Ataxia Telangiectasia Mutated-Dependent Apoptosis after Genotoxic Stress in the Developing Nervous System Is Determined by Cellular Differentiation Status

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Ataxia-telangiectasia (A-T) is a neurodegenerative syndrome resulting from dysfunction of *ATM* (*ataxia telangiectasia mutated*). The molecular details of ATM function in the nervous system are unclear, although the neurological lesions in A-T are probably developmental because they appear during childhood. The nervous systems of *Atm-null* mice show a pronounced defect in apoptosis that is induced by DNA damage, suggesting that ATM may function to eliminate DNA-damaged neurons. Here we show that Atm-dependent apoptosis occurs at discrete stages of neurogenesis. Analysis of γ-irradiated mouse embryos showed that Atm-dependent apoptosis occurred only in the postmitotic populations that were present in the neuroepithelial subventricular zone of the developing nervous system. Notably, Atm deficiency did not prevent radiation-induced apoptosis in multipotent precursor cells residing in the proliferating ventricular zone. Atm-dependent apoptosis required p53 and coincided with the specific phosphorylation of p53 and caspase-3 activation. Thus, these data show that Atm functions early in neurogenesis and underscore the selective requirement for Atm in eliminating damaged postmitotic neural cells. Furthermore, these data demonstrate that the differentiation status of neural cells is a critical determinant in the activation of certain apoptotic pathways.

Key words: ataxia-telangiectasia; ATM; p53; subventricular zone; neurogenesis; apoptosis; ionizing radiation; DNA damage; DNA repair

Individuals with the autosomal recessive disorder ataxia-telangiectasia (A-T) manifest a diverse array of symptoms, including immune deficiency, predisposition to cancer, progressive neurodegeneration, and hypersensitivity to ionizing radiation (IR) (for review, see Sedgwick and Boder, 1991; Lavin and Shiloh, 1997; Crawford, 1998; Gatti et al., 2001). Cell lines derived from A-T individuals also show x-ray sensitivity, cell cycle checkpoint defects, premature senescence, and genomic instability (Lavin and Shiloh, 1997; Rotman and Shiloh, 1998). A-T results from dysfunction of *ATM* (*ataxia telangiectasia mutated*), a 370 kDa protein that has protein kinase activity with specificity for serine and threonine residues and C-terminal sequence similarity to the phosphatidyl-inositol-3-kinase (PI$_3$K) family (Savitsky et al., 1995; Lim et al., 2000).

The most prevalent feature of A-T is progressive neurodegeneration, although the mechanism and the etiological agent that are responsible are unknown. Therefore, understanding ATM signaling in the nervous system is particularly relevant to the neuropathology of A-T. It is likely that ATM dysfunction impacts during development, because the neurological defects in A-T are apparent early in life (Sedgwick and Boder, 1991; Crawford, 1998). Furthermore, Atm is highly expressed in the developing mouse nervous system but expressed only at low levels in the adult CNS (Soares et al., 1998).

Insight into ATM function in the nervous system has come from *Atm-null* mice. These mice recapitulate many of the features of the human disease, including cancer predisposition and severe intestinal toxicity after radiation (Barlow et al., 1996; Elson et al., 1996; Xu et al., 1996; Herzog et al., 1998; Borghesani et al., 2000). Additionally, cells derived from the *Atm-null* mouse have similar characteristics to human A-T cells, such as cell cycle checkpoint defects and replicative senescence (Rotman and Shiloh, 1998). Although overt ataxia is not present in these mice, neurological deficits have been reported. These include behavioral abnormalities, dopaminergic neuron loss in the substantia nigra, and altered brain electrophysiology (Barlow et al., 1996; Eilam et al., 1998; Chiesa et al., 2000). In contrast to the extreme radiosensitivity present in A-T individuals, *Atm-null* mice show a striking resistance to DNA damage-induced apoptosis in the nervous system (Herzog et al., 1998; Chong et al., 2000). These data suggest that ATM may function to eliminate neural cells that have incurred genomic damage (Herzog et al., 1998; Lee and McKinnon, 2000). Additional support for this assertion comes from the prevalence of neurodegeneration in many DNA repair-deficient syndromes (Rolig and McKinnon, 2000).

The current consensus is that ATM functions as a protein kinase, and inactivation of this kinase activity is responsible for A-T. Biochemical and genetic analyses have identified various substrates for ATM (Lim et al., 2000), and some of these will be important for ATM function in the nervous system. One well characterized substrate of ATM is p53 (Kastan et al., 1992; Kastan and Lim, 2000). For example, IR promotes Atm-dependent p53 stabilization leading to G$_1$ arrest. ATM is required for the phosphorylation of serine-15 and serine-20 of p53 in a DNA damage-dependent manner that leads to stabilization and transcriptional activation (Banin et al., 1998; Canman et al., 1998; Khanna et al., 1998; Waterman et al., 1998; Ahn et al., 2000). Whereas serine-20 phosphorylation of p53 occurs via an

ATM-dependent modification of Chk2, serine-15 phosphorylation may be a direct event. Thus, *in vitro*, ATM can affect p53 phosphorylation, and these events may contribute collectively to p53 function (Meek, 1999). Although these Atm-dependent modifications of p53 are uncharacterized in the nervous system, they are likely to be relevant because the defect in IR-induced apoptosis in *Atm-null* neurons is also present in *p53-null* mice (Enokido et al., 1996; Morrison et al., 1996; Herzog et al., 1998; Chong et al., 2000). Therefore, we assessed Atm function in the developing nervous system. Here we report that Atm functions at defined developmental stages and structures in the nervous system to regulate radiation-induced apoptosis.

