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Advances in Brief

Loss of *atm* Radiosensitizes Multiple *p53* Null Tissues[1]

Christoph H. Westphal, Kathryn P. Hoyes, Christine E. Canman, Xin Huang, Michael B. Kastan, Jolyon H. Hendry, and Philip Leder[2]

Department of Genetics and HHMI, Harvard Medical School, Boston, Massachusetts 02115 [C. H. W., P. L.]; CRC Section of Genome Damage and Repair, Paterson Institute for Cancer Research, Manchester, M20 4BX, United Kingdom [K. P. H., J. H. H.]; and Department of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105-2794 [C. E. C., X. H., M. B. K.]

Abstract

An unusual clinical finding in ataxia-telangiectasia, a human disorder caused by mutations in *atm*, is exquisite sensitivity to γ irradiation. By contrast, homozygous deletion of *p53* is marked by radiation resistance in certain tissue compartments. Previous studies (A. J. Levine, Cell, *88:* 323–331, 1997) have shown that, *in vitro*, *p53*-deficient bone marrow cells are resistant to γ irradiation. Furthermore, the gastrointestinal radiosensitization engendered by the loss of *atm* has recently been shown (C. H. Westphal *et al.*, Nat. Genet., *16:* 397–401, 1997) to be independent of *p53*. Expanding on previous work, we have looked at *in vivo* bone marrow resistance in *p53*-deficient mice. Our results indicate that inbred FVB strain *p53* null mice survive lethal irradiation doses because of bone marrow resistance. Moreover, the deletion of *atm* radiosensitizes even *p53* null bone marrow and mouse embryonic fibroblast cells. The results presented here argue that the loss of *atm* radiosensitizes multiple tissues in a *p53*-independent manner. Hence, functional inhibition of *atm* in *p53* null and *p53* wild-type human tumors may be a useful adjunct to γ irradiation-based antitumor therapy.

Introduction

AT[3] is a pleiotropic autosomal recessive disorder (1) caused by mutations in the *atm* gene (2). Impaired *p53* induction after γ irradiation in AT cells indicates that *atm* and *p53* may be linked in a signal transduction cascade (3). Several additional interactions between *atm* and *p53* have been described or hypothesized (4–9). The generation of mouse models of AT and their derivatives have allowed the genetic testing of such hypotheses (10–13). Interactions between *atm* and *p53* have been shown to control cellular proliferation, mediate the G_1-S cell cycle checkpoint, regulate thymocyte apoptosis, and modulate tumorigenesis (9, 14–16). In contrast to previous hypotheses (4, 17), however, *atm* and *p53* do not interact in the acute radiation response of gastrointestinal tissues (15).

Because more than one-half of human tumors are *p53* null and hence may be radioresistant (18), a means for radiosensitizing *p53* null tissues assumes great importance. We were, therefore, interested in studying whether various *p53* null tissues could be radiosensitized by the loss of *atm*. The results presented here indicate that, in addition to gastrointestinal cells, bone marrow cells and MEFs are radiosensitized by the loss of *atm* in a *p53*-independent manner.

Materials and Methods

Mouse Irradiation, Histological Analysis, and Blood Counts. *p53* null mice were obtained from Larry Donehower and back-crossed 10 generations into a pure FVB background. Mice were irradiated with 10 Gy in a ^{137}Cs irradiator at a dose rate of 1.2 Gy/min and were scored for survival up to 30 days. To determine the cause of death, mice of various genotypes were sacrificed 8 days after 10 Gy irradiation, and tissue was analyzed histologically as described previously (15). Blood samples were obtained by tail bleed in tandem, and complete blood counts were performed.

***In Vitro* Hemopoietic CFC Assay.** Bone marrow was harvested from both femora of individual mice and resuspended in Fischer's medium. The suspension was counted to give the number of cells per femur and then divided into four portions for irradiation with 0, 2, 4, or 6 Gy ^{137}Cs γ-rays (dose rate, ~3.5 Gy/minute). Immediately after irradiation, *in vitro* hemopoietic CFCs were cultured as described previously (19). Briefly, appropriate volumes of bone marrow suspension were resuspended in Fischer's medium supplemented with 20% FCS, 0.33% agar, and recombinant murine interleukin 3. The culture mixture (1 ml) was placed in each of three 35-mm Petri dishes and incubated in a humidified incubator at 37°C, with 5% O_2 and 5% CO_2. Granulocyte/macrophage colonies were counted using a stereo microscope after 7 days' growth. Cell numbers required to generate adequate colony counts at each radiation dose were estimated from historical cell survival fractions for BDF_1 mice, as described previously (20). Surviving fractions (cloning efficiencies) were 1.0 (0 Gy), 0.5 (2 Gy), 0.16 (4 Gy), and 0.04 (6 Gy). The cell survival/dose relationships were analyzed using a linear-quadratic function and quasi-likelihood models in an ANOVA. *P* values as mentioned in the text were calculated using standard statistical methods.

