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Heather L. Armata

University of Massachusetts Medical School

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Loss of p53 Ser18 and Atm Results in Embryonic Lethality without Cooperation in Tumorigenesis

Heather L. Armata1, Punita Shroff1, David E. Garlick2, Krista Penta3, Andrew R. Tapper3, Hayla K. Sluss1

1 Division of Endocrinology, Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America, 2 Department of Cancer Biology, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America, 3 Brudnick Neuropsychiatric Research Institute, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America

Abstract
Phosphorylation at murine Serine 18 (human Serine 15) is a critical regulatory process for the tumor suppressor function of p53. p53Ser18 residue is a substrate for ataxia-telangiectasia mutated (ATM) and ATM-related (ATR) protein kinases. Studies of mice with a germ-line mutation that replaces Ser18 with Ala (p53S18A mice) have demonstrated that loss of phosphorylation of p53Ser18 leads to the development of tumors, including lymphomas, fibrosarcomas, leukemia and leiomyosarcomas. The predominant lymphoma is B-cell lymphoma, which is in contrast to the lymphomas observed in Atm-/- animals. This observation and the fact that multiple kinases phosphorylate p53Ser18 suggest Atm-independent tumor suppressive functions of p53Ser18. Therefore, in order to examine p53Ser18 function in relationship to ATM, we analyzed the lifespan and tumorigenesis of mice with combined mutations in p53Ser18 and Atm. Surprisingly, we observed no cooperation in survival and tumorigenesis in compound p53S18A and Atm-/- animals. However, we observed embryonic lethality in the compound mutant animals. In addition, the homozygous p53Ser18 mutant allele impacted the weight of Atm-/- animals. These studies examine the genetic interaction of p53Ser18 and Atm in vivo. Furthermore, these studies demonstrate a role of p53Ser18 in regulating embryonic survival and motor coordination.

Introduction
Ataxia telangiectasia (A-T) is a recessive childhood disease with no cure. Patients with ataxia telangiectasia exhibit pleiotropic symptoms, including ataxia, telangiectasia, early aging, radiosensitivity and susceptibility to cancer [1]. The predominant childhood malignancy is lymphoid malignancies, such as a B-cell non-Hodgkin lymphoma and T-cell lymphoid tumors (T-cell lymphoma and T-cell acute lymphoblastic leukemia) [2]. Mutations in ataxia-telangiectasia mutated (ATM, the gene mutated in A-T) have also been detected in sporadic lymphoid tumors, such as T-cell prolymphocytic leukemia [3,4,5], B-cell chronic lymphocytic leukemia [6,7,8] and mantle cell lymphoma [9]. In addition, other tumors, including brain tumors and certain carcinomas, are also seen in patients with A-T [10]. Studies also indicate a role for malignancy in heterozygous carriers. For example, women who are heterozygous carriers for A-T are at an increased risk for breast cancer [11].

Atm-null mice have been generated and can phenocopy several aspects of the A-T disease. The Atm-null animals develop tumors, predominantly lymphomas [12,13,14,15]. The tumor cell type that develops is mainly immature T-cell thymic lymphoblastic lymphoma [13,15]. Atm-deficient animals have been shown to exhibit gross motor skill impairment [15]. In addition, cells from Atm-deficient mice can exhibit some of the neurodegeneration observed in A-T [16,17]. Additional studies have demonstrated that Atm-deficient animals undergo early aging when crossed into a telomerase deficient background [15,18]. Recently, a knock-in mouse has been generated corresponding to the common human ATM536del6 observed in A-T patients [19]. The ATM536del6 mutation produces an almost full-length protein that lacks kinase activity. These mice had a longer life-span than Atm-null mice as well as a reduction in the number of lymphomas. This observation points to the fact that the outcome of the disease depends on the nature of the ATM mutation in the patients [1].

The tumor suppressor function of ATM has been linked to its role in DNA repair and checkpoint function [20,21,22]. The checkpoint response is coupled in part to the phosphorylation of downstream effector molecules, such as the tumor suppressor p53 [23]. ATM activates p53 directly or indirectly through activation of its downstream kinase chk2, leading to p53-dependent responses such as transient T-cell cycle arrest, senescence or apoptosis.

p53 is a critical tumor suppressor mutated in over 50% of human malignancies. Regulation of p53 can occur through phosphorylation of downstream effector molecules, such as the tumor suppressor p53 [23]. ATM activates p53 directly or indirectly through activation of its downstream kinase chk2, leading to p53-dependent responses such as transient T-cell cycle arrest, senescence or apoptosis.
DNA damage-induced cell cycle arrest [25]. p53\(^{S18A}\) mice developed lymphomas mostly of B-cell origin, which is in contrast to the T-cell lymphomas which develop in Atm-null mice. These mice also developed several malignancies, including fibrosarcoma, leukemia, leiomyosarcoma, and myxosarcoma, which are unusual in p53-null and Atm-null mice.

