Suppression of Apoptosis by PIF1 Helicase in Human Tumor Cells

Mary E. Gagou, Anil Ganesh, Ruth Thompson, Geraldine Phear, Cyril Sanders, and Mark Meuth

Abstract

Defining the processes that sustain telomere maintenance is critical to our understanding of cancer and longevity. PIF1 is a nonprocessive 5'→3' human DNA helicase that exhibits broad substrate specificity. In vitro studies have implicated PIF1 in maintaining telomeres and processing stalled DNA replication forks, but disruption of the murine Pif1 gene did not yield any apparent phenotype. In this study, we evaluated the function of the PIF1 gene in human cells by using siRNA knockdown strategies to gauge its role in the response to DNA replication stress. We found that PIF1 depletion reduced the survival of both p53-deficient and p53-proficient human tumor cells by triggering apoptosis. In contrast, nonmalignant cells were unaffected by PIF1 depletion. Apoptosis induction in tumor cells was augmented by cotreatment with replication inhibitors (thymidine, hydroxyurea, or gemcitabine). When sensitive PIF1-depleted cells were released from a thymidine-induced S-phase arrest, there remained a subpopulation of cells that failed to enter S-phase. This cell subpopulation displayed an increase in levels of cyclin E and p21, as well as a deficiency in S-phase checkpoint markers that were induced with thymidine in PIF1 expressing cells. Specifically, CHK1 activation was suppressed and we detected no consistent changes in ATM S1981 autophosphorylation, γH2AX induction, or RPA hyperphosphorylation. Death in PIF1-depleted cells was detected in late G1/early S-phase and was dependent on caspase-3 activity. Taken together, our findings suggest roles for PIF1 in S-phase entry and progression that are essential to protect human tumor cells from apoptosis. Cancer Res; 71(14): 4998–5008. ©2011 AACR.
cells and mPif1−/− mice have no obvious phenotype (13). However, in vitro studies of purified PIF1 protein indicate that it can bind and unwind DNA structures resembling stalled replication forks to produce free 3′ssDNA ends, suggesting that PIF1 could play a role in the response to DNA replication stress (14). The aim of the work reported here was to investigate the potential role of PIF1 in the cellular response to DNA replication stress. Our results suggest that PIF1 is essential to protect a number of human tumor cell lines from apoptosis, particularly after the inhibition of DNA replication.

Materials and Methods

Cell lines and cultures

Human tumor cell lines and the human embryonic kidney (HEK) 293 line were from American Type Culture Collection. p21−/− and p53−/− derivatives of HCT116 were provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). The immortalized human fibroblast cell line MRC5 VA was from the CRUK London Laboratories Cell Repository. Identity of the lines used in these experiments was confirmed by DNA fingerprinting (LGC Standards). Cells were carried for no longer than 2 months before rethawing from a frozen stock. Cells were routinely tested for contamination. HCT116 strains overexpressing mPif1 and p21−/− derivatives of HCT116 were provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). The immortalized human fibroblast cell line MRC5 VA was from the CRUK London Laboratories Cell Repository. Identity of the lines used in these experiments was confirmed by DNA fingerprinting (LGC Standards). 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siRNA transfection

PIF1 siRNAs, sense-strand sequences GGCCAGAGCAGCUUCUUCATT (siRNA1), GGGAUGCUAUUGCUACATTT (siRNA 2), and CCCUUCAGGCUAACCAATT (siRNA3), were obtained from Applied Biosystems (Ambion). The CHK1 siRNA (sense strand: GAAAGAGGCUGCAAGAAGA) was designed by J. Blackburn and C. Smythe. Control siRNAs, containing nonspecific sequences that do not have homology in the human genome, were provided by Eurogentec (OR-0030-NEG). siRNA duplexes were transfected into cells by using Lipofectamine 2000 (Invitrogen).