MEF Clonogenic Survival Assays. Exponentially growing MEFs were seeded (300 cells/well on six-well tissue culture plates) and then irradiated with the indicated doses the next day. After 1 week, the colonies were fixed in cold CH_3OH and stained with trypan blue. Each experiment was performed in triplicate for each dose. Cloning efficiencies (termed the surviving fractions) were calculated as the number of colonies divided by the number of cells seeded for each treatment, normalized to the control (unirradiated) plating efficiency. A ^{137}Cs irradiation source at a dose rate of 85 cGy/min was used.

Results

During previous irradiation studies, we noticed differences in *p53* null *versus* wild-type *in vivo* radiosensitivity (15). To address this issue in a more rigorous manner, we compared inbred FVB strain mice differing only in their *p53* genotype. As illustrated in Fig. 1*A*, survival after irradiation is dependent on *p53* gene dosage. After 10 Gy irradiation, all of the wild-type mice but only roughly one-half of the *p53* heterozygotic mice and no *p53* null mice succumbed (Fig. 1*A*).

The time course of death, namely between 1 and 2 weeks after irradiation, is consistent with acute bone marrow toxicity. We hence performed histological analyses 8 days after 10 Gy γ irradiation to address this hypothesis (Fig. 1, *B-E*). A striking sparing of hematopoietic tissue is seen in *p53* null (Fig. 1*C*) *versus* wild-type (Fig. 1*B*) bone marrow. Furthermore, an analysis of splenic tissues revealed similarly spared *p53* null (Fig. 1*E*) *versus* wild-type (Fig. 1*D*) tissue.

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[2] To whom requests for reprints should be addressed, at Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115.

[3] The abbreviations used are: AT, ataxia-telangiectasia; CFC, colony-forming cell; MEF, mouse embryonic fibroblast.

