-}$ MEFs and WT MEFs infected with MSCV control (vector), shPU-3, and shp53. In each well, 2,500 cells were plated and cultured for 12 days before staining with crystal violet.

DNA damage checkpoint after irradiation, as indicated by an ≈5-fold decrease in BrdUrd relative to unirradiated controls (Fig. 2 *A* and *B*). In contrast, shp53-expressing MEFs and *p53*-null MEFs showed no significant reduction in BrdUrd incorporation after γ-irradiation (Fig. 2 *A* and *B*).

WT MEFs undergo senescence when plated at clonogenic density, whereas *p53*-deficient MEFs form colonies that are readily immortalized (19). To determine whether PUMA could influence the ability of p53 to promote senescence, MEFs expressing shRNAs targeting *PUMA* or *p53* were plated at low density and examined for colony formation after 2 weeks. In agreement with the acute arrest assays, *PUMA* shRNAs had no impact on colony formation, whereas p53 shRNAs were highly effective (Fig. 2*C*). In this assay, *p53*-deficiency and *p53* shRNAs resulted in the significant enhancement of the ability of untransformed cells to form colonies when plated at clonogenic density (Fig. 2*C*). Therefore, although suppression of *PUMA* can effectively disable p53 apoptotic functions, it has no impact on p53-mediated cell-cycle arrest.

PUMA Can Be a Potent Suppressor of Transformation. p53 tumor suppressor activity has been extensively studied in primary rodent cells, where *p53* mutations can cooperate with the combination of *E1A* and *ras* oncogenes, or oncogenic *ras* alone, in promoting oncogenic transformation (19, 25, 26). To determine the extent to which *PUMA* suppression could mimic *p53* mutations in these assays, we introduced shRNAs targeting *PUMA* or *p53* into WT or $p53^{-/-}$ MEFs, along with retroviruses that coexpressed *E1A*/*ras* or *ras* alone. After transduction, the infected cell populations were injected s.c. into immunocompromised mice, which were monitored for tumor formation at the sites of injection. Because the *PUMA* and *p53* shRNAs also coexpressed a GFP reporter, tumor formation also could be visualized by whole-body fluorescence imaging.

PUMA shRNAs acted as potent inducers of transformation in cells coexpressing *E1A*/*ras*, because the transduced cell populations appeared morphologically transformed (data not shown) and formed rapidly progressing tumors at the majority of injected sites (Fig. 3). Indeed, the ability of PUMA shRNAs to enhance the tumorigenicity of *E1A*/*ras* MEFs was similar to that produced by a *p53* shRNA (Fig. 3*A*), although the tumors progressed at a somewhat slower rate (Fig. 3*B*). Importantly, no tumors occurred in *E1A*/*ras* MEFs infected with the control vector. Furthermore, both *PUMA* shRNAs tested were effective in this assay, indicating that their oncogenic effects were unlikely to result from off-target oncogenic activities. Therefore, PUMA can approximate p53 action in suppressing transformation by *E1A* and *ras*.

The same transforming effects of *PUMA* shRNAs were not observed in the presence of oncogenic *ras* alone. Hence, cell populations expressing *PUMA* shRNAs and *ras* appeared morphologically senescent (data not shown) and, like cells coexpressing *ras* and the control vector, did not form tumors at any of the injected sites (Fig. 3 *A* and *C*). These observations are in stark contrast to WT MEFs expressing a *p53* shRNA or p53-deficient MEFs, where oncogenic *ras* was highly tumorigenic. Therefore, PUMA suppression can approximate p53 loss in promoting transformation by some oncogene combinations, but not others. Because both the *E1A*/*ras* and *ras* transformation assays were performed in the same MEF populations, these differences must reflect a distinct requirement for p53 effectors in different signaling environments.

***PUMA* Loss Accelerates *Eμ-myc* Lymphomagenesis.** *Eμ-myc* transgenic mice express the c-*myc* oncogene from an Ig heavy chain

