

Atm Is Dispensable for p53 Apoptosis and Tumor Suppression Triggered by Cell Cycle Dysfunction

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Both p53 and ATM are checkpoint regulators with roles in genetic stabilization and cancer susceptibility. ATM appears to function in the same DNA damage checkpoint pathway as p53. However, ATM's role in p53-dependent apoptosis and tumor suppression in response to cell cycle dysregulation is unknown. In this study, we tested the role of murine ataxia telangiectasia protein (Atm) in a transgenic mouse brain tumor model in which p53-mediated apoptosis results in tumor suppression. These p53-mediated activities are induced by tissue-specific inactivation of pRb family proteins by a truncated simian virus 40 large T antigen in brain epithelium. We show that p53-dependent apoptosis, transactivation, and tumor suppression are unaffected by Atm deficiency, suggesting that signaling in the DNA damage pathway is distinct from that in the oncogene-induced pathway. In addition, we show that Atm deficiency has no overall effect on tumor growth and progression in this model.

The *p53* tumor suppressor and *ATM* (mutated in human ataxia telangiectasia [AT] disease; *Atm* in mice) are both cancer susceptibility genes with roles in checkpoint regulation (9, 22, 23). Each is associated with distinct human genetic disorders in which patients are prone to cancer. Patients with Li-Fraumeni syndrome carry a mutant *p53* allele and develop a variety of cancers, including mammary adenocarcinomas, sarcomas, brain tumors, and leukemia (21, 33). The *p53* gene is also mutated in about 50% of sporadic human cancers (6, 11). AT is an autosomal recessive disease characterized by cerebellar degeneration, oculocutaneous telangiectasia, retarded growth, infertility, sensitivity to ionizing radiation (IR), and a high incidence of cancers, most commonly lymphoid malignancies (17, 30). The early deaths of most homozygous AT patients preclude an accurate assessment of the full tumor spectrum and the frequency of *ATM* deficiency in humans. Thus, human disease progression alone cannot predict whether *p53* and *ATM* share tumor suppressor pathways.

p53 is involved in the cellular responses to a variety of stress signals, the best characterized of which is DNA damage (7, 32). In response to a given signal, *p53* can induce cell cycle arrest or apoptosis, and these functions appear to be involved in its ability to suppress tumorigenesis. *p53* deficiency can promote tumor growth by a reduction in the level of apoptosis, an event for which there would be substantial selection (12, 25, 34). Alternatively (or in addition), since *p53*-deficient cells are prone to genomic instability (20, 44), the loss of *p53* responses may promote tumor progression through the genetic alteration of other cancer genes (8, 15, 16).

ATM is also involved in checkpoint regulation. It belongs to the phosphatidylinositol-3' kinase superfamily, a family of signal transduction proteins with homology in their carboxyl kinase domains (29). In response to DNA damage, this 350-kDa protein kinase appears to be required for checkpoints in G₁, S,

and G₂ phases (22, 23). Cultured cells derived from AT patients or from *Atm*-deficient mice are highly abnormal. These cells grow slowly and exhibit senescence prematurely (2, 17, 41). They demonstrate high rates of spontaneous apoptosis and a hypersensitivity to IR (22). Genome instability characterized by frequent chromosomal translocations and telomere defects is also commonly observed in AT cells (31, 35). *Atm*-deficient mice display many of the human AT phenotypes, such as retarded growth, infertility, sensitivity to IR, neurological dysfunction (although mild), and tumor proneness (2, 5, 40).

Evidence that *ATM* and *p53* could act in the same pathway comes from studies of cell lines derived from AT patients and of knockout mice. Induction of *p53* and G₁ arrest in response to DNA damage is impaired in AT cell lines (14) and in mouse *Atm*^{-/-} embryonic stem cells (41), embryo fibroblasts, and thymus cells (1, 2), indicating that *ATM* acts upstream of *p53* in response to DNA damage. Both *p53*^{-/-} (4, 13) and *Atm*^{-/-} (2, 5, 40) mice develop thymic lymphoma, also suggesting the possibility of a common thymocyte tumor suppression pathway.

In addition to DNA damage, *p53* is activated by many other signals, such as hypoxia, low ribonucleoside triphosphate levels, and oncogene-induced aberrant proliferation (7, 18). Dysregulated cell cycle activity has been shown to signal *p53*-dependent apoptosis and tumor suppression in vivo (12, 24, 25, 34). It is not clear whether these different stress signals are transduced to *p53* via common or distinct upstream molecular pathways.

