Advances in Brief

Genetic Interactions between atm and p53 Influence Cellular Proliferation and Irradiation-induced Cell Cycle Checkpoints

Christoph Heiner Westphal, Cornelius Schmaltz, Sheldon Rowan, Ari Elson, David Erich Fisher, and Philip Leder

Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School (C. H. W., P. L.), and Department of Pediatric Oncology, Dana-Farber Cancer Institute and Harvard Medical School (C. S. R., D. E. F.), and Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100 Israel (A. E.)

Abstract

Ataxia-telangiectasia and Li-Fraumeni syndrome, pleiotropic disorders caused by mutations in the genes atm and p53, share a marked increase in cancer rates. A number of studies have argued for an interaction between these two genes (for comprehensive reviews, see M. S. Meyn, Cancer Res., 55: 5991–6001, 1995, and M. F. Lavin and Y. Shiloh, Annu. Rev. Immunol., 15: 177–202, 1996). Specifically, atm is placed upstream of p53 in mediating G1-S cell cycle checkpoint control, and both atm and p53 are believed to influence cellular proliferation. To analyze the genetic interactions of atm and p53, mouse embryonic fibroblasts (MEFs) homozgyously deficient for both atm and p53 were used to assess cell cycle and growth control. These double-null fibroblasts proliferate rapidly and fail to exhibit the premature growth arrest seen with atm-null MEFs. MEFs null for both atm and p53 do not express any p21waf1, showing that p53 is required for p21waf1 expression in an atm-null background. By contrast, homozygous loss of either atm, p53, or both results in similar abnormalities of the irradiation-induced G1-S cell cycle checkpoint. Our results suggest two separate pathways of interaction between atm and p53, one linear, involving G1-S cell cycle control, and another more complex, involving aspects of growth regulation.

Introduction

Ataxia-telangiectasia is a multifaceted disorder marked by progressive neurological degeneration, specific immunodeficiencies, telangiectasias, lymphoreticular malignancies, infertility, growth retardation, and premature signs of aging. Recently, three laboratories have reported the generation of mice null for atm (1–3). These mice are small and prone to thymic lymphomas and yield MEFs3 that undergo growth arrest rapidly. Mice deficient in p53 are similarly prone to thymic lymphomas and yield MEFs that undergo growth arrest between passages 3 and 4, whereas the p53 and atm/p53 doubly deficient cell lines continued at a doubling rate of 18–20 h past passage 20.

Irradiation-induced Cell Cycle Checkpoints. Unsynchro- nized early-passage (p2–p4) MEFs, growing on tissue culture dishes, were irradiated from a 137Cs-source (Gammacell 40, Atomic Energy of Canada, Ltd.) at a dose rate of 1 Gy/min. Cells were harvested 16 h after irradiation, fixed in 50% ethanol, and stained for 30 min in a solution of 2.5 µg/ml propidium iodide and 50 µg/ml RNase A. Flow cytometry was carried out on a FACSscan (Becton Dickinson) using CellQuest Software. The data were analyzed subsequently with ModFit LT V1.01 for cell cycle determination.

Protein Analysis. Early-passage wild-type, atm-null, p53-null, and double-null cells were harvested, and 500,000 cells were lysed to make extracts. Entire cell lysates were separated by 13% PAGE and transferred onto a nitrocellulose filter (Schleicher & Schuell). Protein was detected using a polyclonal p21waf1 antibody (C-19; Santa Cruz Biotechnology) or a β-tubulin-specific antibody (TUB 2.1, Sigma Chemical Co.) Filters were incubated with horse-radish peroxidase-coupled secondary antibody (Cappel) and visualized with enhanced chemiluminescence.

Results

Generation of atm(−/−) p53(−/−) MEFs. To produce MEFs null for both the atm and p53 genes, compound heterozygotic atm(+/−) p53(+/−) mice were mated, and pregnant females were sacrificed to derive MEFs between 11.5 and 16.5 days post coitum. A total of 79 primary cell lines were derived in this manner, yielding all possible genotypic combinations, including two deficient for both atm and p53. These cell lines were used for subsequent growth experiments and cell cycle analysis.