MATERIALS AND METHODS

Animals. Mice were housed in an American Association of Laboratory Animal Care-accredited facility and were maintained in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All procedures for animal use were approved by the institutional animal care and use committee at St. Jude Children's Research Hospital. The presence of a vaginal plug was designated as embryonic day 0.5 (E0.5) and the day of birth as postnatal day 0 (P0). All experimental groups contained at least three Atm or p53 knock-out animals along with wild-type littermates. Mice were irradiated with 14 Gy from a cesium irradiator (at a rate of 0.9 Gy/min) and killed at 1, 2, 4, 6, or 18 hr after irradiation. Littermate animals without irradiation were used as controls. Tissues were collected after transcardial perfusion with 4% paraformaldehyde in PBS. E12.5 and E15.5 embryos were submersed into paraformaldehyde solution immediately after dissection. Fixed tissues were cryoprotected in 25% buffered sucrose solution and cryosectioned at 12 μm with a HM500M cryostat (MICROM, Walldorf, Germany).

Histology and immunohistochemistry. Neutral red staining was performed with 1% Neutral Red (Aldrich Chemical, Milwaukee, WI) in 0.1 M acetic acid, pH 4.8, for 1 min, followed by dehydration in ethanol; the slides were coverslipped with Permount (Fisher Scientific, Pittsburgh, PA). For immunohistochemistry the following antibodies were used: CM5 anti-p53 (at 1:1000 for postnatal brains and 1:500 for embryos; Novocastra Laboratories, Newcastle on Tyne, UK), anti-phospho-p53 (Ser15, at 1:250 for postnatal brains and 1:150 for embryos; New England Biolabs, Beverly, MA), anti-P27 (at 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), anti active-caspase-3 (CM1, at 1:1000; IDUN Pharmaceuticals, San Diego, CA), anti-Ki67 (at 1:1000; Novocastra Laboratories), and anti-β-tubulin III (Tuj1, at 1:1000; Babco, Richmond, CA). Antigen retrieval (Midgley et al., 1992) was used for all immunohistochemistry. Cryosections were incubated with antibodies overnight at room temperature after being quenched with endogenous peroxidase, using 0.6% hydrogen peroxide. Immunoreactivity was visualized with the VIP substrate kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's directions after the tissues were treated with biotinylated secondary antibody and avidin DH-biotinylated horseradish peroxidase-H complex (Vectastain Elite Kit, Vector Laboratories). Sections were counterstained with 0.1% methyl green (Vector Laboratories), dehydrated, and mounted in Permount. *In situ* end labeling (ISEL) staining was performed on cryosections with the Klenow-FragEL kit (Oncogene Research Products, San Diego, CA) according to the manufacturer's directions. For fluorescence detection the embryo sections were treated with blocking solution (5% donkey serum and 1% bovine serum) and then incubated with p53 antibody (CM5 at 1:500). Signals of p53 were visualized with TRITC-anti rabbit IgG raised in donkey (1:200). After intensive washing and blocking with 5% goat serum and 1% bovine serum, the sections were incubated with Ki67 antibody (1:500). FITC-anti rabbit IgG raised in goat (1:200) was used to detect Ki67 signals.

Nonradioactive in situ hybridization. pSPORT containing nucleotides 8345–8939 of mouse Atm was used to generate *in situ* probes (Soares et al., 1998). Digoxigenin (DIG)-labeled riboprobes were synthesized by *in vitro* transcription according to the standard DIG-labeling reaction protocol from Roche Bioscience (Palo Alto, CA). T7 RNA polymerase was used to synthesize the sense probe, and SP6 RNA polymerase was used for the antisense probe. Cryosections were washed in PBS [containing (in mM) 140 NaCl, 2.7 KCl, 10 Na_2HPO_4, and 1.8 KH_2PO_4, pH 7.4] and deproteinized with 1 μg/ml proteinase K in Tris-EDTA buffer (100 mM

Tris, 50 mM EDTA, pH 8.0) for 30 min. After brief post-fixation with 4% buffered paraformaldehyde and several washes, the sections were acetylated with acetic anhydride (0.25% in 0.1 M triethanolamine buffer, pH 8.0). Sections then were incubated with hybridization buffer (50% formamide, 50 μg/ml yeast tRNA, 50 μg/ml heparin, 1% SDS, and 5× SSC, pH 4.5) for 2 hr at 56°C. Finally, the sections were incubated with the hybridization buffer containing 0.6 ng/μl of antisense or sense probes at 72°C overnight in a humidified chamber. On the following day the sections were washed sequentially in 5× SSC, 0.2× SSC, and Tris buffer I (100 mM NaCl, 100 mM Tris, pH 7.5). For immunological detection the sections were incubated with 5% BSA and 5% normal sheep serum in Tris buffer I containing 0.1% Triton X-100 and then with a sheep anti-DIG alkaline phosphate antiserum (at 1:500; Roche) overnight at room temperature in a humidified chamber. To visualize the positive signals, we rinsed sections with Tris buffer I and subsequently with Tris buffer II, pH 9.5 (100 mM Tris, 100 mM NaCl, 50 mM $MgCl_2$). The signal for Atm mRNA was developed with NBT/BCIP solution (Roche) in Tris buffer II, and the reaction was stopped by incubating the slides in 10 mM Tris and 1 mM EDTA, pH 8.1.