Based on the established link between *Atm* and *p53* in response to DNA damage, we addressed the role of *Atm* in *p53*-dependent apoptosis and tumor suppression when induced by aberrant cell cycle activities by using the transgenic TgT₁₂₁ brain tumor model. T₁₂₁ is a truncated simian virus 40 large T antigen that binds and inactivates pRb and the related proteins p107 and p130 (27). Tissue-specific expression of this oncoprotein in brain choroid plexus epithelium (CP) induces the aberrant proliferation of these normally quiescent cells, and a measurable *p53*-dependent apoptosis pathway that suppresses tumor growth is activated (Fig. 1A). Inactivation of *p53*

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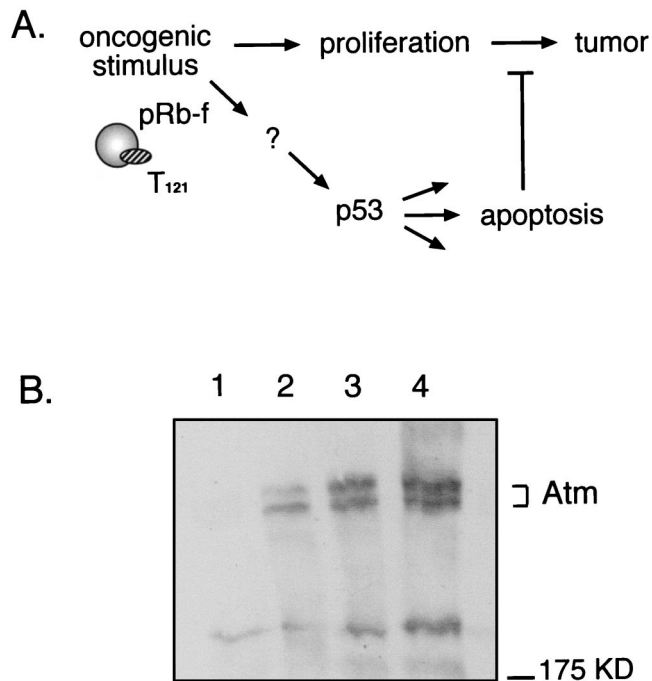


FIG. 1. Is Atm required for p53-dependent apoptosis and tumor suppression in the TgT₁₂₁ brain tumor model? (A) Transgenic expression of T₁₂₁ in CP inactivates pRb family proteins (pRb-f) (including pRb, p107, and p130), resulting in abnormal proliferation and tumorigenesis. As a result, p53 is activated, causing apoptosis and attenuation of tumor growth (34). (B) Atm is expressed in T₁₂₁ CP. Western immunoblotting was performed on tissue extracts by using a monoclonal antibody specific for ATM, 2C6. Lane 1, *Atm*^{-/-} spleen (negative control); lanes 2 and 3, thymus and spleen tissues, respectively, of a wild-type mouse (positive control); lane 4, TgT₁₂₁ CP. Two resolvable Atm-specific bands the size of mouse Atm are detectable in lanes 2 through 4 and are absent from the negative control. The position of a 175-kDa molecular mass marker is shown on the right.

causes an 85% reduction in CP apoptosis and accelerates tumor growth sevenfold (34). This model provides a quantitative test for p53 tumor suppression activities in vivo. In this study we assessed the impact of Atm deficiency on p53-mediated apoptosis and tumor suppression in these mice.

MATERIALS AND METHODS

Mice. TgT₁₂₁ mice carry a transgene that expresses T₁₂₁ specifically in CP under the control of lymphotropic papovavirus transcriptional signals (28). T₁₂₁ consists of the simian virus 40 T-antigen N-terminal 121 amino acids with 11 C-terminal missense residues (27). The *Atm*^{-/-} mice carry a truncation mutation at nucleotide 5790 caused by a PGKneo gene insertion (2). TgT₁₂₁ (C57BL/6 × DBA2) mice were bred with *Atm*^{+/-} mice (C57BL/6 × 129sv) to generate (TgT₁₂₁ × *Atm*^{+/-}) F₁ mice. F₁ mice were further intercrossed to generate TgT₁₂₁ *Atm*^{-/-} mice (C57BL/6 × DBA2 × 129sv). TgT₁₂₁ and *Atm*^{-/-} genotypes were determined by PCR analysis of tail DNA. TgT₁₂₁ screening has been described previously (28). PCR primers were designed for genotyping *Atm*^{-/-} mice. Primer pairs Atm-F (5'-GAC TTC TGT CAG ATG TTG CTG CC-3') and Atm-B (5'-CGA ATT TGC AGG AGT TGC TGA G-3') were used to identify the wild-type *Atm* allele, and Atm-F and Atm-Neo (5'-GGG TGG GAT TAG ATA AAT GCC TG-3') were used to identify the knockout allele by performing 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The Atm-F-Atm-B pair generates a 162-bp PCR product, and the Atm-F-Atm-Neo pair generates a 441-bp PCR product.

Western blotting. Western blotting analysis was carried out as previously described (39). Two hundred micrograms of protein from total cell lysates of fresh tissues was resolved by sodium dodecyl sulfate-5% polyacrylamide gel electrophoresis (cross-linking ratio, 29:1). Two independent anti-human ATM antibodies, 2C6 (3) and 473 (kindly provided by Eva Lee and Michael Kastan, respectively), were used separately to detect Atm protein expression in TgT₁₂₁ CP. The results were the same with both reagents. The enhanced chemilumi-

nescence system (Amersham) was used according to the manufacturer's instructions.

Histology, S-phase, and apoptosis assays. Brain tissues were fixed in 10% formalin, embedded in paraffin, and sectioned as previously described (34). To examine tumor size, 6- μ m sections were taken from 10 successive layers at 100- μ m intervals. For histology assays, sections were stained with hematoxylin and eosin as previously described (34). Bromodeoxyuridine (BrdU) labeling and immunostaining as well as the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) apoptosis assay were completed as previously described (34, 43). Quantification was made by averaging the percentage of positive cells in 10 random fields at a magnification of $\times 400$.