Control of Cellular Proliferation Is Mediated by atm and p53. The effects of atm and p53 on cellular proliferation were studied via cell cycle experiments on early-passage MEFs. As shown in Fig. 1A, and consistent with earlier observations, atm-null MEFs undergo fewer than four doublings before undergoing growth arrest (1–3). By contrast, homozygous deletion of p53 in an atm-null background...
atm AND p53 MODULATE PROLIFERATION AND G1-S CHECKPOINTS

overcomes this growth arrest (two independently derived MEFs, ap38 and ap24, are depicted in Fig. 1A). The signal for growth arrest seen in atm-null MEFs is hence dependent on p53 function. Fig. 1B shows a representative Southern blot of MEFs derived in the present cross, including ap38, one of the double-null MEFs used for the growth experiments shown in Fig. 1A. atm(—/—) p53(—/—) cells have been monitored for more than 20 passages without signs of the slowed growth indicative of crisis (data not shown). atm(+/+) p53(—/—) cells were monitored in parallel and proliferated at the same rate over 20 passages.

To address the role of p21cip/waf1 in the control of cellular proliferation, protein expression was analyzed in wild-type, single-null, and double-null cells. p21cip/waf1 protein is present at equivalent levels in both proliferating and nonproliferating atm-null MEFs and is expressed at lower levels in wild-type cells (Fig. 2, Lanes 1, 2, and 5; see also Ref. 3). Interestingly, neither atm(—/—) p53(—/—) nor atm(+/+) p53(—/—) cells express p21cip/waf1, as measured by Western blot (Fig. 2, Lanes 3 and 4, respectively). These observations support the view that p53 is the dominant modulator of p21cip/waf1 expression, whereas loss of atm leads to up-regulation of p21cip/waf1 protein levels via direct or indirect mechanisms (see also Fig. 4A).

atam(—/—) p53(—/—) MEFs Have an Impaired Irradiation-induced G1-S Cell Cycle Checkpoint. Patients with ataxia-telangiectasia and Li-Fraumeni syndrome are known to develop cancers at an increased rate. In both cases, the pathogenic role of increased genomic fragility has been implicated (9, 10). Deficiencies in either atm or p53 lead to profound defects in gamma-irradiation-induced cell cycle checkpoints, which likely play a role in the predisposition to cancer seen in both diseases (11). p53 activation of p21cip/waf1 plays a central role in the G1-S checkpoint after certain types of DNA damage, such as that caused by ionizing radiation (8, 12). The G2-M damage-activated checkpoint, on the other hand, appears to be partially p53 independent (13). Loss of atm has a complex effect on the G2-M cell cycle checkpoint (14–17), which is felt to be independent of p53. A more detailed analysis of the G2-M checkpoint is hence not presented here.

To understand the epistatic relationship between atm and p53 in cell cycle control, atm/p53 double-null cells were compared to littermate control MEFs with respect to the function of their G1-S cell cycle checkpoint in response to gamma irradiation. The primary data are presented in Fig. 3, A–D. Because atm homozygously deficient cell lines undergo growth arrest quite rapidly, Southern analysis of embryos was completed within 2.5 days after harvesting, and atm-null cells were subjected immediately to cell cycle analysis. The cell cycle analysis involved gamma-irradiating cells at the indicated doses and harvesting cells 16 h later for fixation, staining, and analysis by flow cytometry. As shown in Fig. 3A, wild-type cells show a functional checkpoint at G1-S. By contrast, the G1-S checkpoint is impaired significantly in p53-null cells (Fig. 3B). Similarly, in the case of the atm-null MEFs (Fig. 3C), there is a clear deficiency in the G1-S checkpoint, which is likely underestimated because a significant proportion of these cells are already growth arrested and residing in G0. This premature growth arrest has been documented extensively (1–3). Cells null in both atm and p53 again show loss of the G1-S checkpoint (Fig. 3D).

Fig. 3E represents the percentage change seen in G1-G0 after 5 or 10 Gy of irradiation. These data are consistent at 5, 10, and 20 Gy, showing that wild-type MEFs manifest an increasing percentage of cells in G1-G0 after irradiation, whereas single- and double-null cells show a decreasing percentage over the range of doses analyzed. Note that both double-null MEFs derived in this cross are shown here, and that the percentage decrease in G1-G0 is similar in these two primary lines. The results presented in Fig. 3A–E support a previously proposed model in which atm and p53 assume equivalent roles in mediating irradiation-induced G1-S cell cycle arrest, consistent with their participation in a linear regulatory pathway (Fig. 4B; Refs. 14 and 15).