Cell-cycle analysis

Cell-cycle analysis was done as described previously (15). Stained nuclei were analyzed on a FACScan (BD Biosciences) by using CellQuest software. Bromodeoxyuridine (BrdU) incorporation was analyzed as previously described (16).

Detection of apoptosis

Caspase-3 activation was analyzed by flow cytometry as described previously (15). Annexin V assays were done as directed by the manufacturer (BD Biosciences).

Cyclin E and p21 analysis

After indicated treatments, cells were fixed with 70% ice-cold ethanol, washed twice with PBS, and incubated for 10 minutes in PBS-T (PBS/0.1% BSA/0.2% Tween 20) before incubation with a 1:50 dilution of the primary antibody in PBS-T (anti-cyclin E; Invitrogen 32-1600, or anti-p21 Pharmingen) for 30 minutes. After 2 washes with PBS-T, cells were incubated with fluorescein isothiocyanate–conjugated anti-mouse immunoglobulin G (1:10; Dako) for 30 minutes, washed with PBS, and resuspended in PBS containing 5 μg/mL PI (Sigma) and 100 μg/mL RNase A (Sigma). After a 30-minute incubation, cells were analyzed by flow cytometry.

Protein extraction and Western blotting

Western blots were done as described (17). Proteins were detected with the ECL Detection System (GE Healthcare) by using the following antibodies: γH2AX (2577; Cell Signaling), CHK1 (2360; Cell Signaling), β-actin (A-5060; Sigma-Aldrich), RPA34 (NA19L; Calbiochem), cleaved caspase-3 (ab32042; Abcam), phospho-ATM (Ser181; 2152;– Epitomics), phospho-CHK1 (Ser345; 2349; Cell Signalling), and FLAG M2 (F1804; Sigma). Antibodies to PIF1 were raised in rabbits. Amino acids 1 to 205 of PIF1 were expressed as a GST fusion protein, using pET11c in E. coli BL21(DE3). The fusion protein was bound to GST sepharose and the PIF1 moiety cleaved off the beads by using thrombin after extensive washing of the beads. Further purification was achieved by size exclusion chromatography (Superdex 75). Band intensities on the Western blots were quantified by densitometry by using the ImageJ software (18).

RNA purification and quantitative analysis of total mRNA levels

RNA was extracted from adherent cells by using the RNeasy Plus Kit (QIAGEN). RNA was treated with DNase I RNase-free (Roche) and purified over an RNeasy column. Two micrograms of RNA were used for first-strand cDNA synthesis by using oligo(dT)18 (Bioscript kit; Bioline). qPCRs (quantitative PCR; Quantace) were run on a 7900HT Fast Real-Time PCR system (Applied Biosystems). Samples were heated for 10 minutes at 95°C and amplified for 45 cycles (15 sec 95°C/15 sec 59°C/25 sec 72°C). All samples were analyzed in duplicate. Data were evaluated by using SDS 2.2.1 data analysis software (Applied Biosystems) and the Pfaffl method (19). PIF1 primers were designed by using Primer3-plus (20) and NCBI (21) programs: PIF1 5′-ccctggattgtgac-3′ (forward) and 5′-actccagactgaggcag-3′ (reverse). Primers for the U1 snRNA internal control were from Hautbergue (22).

Immunofluorescence analysis

RPA foci were analyzed as described previously (16).
**Results**

**PIF1 depletion sensitizes HCT116 cells to DNA replication stress**

Given our previous work showing that static fork-like structures could serve as efficient substrates for purified PIF1 (14), we investigated the effects of siRNA-mediated depletions of PIF1 on cells exposed to DNA replication stress. The effect of the siRNA treatment on PIF1 levels was first determined by Western blotting. PIF1 is present at very low levels in mammalian cells (11) and large amounts of extracts had to be loaded onto gels to visualize the protein by Western blotting. At 24 hours posttransfection, PIF1 levels were reduced 3-fold relative to control siRNA-transfected cells and this level of depletion was maintained through 48 hours (Fig. 1A). At 72 hours, PIF1 levels remained low in PIF1 siRNA-transfected cells although the levels of this protein also decreased in cultures treated with the control siRNA as these approached confluence. In addition, DNA expression array analysis showed a highly significant ($P = 2.69 \times 10^{-4}$) 3-fold depletion of the PIF1 transcript 48 hours after transfection of the siRNA. To further confirm the effectiveness of the siRNA, HCT116 cells overexpressing FLAG-tagged PIF1 were transfected with PIF1 siRNA. Western blot analysis showed a