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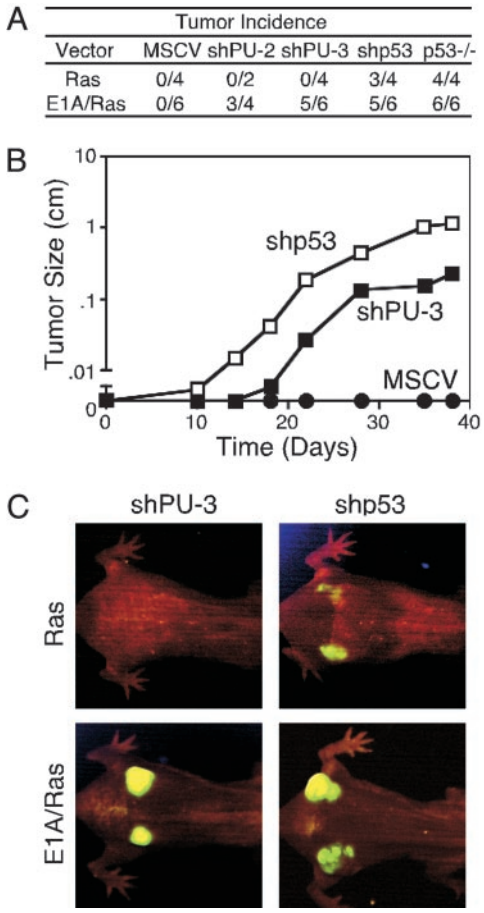

Fig. 3. *PUMA* loss transforms *E1A/ras* MEFs but not *ras*-transduced MEFs. (*A*) *E1A/ras* or *ras* MEFs coexpressing a control vector, PUMA shRNAs, or a p53 shRNA were injected s.c. into athymic nude mice and monitored for tumor formation. Shown is the number of tumors per injected site. The tumor incidence from $p53^{-/-}$ MEFs expressing *E1A/ras* or *ras* is also displayed. (*B*) Relative growth rate of s.c. tumors induced by MSCV-, shPU-3-, and shp53-infected *E1A/ras* MEFs. (*C*) GFP imaging of tumors arising from *ras* and *E1A/ras* MEFs transduced with *PUMA* and *p53* shRNAs.

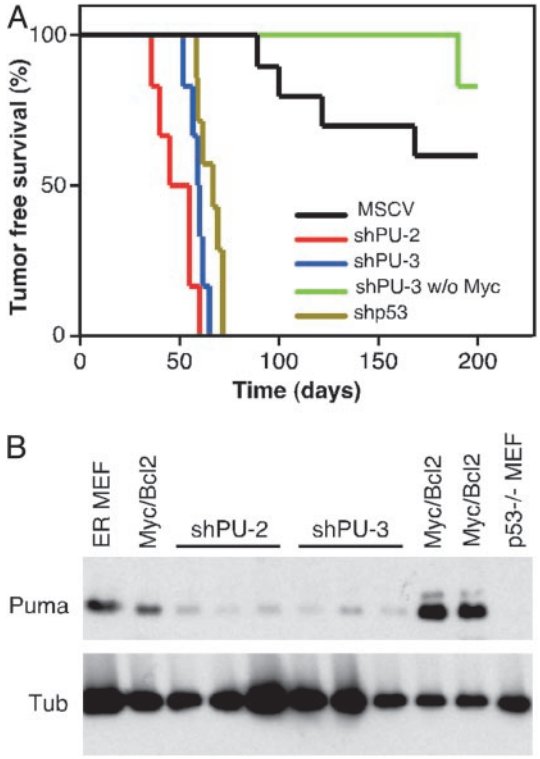

Fig. 4. Acceleration of *myc*-induced lymphomagenesis by *PUMA* shRNAs. (*A*) Mice reconstituted with stem cells infected with the indicated *PUMA* shRNAs were monitored for tumor onset and illness until they reached a terminal stage and were killed. The data are presented in a Kaplan–Meier format showing the percentage of mouse survival at various times postreconstitution. The shp53 survival data represents an updated cohort of mice, including previously published shp53-recipient mice (16). (*B*) Western blot of shPUMA-induced tumors showing decreased levels of PUMA relative to control *Eμ-myc bcl2* lymphomas and MEFs infected with *E1A/ras*. Tubulin is shown as a loading control.

enhancer and develop B cell lymphomas between 3 and 6 months of age (27). However, *Eμ-myc* lymphomas harboring *p53* deletions arise much more rapidly and typically display a more aggressive and disseminated pathology (28). Hematopoietic stem cells (HSCs) from *Eμ-myc* transgenic mice also give rise to lymphomas upon adoptive transfer into normal recipients, and these lymphomas can be greatly accelerated by *p53* suppression by RNAi (16). Given the ability of PUMA to mediate p53-dependent apoptotic activity in MEFs, we investigated whether stable suppression of PUMA in *Eμ-myc* hematopoietic stem cells by RNAi could recapitulate the effects of *p53* deletions and accelerate lymphomagenesis in recipient animals.

