Stress-induced Premature Senescence in hTERT-expressing Ataxia Telangiectasia Fibroblasts*

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In addition to replicative senescence, normal diploid fibroblasts undergo stress-induced premature senescence (SIPS) in response to DNA damage caused by oxidative stress or ionizing radiation (IR). SIPS is not prevented by telomere elongation, indicating that, unlike replicative senescence, it is triggered by non-specific genome-wide DNA damage rather than by telomere shortening. ATM, the product of the gene mutated in individuals with ataxia telangiectasia (AT), plays a central role in cell cycle arrest in response to DNA damage. Whether ATM also mediates signaling that leads to SIPS was investigated with the use of normal and AT fibroblasts stably transfected with an expression vector for the catalytic subunit of human telomerase (hTERT). Expression of hTERT in AT fibroblasts resulted in telomere elongation and prevented premature replicative senescence, but it did not rescue the defect in G1 checkpoint activation or the hypersensitivity of the cells to IR. Despite these remaining defects in the DNA damage response, hTERT-expressing AT fibroblasts exhibited characteristics of senescence on exposure to IR or H2O2 in such a manner that triggers SIPS in normal fibroblasts. These characteristics included the adoption of an enlarged and flattened morphology, positive staining for senescence-associated β-galactosidase activity, termination of DNA synthesis, and accumulation of p53, p21WAF1, and p16 INK4A. The phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK), which mediates signaling that leads to senescence, was also detected in both IR- or H2O2-treated AT and normal fibroblasts expressing hTERT. These results suggest that the ATM-dependent signaling pathway triggered by DNA damage is dispensable for activation of p38 MAPK and SIPS in response to IR or oxidative stress.

Culture of primary cells for many generations eventually results in a loss of proliferative potential, a phenomenon referred to as replicative senescence or, more generally, as cellular senescence. Cellular senescence can also be induced by stressful conditions (1). Replicative senescence likely results from the shortening of telomeres to such an extent that the chromosome ends are not fully masked from recognition by the proteins responsible for double strand break repair. Whereas primary human fibroblasts, which lack telomerase activity, normally exhibit a finite life span in culture (2), ectopic expression of the catalytic subunit of human telomerase (hTERT) in these cells restores telomerase activity, stabilizes telomere length, and prevents replicative senescence (3). Although somatic cells in culture also undergo senescence, this phenotype is attributable to unsuitable conditions (culture shock) or to growth arrest mediated by p53 and p19ARF, not to telomere shortening per se. In contrast, the major signaling pathway responsible for senescence in human cells, including that due to telomere shortening, is mediated by RB and p16INK4A (4, 5).

The introduction of activated oncogenes into primary cells triggers defense responses that prevent cell proliferation. Some oncogenes, such as those for c-Myc and E2F1, trigger apoptosis (6, 7), whereas others, such as those for Ras and Raf, trigger a permanent and irreversible cell cycle arrest that is reminiscent of replicative senescence and termed oncogenic stress-induced senescence (8, 9). Such senescence-like growth arrest induced by oncogenic Ras is accompanied by the accumulation of growth inhibitors such as p53 and p16INK4A (8). The induction of premature senescence or apoptosis is important for tumor suppression in the presence of oncogenic stimuli.

Cells subjected to other types of sublethal stress also enter a state that closely resembles replicative senescence and is referred to as stress-induced premature senescence (SIPS) (10). Cells subjected to DNA damage induced by ultraviolet or x-radiation (10–13), to oxidative stress (induced by H2O2 or hy- peroxia) (14–16), or to treatment with a histone deacetylase inhibitor (17) thus enter SIPS. Cells undergoing SIPS manifest all the major characteristics of replicatively senescent cells, including an enlarged flattened morphology and positive staining for senescence-associated (SA) β-galactosidase activity. Rapid shortening of telomeres is also associated with SIPS (10).