RESULTS

Atm-dependent apoptosis is a feature of differentiating cells in the retina

We used ISEL staining of P5 retina from *Atm-null* and wild-type (WT) mice 18 hr after radiation treatment to identify the cells that were susceptible to apoptosis in this tissue. Although WT retina was susceptible to radiation-induced apoptosis, there was a pronounced resistance in *Atm-null* retina (Fig. 1*A*). However, the resistance to apoptosis in the *Atm-null* retina was confined to the central region, because many neuroblasts in the periphery of the *Atm-null* P5 retina underwent apoptosis after radiation (Fig. 1*A*). A distinguishing characteristic of this peripheral cell population is that they are proliferating, undifferentiated cells, as distinct to the postmitotic central neuroblasts (Fig. 1*B*) (Young, 1985a,b). Thus, Atm is dispensable for radiation-induced apoptosis in cycling cells in the retina.

To define Atm involvement in retinal cell populations further, we examined apoptosis after radiation at embryonic stages of retinal development. We used markers for proliferating cells, such as Ki67 (Gatter et al., 1986), and differentiating neurons, such as Tuj1 (a neuron-specific β-tubulin III), to distinguish these populations in the retina (Fig. 1*Ca,Cb*). Although WT retina showed extensive apoptosis after radiation (Fig. 1*Ce,Cf*), Atm deficiency resulted in a marked abrogation of IR-induced apoptosis in the neuroblasts of the developing E15.5 retina (Fig. 1*Cg,Ch*). However, consistent with the P5 situation, some apoptosis was observed in the proliferative layer of the $Atm^{-/-}$ retina (Fig. 1*Cg,Ch*). Essentially no apoptosis, as judged by ISEL staining, was seen without radiation treatment in the WT (Fig. 1*Cc,Cd*) or $Atm^{-/-}$ or $p53^{-/-}$ (results not shown). There was a pronounced lack of apoptosis after IR in the Tuj1-positive layer of *Atm-null* neuroblasts compared with extensive death through this layer in WT retina. Apoptosis in the $Atm^{-/-}$ was confined to regions harboring cells that reside in the proliferative layer. Consistent with a requirement of p53 for radiation-induced apoptosis, there was no cell death in the E15.5 *p53-null* retina after IR (Fig. 1*Ci,Cj*; see below).

Serine-15 phosphorylation of p53 after ionizing radiation requires Atm

Atm signaling to p53 is a critical step in IR-induced apoptosis in the postnatal cerebellum (Herzog et al., 1998); accordingly, p53 stabilization and apoptosis after IR are reduced greatly in the P5 cerebellum of Atm-deficient animals (Fig. 2). However, p53 stabilization still occurs, although at very reduced levels compared
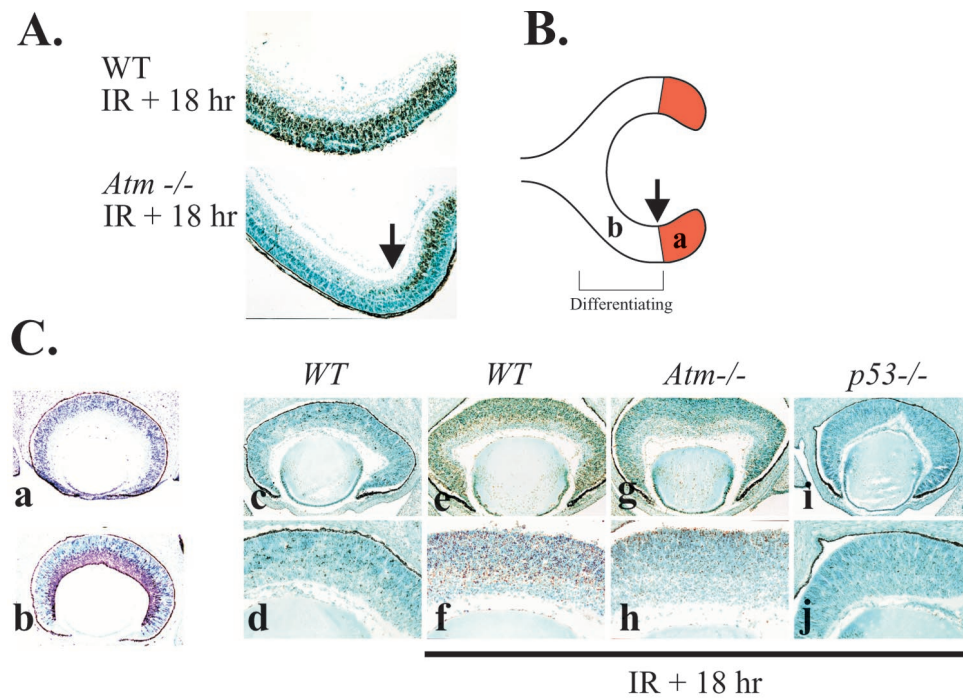
Figure 1. Atm is required for apoptosis that is induced by ionizing radiation in the differentiating zone of the retina. *A*, ISEL-positive signal occurs throughout the P5 wild-type (*WT*) retina but only in the undifferentiated field of the *Atm-null* retina 18 hr after radiation. *B*, Schematic diagram shows the stage of cell differentiation in the P5 retina; *a* is proliferative, whereas *b* is the differentiating region. In both *A* and *B* the *arrow* demarcates the boundary between proliferation and differentiation. *C*, At E15.5, cell death, shown by ISEL labeling, is widespread in the WT (*e, f*) but markedly reduced in the *Atm*$^{-/-}$ animals (*g, h*). The irradiated *p53*$^{-/-}$ E15.5 retina (*i, j*) is indistinguishable to the unirradiated WT (*c, d*). Proliferative populations in the E15.5 retina are shown by Ki67 (*a*), and differentiating fields are shown by TuJ1 immunoreactivity (*b*).