Thus, the phosphorylation site Ser18 on p53 contributes to tumor suppression and regulation of lifespan in vivo. The p53Ser18 moity can be phosphorylated by additional kinases other than ATM, suggesting there may be ATM-independent roles for p53Ser18. Further support for this hypothesis is that p53Ser18 deficient animals (p53\(^{S18A}\)) develop mostly B-cell tumors [26], which are not observed in Atm\(^{-/-}\) mice. In addition, it has also been shown that p53 can have a tumor suppressive role in Atm-deficiency [26].

In order to examine the function of p53Ser18 in the process of tumor suppression independent of ATM, we generated and characterized compound p53\(^{S18A}\) and Atm-deficient animals. Surprisingly, the status of p53Ser18 did not alter the survival of Atm-null or Atm\(^{-/-}\) mice. The tumor onset or profile of Atm-null mice was also not affected by p53Ser18 status. However, we observed embryonic lethality in the compound mutant animals. Furthermore, cell cycle was greatly affected in cells from these animals. Interestingly, we observed a decrease in weight in compound Atm\(^{-/-}\); p53\(^{S18A/+}\) mice compared to Atm\(^{-/-}\) mice. We present here our findings of the contribution of p53Ser18 to ATM-mediated tumor suppression. Furthermore, these studies confirm the importance of p53Ser18 in regulating tumorigenesis in vivo.

Materials and Methods

Ethics statement

All mice were housed in a pathogen-free facility accredited by the American Association for Laboratory Animal Care (AALAC). Mice were under protocol number 1032. Mice were bred under standard conditions with a 12-hour light/dark cycle, and were fed ad libitum with standard chow (Iso-Pro3000, Prolab). The Institutional Animal Care and Use Committee of the University of Massachusetts approved all studies using animals.

Mouse strains and tumor analysis

The generation and genotyping of the p53\(^{S18A}\) mice [25], p53\(^{-/-}\) mice [27], Atm\(^{-/-}\) and Atm\(^{-/-}\); p53\(^{S18A}\) mice [15] have been previously described. Because Atm\(^{-/-}\) mice are sterile, Atm\(^{-/-}\); p53\(^{S18A}\) mice were interbred to obtain the genotype Atm\(^{-/-}\); p53\(^{S18A/+}\). Similar age-matched cohorts of Atm\(^{-/-}\), Atm\(^{-/-}\); p53\(^{S18A/+}\), Atm\(^{-/-}\); p53\(^{S18A/+}\), Atm\(^{-/-}\); p53\(^{S18A/+}\), Atm\(^{-/-}\); p53\(^{S18A/+}\), and Atm\(^{-/-}\); p53\(^{S18A/+}\) mice were established. All mice were on a mixed 129SvEv/C57Bl6 background. Litters with greater than 5 animals were included in the offspring analysis. The survival and tumor data in the control (wild-type) mice has been published [26]. The mice in the survival analysis were observed twice a week for any signs of tumors or distress. Mice were sacrificed when a tumor was apparent or when the mice became unhealthy (severe weight loss, severe dermatitis, or pronounced lordosis). Some animals were included in the survival but not the tumor analysis because of post-mortem autolysis. The mice were examined by necropsy to detect tumors or other gross pathology and tissues were fixed in 10% formalin. Fixed tumors or organs were embedded in paraffin and sectioned. Sections were mounted on slides and stained with hematoxylin and eosin. Slides were examined by a board-certified veterinary pathologist.

Cell culture and proliferation assays

Marine embryonic fibroblasts (MEFs) were generated from day 13.5 embryos. Since Atm\(^{-/-}\) mice are sterile the compound mutant MEFs were obtained from an Atm\(^{-/-}\); p53\(^{S18A/+}\) intercross. The MEFs were maintained in Dulbeccos’ Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum, 5 mM glutamine, and penicillin and streptomycin (Invitrogen). The MEFs were cultured at sub-confluence and were passaged no more than 4 times, unless otherwise indicated. Cellular proliferation/survival analysis was performed as described [25] with pass 2 MEFs. Briefly, 2\(\times10^{3}\) MEFs were plated onto each well of a 6-well plate, and each day after plating MEFs from three plates of each genotype were fixed, stained with trypan blue and absorbance was determined. MEFs from two different intercrosses were used for the experiments. Experiments were performed in triplicate for each line and are presented as mean values with standard deviations.