Overexpression of antioxidant proteins in human fibroblasts slows the rate of telomere shortening and extends their life span (18). Accelerated telomere shortening caused by oxidative stress has thus been suggested as a cause of SIPS (10, 19, 20). However, no difference in SIPS induction was detected between parental and hTERT-expressing human fibroblasts after exposure to H2O2 or to ultraviolet or x-radiation, and telomere shortening was not apparent in the hTERT-expressing cells.

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‡ The abbreviations used are: hTERT, catalytic subunit of human telomerase; SIPS, stress-induced premature senescence; SA, senes-
cence-associated; MAPK, mitogen-activated protein kinase; AT, ataxia telangiectasia; IR, ionizing radiation; PD, population doubling; TRAP, telomeric repeat amplification protocol; TRF, terminal restric-
tion fragment; BrdUrd, bromodeoxyuridine; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; RB, retinoblastoma.
during SIPS induction (21). These observations thus indicate that, in contrast to replicative senescence, SIPS cannot be prevented by hTERT-mediated telomere elongation (21). They further suggest that SIPS is triggered not only by telomere shortening but also by nonspecific genome-wide DNA damage. Senescence induced by oncogenic Ras is prevented by inhibition of the activity of the p38 (stress-activated) isoform of mitogen-activated protein kinase (p38 MAPK), suggesting that activation of p38 MAPK is essential for oncogenic stress-induced senescence (22). The activation of p38 MAPK also contributes to the onset of senescence induced by telomere shortening, oxidative stress, culture shock, or activation of Ras-Raf signaling (23). However, the signaling pathway responsible for p38 MAPK activation during SIPS induction is not well characterized.

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by progressive neurological degeneration, telangiectasia, growth retardation, specific immunodeficiency, high sensitivity to ionizing radiation (IR), an increased incidence of malignancy, and premature aging of the skin and hair (24, 25). Mitotic cells, such as fibroblasts and lymphoblasts, from individuals with AT exhibit a variety of anomalies in culture including c-Abl, NBS1, BRCA1, Chk2, p53, MDM2, FANCD2, ATM, the product of the gene mutated in AT patients, regulates checkpoints triggered by DNA damage and in DNA repair, including c-Abl, NBS1, BRCA1, Chk2, p53, MDM2, FANCD2, and SMC1 (32–34). ATM thus plays a central role in the response to DNA damage by contributing to the G1, S, and G2-M cell cycle checkpoints (33–35). For example, ATM phosphorylates and activates Chk2 in response to IR, resulting in the stabilization and activation of p53, the induction of p21\(^\text{WAP1}\) (p21\(^\text{CTP}\) expression, and cell cycle arrest at G1 phase (36–40).