HSCs derived from *Eμ-myc* fetal livers were infected with retroviruses encoding a control vector, shPU-2, shPU-3, or a *p53* shRNA, and the resulting populations were used to reconstitute the hematopoietic system of lethally irradiated mice. Whereas only 40% of mice reconstituted with control HSCs developed lymphomas (four of 10, average survival of 120 ± 29 days), 100% of the mice receiving shPUMA-expressing HSCs developed lymphomas with dramatically reduced latency ($n = 12$, average survival 49 ± 7 and 59 ± 4 for shPU-2 and shPU-3, respectively; $P < 0.001$ relative to controls). Importantly, lymphomas arising from shPUMA-transduced shRNAs contained the shPUMA retrovirus and suppressed PUMA protein because all 12 of these lymphomas were GFP-positive (compared to only 1 of 4 controls) and showed substantially reduced PUMA expression relative to *E1A/ras* MEFs and *bcl2*-expressing lymphomas (Figs. 4*B* and 5*A*). Importantly, *PUMA* shRNAs were as potent as a *p53* shRNA in promoting lymphomagenesis (Fig. 4*A*) (16). Moreover, *myc* was required for these effects because only one-sixth of mice reconstituted with WT HSCs expressing *PUMA* shRNAs formed a lymphoma of T cell origin. Therefore, like *p53* loss, PUMA suppression can potently cooperate with *myc* during lymphomagenesis.

shPUMA Lymphomas Are Aggressive Pre-B Cell Lymphomas That Retain p53-Dependent Cell-Cycle Checkpoints. To further characterize tumorigenesis produced by *PUMA* suppression, we monitored lymphoma manifestation in recipient animals and conducted a variety of pathological analyses. In agreement with the survival data, whole-body fluorescence imaging of GFP expression in developing lymphomas revealed that the onset and overall distribution of shPUMA lymphomas closely resembled that occurring in shp53 lymphomas. Specifically, as was shown for *Eμ-myc* shp53 tumors in ref. 16, *Eμ-myc* shPUMA lymphomas typically involved many of the peripheral lymph nodes, including cervical, inguinal, brachial, and mesenteric lymphomas (Fig. 5*A*) (data not shown), with significant dissemination into the liver and spleen (Fig. 5*B*) (data not shown). Consistent with previous studies on *p53*-null lymphomas, both shPUMA and shp53 lymphomas rarely displayed the "starry sky" histology