Rota Rod Experiment

The rotarod apparatus (Stoelting) was used to measure motor coordination and balance, as well as the ability of mice to improve motor skill performance with training. During training, mice were placed on a rotarod accelerating from 4–40 RPM over 5 minutes. Each mouse received ten trials (one trial every five minutes) and the latency to fall off the rotarod in each trial was measured. The following day, mice received three test trials on the accelerating rotarod with each trial separated by one minute.

Data Analysis

Survival of mice was determined by Kaplan Meier analysis using JMP software (SAS, Cary, NC). Pair-wise comparison of the Kaplan Meier analysis was done using the Log Rank test. Statistical analysis on perinatal lethality was done comparing expected #/genotype for each litter compared to actual #/genotype for each litter using a student’s t-test. MEF analysis was done using student’s t-test on triplicate readings per time point. Average latencies to fall from the rotarod were compared using One-way Analysis of Variance followed by Tukey post-hoc tests. Statistical significance was set at \(P<0.05\).

Results

Growth Properties of Atm\(^{-/-}\); p53\(^{S18A/+}\) Cells and Animals

Cells from Atm-deficient animals exhibit decreased growth properties, characterized by decreased cell proliferation [12]. We compared the growth properties of Atm\(^{-/-}\); p53\(^{S18A/+}\) and compound Atm\(^{-/-}\); p53\(^{S18A/+}\) MEFs. Figure 1A demonstrates the results from a representative experiment, and cell lines were plated in triplicate. Atm\(^{-/-}\); p53\(^{S18A/+}\) MEFs exhibited a decrease in trypan blue staining, which is correlative of slower proliferation, compared to wild-type cells, as previously described [12,15]. p53\(^{S18A/+}\) MEFs also exhibited increased growth compared to wild-type cells as previously described [25]. Cell lines generated from heterozygous mice (p53\(^{S18A/+}\) and Atm\(^{-/-}\); p53\(^{S18A/+}\)) grew similar to wild-type cells (data not shown). The p53\(^{S18A/+}\) MEFs exhibited a greater rate of growth than Atm\(^{-/-}\); p53\(^{S18A/+}\) cells (Figure 1A). Importantly, the cells used were early pass MEFs which do not exhibit the significant number of senescent cells observed in higher pass cells. Interestingly, the compound MEFs exhibited a proliferation rate that was slower than p53\(^{S18A/+}\) MEFs, but not as slow as Atm\(^{-/-}\) MEFs (Figure 1A). This suggested there was no cooperation in the cell growth defects observed in the compound MEFs as they did not grow slower than single mutant (Atm\(^{-/-}\) ) MEFs. However, there was a decrease on the growth of p53\(^{S18A/+}\) MEFs with the loss of Atm, suggesting an additional site of ATM regulation on the p53\(^{S18A}\) protein.
**Prenatal Lethality in Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> Animals**

It has been reported that loss of p53 in an Atm-null background leads to embryonic lethality [28]. The frequency of Atm<sup>−/−</sup> offspring from 13 Atm<sup>−/−</sup> intercrosses was not significantly reduced (Table 1, Cross A, confirming that loss of Atm does not lead to embryonic lethality. Importantly, we previously observed no embryonic lethality due to the p53<sup>S18A</sup> mutation [26]. To determine whether p53Ser18 loss would lead to perinatal lethality in an Atm<sup>−/−</sup> background, Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> mice were intercrossed and genotype of the offspring was analyzed (Table 1, Cross B). The number of Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> offspring was significantly reduced in 25 litters, from the expected 25% to 16.9%. Thus, 30 out of the predicted 45.75 Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> mice were born, indicating about 53% of Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> offspring die perinatally. This percent is less than the 60% of Atm<sup>−/−</sup>; p53<sup>−/−</sup> mice which have been reported to die perinatally [28]. However, it has been reported up to 15% of p53<sup>−/−</sup> mice exhibit embryonic lethality [29]. Thus, although there is a perinatal lethality associated with p53<sup>S18A</sup>, it is not as robust as that with loss of p53.