with WT (Fig. 2*e*). Because ATM is known to modify serine-15 of p53 selectively after DNA damage (Banin et al., 1998; Canman et al., 1998), we assessed serine-15 phosphorylation of p53 after IR as a measure of Atm activation. We used phospho-specific antisera against phosphorylated serine-15 of p53 to detect phosphorylation of the equivalent residue (serine-18) of mouse p53. We compared stabilization and phosphorylation of p53, before and 1 hr after IR, in the P5 cerebellum in WT and *Atm-null* mice (Fig. 2). We found that serine-18 phosphorylation of p53 was absent 1 hr after IR in *Atm-null* animals, compared with controls (Fig. 2*d,f*). Thus, phosphorylation of p53 serine-18 after radiation is Atm-dependent in the developing cerebellum and reflects Atm signaling to p53. These data also indicate a portion of p53 stabilization after IR is independent of serine-18 phosphorylation (compare Fig. 2*e,f*). Therefore, we also included anti-serine-15 phospho-specific immunohistochemistry in the following experiments to evaluate specific Atm signaling precisely in the developing nervous system.

Atm is required for IR-induced apoptosis in cell populations of the subventricular zone

Analysis of Atm in the developing retina indicates a distinct role in mitotic compared with differentiating cells in radiation-induced apoptosis. To determine the extent of the relationship of Atm to cellular differentiation, we examined Atm function after IR during neural development. To do this, we irradiated embryos at either E12.5 or E15.5 and assessed Atm function via p53 stabilization and serine-18 modification of p53. In the developing nervous system at E12.5 there is extensive cellular proliferation, but only limited differentiation. At this stage the majority of the CNS shows immunoreactivity for the proliferation marker Ki67 (Fig. 3*a*) but a restricted staining for neuronal differentiation markers such as Tuj1 (data not shown). As reported previously (Soares et al., 1998) and shown here in Figure 3*b*, *Atm* expression as determined by *in situ* hybridization is particularly abundant in the ventricular layers of the neuroepithelium during this stage of development.

After radiation at E12.5, p53 stabilization and serine-18 phosphorylation were found throughout the ventricular zones (VZ) of the CNS in both WT and *Atm-null* animals (Fig. 3*e–h*). No detectable p53 stabilization or serine-18 phosphorylation was seen without radiation (Fig. 3*c,d*). Cell death measured by ISEL was also apparent throughout the CNS 4 hr after radiation and was confined to regions that showed serine-18 phosphorylation, p53 stabilization, and activated caspase-3 immunoreactivity (data not shown). Because the extent of p53 activation occurring in WT and *Atm-null* embryos was indistinguishable, it is unlikely that Atm is required for IR-induced apoptosis in these proliferative zones. Thus, in multipotential proliferating cells in the E12.5 VZ, Atm status is unimportant for radiation-induced apoptosis.

However, in contrast to E12.5, at E15.5 there was a clear Atm dependency for IR-induced death in the subventricular zone (SVZ) of the developing nervous system. Atm was required for p53 stabilization, serine-18 phosphorylation, and caspase-3 activation in the SVZ of the neopallial cortex (Fig. 3*q,r,t*), whereas radiation led to widespread apoptosis in WT embryos (Fig. 3*o,p,s*). However, like E12.5, cell populations in the VZ of the E15.5 neopallial cortex in either WT or *Atm-null* are equally susceptible to radiation. Although Atm is not required for radiation-induced apoptosis in the VZ, p53 is essential in both this region and the SVZ (Fig. 3*i,j*). We also found an absence of radiation-induced apoptosis throughout the nervous system of *p53-null* embryos at developmental stages between E12.5 and E18.5 (results not shown).