![Western blot images](image1)

- **A**
  - siRNA: PIF1
  - Control
  - % remaining: 34.5 35.5 50

- **B**
  - siRNA: PIF1
  - Control
  - β-Actin

- **C**
  - No thymidine
  - Control
  - 0 24 h 48 h

- **D**
  - HCT116
  - PIF1
  - % cells

- **E**
  - Annexin V

Figure 1. Apoptosis is increased in PIF1-depleted HCT116 cells in the presence or absence of DNA replication inhibitors. A, HCT116 cells were transfected with control or PIF1 siRNAs and harvested at 24, 48, and 72 hours for Western blot analysis of PIF1. Cells transfected with PIF1 siRNA show a 2.9-fold knockdown of the target protein level at 24 or 48 hours. At 72 hours, this low level of PIF1 was maintained whereas it declined in control siRNA-treated cells as they reached confluence. β-Actin levels are presented as loading controls. The % remaining values presented were corrected for loading. The % remaining values presented were corrected for loading. B, HCT116 cells transfected with control or PIF1 siRNAs were plated in medium containing increasing concentrations of thymidine (TdR), HU, or gemcitabine. Surviving cells were allowed to form colonies that were scored. Surviving fractions presented are normalized to the survival obtained in the control siRNA-transfected cells in the absence of the replication inhibitors. Under these conditions, surviving fractions of PIF1 siRNA-treated HCT116 cultures were about 10% of those obtained with control siRNA. C and D, HCT116 cells transfected with control or PIF1 siRNAs and treated or not treated with 2 mmol/L thymidine were harvested at 24 or 48 hours for analysis of DNA content by flow cytometry. Representative cell-cycle profiles (C) and cell-cycle distributions (D) determined from these profiles are presented. E, Annexin V levels in PIF1-depleted HCT116 cells treated as indicated. Results in D and E represent means of at least 3 independent experiments with SDs indicated by error bars. ns, not significant.
pronounced reduction of the tagged protein at 24 or 48 hours posttransfection by using either PIF1 or anti-FLAG antibodies (Supplementary Fig. S1).

To determine the effect of PIF1 depletion on cell survival during replication stress, HCT116 cells were treated with control or PIF1 siRNAs for 24 hours before plating in the presence of the replication inhibitors thymidine or hydroxyurea (HU) or the therapeutic deoxycytidine analog gemcitabine for colony formation. Interestingly, cells depleted of PIF1 showed reduced frequencies of colony formation relative to cells treated with control siRNA and these were further reduced by exposure to the replication inhibitors (Fig. 1B). Most notably, PIF1-depleted cells showed a 270-fold decrease in survival in 1.0 mmol/L gemcitabine relative to cells treated with the control siRNA.

We next determined the effect of the replication inhibitors on cell-cycle distribution following PIF1 depletion. HCT116 cells transfected with control or PIF1 siRNAs were treated with thymidine and analyzed for DNA content by flow cytometry (Fig. 1C and D). PIF1-depleted cultures that were not treated with thymidine showed a significant increase in the level of cells with a subG1 DNA content by 24 hours relative to control. Cells transfected with the control siRNA before thymidine treatment accumulated in early S-phase at 24 hours and progressed slowly through S-phase, characteristic of the slowing of DNA replication during thymidine-induced dCTP starvation. PIF1-depleted cells showed a similar accumulation in early S-phase after the 24-hour thymidine treatment but after 48 hours, there was a marked decrease in the proportion of cells in S-phase, accompanied by a significant increase of cells with a subG1 DNA content. The fraction of "subG1 cells" in PIF1-depleted cultures after exposure to thymidine was significantly greater than that seen in cells depleted of PIF1 alone. Similar increases in the proportion of cells showing the early apoptotic marker Annexin V were found in PIF1-depleted cells in the presence or absence of thymidine (Fig. 1E).