**Analysis of mouse survival**

To examine the role of p53Ser18 in an ATM-independent survival, we performed a survival analysis to examine the effects of p53<sup>S18A</sup> in the survival of Atm<sup>−/−</sup> and Atm<sup>−/−</sup> mice. The Atm<sup>−/−</sup> mice had a rapid demise compared to wildtype mice (Figure 2A). Of note, we observed a median survival of the Atm<sup>−/−</sup> mice of 41 weeks, which has been reported for the mice in the same genetic background and mouse facility conditions [30]. Surprisingly, Kaplan Meier analysis indicated that the presence of the homozygous p53<sup>S18A</sup> mutation had no significant effect on the survival of Atm<sup>−/−</sup> mice (Figure 2A). However there was a reduction in the survival of Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> mice compared to p53<sup>S18A/S18A</sup> mice [26]. The presence of a heterozygous p53<sup>S18A</sup> mutation also had no effect on the overall survival of Atm<sup>−/−</sup> mice, although the median was delayed (but not significantly) (Figure 2A). The lack of cooperation in tumorigenesis is in contrast to that observed for p53<sup>−/−</sup> animals, which demonstrate cooperation in survival and tumorigenesis with Atm<sup>−/−</sup> mice [28].

We observed a significant decrease in the overall survival of Atm<sup>−/−</sup> mice compared to wildtype mice (Figure 2B). In addition, there was a significant decrease in the median survival. The wildtype median survival age was 98 weeks, whereas the Atm<sup>−/−</sup> median survival age was 81 weeks. The mutation of one or two alleles of p53Ser18 had no significant effect on the overall survival.

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Table 1. Genotype of Offspring from Intercrosses.

| Cross A: Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> × Atm<sup>−/−</sup>; p53<sup>−/−</sup> Total n = 202 |
|---|---|---|---|
| Genotype: | Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> | Atm<sup>−/−</sup>; p53<sup>−/−</sup> | Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> |
| Obtained | 46 (22.8%) | 116 (57.4%) | 40 (19.8%) |
| Expected | 50.5 (25%) | 101 (50%) | 50.5 (25%) |
| T-Test | 0.43 | 0.08 | 0.12 |

| Cross B: Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> × Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> Total n = 183 |
|---|---|---|---|
| Genotype: | Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> | Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> | Atm<sup>−/−</sup>; p53<sup>S18A/S18A</sup> |
| Obtained | 50 (27.32%) | 103 (56.28%) | 30 (16.39%) |
| Expected | 45.75 (25%) | 91.5 (50%) | 45.75 (25%) |
| T-Test | 0.58 | 0.34 | 0.007 |

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of Atm\(^{-/}\) mice (Figure 2B). The median survival age for Atm\(^{-/}\); p53\(^{S18A/+}\) and Atm\(^{-/}\); p53\(^{S18A/S18A}\) was 90 and 81 weeks respectively. Interestingly, we previously observed the median survival age for p53\(^{S18A/+}\) mice was 94 weeks [26], thus, the loss of one allele of Atm further decreased lifespan of p53\(^{S18A/+}\) mice. This suggested that the residual ATM in Atm\(^{-/}\); p53\(^{S18A/+}\) mice could be phosphorylating the remaining p53Ser18 moiety, which would explain the increased viability of p53\(^{S18A/+}\) animals compared to p53\(^{S18A/S18A}\) animals [26].

**Analysis of Spontaneous Tumorigenesis in Atm and p53Ser18 Deficient Mice**

We analyzed spontaneous tumor development in Atm\(^{-/-}\) and Atm\(^{-/-}\); p53\(^{S18A/S18A}\) animals. The predominant tumor observed
in these animals the lymphomas. One animal had a hemangioma in the liver. In addition, one animal had an abdominal mass that was a histiocytic sarcoma (Figure 2G) that had spread to the kidney (Figure 2H). The tumor penetrance for Atm−/+; p53S18A/S18A animals was 16%. Interestingly, very few lymphomas were observed in Atm−/+; p53S18A/S18A mice. This observation was in contrast with what we had observed in p53S18A/S18A animals where 35.7% of animals had tumors, and 81% of tumors were lymphomas [26]. There was one incipient lymphoma present in the thymus of one Atm−/+; p53S18A/S18A animal, visible as a diffusely sheet of neoplastic lymphocytes (Figure 2F). In addition, other animals developed an adenoma, a fibrosarcoma, and a hemangiosarcoma in the spleen. The hemangiosarcoma almost completely effaced the spleen (Figure 2J,K).

We previously observed tumor-free p53S18A animals that presented with cellular alterations at the time of death that were consistent with accelerated lifespan [26]. We observed some of the same alterations in cellular composition in the compound Atm−/+; p53S18A/S18A animals [26]. In the Atm−/+; p53S18A/S18A mice we observed alterations in the kidney, such as increased inflammation, tubular cysts, hematomas and fibrosis. The Atm−/+; p53S18A/S18A mice had liver inflammation and degeneration. Kidneys also had increased inflammation as well as glomerulonephropathy. Further analysis of a larger cohort of animals will be required to determine if the animals are presenting with accelerated aging, as we observed in the p53S18A animals [26].