In situ RNA hybridization. Sections (6 μ m) were hybridized with p21 or Bax antisense probes as previously described (26, 43). Probes were radiolabeled with α -³⁵S-UTP and used at a concentration of 5×10^4 cpm ml⁻¹. Autoradiography was conducted at 4°C for 3 days for the p21 probe and at 4°C for 3 weeks for the Bax probe. Signal densities were quantified with NIH Image 1.58. The threshold was set by using the sense probe signals as the background. Means were determined from 10 random areas of CP for each sample.

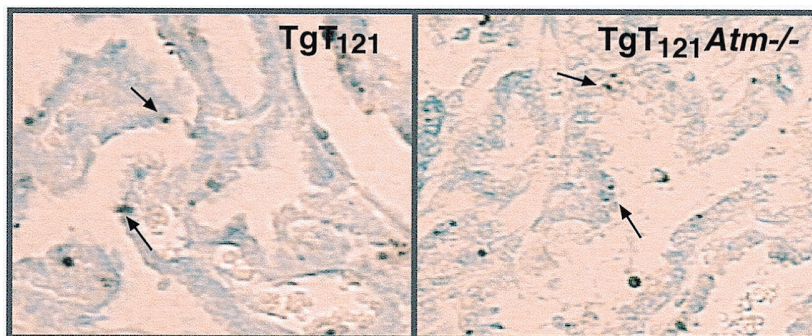
RESULTS

p53-dependent apoptosis does not require Atm. A genetic approach has been taken to determine the pathway by which p53-dependent apoptosis and tumor suppression proceed in TgT₁₂₁ mice. For example, by assessing the rates of apoptosis and tumor growth in a background with specific deficiencies, we previously determined that E2F1 is upstream (26) and Bax is downstream (43) of p53 in this system. The possibility that Atm could be active in TgT₁₂₁ CP is demonstrated by the detection of Atm in this tissue by Western blotting analysis (Fig. 1B). A spleen extract from an *Atm*^{-/-} mouse served as a negative control and showed no specific proteins the size of Atm (Fig. 1B, lane 1). In contrast, spleen and thymus tissues from a wild-type mouse (lanes 2 and 3) and CP from a TgT₁₂₁ mouse (lane 4) contained a 350-kDa Atm-specific doublet detectable with two independent ATM-specific antibodies (the results presented in Fig. 1B were obtained with the 2C6 monoclonal antibody).

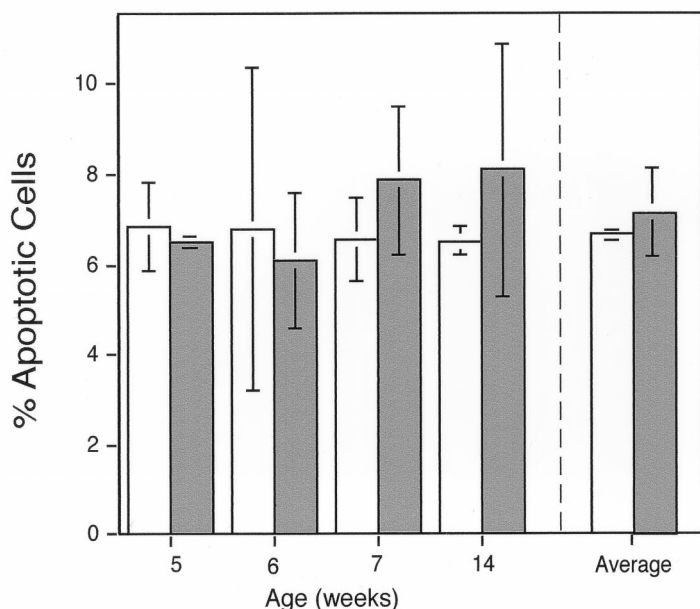
To examine whether Atm deficiency has a role in p53 tumor suppression activities, we generated TgT₁₂₁ *Atm*^{-/-} mice through a series of crosses by using TgT₁₂₁ (28) and *Atm*^{+/-} (2) mice. We first assessed the effect of Atm deficiency on T₁₂₁-induced p53-dependent apoptosis by using the in situ TUNEL assay (Fig. 2A). The CP apoptotic index (AI) was determined for brain sections from TgT₁₂₁ *Atm*^{-/-} mice, which were compared to TgT₁₂₁ *Atm*^{+/+} littermates. Previously, TgT₁₂₁ CP cells were shown to have an average AI of 7.3%, 85% of which requires functional p53 (34). In the present study, the CP AI of TgT₁₂₁ *Atm*^{+/+} littermate controls was similar, averaging $6.69\% \pm 0.17\%$ (Fig. 2B). The CP of control *Atm*^{-/-} mice appeared to be normal, with no apoptotic activity (Table 1). The AI in TgT₁₂₁ *Atm*^{-/-} CP was similar to that of TgT₁₂₁ *Atm*^{+/+} mice ($7.17\% \pm 0.99\%$) (Fig. 2; Table 1). This is in stark contrast to the dramatic decrease in AI caused by p53 deficiency (Table 1; Fig. 2C) (34). Thus, in this system, p53-dependent apoptosis in response to abnormal proliferation does not require Atm.