The premature replicative senescence of AT fibroblasts is rescued by telomere elongation achieved by ectopic expression of hTERT (41, 42), as is that of fibroblasts from individuals with Werner’s (premature aging) syndrome (43) or with Nijmegen breakage syndrome (44). ATM has recently been shown to contribute to telomere maintenance and to senescence signaling originating from telomeres (45, 46). In addition to its role as a damage sensor for cell cycle checkpoints and DNA repair, ATM mediates up-regulation of ATP3, a target of p38 MAPK, in cells exposed to IR (47), suggesting that it acts as a trigger for stress-related MAPK signaling by activating c-Abl (32). ATM has thus been proposed to function as a stress sensor in SIPS (48). It has not been possible to study SIPS in AT cells, however, because of their premature replicative senescence. To evaluate the possible role of ATM in SIPS, we therefore established hTERT-expressing AT fibroblasts and examined whether SIPS is induced in these cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Infection**—Primary human dermal fibroblasts established from a normal individual (YMM) or from individuals with AT (AT1KY, AT2KY, AT4KY, AT5KY, AT6KY, and AT1OS) were cultured under a humidified atmosphere of 5% CO\(_2\) at 37 °C in Dulbecco’s modified Eagle’s medium (Invitrogen, Rockville, MD) supplemented with 15% heat-inactivated fetal bovine serum (Invitrogen) (49). The retroviral vector pMX-puro-hTERT (23) and Phenix-A retroviral packaging cells were kindly provided by F. Ishikawa (Kyoto University), T. Kitamura (University of Tokyo), and G. P. Nolan (Stanford University). For retrovirus production, we transfected Phenix-A cells with pMX-puro-hTERT with the use of FuGENE 6 (Roche Applied Science, Mannheim, Germany). The resulting retroviruses were used to infect fibroblasts, which were then selected in medium containing puromycin (0.5 µg/ml) (Sigma, St. Louis, MO). To examine the kinetics of cell proliferation, we plated hTERT-expressing and parental primary fibroblasts (2 × 10\(^5\) cells) in 95-mm-diameter culture dishes and determined the cell number originating from telomeres (45, 46). In addition to its role as a damage sensor for cell cycle checkpoints and DNA repair, ATM mediates up-regulation of ATP3, a target of p38 MAPK, in cells exposed to IR (47), suggesting that it acts as a trigger for stress-related MAPK signaling by activating c-Abl (32). ATM has thus been proposed to function as a stress sensor in SIPS (48). It has not been possible to study SIPS in AT cells, however, because of their premature replicative senescence. To evaluate the possible role of ATM in SIPS, we therefore established hTERT-expressing AT fibroblasts and examined whether SIPS is induced in these cells.
Telomere length was measured with a terminal restriction fragment (TRF) assay. Genomic DNA (5 μg) was thus digested with HinfI and RsaI, and the resulting fragments were subjected to Southern blot analysis with a 32P-labeled telomeric oligonucleotide probe, (TTAGGG)₄; hybridization was performed for 12–15 h at 37 °C in a solution containing 0.75 M NaCl, 30 mM sodium citrate, and 1% SDS. Signals were visualized by autoradiography.

**Cell Cycle Analysis—** Cells were labeled for the indicated times with 10 μM bromodeoxyuridine (BrdUrd), fixed with 70% ethanol, and stained with a fluorescein isothiocyanate-conjugated mouse monoclonal antibody to BrdUrd (BD Pharmingen, San Diego, CA) and with propidium iodide (Sigma). The cellular content of DNA was determined by flow cytometry.

**Fig. 3.** Defective G₁ checkpoint response to IR in hTERT-expressing AT fibroblasts. A, cell cycle analysis. Asynchronous normal/TERT or AT/TERT cells were exposed (or not) to 4 Gy of x-radiation (1.6 Gy/min) and then cultured for 7 h in the absence and 1 h in the presence of 10 μM BrdUrd. The cells were then fixed, stained with both fluorescein isothiocyanate-conjugated antibodies to BrdUrd and propidium iodide, and analyzed by flow cytometry. B, analysis of signaling via the ATM-Chk2-p53-p21WAF1 pathway. hTERT-expressing normal and AT fibroblasts were irradiated as in A and, after incubation for 2 h, lysed and subjected to immunoblot analysis with antibodies to the indicated proteins.
Parental (open symbols) or hTERT-expressing (closed symbols) normal (squares), AT2KY (circles), or AT4KY (triangles) fibroblasts were exposed to the indicated doses of \( \gamma \)-radiation. Two weeks thereafter, the cells were fixed and stained by crystal violet, and colonies containing \( \geq 50 \) cells were scored as being derived from viable clonogenic cells.

**Clonogenic Assay**—Clonogenic assays were performed as described previously (26). Cells in the exponential phase of growth were plated on 95-mm culture dishes, incubated for 24 h, and then irradiated at room temperature, at the indicated doses, with a \( ^{137} \text{Cs} \) \( \gamma \)-ray source at a rate of 7.32 Gy/min (21). For \( \text{H}_2\text{O}_2 \) treatment, cells were seeded in 95-mm dishes, incubated for 3 days, and exposed to 55 mM \( \text{H}_2\text{O}_2 \) for 2 h (C, F, I, and L). After 10 days, the cells were stained for SA \( \beta \)-galactosidase activity (A–C and G–I) and counterstained with propidium iodide (D–F and J–L).