Performance on the rotarod apparatus

ATM has been shown to contribute to motor coordination [15]. We therefore tested motor coordination in p53S18A animals to determine if Ser18 contributes to this function. The accelerated rotarod assay was used to measure motor coordination in p53S18A mice. All data were collected on age- and gender-matched naive animals. Immediately prior to training, mice were weighed. Female wildtype and p53S18A mice did not significantly differ in weight; whereas male p53S18A mice were modestly but significantly heavier than wildtype mice (Figure 3A, B). During the training phase, mice were placed on the accelerating rotarod for ten consecutive trials over the course of one hour and latency to fall off of the rotarod was recorded. Latency to fall off of the rotarod in

| Table 2. Tumorigenesis in Atm and p53Ser18 deficient animals. |
|---------------------------------------------------------------|
| **Atm**−/+ p53+/+ | **Atm**−/+ p53S18A/+ | **Atm**−/+ p53Ser18A/S18A | **Atm**−/+ p53+/+ | **Atm**−/+ p53S18A/+ | **Atm**−/+ p53Ser18A/S18A |
|-------------------|---------------------|---------------------------|-------------------|---------------------|---------------------------|
| Mice Analyzed     | 11                  | 16                        | 12                | 15                  | 14                        | 18                        |
| Mice with tumors  | 8                   | 12                        | 12                | 3                   | 5                         | 3                         |
| Total tumors      | 8                   | 13                        | 13                | 3                   | 5                         | 4                         |
| Tumor Classification |
| Lymphoma          | 6                   | 12                        | 12                | 2                   | 3                         | 1                         |
| Histiocytoma      | 0                   | 1                         | 0                 | 0                   | 0                         | 0                         |
| Histiocytic sarcoma | 0                | 0                         | 0                 | 0                   | 0                         | 0                         |
| Hemangiosarcoma   | 0                   | 0                         | 0                 | 1                   | 0                         | 1                         |
| Hemangioma        | 0                   | 0                         | 0                 | 1                   | 0                         | 1                         |
| Fibrosarcoma      | 0                   | 0                         | 0                 | 0                   | 0                         | 0                         |
| Carcinoma         | 1                   | 0                         | 0                 | 0                   | 0                         | 0                         |
| Adenoma           | 1                   | 0                         | 0                 | 0                   | 0                         | 1                         |

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both wildtype and p53S18A mice increased with successive trials and reached a plateau by the tenth trial (Figure 3C, D). Twenty-four hours after training, mice were again placed on the accelerating rotarod and their performance was measured. Compared to wildtype female animals, female p53S18A animals exhibited an increased latency to fall off of the rotarod (Figure 3E, F(1, 9) = 31.6, p < 0.001). Conversely, male p53S18A mice exhibited a significant decreased latency to fall off of the rotarod compared to male wildtype mice (Figure 3F, F(1, 9) = 5.8, p < 0.05).

Discussion

ATM has been shown to have a role in DNA repair, DNA-damage induced checkpoints, and telomere maintenance. p53 is a substrate of ATM that is intimately linked with its role in DNA repair and checkpoint function. We previously generated mice with a germ-line mutation that replaces Ser18 with Ala (p53S18A mice). Studies with these mice and the same mice generated by another lab have demonstrated that phosphorylation of p53Ser18 is required for normal DNA damage-induced PUMA expression and apoptosis, but not for DNA damage-induced cell cycle arrest [25,26,31]. In addition, p53S18A mice developed late-onset lymphomas at 18 months of age [26]. Studies indicate an ATM-independent tumor-suppression function of p53 [32]. Our observation that p53S18A mice develop predominantly B-cell tumors whereas Atm−/− mice develop T-cell tumors lead us to hypothesize that there is an ATM-independent growth suppressive role for p53Ser18. We investigated the role of p53Ser18 in the tumor suppressor function of ATM by generating and characterizing compound mutant animals. Cells generated from Atm−/− and Atm−/−; p53S18A/S18A animals grew slower than wildtype cells. In addition, p53S18A/S18A cells grew significantly faster than Atm−/− cells. However, there was no synergy in the Atm−/−; p53S18A/S18A cells compared to the single mutant T-cells (Figure 1A). This result was surprising, as there are additional kinases that can phosphorylate p53Ser18 in an Atm−/− background. If the decreased proliferation induced by loss of Ser18 is due to regulation by an ATM-independent pathway in MEFs, one could observe that the compound MEFs would have a combined reduced proliferation. We observed a decrease in the proliferation of cells from Atm−/−; p53S18A/S18A mice compared to cells from p53S18A/S18A mice. One possibility is that alternative ATM sites are phosphorylated in the p53S18A protein that contribute to cell proliferation. Alternatively, in the absence of ATM, another kinase can compensate at a secondary site of phosphorylation, but not as efficiently. In addition to growth defects, Atm−/− mice exhibit somatic growth defects [15]. Interestingly, we observed a significant reduction in the weight...
of Atm−/−; p53S18A/18A mice compared to Atm−/− animals at 5–6 months (Figure 1B). This is in contrast to our observations that older p53S18A/18A animals are obese [33]. In addition, we observed embryonic lethality in Atm−/−; p53S18A/18A animals (Table 1), although not to the same extent seen in Atm−/−; p53/− mice.