In other regions of the CNS, such as the cingulate cortex, a similar requirement of Atm for IR-induced death in postmitotic cells (p27- and Tuj1-immunopositive regions) of the SVZ was observed (Fig. 4). Throughout the WT cingulate cortex IR induced p53 stabilization, caspase-3 activation, and cell death (Fig. 4*d,h–j*). However, in the Atm-deficient embryos, the activation of p53 and caspase-3 after radiation occurred only in the VZ (Fig. 4*f,k–m*), with no significant increase in immunoreactivity for p53 or active caspase-3 in the SVZ of the *Atm-null* embryos.
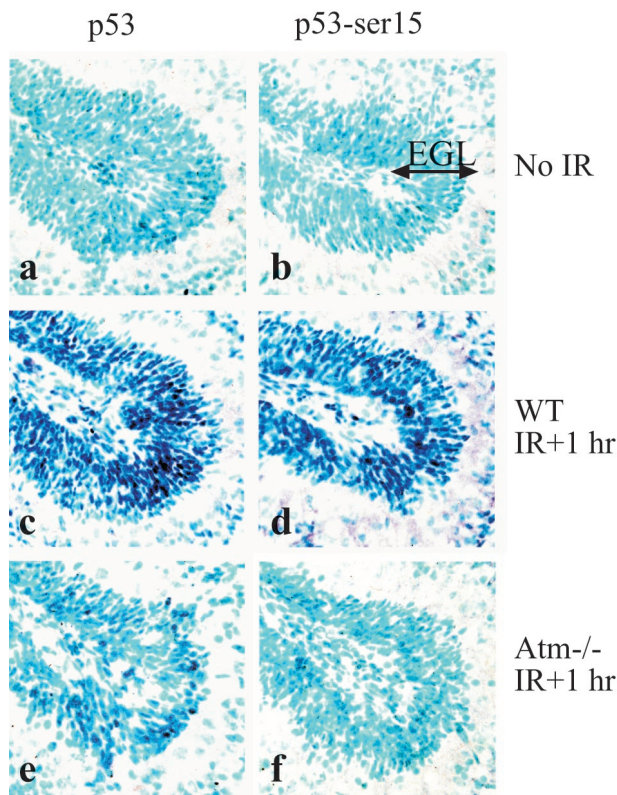
Figure 2. Serine-18 phosphorylation of p53 after radiation in the P5 cerebellum requires Atm. The external granule layer (*EGL*) of the wild-type P5 cerebellum shows increased amounts of p53 (*c*) and phosphorylated p53 (serine-18; *d*) 1 hr after radiation. In the Atm-deficient P5 cerebellum, p53 stabilization is reduced dramatically (*e*), and serine-18 phosphorylated p53 is not detected (*f*). No p53 or serine-18 phosphorylated p53 is detected in unirradiated tissue (*a, b*).

Although Atm-dependent apoptosis coincided only with Tuj1- or p27-immunopositive regions in the SVZ, we used comparative colocalization of Ki67 and p53 after radiation in WT and *Atm-null* E15.5 embryos. Figure 5 shows that the p53-positive signal in the *Atm-null* caudate nucleus was confined to regions that harbor Ki67-immunoreactive cells and was not present in the Ki67-negative cells (Fig. 5*d–f*), whereas the WT caudate nucleus showed a strong p53 signal in Ki67-negative regions (Fig. 5*a–c*).

Thus, Atm is required for p53 activation in the SVZ, but not mitotic cells of the VZ. This is despite high *Atm* expression in these proliferating areas. Consequently, whereas Atm is important for IR-induced apoptosis in the SVZ, it does not appear to play a role in IR-induced apoptosis of the proliferative populations of the VZ.

Atm is required for IR-induced apoptosis in the developing peripheral nervous system

High levels of *Atm* expression have been found in dorsal root ganglia (DRG) throughout development and adulthood (Soares et al., 1998). However, no function has been reported for Atm in the PNS. To determine whether Atm function in the PNS is similar to that in the CNS, we examined E12.5 DRG after radiation. In the *Atm-null* DRG there was a pronounced reduction in cells undergoing IR-induced apoptosis compared with WT tissue (Fig. 6*b,d*). Consistent with the report of heterogeneity of *Atm* expression in the DRG (Soares et al., 1998), the apoptotic response is not entirely uniform because there are some ISEL+
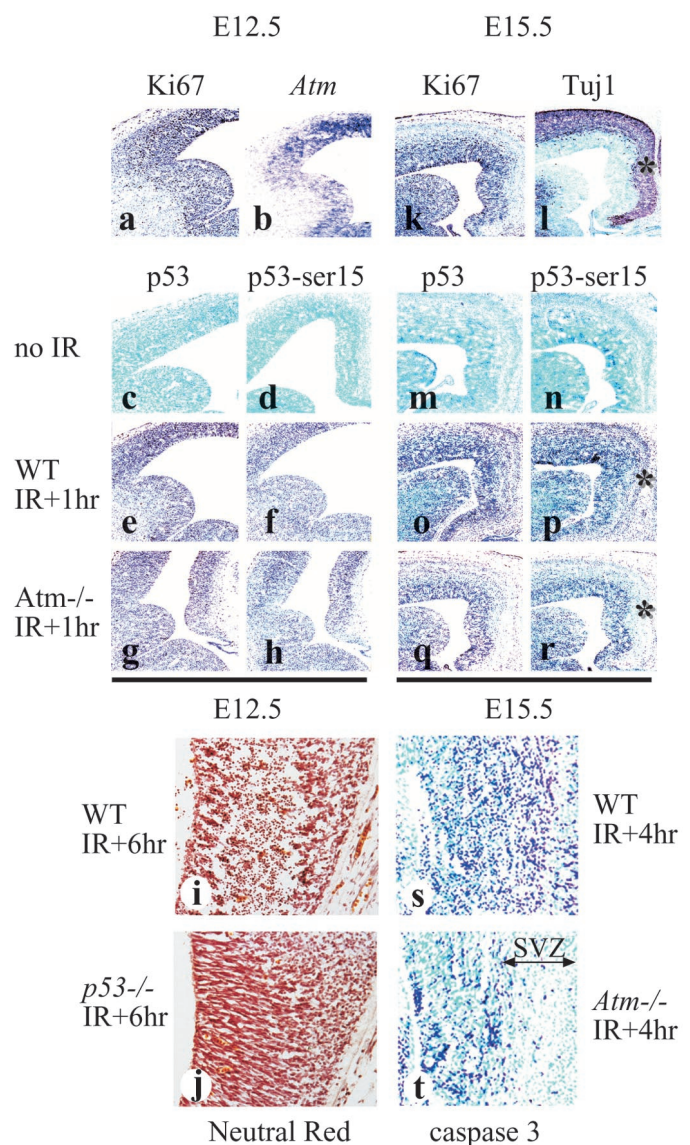