To further examine the effect of PIF1 depletion on S-phase transition, HCT116 cells transfected with control or PIF1 siRNAs were treated with thymidine for 24 hours before release into thymidine-free medium. Cells were labeled with BrdU for 1 hour before transfer to deoxynucleoside-free medium. At various times after release, cells were harvested and analyzed for DNA and BrdU content by flow cytometry (Fig. 2A). A lower fraction of released PIF1-depleted cells incorporated BrdU relative to cells treated with the control siRNA (Fig. 2B and C). Noticeably, about 20% of PIF1-depleted cells failed to enter S-phase and incorporate BrdU (Fig. 2C). Unlike cells treated with the control siRNA, PIF1-depleted cells incorporating BrdU also accumulated in late S-phase at 4 to 6 hours.

### Table 1. Effects of PIF1 depletion on the induction of apoptosis in tumor and nontumor cell lines in the presence or absence of thymidine

<table>
<thead>
<tr>
<th>siRNA</th>
<th>Control 0</th>
<th>PIF1 0</th>
<th>Control 48</th>
<th>PIF1 48</th>
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<tbody>
<tr>
<td>2 mmol/L TdR (h)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HEK293</td>
<td>4.32 ± 4.00</td>
<td>4.55 ± 3.05</td>
<td>4.04 ± 1.79</td>
<td>7.26 ± 5.62</td>
</tr>
<tr>
<td>MRC5VA</td>
<td>3.39 ± 1.72</td>
<td>4.85 ± 3.26</td>
<td>3.87 ± 2.35</td>
<td>5.24 ± 2.42</td>
</tr>
<tr>
<td>SW480</td>
<td>6.47 ± 2.14</td>
<td>15.39 ± 6.12</td>
<td>8.10 ± 2.99</td>
<td>18.93 ± 6.10</td>
</tr>
<tr>
<td>HCT116</td>
<td>2.15 ± 0.50</td>
<td>15.95 ± 4.00</td>
<td>4.57 ± 1.44</td>
<td>28.74 ± 4.13</td>
</tr>
<tr>
<td>MCF7</td>
<td>20.88 ± 9.32</td>
<td>63.41 ± 9.35</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