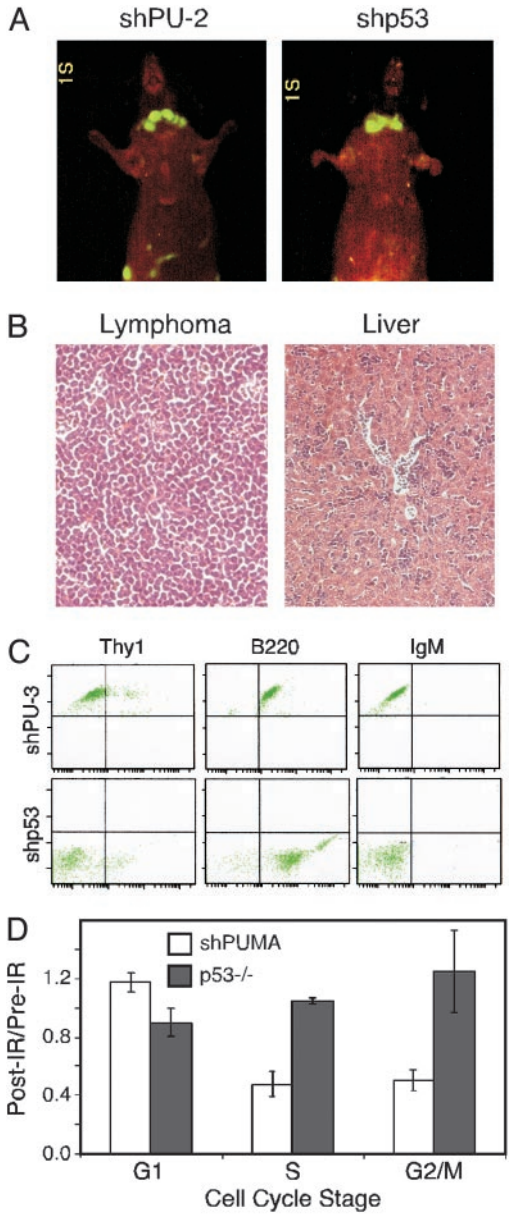

Fig. 5. shPUMA lymphomas phenocopy shp53 lymphomas. (*A*) *In vivo* GFP imaging showing cervical lymphomas in mice reconstituted with *Eμ-myc* HSCs transduced with PUMA and p53 shRNAs. (*B*) Hematoxylin/eosin staining of shPUMA lymphoma and liver sections, showing an absence of apoptotic cell clusters and perivascular and perenchymal infiltration of tumor cells, respectively. (*C*) Immunophenotyping of shPUMA and shp53 tumors by flow cytometry reveals that both tumors are pre-B cell lymphomas. Unlike the shPUMA vector, the shp53 vector lacks GFP expression. Thus, the resulting tumors are GFP-negative. (*D*) Mice harboring shPUMA and $p53^{-/-}$ lymphomas were left untreated or irradiated at 6 Gy, and DNA content analysis was performed on extracted lymphoma cells 36 h later. The relative ratios of the percent of irradiated vs. nonirradiated shPUMA and $p53^{-/-}$ lymphoma cells in each cell-cycle stage are shown.

indicative of the extensive apoptosis observed in *Eμ-myc* lymphomas (Fig. 5*B*) (6), and both were classified as pre-B cell lymphomas by immunophenotyping (Fig. 5*C*). Thus, the effects of p53 loss and *PUMA* suppression on *myc*-induced lymphomagenesis are strikingly similar.

The data described above suggest that disruption of apoptosis through *PUMA* suppression can mimic *p53* loss during *myc*-induced lymphomagenesis. To determine whether shPUMA lymphomas have acquired defects in p53-dependent arrest functions, we examined the integrity of the radiation-induced cell-cycle checkpoint in shPUMA lymphomas. Tumor-bearing mice were subjected to whole body γ-irradiation, and, 36 h later, the lymphomas were harvested and subjected to DNA content analysis by using flow cytometry. In contrast to $p53^{-/-}$ lymphomas but comparable with control and Bcl2-expressing lymphomas with intact p53 (8), shPUMA lymphomas accumulated in G_1 and showed a significant reduction in *S* phase after irradiation (Fig. 5*D*) (data not shown), implying that the effects of *PUMA* shRNAs on tumorigenesis do not require secondary mutations that compromise p53-dependent cell-cycle checkpoints. Interestingly, unirradiated p53-deficient lymphomas displayed a substantially increased S-phase population relative to shPUMA lymphomas at the time of isolation (data not shown). Therefore, although the increased proliferative rate associated with *p53* mutations may confer advantages over *PUMA* suppression at later stages of tumor evolution, our data indicate that loss of apoptosis through *PUMA* disruption phenocopies *p53* loss in promoting *Eμ-myc* lymphomagenesis.

Discussion

Our studies indicate that suppression of *PUMA* can approximate the effects of *p53* loss during *E1A/ras*-mediated transformation of primary MEFs and during *Myc*-induced lymphomagenesis. As such, they demonstrate that *PUMA* is an important component of the p53 tumor suppressor network and highlight the utility of stable RNAi technology to evaluate the activity of candidate tumor-suppressor genes.

These results demonstrate that *PUMA* can function as a *bona fide* tumor suppressor in mice. The effects of *PUMA* suppression were equivalent to *p53* loss in promoting lymphoma onset and were nearly as effective as *p53* loss during *E1A/ras*-induced transformation. Although mutations in BH3-only proteins have yet to be identified in human tumor specimens, our work predicts that *PUMA* suppression, through loss of p53 transactivating functions or direct mutations, may contribute to human cancer. Interestingly, *PUMA* maps to chromosome 19q13.3, which is altered in human gliomas, neuroblastomas, and B cell lymphomas (29–31). Notably, complete inactivation of *PUMA* may not be necessary to promote tumor phenotypes because, although *PUMA* expression was substantially reduced in shPUMA-expressing lymphomas, it was still detectable (Fig. 4*B*).