Intact p53 transactivation activity in the absence of Atm. To further assess the impact of Atm deficiency on p53 function, we examined p53 transactivation activity in TgT₁₂₁ *Atm*^{-/-} CP. We previously showed that T₁₂₁ expression in the CP induces the p53-regulated genes *bax* (43), *p21* (26), and *mdm2* (26) in a p53-dependent manner. To assess the p53 transactivation activity in the absence of Atm, *bax* and *p21* transcript levels were measured in TgT₁₂₁ *Atm*^{-/-} and TgT₁₂₁ *Atm*^{+/+} brain sections by in situ RNA hybridization (Fig. 3). Samples from young mice (5 to 7 weeks) were analyzed to avoid the influence of spontaneous genetic changes during tumor progression. Quantitative analysis indicated that p53-dependent *p21* and

A.



B.



C.

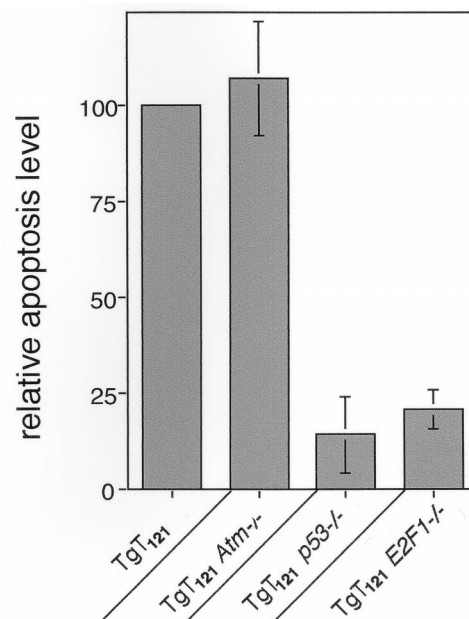


FIG. 2. p53-mediated apoptosis does not require Atm. (A) Apoptotic cells in CP (arrows) were detected by the TUNEL assay. No qualitative changes were detected between TgT₁₂₁ and TgT₁₂₁ Atm^{-/-} CP tumor cells. (B) Quantitative apoptotic indices in TgT₁₂₁ and TgT₁₂₁ Atm^{-/-} CP. The percentages of apoptotic cells were determined for TgT₁₂₁ (open bars) and TgT₁₂₁ Atm^{-/-} (solid bars) littermates at different ages. Each bar in the left panel represents the averaged counts from 10 fields of one sample from one mouse at the specified age. The averages of all four mice are shown at the right. The error bars represent the standard deviations among microscopic fields (left) or among mice (right). (C) Impact of Atm deficiency on apoptosis compared with impact of p53 and E2F1 deficiency. Atm deficiency has no effect on apoptosis in TgT₁₂₁ CP cells, while p53 and E2F1 account for 85 and 80% of the apoptosis, respectively (26, 34).

bax RNA levels in the CP remain unchanged in the absence of Atm (Fig. 3A through D). The levels of *p21* and *bax* transcripts in TgT₁₂₁ Atm^{-/-} CP were 99% ± 4.8% and 105% ± 8%, respectively, of those of TgT₁₂₁ Atm^{+/+} littermates (Fig. 3G).

All *p21* expression in these cells was dependent on p53 activity (Fig. 3E), as was much of the *bax* expression, as previously observed (Fig. 3F). In this system, Bax is involved in p53-dependent apoptosis (43), while p21 is not (our unpublished