Figure 3. Atm function is distinct between the ventricular and subventricular zones. Comparative analysis of WT and $Atm^{-/-}$ embryos at E12.5 and E15.5 showed a marked reduction in radiation-induced death in the $Atm^{-/-}$ SVZ, as indicated by the *asterisks*. Regions of proliferation at E12.5 and E15.5 are indicated by Ki67 (*a, k*) and differentiation at E15.5 by Tuj1 (*l*) immunostaining. *Atm* expression at E12.5 is shown by *in situ* hybridization in *b*. In the ganglionic eminence and primordial plexiform layer at E12.5 (*c–h*), no differences in p53 immunostaining between WT and $Atm^{-/-}$ are observed: no irradiation (*c, d*), irradiated WT (*e, f*), or irradiated $Atm^{-/-}$ (*g, h*). In the WT E15.5 neopallial cortex (*m–r*) p53 stabilization and serine-18 phosphorylation are widespread (*o, p*), whereas they are restricted to the ventricular zone in $Atm^{-/-}$ (*q, r*). Apoptosis is present in WT (*i*), but not in the ventricular zone of the E12.5 $p53^{-/-}$ embryo (*j*). Regions of caspase-3 activation in E15.5 WT (*s*) and $Atm^{-/-}$ (*t*) coincided with p53 stabilization and phosphorylation.

cells in the *Atm-null* DRG (Fig. 6*d*). The resistance of *Atm-null* DRG to radiation-induced apoptosis occurred at all levels of the spinal cord (lumbar to sacral) in an indistinguishable manner to that shown in Figure 6 (results not shown). Consistent with other CNS regions, there was also widespread apoptosis in the spinal cord of the WT animal 18 hr after radiation, but not in the spinal cord of *Atm-null* mice (Fig. 6*a,b*). Other PNS regions, such as the trigeminal ganglion, also showed increased cell death in the
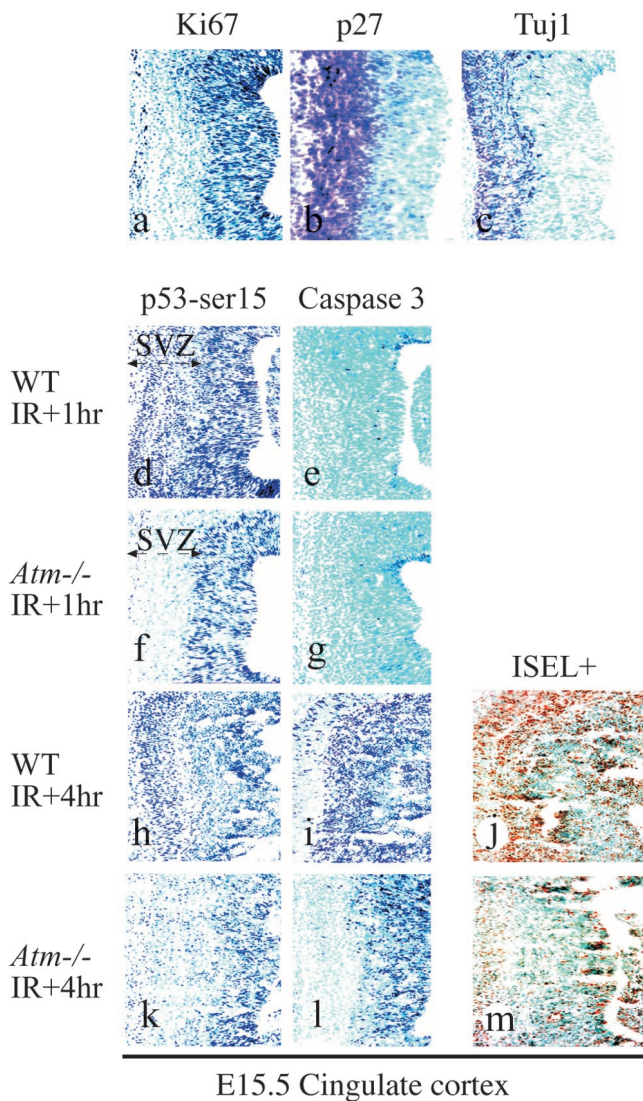
Ki67   p27   Tuj1

p53-ser15   Caspase 3

WT
IR+1hr

Atm-/-
IR+1hr

WT
IR+4hr

Atm-/-
IR+4hr

ISEL+

E15.5 Cingulate cortex

Figure 4. Postmitotic regions in the $Atm^{-/-}$ cingulate cortex are resistant to radiation-induced apoptosis. In the $Atm^{-/-}$ cingulate cortex at E15.5, the subventricular zone (*SVZ*) was markedly resistant to radiation-induced apoptosis. Proliferating cells were identified by Ki67 (*a*); to identify postmitotic regions that define the SVZ, we used p27 (*b*) and Tuj1 (*c*). The SVZ of WT embryos shows radiation-dependent serine-18 phosphorylation of p53 (*d, h*), whereas the $Atm^{-/-}$ SVZ does not (*f, k*). Activated caspase-3 immunoreactivity is shown at 1 hr (*e, g*) and 4 hr (*i, l*) after radiation. ISEL+ staining indicates that apoptosis occurs in WT (*j*), but not in $Atm^{-/-}$ (*m*), cingulate cortex SVZ.