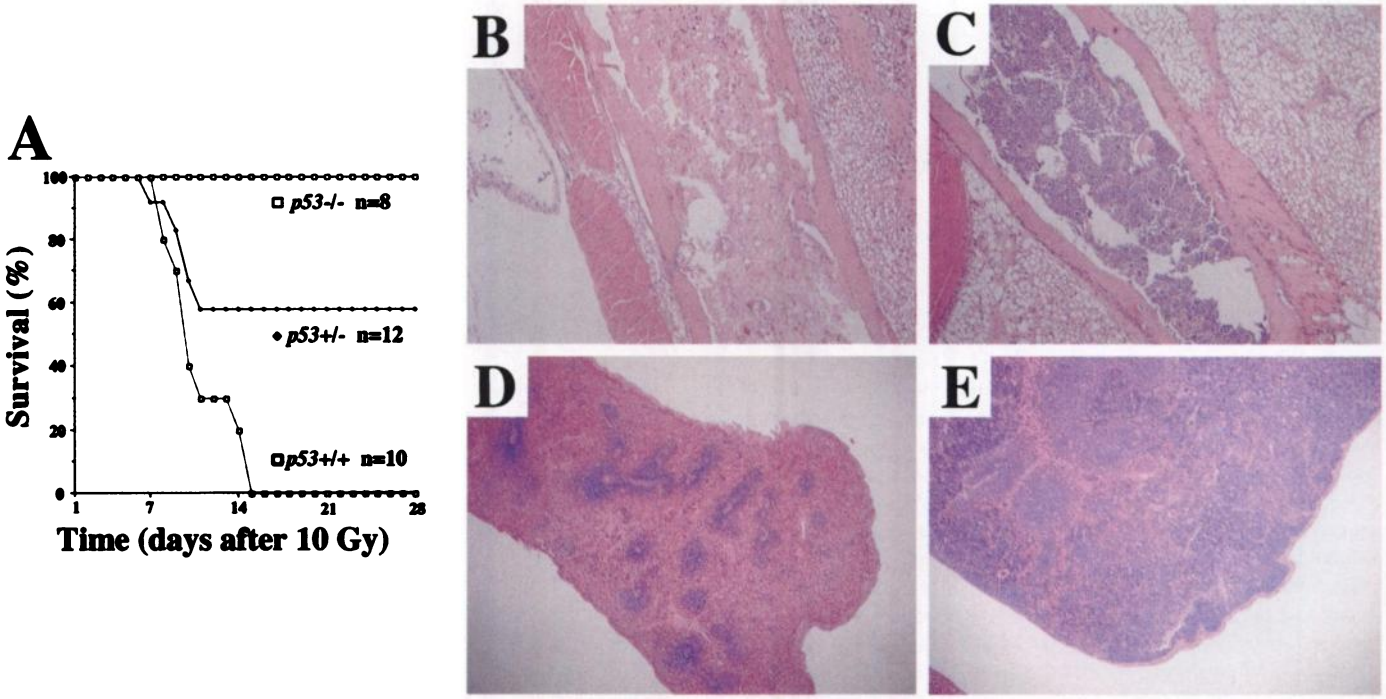

Fig. 1. Selective survival of *p53* null mice after 10 Gy γ irradiation, and histological analysis. *A* shows that all of the *p53* null mice, one-half of the *p53* heterozygotic mice, and none of the *p53* wild-type mice survived this irradiation dose. The absence of hematological precursors after irradiation is shown 8 days after irradiation in *p53* wild-type mice, both in bone marrow (*B*; ×10) and spleen (*D*; ×5). In sharp contrast, *p53* null mice show the relative sparing of hematological precursors in both bone marrow (*C*; ×10) and spleen (*E*; ×5).

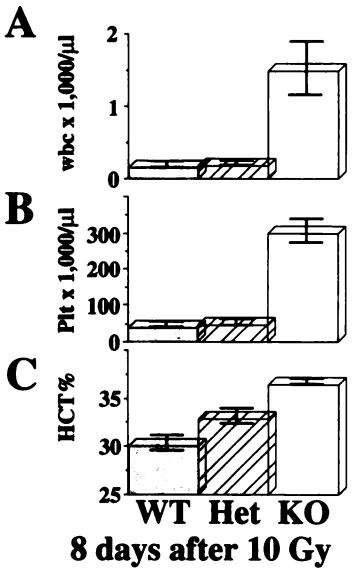

Fig. 2. Sparing of *p53* null blood cell counts 8 days after 10 Gy γ irradiation (*bars*, SE). *A* shows that *p53* null WBCs are radioresistant. *B* indicates that *p53* null platelets are radioresistant. *C* demonstrates the sparing of hematocrit after irradiation in *p53* null mice. ■ (*WT*), *p53*+/+; ▨ (*Het*), *p53*+/−; □ (*KO*), *p53*−/−.

The histological data are, therefore, consistent with increased *in vivo* resistance of *p53* null hematological precursor cells to γ irradiation.

To confirm these observations, we performed complete blood counts of mice 8 days after 10 Gy γ irradiation (Fig. 2). These data indicate that, *in vivo*, WBC (Fig. 2*A*) and platelets (Fig. 2*B*) are made radioresistant by the deletion of *p53*. The relative sparing of hematocrit seen in *p53* null mice (Fig. 2*C*) further supports this notion. The data shown in Figs. 1 and 2 argue that the loss of *p53* engenders *in vivo* radioresistance via protective effects on blood cell precursors.

atm and *p53* do not interact in the acute radiation response of gastrointestinal tissues (15). We were interested to expand these observations to bone marrow cells to see how *atm* and *p53* interact in the radiation response in this important tissue compartment. Consistent with previous *in vitro* studies (20–23), the loss of *p53* renders bone marrow cells radioresistant *in vitro* as compared with wild-type cells (Fig. 3, *top curve*; $P < 0.01$). In sharp contrast, the loss of *atm* radiosensitizes bone marrow cells as compared with wild-type cells (Fig. 3, *bottom curve*; $P < 0.01$). Strikingly, the *atm* null radiosensitization is present even in *p53* null bone marrow cells, such that *atm/p53* double null bone marrow cells are significantly more sensitive to irradiation than wild-type cells. Our data indicate that the loss of *atm* is dominant to the loss of *p53* in radiosensitizing bone marrow cells. Furthermore, there is no significant interaction between *atm* and *p53* in the control of bone marrow radiosensitization ($P = 0.96$).