TABLE 1. Atm impact on tumor growth

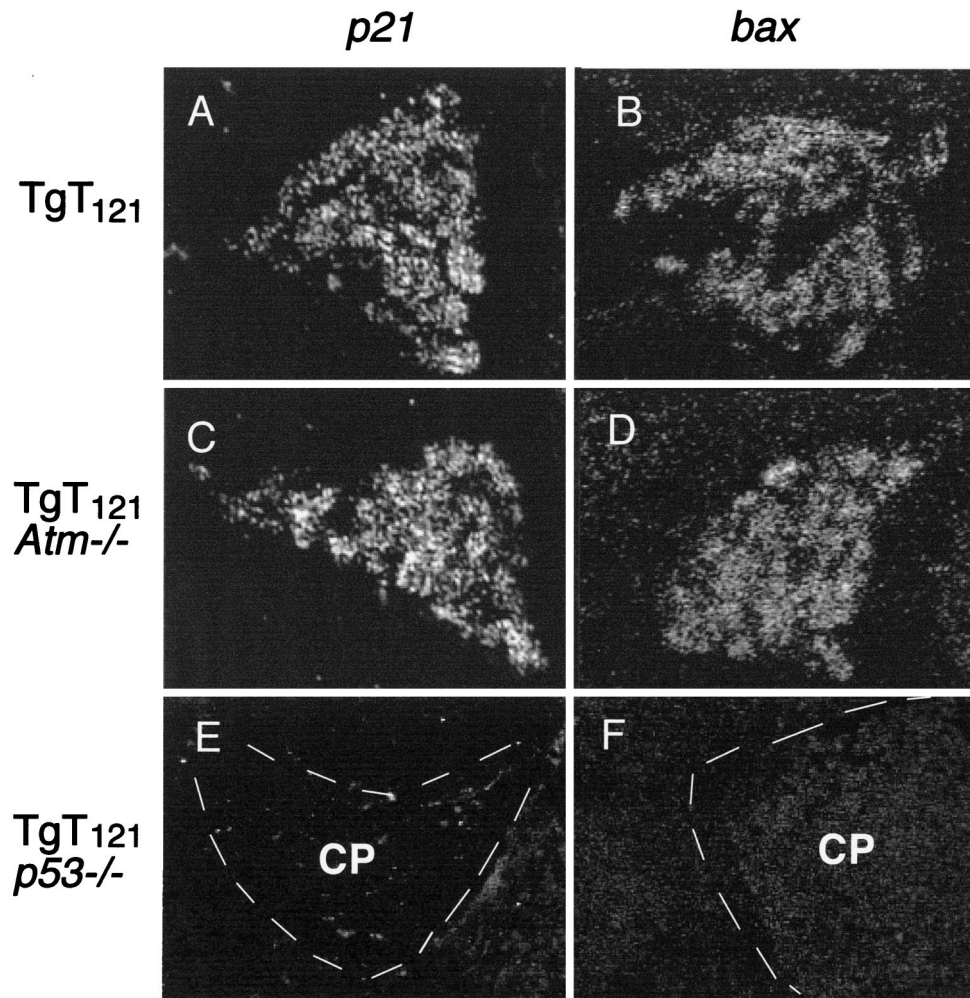
| Genotype | % of apoptotic cells ^a | % of S-phase cells ^a | Type of CP neoplastic growth | No. of wks of survival (mean ± SE) ^b | Cause of death |
|----------------------------------------|-----------------------------------|---------------------------------|------------------------------|-------------------------------------------------|----------------|
| Atm ^{-/-} | 0 ^c | 0 ^c | None | 20.2 ± 3.9 (6) | Thymoma |
| TgT ₁₂₁ | 100 | 100 | Slow | 31.0 ± 1.6 (16) | CP tumor |
| TgT ₁₂₁ Atm ^{+/-} | 98 ^c | 120 ^c | Slow | 37.2 ± 4.21 (6) | CP tumor |
| TgT ₁₂₁ Atm ^{-/-} | 107 ± 15 | 103 ± 33 | Slow | 22.7 ± 1.8 (15) | Thymoma |
| TgT ₁₂₁ p53 ^{-/-d} | 14 ± 10 | 109 ^c | Rapid | 4 ± 0.2 (4) | CP tumor |

^a Relative to the level in TgT₁₂₁ CP.

^b Values in parentheses are numbers of mice tested. Mean survival times of TgT₁₂₁ and TgT₁₂₁ Atm^{+/-} mice are not significantly different (*P* = 0.1026 in an unpaired two-tailed *t* test). The difference may reflect background strain differences (TgT₁₂₁ mice are C57BL/6 × DBA2; TgT₁₂₁ Atm^{+/-} mice are C57BL/6 × DBA2 × 129sv).

^c From a single sample.

^d Data from reference 34; TgT₁₂₁ p53^{-/-} mice are of a C57BL/6 × DBA2 × 129sv background.



G. Relative RNA levels

| | TgT ₁₂₁ | TgT ₁₂₁ <i>Atm</i> ^{-/-} | TgT ₁₂₁ <i>p53</i> ^{-/-} |
|------------|--------------------|----------------------------------------------|----------------------------------------------|
| <i>p21</i> | 100 | 99 | 4 |
| <i>bax</i> | 100 | 105 | 13 |

FIG. 3. *Atm* deficiency does not affect p53 transactivation function in CP. Representative in situ RNA hybridization signals are shown for *p21* (A, C, and E) and *bax* (B, D, and F) expression. *p21* RNA is readily detectable in TgT₁₂₁ (A) as well as in TgT₁₂₁ *Atm*^{-/-} (C) CP. *bax* RNA levels are also equally expressed in TgT₁₂₁ (B) and TgT₁₂₁ *Atm*^{-/-} (D) cells. *bax* and *p21* expression in TgT₁₂₁ CP are both p53 dependent, as seen in TgT₁₂₁ *p53*^{-/-} cells (E and F). (G) Quantitative comparisons of RNA levels; signals in TgT₁₂₁ are designated as 100%. The signal detected in CP by using a *bax* or *p21* sense probe was at about the background level, ensuring the specificity of the assay. Ten fields of each sample were analyzed. Standard errors in each field are less than 10% of the relative mean value.