NOTE: The indicated cells were transfected with control or PIF1 siRNAs and 24 hours later were exposed or not exposed to 2 mmol/L thymidine. After a 48-hour treatment, cells were harvested for analysis of DNA content by flow cytometry. Proportions of cells with a subG1 DNA content are presented. Results presented are an average of at least 3 independent experiments ± SDs. Abbreviation: NT, not tested.
Figure 2. PIF1 is required for efficient entry into S-phase and S-phase progression. A, HCT116 cells, transfected with control or PIF1 siRNAs, were treated with 2 mmol/L thymidine for 24 hours. Thymidine-containing medium was removed and cultures were washed (W) with thymidine-free medium before transfer to BrdU (10 μmol/L)-containing medium. After 1 hour, cultures were washed with BrdU-free medium and restored to deoxynucleoside-free medium. At indicated times postrelease, cells were harvested and analyzed for DNA content and BrdU incorporation by flow cytometry. B and C, top panels in each present scatter plots of analysis of BrdU incorporation and DNA content. Middle panels present DNA content of all the cells analyzed, whereas the bottom panels present the DNA content of cells labeled with BrdU (gated into cells with G1, S, or G2/M DNA contents in top panels). Percentages presented in bottom panels represent the proportion of cells incorporating BrdU. Left-most panels present randomly growing cultures labeled for 1 hour with BrdU before harvest. The next panel from left presents the cells labeled for 1 hour with BrdU after thymidine release. The remaining panels present cells released from both deoxynucleosides for the indicated times. Closed arrowheads in C indicate PIF1-depleted cells that do not enter S-phase after release, whereas open arrowheads indicate those delayed from entering G2-M. The data presented are representative of 3 independent experiments. Values for percentages of cells incorporating BrdU represent means of values obtained from 3 experiments ± SDs.
Figure 3. PIF1-depleted cells arrested at G1/S show increased cyclin E and p21. A and B, HCT116 cells transfected with control (A) or PIF1 (B) siRNAs were exposed to 2 mmol/L thymidine for 24 hours before release into thymidine-free medium. Control (thymidine-untreated), thymidine-treated, and thymidine-treated cultures released into thymidine-free medium for 2 hours were harvested and analyzed for cyclin E and DNA content by flow cytometry. Top panels present cyclin E levels and DNA content whereas bottom panels present DNA content of all cells. Closed arrowhead in B indicates cyclin E level in PIF1-depleted cells arrested at G1/S. Cells gated in top panels indicate those with elevated cyclin E levels relative to control cultures. C and D, HCT116 cultures, transfected with control (C) or PIF1 (D) siRNAs, treated with thymidine and released for 6 hours as above, were analyzed for p21 and DNA content. Top panels in each present p21 and DNA content whereas bottom panels present DNA content of all cells. Closed arrowhead in D indicates the p21 level of cells that fail to enter S-phase. E, percentages of cells in the indicated phases of the cell cycle containing elevated levels of p21 (gated in C and D). Results represent means of 3 independent experiments ± SDs. F, p21−/− HCT116 cells depleted of PIF1 show enhanced apoptosis. Cells transfected with control or PIF1 siRNAs and treated or not treated with 2 mmol/L thymidine for 48 hours were analyzed for DNA content by flow cytometry for the fraction of subG1 cells. Average percentages of cells with subG1 DNA contents from 3 independent experiments are presented ± SDs.
hours after release from thymidine, suggesting a delay in S-phase progression. Similar cell-cycle delays at the G1/S border and in late S-phase were observed with PIF1-depleted SW480 cells (Supplementary Fig. S5). To clearly distinguish cells entering S-phase from those that did not, thymidine-released HCT116 cells were labeled with BrdU for 6 hours. Under these conditions, unlabeled G1 cells persisted at least 24 hours. There was a weak increase in the level of unlabelled cells with a subG1 DNA content (Supplementary Fig. S6). However, the level of BrdU-labeled cells with a subG1 DNA content increased more rapidly, suggesting that cycling cells may be more prone to apoptosis.

Flow cytometric measurements of DNA content and cyclin E in PIF1-depleted HCT116 cells released into thymidine-free medium indicate that cells failing to enter S-phase have an elevated level of cyclin E (Fig. 3A and B). However, levels of the cyclin-dependent kinase inhibitor p21 were also high in the PIF1-depleted cells relative to controls, particularly in cells retained at the G1/S border (Fig. 3C–E). To further address the potential role of p21 in the induction of death in PIF1-depleted cells, p21–/– HCT116 cells were depleted of PIF1 in the presence or absence of thymidine. The level of subG1 cells in PIF1-depleted p21–/– cultures increased relative to control siRNA-treated cells (Fig. 3F). Furthermore, the level of death in PIF1-depleted p21–/– HCT116 cells was higher than that found in p21+/+ HCT116 cells. Taken together, these results suggest that p21 protects cells from entry into S-phase and apoptosis triggered by PIF1 depletion.