wild-type, but not in the *Atm-null*, embryos after radiation (data not shown). Thus, consistent with Atm expression in the DRG, we demonstrate here that Atm is also important for radiation-induced apoptosis during development in this structure, broadening the Atm function to a role in the developing PNS.

DISCUSSION

In this report we showed that the developmental stage and differentiation status of the nervous system determine Atm-dependent apoptosis after radiation. Furthermore, Atm function appears to be important only in the SVZ, where it is required for apoptosis of neural cells after radiation. It is surprising that, whereas *Atm* is expressed in the VZ, it is not required for radiation-induced apoptosis in this region. Although it is possible
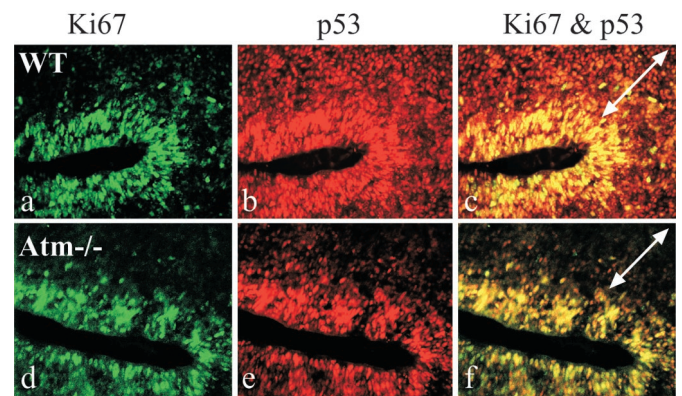

Ki67   p53   Ki67 & p53

WT

Atm-/-

Figure 5. Radiation-induced stabilization of p53 occurs in proliferating cells. At 1 hr after radiation, p53 stabilization in the caudate nucleus from WT E15.5 is widespread (*a–c*), but in $Atm^{-/-}$ it is restricted to Ki67-expressing regions (*d–f*). *c* and *f* are merged images of *a, b* and *d, e*, respectively. The *arrows* in *c* and *f* indicate the subventricular zone.

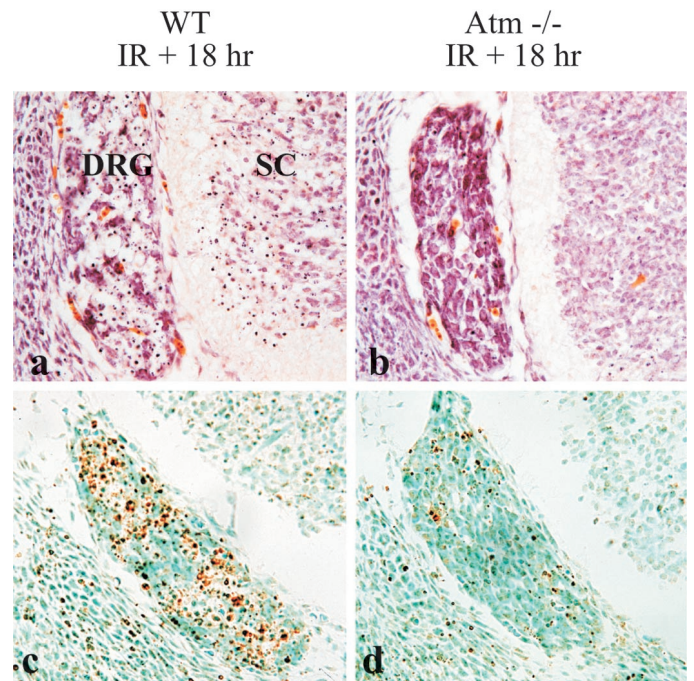WT
IR + 18 hr

Atm -/-
IR + 18 hr

DRG   SC

Figure 6. Atm is required for radiation-induced apoptosis in the peripheral nervous system. Widespread apoptosis in the WT (*a, c*), but not in $Atm^{-/-}$ (*b, d*), dorsal root ganglia (*DRG*) is shown both as neutral red staining (*a, b*) and ISEL staining (*c, d*). Neutral red-stained pyknotic nuclei are also apparent in the WT (*a*), but not in $Atm^{-/-}$ (*b*), spinal cord (*SC*).

that the death observed in the $Atm^{-/-}$ VZ was attributable to excessive radiation dose, coupled with the extreme sensitivity of proliferating cells to radiation, the striking resistance in the $Atm^{-/-}$ SVZ makes this explanation seem unlikely. Moreover, there is still a genetic basis for this apoptosis, because $p53^{-/-}$ VZ cells do not undergo radiation-induced death. Therefore, an alternative effector must operate in the VZ that activates p53. Although the identity of this effector is speculative, it may be the ATM-related protein, ataxia telangiectasia Rad 3-related (ATR), which also can signal to p53 via serine-15 phosphorylation (Tibbetts et al., 1999; Shiloh, 2001). In contrast to the nervous system, during early development (~E6.5) multipotential precursors in

the mouse embryo show Atm-dependent apoptosis after IR (Heyer et al., 2000). This implicates a role for Atm during early stages of development and may have some bearing on the recent reports of early lethality in double knock-outs of Atm and DNA-PK$_{cs}$ (Gurley and Kemp, 2001), Atm and Ku (Sekiguchi et al., 2001), and Atm and PARP (Menisser-de Murcia et al., 2001).