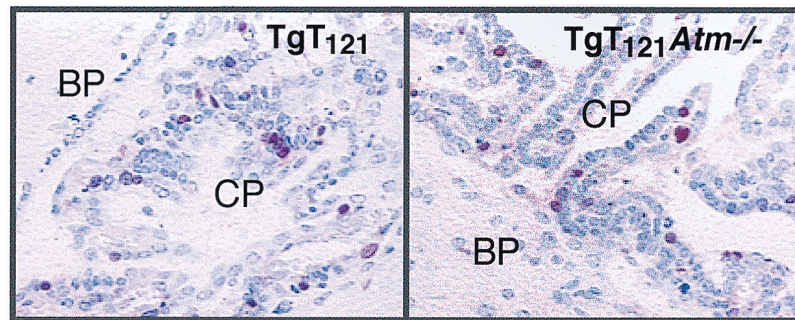
results). Thus, *Atm* is dispensable for T₁₂₁-induced p53 transactivation functions that are apoptosis dependent as well as independent.

Effects of *Atm* deficiency on tumor cell proliferation and overall tumor growth. Although the above results show that *Atm* is not required for p53-dependent apoptosis or transactivation, *Atm* could affect CP tumor growth by other mechanisms. For example, *Atm* deficiency could diminish tumor

growth since *Atm*^{-/-} fibroblasts exhibit poor growth and a reduced life span in culture (2, 41). Alternatively, *Atm* inactivation could accelerate tumor growth through genetic instability, as proposed for *Atm* deficiency-induced lymphoma and leukemia (35).

To determine whether *Atm* deficiency has an impact on CP tumor cell proliferation, we measured S-phase indices by in vivo BrdU incorporation (Fig. 4A). Mice were analyzed at

A.



B.

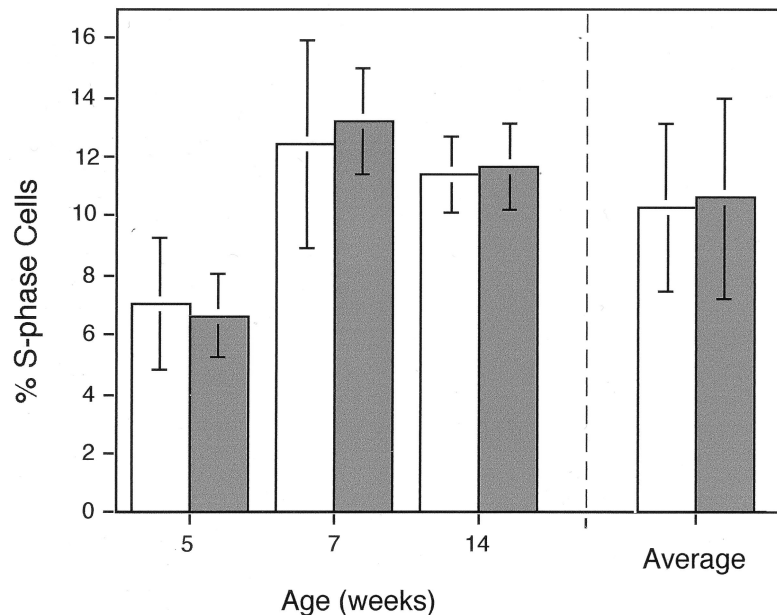


FIG. 4. Effects of *Atm* deficiency on tumor cell proliferation. TgT₁₂₁ and TgT₁₂₁ *Atm*^{-/-} CP were stained for BrdU incorporation to measure proliferation rates. (A) Representative views of CP from TgT₁₂₁ and TgT₁₂₁ *Atm*^{-/-} 7-week-old littermates stained for BrdU (red), with nuclei counterstained by hematoxylin (blue). No BrdU-staining cells are present in the surrounding brain parenchyma (BP). (B) Percentage of S-phase cells in TgT₁₂₁ (open bars) and TgT₁₂₁ *Atm*^{-/-} (solid bars) CP. Left, percentage of proliferating cells in paired littermates (each bar represents brain sections from one mouse; error bars represent the standard deviations among sets of 10 microscopic fields); right, averaged percentage of S-phase cells in these age-matched groups shown at the left (error bars represent standard deviations among individual mice).

various times during early tumor growth (5 to 14 weeks) to circumvent selective changes during tumor progression. No difference was detected in the percentage of S-phase CP in TgT₁₂₁ *Atm*^{-/-} and TgT₁₂₁ *Atm*^{+/+} littermates (Table 1). TgT₁₂₁ *Atm*^{-/-} CP averaged 10.6% ± 3.4% of cells in S phase, while TgT₁₂₁ *Atm*^{+/+} CP averaged 10.3% ± 2.8% of cells in S phase (Fig. 4B). Therefore, unlike fibroblast growth in culture, *Atm* deficiency had no measurable impact on CP tumor cell proliferation in vivo.

Although the apoptotic and proliferative indices of TgT₁₂₁ CP were not affected by *Atm* inactivation, an effect on tumor morphology and progression was possible. To determine the overall impact of *Atm* deficiency on tumor growth, the survival times, tumor sizes, and morphologies of TgT₁₂₁ *Atm*^{-/-} mice were compared to those of TgT₁₂₁ *Atm*^{+/+} littermates. Control

TgT₁₂₁ and *Atm*-deficient mice developed different tumor types and had different average survival times. TgT₁₂₁ mice died of brain tumors with an average survival time of 31 weeks, whereas *Atm*-deficient mice died of thymoma with an average survival time of 20 weeks (Table 1). Like *Atm*^{-/-} mice, all TgT₁₂₁ *Atm*^{-/-} mice developed thymic lymphoma (average survival time of 23 weeks [Table 1]). The difference between thymoma development in *Atm*^{-/-} mice and that in TgT₁₂₁ *Atm*^{-/-} mice is not statistically significant ($P = 0.5115$ in an unpaired two-tailed *t* test) and could be due to background strain differences (C57BL/6 × 129sv versus C57BL/6 × DBA2 × 129sv) or due to T₁₂₁ expression in thymocytes (28).