**Immunoblot Analysis**—Cells were lysed in a solution containing 50 mM Tris-HCl (pH 7.4), 125 mM NaCl, 0.1% Nonidet P-40 (Sigma), 5 mM EDTA, 0.1 mM NaF, and a mixture of protease inhibitors (Complete, Roche Applied Science). The protein concentration of the lysate was determined with the BCA protein assay reagent (Pierce, Rockford, IL), after which samples (50 \( \mu \)g of protein) were subjected to SDS-polyacrylamide gel electrophoresis and immunoblot analysis with mouse monoclonal antibodies to p21WAF1 (F-5; Santa Cruz Biotechnology, Santa Cruz, CA), mouse monoclonal antibodies to p53 (Ab-6; Oncogene Research, Cambridge, MA), mouse monoclonal antibodies to p21\(^{\text{MAPK}} \) (C-20, Santa Cruz Biotechnology), rabbit polyclonal antibodies to p16\(^{\text{INK4A}} \) (C-20, Santa Cruz Biotechnology), rabbit polyclonal antibodies to p38\(^{\text{MAPK}} \) (Phospho-p38\(^{\text{MAPK}} \); Cell Signaling Technology), and sheep polyclonal antibodies to Chk2 (Upstate Biotechnology). Immune complexes were detected with horseradish peroxidase-conjugated secondary antibodies and the ECL Plus system (Amersham Bioscience, Piscataway, NJ).

**Establishment of hTERT-expressing AT Fibroblasts**—Primary fibroblasts derived from individuals with AT exhibit a variety of abnormalities, including defective cell cycle checkpoint function in response to DNA damage as well as rapid shortening of telomere length and consequent premature replicative senescence. To examine the role of ATM in SIPS, we established primary fibroblasts from a normal individual (normal/TERT) and from AT patients (AT/TERT) that stably express hTERT as a result of infection with a recombinant retrovirus. The cells were selected and expanded as polyclonal populations. Telomerase activity was detected by the TRAP assay in AT1KY/TERT, AT2KY/TERT, AT4KY/TERT, AT5KY/TERT, or AT6KY/TERT cells were exposed (or not) to \( \text{H}_2\text{O}_2 \) as in Fig. 5 and then cultured for 36 h in the absence and 36 h in the presence of BrdUrd. Cells were fixed, and BrdUrd incorporation was determined by flow cytometry. Data are means ± S.D. from three independent experiments.
TERT, AT6KY/TERT, and AT1OS/TERT cells as well as in normal/TERT cells but not in the corresponding parental cells (Fig. 1A; data not shown). Reverse transcription and polymerase chain reaction analysis also revealed the presence of hTERT mRNA in AT/TERT and normal/TERT cells but not in the corresponding parental cells (data not shown). The TRF assay showed that the telomere length of AT/TERT cells was extended to >12 kb, compared with 4–11 kb for parental AT cells (Fig. 1B). Whereas parental AT cells underwent replicative senescence after ~24 PDs (Fig. 2A), AT/TERT cells survived for >70 to 100 PDs (Fig. 2B, data not shown). These results indicated that the telomere elongation induced by expression of hTERT allowed AT cells to overcome premature replicative senescence.