Interestingly, the overall survival of Atm-null mice was not affected by the presence of a heterozygous or homozygous p53S18A allele. Of note, the median death of the Atm−/− mice we observed is longer than reported for some studies [13,14,15], but similar to animals maintained in comparable housing conditions [30]. This observation confirms that the conditions of the mouse facility and food can greatly affect the survival of Atm-deficient mice.

There was no cooperation in tumorigenesis observed in the compound genotypes compared to Atm−/− mice (Figure 2A, B). The majority of the p53S18A animals in the Atm−/− background succumbed to tumors and the tumors the animals developed were similar (Table 2). The main tumor developed was thymic lymphoma, which was either lymphoblastic or poorly differentiated in appearance (Figure 2). The observation of predominantly lymphoblastic lymphomas in Atm−/−; p53S18A/18A animals is in contrast to the B-cell lymphomas (follicular and centroblastic) observed in p53S18A/18A animals [26]. In addition, the thymus was rarely involved in p53S18A/18A animals.

The lack of cooperation in tumorigenesis suggests the Atm−/−; p53S18A/18A mice are still able to undergo tumor suppression in B-cells in young animals, which is where p53S18A/18A mice develop tumors with a greater latency. It is interesting that the time frame is longer for tumors to arise in p53S18A/18A mice than in Atm−/− or Atm−/−; p53S18A/18A mice, suggesting the mechanism is different. Indeed, tumors in Atm−/− thymocytes arise from translocations [1], whereas as we linked the tumor suppression by p53Ser18 in B-cells to defective apoptosis [26]. We have also linked apoptosis effects of p53Ser18 to prevention of tumorigenesis by the Myc oncogene [34]. An alternative explanation for the lack of synergy in Atm-null and p53S18A/18A mutant animals may be that the phosphorylation of Ser18 may be redundant in certain cell types and at certain stages, but that later it plays a more critical role.

The synergy observed in compound Atm-null and p53-null mice may be due to the fact that there are additional functions of p53 which are lost in p53-null animals but still retained in p53S18A/18A cells. Nonetheless, the rapid demise of the Atm−/−; p53S18A/18A compound animals and the similar tumor type suggests that the loss of Atm is dominant over the mutation in p53Ser18.

It has been reported that Atm−/− mice do not develop spontaneous tumors, whereas Atm−/− mice have increased cancer risk [35]. In our study, we found that the Atm−/− mice had a decreased lifespan (Figure 2B) and developed tumors (Table 2). The tumor penetrance was 23% for Atm−/− mice and 8% for wild-type mice. Our observation for tumors in Atm−/− mice is different than that reported, and may be due to a combination of different genetic backgrounds and housing specifications. Different observations have also been reported for studies with radiation-induced tumorigenesis: whereas some studies have reported an increased incidence in radiation-induced tumors in Atm−/−/− mice [36,37,38], another study has shown no effect of Atm heterozygosity on radiation-induced tumors [39]. Our observation is consistent with reports of increased risk of cancer in A-T heterozygous carriers [40].

In addition to a role in tumor suppression, ATM also has a role in motor function. A characteristic of A-T is ataxia. Although Atm−/− mice do not display overt ataxia, they display defects in motor behavior [15]. In order to assess motor coordination in p53S18A/18A mice, we examined the performance of the mice on an accelerating rotarod. We used animals at 4–5 months of age (Figure 3). All mice used in the study improved rotarod performance over successive trials during the training phase of the experiment as evidenced by an increase in the mean time spent on the rotarod before falling. Thus, mutant and wildtype mice did not significantly differ in motor learning skills (Figure 3C, D). Interestingly, during the test trial, female p53S18A/18A mice showed a significant improved performance on the rotarod (Figure 3E).