Although TgT₁₂₁ *Atm*^{-/-} mice succumbed to thymic lymphoma prior to the terminal age anticipated for mice with brain tumors, their lengthy survival indicates that *Atm* defi-

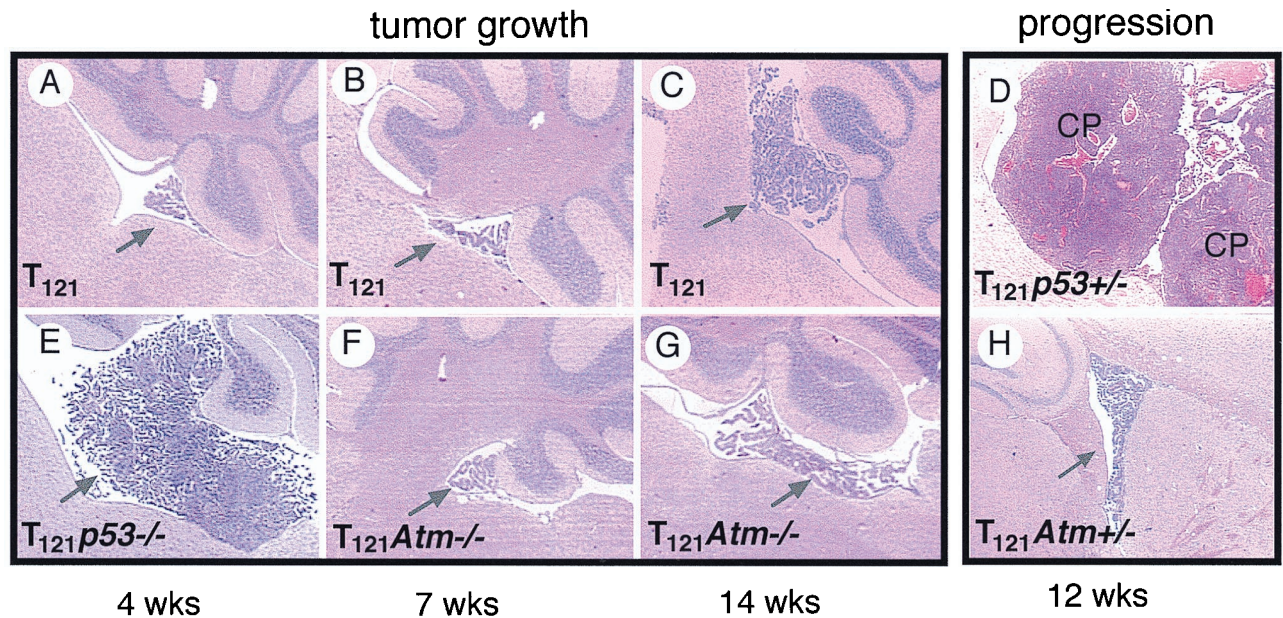


FIG. 5. Effects of *Atm* deficiency on CP tumor growth and progression. Sizes of the CP masses (arrows) are shown. TgT_{121} mice at 7 weeks (B) and 14 weeks (C) are compared to $TgT_{121} Atm^{-/-}$ littermates (F and G, respectively). Note that *p53* deficiency greatly accelerated tumor growth as indicated by the mass size at 4 weeks (E versus A). CP tumor progression to aggressive angiogenic states was observed in $TgT_{121} p53^{+/-}$ mice (D) but not in $TgT_{121} Atm^{+/-}$ mice (H). These views represent the largest mass present among step sections sampled from the full brain to ensure a fair comparison of tumor sizes.

ciency did not substantially accelerate the growth of T_{121} -induced brain tumors. CP tumors present in $TgT_{121} Atm^{-/-}$ mice at the time of autopsy were comparable in size and morphology to those in TgT_{121} mice of similar ages (data not shown). Furthermore, assessment of tumor sizes at 4 to 20 weeks showed no significant differences between $TgT_{121} Atm^{+/+}$ and $TgT_{121} Atm^{-/-}$ littermates (Fig. 5). The CP of $Atm^{-/-}$ mice was morphologically normal. These results are consistent with the observation that both apoptosis and proliferation were unaffected by *Atm* deficiency. In sharp contrast, $TgT_{121} p53^{-/-}$ mice develop large tumor masses within 4 weeks (Fig. 5E; Table 1) (34). Analysis of $TgT_{121} Atm^{+/-}$ mice also showed that *Atm* deficiency did not promote tumor progression. Tumor growth in $TgT_{121} p53^{+/-}$ mice progresses from slow growth to highly aggressive and angiogenic growth within 7 weeks, leading to the death of the mice by 12 weeks of age (Fig. 5D) (34). This occurs in all mice and correlates with a high frequency of *p53* loss of heterozygosity. In contrast, no such acceleration was observed in $TgT_{121} Atm^{+/-}$ mice (Fig. 5H).