Finally, we analyzed the interactions between *atm* and *p53* in

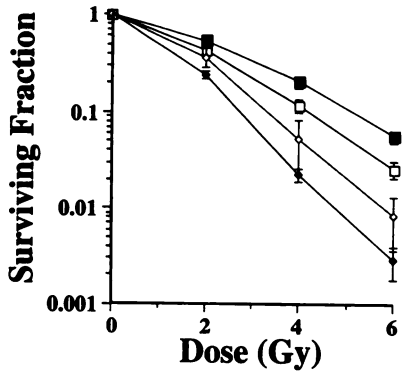

Fig. 3. The effect of γ irradiation on *in vitro* hemopoietic colony formation is dependent on *atm* and *p53* genotype. Fitted linear-quadratic radiation survival curves for *in vitro* CFCs in wild-type, *p53* null, *atm* null, and atm/p53 double null mice. Data points are the mean ± SE for three to five individual mice per point. The loss of *atm* (see *bottom two curves*) radiosensitizes even *p53* null bone marrow cells. ■, *atm*+/+*p53*−/−; □, wild type; ◇, *atm*−/−*p53*−/−; ◆, *atm*−/−*p53*+/+.

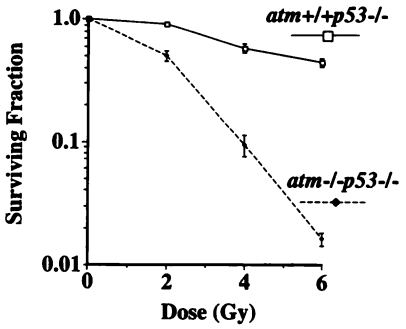

Fig. 4. MEF clonogenic survival after γ irradiation depends on *atm* and *p53* genotype. *p53* null MEFs (*upper curve*) are resistant to the irradiation doses used, whereas *atm/p53* double null MEFs (*lower curve*) are radiosensitive. Each curve represents the mean value per point ± SE.

fibroblasts, inasmuch as epithelial cells represent an important tissue compartment for human cancers. In a comparison of the radiation response in *atm/p53* double null MEFs *versus p53* null fibroblasts (14), the loss of *atm* radiosensitized *p53* null cells (Fig. 4). In summary, our data argue that in multiple tissues (gastrointestinal cells, bone marrow cells, and fibroblasts), the loss of *atm* leads to *p53*-independent radiosensitization.

Discussion

A majority of human tumors are *p53* null and hence presumed to be resistant to therapeutic regimens (18). In an initial study (15), we had shown that the loss of *atm* radiosensitizes even *p53* null mouse gastrointestinal tissues. We have now expanded upon this work, confirming earlier *in vitro* observations (20–23) by demonstrating that *p53* null mice manifest *in vivo* resistance of hematological precursor cells to γ irradiation. This radioresistance is overcome by concurrent deletion of *atm*. Furthermore, the deletion of *atm* radiosensitizes a third *p53* null tissue compartment, namely MEFs.

Gene defects can be expected to affect cellular radiosensitivity in different ways, depending upon the mode of cell killing. Cell death may occur via numerous mechanisms, *e.g.*, apoptosis, permanent G_1 arrest, or aberrant mitosis (18). Regarding the relationship between *p53* and *atm*, *p53* may be expected to have a dominant role in cell types with a high propensity to radiation-induced apoptosis, such as lymphohemopoietic cell types (24). Conversely, *atm* might be more important in cell types where nonapoptotic modes of cell death predominate, for example in cells and cancers of epithelial origin (7).

It is important to note that *atm* null nervous tissue has recently been shown to be radioresistant (25) in a manner similar to *atm* null thymocytes (15), which indicates that specific tissues are not radiosensitized by the loss of *atm*. These differences probably reflect tissue-specific radiation responses, which would need to be taken into account when designing radiosensitization therapies.

In summary, our data indicate that multiple *p53* null tissues are radiosensitized by the loss of *atm*. Because atm is a protein kinase (26), tumor-targeted functional inhibition of this protein may prove valuable as an adjunct to γ irradiation or other DNA-damaging therapies in the treatment of both *p53* null and *p53* wild-type human tumors.

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