Conversely, male p53S18A/18A mice exhibited a significant decrease in performance on the rotarod during training (Figure 3F). A significant, but modest, increase was seen in the weight of the p53S18A/18A males, potentially influencing rotarod performance. No weight difference was observed that would affect the results for the female mice. This gender difference suggested the tumor profile could exhibit gender differences. However, we examined the tumor onset and type for the various genotypes and found no gender difference for Atm mice on a p53S18A/18A background. Nevertheless, these observations are the first to indicate a gender difference associated with p53 phosphorylation and motor coordination.

In summary, these studies further confirm the essential role of the ATM/p53 pathway in tumor suppression. While loss of p53Ser18 had no increased effects in survival and tumor distribution in Atm-null mice, there appears to be a function of p53Ser18 in mediating some of the role of ATM in embryonic survival.

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Author Contributions

Conceived and designed the experiments: HA AT HS. Performed the experiments: HA PS KP AT HS. Analyzed the data: HA PS DG KP AT HS. Wrote the paper: HA DG AT HS.

References

1. Taylor AM, Byrd PJ (2005) Molecular pathology of ataxia telangiectasia. J Clin Pathol 58: 1009–1015.

2. Taylor AM, Metcalfe JA, Thick J, Mak YF (1996) Leukemia and lymphoma in ataxia telangiectasia. Blood 87: 423–443 200.

3. Vorobetsky I, Luo L, Dyer MJ, Catovksy D, Amlot PL, et al. (1997) Clustering of nonsense mutations in the ataxia-telangiectasia gene in a sporadic T-cell leukemia. Nat Genet 17: 96–99.

4. Stolzenbauer S, Schaffer C, Litterer A, Liebisch P, Gaid S, et al. (1997) Biallelic mutations in the ATM gene in T-prolymphocytic leukemia. Nat Med 3: 1155–1159.

5. Stoppa-Lyonnet D, Soulier J, Laugier A, Dastot H, Garand R, et al. (1998) Inactivation of the ATM gene in T-cell prolymphocytic leukemias. Blood 91: 3920–3926.

6. Stankovic T, Stewart GS, Byrd P, Fegan C, Moss PA, et al. (2002) ATM mutations in sporadic lymphoid tumours. Leuk Lymphoma 43: 1563–1571.

7. Bullrich F, Rasio D, Kitada S, Starostik P, Kipps T, et al. (1999) ATM mutations in B-cell chronic lymphocytic leukemia. Cancer Res 59: 24–27.

8. Schaffer C, Stolzenbauer S, Rappold GA, Dohner H, Lichter P (1999) Somatic ATM mutations indicate a pathogenic role of ATM in B-cell chronic lymphocytic leukemia. Blood 94: 748–753.

9. Schaffer C, Iller I, Stolzenbauer S, Dohner H, Lichter P (2000) Mandle cell lymphoma is characterized by inactivation of the ATM gene. Proc Natl Acad Sci USA 97: 2773–2778.

10. Stankovic T, Kidd AM, Stolzenbauer S, McGuire GM, Robinson P, et al. (1998) ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. Am J Hum Genet 62: 334–345.

11. Swift M, Reitnauer PJ, Morrell D, Chase CL (1987) Breast and other cancers in families with ataxia-telangiectasia. N Engl J Med 316: 1289–1294.
12. Xu Y, Baltimore D (1996) Dual roles of ATM in the cellular response to radiation and in cell growth control. Genes Dev 10: 2401–2410.

13. Xu Y, Ashley T, Brainerd EE, Bronson RT, Meyn MS, et al. (1996) Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev 10: 2411–2422.

14. Elson A, Wang Y, Daugherty CJ, Morton GC, Zhou F, et al. (1996) Pleiotropic defects in ataxia-telangiectasia protein-deficient mice. Proc Natl Acad Sci U S A 93: 13804–13809.

15. Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, et al. (1996) Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Proc Natl Acad Sci U S A 93: 13804–13809.

16. Kuljis RO, Xu Y, Aguila MC, Baltimore D (1997) Degeneration of neurons, synapses, and neuropil and glial activation in a murine Atm knockout model of ataxia-telangiectasia. Proc Natl Acad Sci U S A 94: 12688–12693.

17. Borghesani PR, Alt FW, Bottaro A, Davidson L, Aksoy S, et al. (2000) Abnormal development of Purkinje cells and lymphocytes in Atm mutant mice. Proc Natl Acad Sci U S A 97: 3330–3334.

18. Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, et al. (2003) Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates aging. Nature 421: 643–648.

19. Spring K, Cross S, Li C, Watters D, Ben-Senior L, et al. (2001) Atm knock-in mice harboring an in-frame deletion corresponding to the human ATM 7636del9 common mutation exhibit a variant phenotype. Cancer Res 61: 4561–4568.

20. O'Driscoll M, Jeggo PA (2006) The role of double-strand break repair - insights from human genetics. Nat Rev Genet 7: 45–54.

21. Lombard DB, Chua KF, Mostoslavsky R, Franco S, Gostissa M, et al. (2005) DNA repair, genome stability, and aging. Cell 120: 497–512.

22. Harper JW, Elledge SJ (2007) The DNA damage response: ten years after. Mol Cell 28: 739–745.

23. Appella E, Anderson CW (2001) Post-translational modifications and activation of p53 by genotoxic stresses. Eur J Biochem 268: 2764–2772.

24. Giaccia AJ, Kastan MB (1998) The complexity of p53 modulation: emerging patterns from divergent signals. Genes Dev 12: 2973–2983.

25. Sluss HK, Arneta H, Gallant J, Jones SN (2004) Phosphorylation of serine 18 regulates distinct p53 functions in mice. Mol Cell Biol 24: 976–984.

26. Armata HL, Garlick DS, Jones SN, Shao HK (2007) The ataxia telangiectasia-mutated target site Ser18 is required for p53-mediated tumor suppression. Cancer Res 67: 11696–11703.

27. Donoghue LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr., et al. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356: 215–221.

28. Xu Y, Yang EM, Brugarolas J, Jacks T, Baltimore D (1996) Involvement of p53 and p21 in cellular defects and tumorigenesis in Atm−/− mice. Mol Cell Biol 16: 4365–4390.

29. Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, et al. (1994) Tumor spectrum analysis in p53-mutant mice. Curr Biol 4: 1–7.

30. Reliene R, Schissel RH (2007) Antioxidants suppress lymphoma and increase longevity in Atm-deficient mice. J Nutr 137: 2298–2328.

31. Chao C, Hergenhahn M, Kaeber MD, Wu Z, Saito S, et al. (2003) Cell type- and promoter-specific roles of Ser18 phosphorylation in regulating p53 responses. J Biological Chem in press.

32. Bailey SL, Gurlay KE, Hoon-Kim K, Kelly-Spratt KS, Kemp CJ (2008) Tumor suppression by p53 in the absence of Atm. Mol Cancer Res 6: 1183–1192.

33. Arneta HL, Goldebowa D, Jung DV, Ko Hj, Kim JK, et al. (2010) Requirement of the ATM/p53 tumor suppressor pathway for glucose homeostasis. Mol Cell Biol 30: 5787–5794.

34. Sluss HK, Gannon H, Coles AH, Shen Q, Eischen CM, et al. (2010) Phosphorylation of p53 serine 18 upregulates apoptosis to suppress Myc-induced tumorigenesis. Mol Cancer Res 8: 216–222.

35. Spring K, Ahangari F, Scott SP, Waring P, Purdie DM, et al. (2002) Mice heterozygous for mutation in Atm, the gene involved in ataxia-telangiectasia, have heightened susceptibility to cancer. nature genetics 32: 185–190.

36. Barlow C, Eckhaus M, Schaffer A, Wynshaw-Boris A (1999) Atm haploinsufficiency results in increased sensitivity to sublethal doses of ionizing radiation in mice. Nature 4: 359–360.

37. Smilenov LB, Brenner DJ, Hale JJ (2001) Modestly increased sensitivity to radiation oncogenesis in ATM heterozygous versus wild-type mammalian cells. Cancer Res 61: 5710–5713.

38. Worgul BV, Smilenov L, Brenner DJ, Junk A, Zhou W, et al. (2002) Atm heterozygous mice are more sensitive to radiation-induced catacaustics than are their wild-type counterparts. Proc Natl Acad Sci U S A 99: 9836–9839.

39. Mao JH, Wu D, DelRosario R, Castellanos A, Balmain A, et al. (2008) Atm heterozygosity does not increase tumor susceptibility to ionizing radiation alone or in a p53 heterozygous background. Oncogene 27: 6596–6600.

40. Thompson D, Duedal S, Kirner J, McGuigou L, Last J, et al. (2005) Cancer risks and mortality in heterozygous ATM mutation carriers. J Natl Cancer Inst 97: 813–822.