The presence of apoptosis in the SVZ, but not in the VZ, after DNA damage has been reported in a number of different mouse models with inactivated DNA repair genes (Barnes et al., 1998; Gao et al., 1998; Deans et al., 2000; Gilmore et al., 2000; Sugo et al., 2000). However, it is likely that the actual DNA damage occurred in the proliferating cell populations of the VZ, possibly during S-phase when there is a greater opportunity for DNA strand breakage to occur (Haber, 1999). In these situations it follows that some sensor recognizes the incurred damage at an early stage after cell cycle exit. Furthermore, at least in some cases, this sensor is Atm (Lee et al., 2000; Sekiguchi et al., 2001). If endogenous DNA damage does occur in proliferating cells, then it is surprising that there is no apparent readout, such as p53 stabilization, until cell cycle exit. Perhaps this reflects relatively low levels of damage compared with radiation, in which the presence of p53 stabilization and apoptosis within the VZ points to a greater genomic insult than endogenous DNA damage.

The role of ATM in the nervous system is unresolved. However, one consistent feature of ATM is involvement in the response to selective DNA damage, such as double strand breaks. Indeed, as mentioned above, recent genetic data have highlighted the importance of the DNA damage response in nervous system development (Barnes et al., 1998; Gao et al., 1998; Deans et al., 2000; Gilmore et al., 2000; Sugo et al., 2000) and the essential role of Atm for apoptotic signal transduction associated with this response (Lee et al., 2000; Sekiguchi et al., 2001). Additionally, human diseases resulting from DNA repair or response abnormalities often are characterized by neurological lesions (Rolig and McKinnon, 2000). Given this clear requirement of DNA repair or DNA damage response for nervous system homeostasis, it seems likely that ATM is important in this capacity in the nervous system. The data reported here suggest a defined developmental period as critical for ATM function and suggest that ATM probably is involved in nervous system maintenance as early as initial neural differentiation. Less clear from these data is a role for ATM at later stages in the life of the nervous system. We have suggested previously that the progressive neurodegeneration seen in A-T is a result of cumulative damage during development, which impacts progressively as the nervous system ages (Herzog et al., 1998; Lee and McKinnon, 2000; Lee et al., 2000). However, it is also possible that ATM performs a non-DNA damage role in the nervous system and that this aspect of ATM function may contribute to nervous system maintenance later in life. ATM function in the nervous system has been linked to regulation of the oxidative load in the brain (Barlow et al., 1999), to a role in neurogenesis (Allen et al., 2001), to survival of dopaminergic neurons (Eilam et al., 1998), and to a possible involvement in vesicular transport (Lim et al., 1998) although, in each of these cases, the Atm-dependent mechanism is unclear.

The progression of neurodegeneration in individuals with A-T is apparent during early childhood and becomes increasingly severe with age, resulting in a requirement for a wheelchair before the early teens. If our hypothesis of accumulated genetic damage as the primary lesion in the nervous system of A-T individuals is correct, then it is likely that the progressive nature of A-T results from a dysfunction of damaged cells over time.

However, given that all A-T individuals have an affected cerebellum, then there is some selectivity in the tissues that are affected, and the dysfunction may not be simply a stochastic event in the nervous system. There is still a need for a more precise description of the progressive pathology associated with A-T, because most data to date have been derived from autopsy samples, which provide limited details about disease progression. Advances in imaging techniques are likely to provide a detailed analysis of the progressive nature of A-T and insight into the neuropathology. These types of insight will generate a useful framework for resolving the progression and relative sensitivities of nervous system compartments affected by ATM inactivation.

Our data also highlight the selective nature of apoptosis as it relates to differentiation status. For example, p53 is required for the completion of apoptosis in proliferative and postmitotic cells after radiation, whereas Atm appears to be required for apoptosis only in postmitotic neural cells. However, between populations of different postmitotic neural cells that are Atm-dependent for apoptosis after radiation, different death effectors can be used. This is illustrated by comparing two regions that require Atm for IR-induced apoptosis, the developing retina and cerebellum, where Bax is essential for death in the cerebellum but is not required for death in retinal neuroblasts (Chong et al., 2000). Therefore, our data describing radiation-induced apoptosis in the nervous system have revealed a number of different levels of genetic control that are cell type-specific and cell stage-specific.

In summary, Atm function after DNA damage is linked intimately to the differentiation status of immature neural cells. Taken together, these data underscore an important role for Atm during early phases of development in response to select types of DNA damage. We hypothesize that this stage-specific function is a developmental checkpoint that monitors genomic integrity of neural cells as they exit the cell cycle and begin to differentiate. This damage, if allowed to consolidate during development, will lead to the progressive neurodegeneration that is seen in ataxia-telangiectasia.

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