**Defective Checkpoint Function in AT/TERT Cells Exposed to IR**—AT fibroblasts show defective cell cycle checkpoint responses to DNA damage induced by IR (51) or to oxidative stress (52). To examine whether the abnormality of the G1 cell cycle checkpoint in AT fibroblasts was rescued by hTERT expression, we exposed asynchronous normal/TERT or AT/TERT cells to IR and determined the numbers of cells in G1, S, and G2/M phases of the cell cycle 8 h thereafter by flow cytometry. The proportion of normal/TERT cells in S phase (BrdUrd-positive) was greatly reduced 8 h after irradiation, whereas IR had no effect on the proportion of AT/TERT cells in S phase (Fig. 3A). The IR-induced G1 checkpoint is triggered by sequential signaling by ATM, Chk2, p53, and p21WAF1 (40). Although ATM was detected in normal/TERT cells, this protein was not apparent in AT/TERT cells by immunoblot analysis (Fig. 3B). The IR-induced phosphorylation and activation of Chk2, as revealed by a decrease in the electrophoretic mobility of the protein, were also evident in normal/TERT cells but not in AT/TERT cells (Fig. 3B). Consistent with these observations, up-regulation of p21WAF1 in response to IR was apparent in normal/TERT cells but not in AT/TERT cells (Fig. 3B). Activation of the ATM-Chk2-p53-p21WAF1 signaling pathway by IR was thus impaired in the AT/TERT cells. Like primary AT fibroblasts, AT/TERT cells also exhibit a defective G1 cell cycle checkpoint as a result of their ATM deficiency.

**Radiosensitivity of AT/TERT Cells**—AT fibroblasts are markedly more sensitive to IR than are normal cells. To examine whether hTERT expression affected the radiosensitivity of AT cells, we performed a clonogenic survival assay after exposure of AT/TERT, normal/TERT, and the corresponding parental cells to various doses of IR. Consistent with previous report, both AT/TERT cells and primary AT fibroblasts were hypersensitive to IR in comparison with normal fibroblast and normal/TERT cells (Fig. 4) (41). ATM is thus essential for signaling that results in DNA repair after exposure of cells to IR.

**SIPS in AT/TERT Fibroblasts**—We have shown that, like that of the parental cells, the response of AT/TERT cells to DNA damage is impaired. If the DNA damage signaling pathway also contributes to SIPS, then AT/TERT cells would also be expected to be resistant to SIPS. To examine this possibility, we compared the responses of normal/TERT and AT/TERT fibroblasts to SIPS inducers (21). 5 or 10 days after exposure to IR or H2O2, normal/TERT cells exhibited a senescence phenotype, including a flattened and enlarged morphology (Fig. 5, A–F, data not shown). Similar morphological changes were also evident in AT4KY/TERT and AT6KY/TERT cells (Fig. 5, G–L, data not shown). Furthermore, normal/TERT cells stained intensely for SA β-galactosidase activity 10 days after exposure to IR or H2O2 (Fig. 5, A–C), as did AT4KY/TERT and AT6KY/TERT cells (Fig. 5, G–I, data not shown).

We also examined DNA synthesis in the treated cells by measuring BrdUrd incorporation. The proportion of BrdUrd-positive cells was reduced by >95% 3 days after exposure of normal/TERT, AT4KY/TERT, or AT6KY/TERT cells to IR or to H2O2 (Fig. 6). These results thus indicate that, despite the defects in cell cycle checkpoint and other DNA damage responses in AT/TERT cells, SIPS was induced similarly in AT/TERT cells and normal/TERT cells.

**Induction or Phosphorylation of Senescence-associated Proteins in AT/TERT Cells**—To investigate the molecular mechanism of SIPS induction, we examined the expression and modification of senescence-associated proteins in normal/TERT and AT/TERT fibroblasts. The abundance of p53, p21WAF1, and p16INK4A has been known to be increased in senescent cells. The accumulation of p53 and p21WAF1 was also observed in normal/TERT cells 6 days after the induction of SIPS by IR or H2O2 (Fig. 7). Furthermore, the accumulation of p53 and p21WAF1 was evident in AT4KY/TERT and AT6KY/TERT cells after exposure to these SIPS inducers, albeit to a slightly lesser extent than that apparent in normal/TERT cells. Although p16INK4A plays an important role in cellular senescence in human cells, the accumulation of this protein was not previ-