Discussion

Loss of p53 Suppresses the Growth Arrest Seen in atm-null MEFs. All previously described human and mouse atm-deficient fibroblast cell lines show significant growth defects. In the mouse, the growth...
arrest is especially severe, leading to a senescent appearance at the third passage in tissue culture (1–3). In contrast, deletion of either p21<sup>WAF1/CIP1</sup> or p53 increases MEF proliferative rates (8). Here we have shown that the homozygous genetic deletion of p53 in an atm-deficient background overcomes the growth arrest seen in atm-null MEFs. These results argue for a dominant role for p53 in mediating MEF growth arrest, because MEFs null for atm and p53 proliferate much more rapidly than MEFs deficient in atm alone. These double-null cells grow as rapidly as cells null for p53. Although the exact role, if any, of p21<sup>WAF1/CIP1</sup> is presently not clear, it is possible that a portion of the growth arrest seen in atm-null cells is mediated by a p21<sup>WAF1/CIP1</sup>-dependent mechanism, because p21<sup>WAF1/CIP1</sup> is overexpressed in atm-null cells. These elements can be brought together in the simplified model outlined in Fig. 4A. It is important to note that suppression of
A) atm ——> endogenous damage ——> p53 ——> p21 and others ——> CELL PROLIFERATION

B) Gamma Irradiation ——> atm ——> p53 ——> p21 and others ——> G1/S CELL CYCLE ARREST

Fig. 4. Diagrammatic representation of the putative actions of atm and p53 as they affect MEFS cellular proliferation and the G1-S cell cycle checkpoint. These diagrams are based on previous models (6, 14, 15, 18) and the observations described in this study. A indicates that loss of atm leads to the sensing and/or production of endogenous cellular damage, which signals MEFS growth arrest via p53 and p21. Note that signals through p53 and p21 may be direct or indirect. Here, homozygous loss of atm leads to growth arrest, whereas homozygous loss of p53 or both atm and p53 leads to relief of inhibition and rapid cellular proliferation. B shows that γ-irradiation induces a G1-S cell cycle arrest directly via atm and p53. In this case, homozygous loss of either atm or p53, or both results in similar defects in the G1-S cell cycle checkpoint.

the atm-null growth arrest by genetic deletion of p53 could also be consistent with a parallel or downstream effect of p53.

atm and p53 Show Equivalent Effects in Facilitating Irradiation-induced Cell Cycle Checkpoints. Because cells deficient in either atm or p53 are defective in the radiation-induced G1-S cell cycle checkpoint, it is possible to imagine that they act along the same or parallel pathways to induce this checkpoint. If the pathways were parallel, the collective effects of both pathways might be additive and their mutual loss would lead to a more severe loss of checkpoint control. To begin to distinguish between these possibilities, we have assessed radiation-induced checkpoint control in MEFS homozygously deficient for either p53 or atm and have compared their response to MEFS homozygously deficient in both atm and p53.

Homozygous loss of either gene clearly impairs the irradiation-induced G1-S checkpoint, whereas deletion of both genes in tandem does not appear to significantly exacerbate this phenotype. These and previous observations argue for an equivalent role for atm and p53 in a common pathway mediating the irradiation-induced G1-S checkpoint (Fig. 4B; Refs. 14 and 15).

Interestingly, loss of p21<sup>WAF1/CIP1</sup> similarly perturbs the G1-S checkpoint (8, 12). Moreover, p21<sup>WAF1/CIP1</sup>-null mice do not appear to develop cancer at increased frequencies (8). Inasmuch as loss of either atm or p53 predisposes to malignancy in both mouse and human, this phenotype seems less likely to involve a p21<sup>WAF1/CIP1</sup>-mediated signal transduction pathway. The studies reported here highlight the paradox that atm function may be either growth-inhibiting (at the G1-S radiation-induced cell cycle checkpoint) or growth-promoting (in MEFS grown in vitro), and that p53 may modulate both of these effects.

References

Genetic Interactions between atm and p53 Influence Cellular Proliferation and Irradiation-induced Cell Cycle Checkpoints

Christoph Heiner Westphal, Cornelius Schmaltz, Sheldon Rowan, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/9/1664

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.