DISCUSSION

***Atm* and *p53*.** In this study we explored the possibility that *Atm* functions in a *p53* apoptosis and tumor suppression pathway that is induced via aberrant proliferation rather than DNA damage. The molecular signals involved in *p53* induction in this case are largely unknown. One possibility is that aberrant S-phase activity generates DNA damage-like signals, in which case the pathways could be coincident. Here, we show that *Atm* deficiency does not impair *p53* activation by inappropriate cell cycle activity in brain epithelium. The approach used in this study to study *Atm* has been used in previous studies to identify both upstream (26) and downstream (43) effectors in this pathway. *E2F1* deficiency causes an 80% inhibition of *p53*-dependent apoptosis (Fig. 2C) as well as inhibition of *p53* transactivation function, indicating that *E2F1* lies upstream of *p53* (26). Therefore, inactivation of pRb proteins by T_{121} leads

to *E2F1* activation, which then induces *p53* function (26). In contrast, this report shows that *Atm* is not required in this pathway—*p53*-dependent apoptosis, transactivation, and tumor suppression are fully active in the absence of *Atm*. Together, these data indicate that different stress signals are transduced to *p53* via distinct pathways.

Comparison of these results with those of previous reports addressing the role of *ATM* in *p53*-dependent activities largely supports the idea that the distinction observed here is based on the inducing signal rather than cell type or biological response (i.e., apoptosis or growth arrest). So far, *ATM* has been implicated only in DNA damage-induced *p53* responses, including the induction of both growth arrest and apoptosis. *Atm* is required for DNA damage-induced *p53* induction and G_1 arrest in human (23) and mouse (2) fibroblasts and in mouse embryonic stem cells (41) and thymus tissue (1). It is also required for IR-induced *p53*-dependent apoptosis of neurons in the developing central nervous system, since the irradiation of newborn mice induces widespread central nervous system apoptosis, coincident with *p53* induction. This apoptosis is dramatically reduced in both *p53*-deficient and *Atm*-deficient mice (10).

In the thymus, IR-induced *p53*-dependent apoptosis is not *Atm* dependent *in vivo* (1, 10), although the *p53*-dependent G_1/S checkpoint does require *Atm* (1). Therefore, within a given tissue, distinct *p53*-mediated biological effects are induced by apparently distinct pathways upstream of *p53* (1). Two reports show a partial reduction in the IR-induced *p53*-dependent apoptosis of *Atm*^{-/-} thymus cells (41) or thymocytes (37). This contradiction could indicate differences in thymocyte subpopulations or could result from *in vitro* versus *in vivo* approaches. In the present study, we have examined *in vivo* apoptotic responses to show that *Atm* is dispensable for *p53*-mediated apoptosis and tumor suppression in brain epithelium. We cannot exclude the possibility that *Atm*-related factors fully compensate for the absence of *Atm*.

Whether *Atm* and *p53* participate in the same tumor sup-

pression pathways in other cell types is not known. Although both Atm- and p53-deficient mice develop thymic lymphomas with high frequency, in *Atm* p53 double-null mice, tumor growth is accelerated (37, 42), indicating that Atm and p53 cooperate in thymocyte tumor suppression, rather than fall within a linear pathway. In addition, V(D)J recombination-associated translocations are frequent in thymomas from Atm-deficient mice (2) but not in thymomas from p53-deficient mice (19). Therefore, in mouse thymocytes, where both Atm and p53 are proven tumor suppressors, the pathways may be distinct.

Atm and tumor growth. In the present study Atm deficiency did not affect CP tumor cell proliferation or tumor growth despite the fact that the proliferation of *Atm*^{-/-} fibroblasts was impaired and senescence occurred prematurely. This lack of Atm effect on CP tumor cell proliferation could reflect cell-type-specific differences. In *Atm*^{-/-} fibroblasts, proliferative defects can be rescued by the inactivation of either *p21* or *p53* (36, 38, 42). CP tumor cells express *p21* abundantly and possess functional p53. However, pRb (a known target of cyclin-dependent kinases inhibited by p21) is inactivated by T₁₂₁ in CP tumor cells, possibly masking any growth-inhibitory effects of Atm deficiency. Finally, we considered the possibility that genetic instability induced by Atm deficiency could result in measurable effects on tumor progression. TgT₁₂₁ p53^{+/-} CP tumors progress to highly aggressive states with frequent p53 loss of heterozygosity (34) and widespread aneuploidy (our unpublished results). No such accelerated progression was observed in TgT₁₂₁ *Atm*^{+/-} mice.

In summary, our results suggest that distinct signal transduction pathways may induce p53, depending on the cellular insults. While Atm appears to be involved in p53 activation by DNA damage, it is not required for p53 activation by aberrant cell cycle activity in brain epithelium. Furthermore, when these tumors are initiated by the inactivation of the pRb family, Atm deficiency has no further impact on tumor growth and progression.

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