**Fig. 7. Expression and phosphorylation of senescence-related proteins in hTERT-expressing normal and AT fibroblasts after exposure to SIPS inducers.** Cells were exposed (or not) to x-radiation or H2O2 as in Fig. 5, cultured for 6 days, lysed, and subjected to immunoblot analysis with antibodies to the indicated proteins.
ously detected during SIPS in human fibroblasts (21, 53). Similarly, we detected only a small increase in the abundance of p16INK4A in normal/TERT fibroblasts 6 days after SIPS induction (Fig. 7). The expression of p16 INK4A was increased to a greater extent in AT/TERT fibroblasts than in normal/TERT cells after exposure to IR or H2O2. The activation of p38 MAPK mediates replicative senescence and senescence induced by Ras-Raf signaling (23). p38 MAPK is phosphorylated on Thr180 and Tyr 182 and thereby activated by the kinases MKK3 and MKK6 (54). We therefore examined the phosphorylation status of p38 MAPK in normal/TERT and AT/TERT cells with antibodies specific for the enzyme phosphorylated on these residues. The phosphorylation of p38 MAPK was induced in normal/TERT, AT4KY/TERT, and AT6KY/TERT cells by IR or H2O2 treatment (Fig. 7). These SIPS inducers did not affect the abundance of p38 MAPK. Our results thus indicate that the signaling pathways responsible for SIPS are intact in AT/TERT cells.

### DISCUSSION

Cells that have been subjected to sublethal stress, such as that caused by radiation-induced DNA damage or oxidants, undergo SIPS. The molecular mechanisms by which cells monitor the effects of such stress and decide to undergo SIPS,
however, have remained unclear. Given that ATM plays a pivotal role as a sensor of DNA damage for cell cycle checkpoints and DNA repair, it has also been a prominent candidate for the stress sensor in SIPS (48). We have examined this possibility in the present study by characterizing SIPS in hTERT-expressing AT fibroblasts.

Expression of certain oncoproteins, such as E2F1 and c-Myc, promotes the accumulation of reactive oxygen species and consequent DNA damage (55, 56). These cellular oncoproteins, as well as the adrenoviral oncoprotein E1A, induce apoptosis by a p53-dependent pathway (6, 7, 57). Such apoptosis is blocked by treatment of the cells with caffeine, an inhibitor of the kinase activities of ATM and the related protein ATR, suggesting that the oncoprotein-induced damage is recognized by ATM or ATR (58). These observations thus also suggested that ATM or a related protein might be the stress sensor for SIPS (48).

Consistent with previous observations (41, 42), we have now shown that expression of hTERT in AT fibroblasts rescued the premature replicative senescence phenotype of these cells. However, like the parental cells, the hTERT-expressing AT fibroblasts still exhibited hypersensitivity to IR, probably because of an impairment in DNA repair activity due to the loss of ATM. Moreover, in contrast to telomerase-positive normal fibroblasts, hTERT-expressing AT fibroblasts did not arrest in G1 phase of the cell cycle in response to DNA damage. Consistent with these observations, IR did not induce either a shift in the electrophoretic mobility of Chk2, which reflects phosphorylation on Thr68 and kinase activation (59), or expression of p21WAF1 in AT/TERT fibroblasts, indicating that the Chk2-dependent activation of p53 in response to DNA damage was not rescued in AT fibroblasts by telomere elongation. These results thus suggested that the replicative senescence induced by telomere shortening and growth arrest in response to DNA damage are mediated by different sensors.