Interestingly, *PUMA*-null mice are not overtly tumor-prone (14, 15). Although, at first glance, these results are contradictory to our findings, they suggest that the ability of PUMA to mediate apoptosis and tumor suppression is context-dependent. In fact, our studies demonstrate that the ability of PUMA to act as a tumor suppressor can be dependent on other oncogenic events. Hence, although *PUMA* shRNAs cooperated effectively with *E1A/ras* and *myc* to promote tumorigenesis *in vivo*, they did not cooperate with *ras* alone. By analogy, *bcl2*, a PUMA antagonist, efficiently cooperates with *myc* during lymphomagenesis but is not a potent oncogene on its own.

Our study has important implications for understanding p53 action in tumor suppression. We previously showed that *bcl2* overexpression could mimic *p53* loss during *myc*-induced lymphomagenesis and suggested that disruption of apoptosis was sufficient to explain the tumorigenic advantage conferred by *p53* mutations in this context. Here, we show that PUMA, a p53 effector that promotes apoptosis but not cell-cycle arrest, can account for p53 action in at least some settings where p53 acts as a tumor suppressor. These data reinforce the notion that, despite the diversity of p53 activities, its action in tumor suppression can be mediated by only one effector function. Consequently, although *p53*-null lymphomas may have many defects, our data imply that some are byproducts of tumorigenesis and do not provide any immediate advantage to the developing tumor.

Importantly, PUMA does not approximate p53 action in modulating all tumor phenotypes. Whereas *p53* loss is an effective initiator of T cell lymphomagenesis (7), PUMA suppression is not, based on our results and the findings in refs. 14 and 15. Additionally, although *p53* and *PUMA* shRNAs transformed primary MEFs with *E1A/ras*, only *p53* shRNAs or *p53* deletions cooperated with *ras*-induced transformation. This context dependence can be understood in light of the underlying p53 biology. The *E1A* and *myc* oncogenes induce proliferation but also activate p53 to promote apoptosis (26, 32, 33). Hence, disruption of the p53-dependent apoptotic activity provides an immediate and potent advantage to the oncogene-expressing cells but provides little benefit to proliferation-restricted normal cells not subject to an apoptotic stimulus. In contrast, oncogenic *ras* promotes proliferation but also activates p53 to promote cellular senescence (20). Here, p53's arrest functions may be crucial for its tumor suppressor activity. Because PUMA does not mediate these functions, its inactivation does not mimic *p53* loss in this setting. The differential relevance of apoptosis in p53-mediated tumor suppression also may explain why certain *p53* mutants, defective in apoptosis but not cell-cycle arrest, are impaired in their ability to initiate T cell lymphomagenesis (10).

In summary, our results imply that the p53 functions underlying its tumor-suppressor activity are context-dependent and may be influenced by cell type, microenvironment, and oncogenic events acquired during the course of tumor evolution. In some settings, such as in *E1A/ras* transformation and *myc*-induced lymphomagenesis, disruption of apoptosis by *PUMA* loss or defects in other apoptotic regulators is sufficient to promote tumorigenesis, whereas defects in cell-cycle checkpoints and genomic instability are apparently byproducts of *p53* loss. However, these byproducts may provide new capabilities that become relevant later during tumor progression or cancer therapy, producing tumors that are more aggressive than those acquiring strictly antiapoptotic lesions (17). It is noteworthy that *p53*-deficient lymphomas, once established, progress to a lethal stage more rapidly than those expressing *PUMA* shRNAs, perhaps because of their higher proliferative capacity or increased genomic instability (data not shown). Undoubtedly, in other settings, defects in cell-cycle checkpoints provide the driving force for *p53* mutations, with apoptotic defects being byproducts of *p53* loss. Because essential p53 tumor-suppressor functions are context-dependent, effective strategies to treat *p53* mutant tumors also may depend on context. Understanding which p53 function(s) are key to the evolution of different tumor types may ultimately identify activities required for tumor maintenance and suggest targets for therapeutic intervention.

We thank L. Bianco for histology; R. Sachidanandam for bioinformatics support; C. Rosenthal, M.-M. Yang, and S. Ray for technical assistance; and R. Dickins and other members of the Lowe laboratory for advice and critical reading of the manuscript. This work was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation (to M.T.H.), a postdoctoral training grant (to J.T.Z.), National Cancer Institute Project Grants CA13106 (to G.J.H. and S.W.L.) and CA87497 (to S.W.L.), and a generous gift from the Laurie Strauss Leukemia Foundation. D.J.B. is the recipient of a Watson School of Biological Sciences Engelhorn Fellowship, and S.W.L. is an American Association for Cancer Research–National Foundation for Cancer Research Research Professor.

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