In contrast to the defective response to DNA damage in AT/TERT fibroblasts, the elicitiation of SIPS in these cells by either H2O2 or x-radiation did not differ substantially from that in normal/TERT cells, suggesting the existence of an ATM-independent pathway for SIPS. We therefore propose the existence of distinct mechanisms for the immediate cell cycle arrest and for SIPS in response to DNA damage (Fig. 8). Upon experiencing DNA damage, cells initially arrest cell cycle progression by ATM-dependent activation of the G1 checkpoint to gain time for DNA repair (Fig. 8A). The major pathway responsible for triggering the G1 checkpoint involves the activation of p53 by ATM, MDM2, and Chk2; the p53-mediated induction of the gene for the Cdk inhibitor p21WAF1; and a reduction in the level of RB phosphorylation that results from Cdk inhibition. If the DNA damage cannot be repaired within a relatively short time window (several days), the cells undergo SIPS, which is independent of ATM, to prevent accumulation of genetic mutations (Fig. 8B). The unrepaired DNA damage is thus recognized by an unknown sensor, and the stress signal is then transduced by a MAPK cascade, possibly mediated by MEK, MKK3 or MKK6, and p38 MAPK (22), resulting in the induction of ATF3 (60). ATF3 induced by p38 MAPK has been shown to repress transcription of the gene for Id1 (61). Activated MEK also induces p16INK4A expression through Ets1- or Ets2-dependent transcription (62). Given that Id1 inhibits the induction of p16INK4A through direct interaction with Ets1 or Ets2 (62), the p38 MAPK pathway also likely contributes to p16INK4A induction during stress signaling. Indeed, activation of p38 MAPK by MKK3 or MKK6 has been shown to increase the abundance of p16INK4A mRNA (22). Consistent with previous observations (21, 53), we detected only a small increase in the abundance of p16INK4A in normal/TERT fibroblasts undergoing SIPS. In contrast, the increase in the amount of p16INK4A was markedly greater in similarly treated AT/TERT fibroblasts. Therefore, the defect in the induction of p21WAF1 due to the lack of ATM in AT/TERT cells might be compensated for by p38 MAPK-dependent induction of p16INK4A. Although up-regulation of ATF3 was not previously observed within 8 h after exposure of AT fibroblasts to IR (47), cells in which the ATM signaling pathway is inoperative appear to be capable of undergoing SIPS in response to severe DNA damage in a manner dependent on the p38 MAPK-p16INK4A pathway.

The accumulation of p53 was apparent in AT/TERT fibroblasts during SIPS. An alternative, ATM-independent pathway, possibly mediated by ATR or a related kinase, might thus be responsible for this p53 accumulation. Given that p38 MAPK also phosphorylates p53 on Ser33 and Ser66 in the NHL2-terminal activation domain and thereby regulates the transactivation activity of p53 (63, 64), p38 MAPK might contribute to the p53 accumulation in AT/TERT fibroblasts during SIPS. In addition, p38 MAPK has been shown to induce the stabilization of p21WAF1 by phosphorylation on Ser130 (65). The accumulation of the Cdk inhibitors p16INK4A and p21WAF1 induced by p38 MAPK might thus contribute to SIPS and to tumor suppression. Indeed, disruption of the p38 MAPK gene by homologous recombination or inactivation of p38 MAPK by overexpression of Ppm1D, a phosphatase that inhibits the activity of p38 MAPK, increases tumor formation in mice (66). Furthermore, mice that lack both MKK3 and MKK6 and are therefore not able to activate p38 MAPK are also prone to tumorigenesis (67). These observations thus highlight the importance of p38 MAPK-mediated signaling for tumor suppression.

Consistent with our observations on SIPS, telomere dysfunction induced by inhibition of telomeric repeat binding factor 2 has also been shown to result in replicative senescence through an ATM-independent pathway in AT fibroblasts, indicating that ATM is not required for telomere-derived senescence signaling (68). Our results indicate that the ATM-mediated signaling pathway triggered by DNA damage is not required for p38 MAPK activation and consequent cellular senescence. The cell cycle checkpoint function of ATM is thus dispensable for the major pathway responsible for the triggering of SIPS.

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