**S-phase checkpoints in PIF1-depleted cells after thymidine treatment**

Cells exposed to DNA-damaging agents or replication stress rapidly activate ATR- and ATM-mediated signaling cascades. CHK1 is a key component of the ATR-mediated cascade and is quickly activated in response to ssDNA that is formed by the disruption of DNA replication (23) to suppress inappropriate origin firing, facilitate replication restart, and prevent apoptosis. Given our in vitro data suggesting a potential role for PIF1 in the generation of ssDNA at stalled forks (14), we next determined the effect of PIF1 depletion on signaling triggered by thymidine treatment. Cell-free extracts prepared from PIF1-depleted HCT116 cells were assayed for checkpoint activation by Western blotting by using antibodies for phosphorylated forms of CHK1, ATM, or γH2AX. pSer345 CHK1 was detected in cells treated with the control siRNA by 1 hour after thymidine treatment, whereas pSer1981 ATM and γH2AX were only weakly detected (Fig. 4A) as previously reported (16, 24). Despite the increased level of apoptosis in PIF1-depleted cells, phosphorylated forms of these checkpoint proteins were not detected in the absence of the replication inhibitor (Fig. 4A). After thymidine treatment, the level of pSer345 CHK1 was noticeably lower at all times in the PIF1-depleted cells relative to cells transfected with the control siRNA. Interestingly, the fraction of cells showing RPA foci was also significantly decreased in PIF1-depleted cells treated with the replication inhibitor aphidicolin (Fig. 4B) relative to cells treated with the control siRNA, suggesting that ssDNA formation may be suppressed in PIF1-depleted cells. RPA hyperphosphorylation (that occurs after some forms of DNA damage or replication stress; ref. 25) was not evident. pSer1981 ATM and γH2AX formation were not significantly increased at 24 hours in PIF1-depleted cells relative to controls.
Apoptosis in PIF1-depleted cells is caspase-3 dependent

In CHK1-depleted cells, apoptosis has been shown to proceed by either caspase-3 or caspase-2-dependent pathways following replication inhibition (15) or DNA damage (26). PIF1-depleted HCT116 cells showed detectable levels of activated caspase-3 at 24 hours in the presence or absence of thymidine (Fig. 5A). Levels of activated caspase-3 were further increased in PIF1-depleted cells after a 48-hour treatment with thymidine. Activated caspase-2 was not detected under any of these conditions (data not presented).

Given the effects of the PIF1 depletion on thymidine-treated cells in G1 or S-phase, we next determined where caspase-3 activation occurred in the cell cycle by flow cytometry. The level of PIF1-depleted cells showing caspase-3 activation was significantly elevated after a 48-hour-thymidine treatment (Fig. 5B and C). The percentage of PIF1-depleted cells showing activated caspase-3 was also increased at 48 hours posttransfection in the absence of thymidine treatment, but this did not reach significance after 72 hours ($P = 0.06$) relative to cells treated with the control siRNA. PIF1-depleted cells having increased levels of activated caspase-3 predominantly showed a G1/early S-phase DNA content. In addition, the caspase-3 inhibitor II (Z-DEVD-FMK) suppressed the accumulation of cells with a subG1 DNA content in PIF1-depleted cultures in the presence or absence of thymidine (Fig. 5D).

Codepletion of CHK1 and PIF1 does not enhance or suppress apoptosis in response to replication inhibitors

Given the importance of CHK1 in the apoptotic response to replication inhibitors (27), we next determined the effect of codepletion of these 2 proteins. HCT116 cultures, transfected with CHK1 or PIF1 siRNAs singly or in combination and then treated or not treated with thymidine for 48 hours, were analyzed for DNA content by flow cytometry. The resulting cell-cycle distributions were compared with those of cells treated with the control siRNA (Fig. 6A and B). Cultures depleted of CHK1 or PIF1 showed increased levels of cells with a subG1 DNA content and a corresponding decrease in the level of S-phase cells after thymidine treatment relative to cells treated with the control siRNA. Codepleted cultures showed a virtually identical response to those depleted of either protein alone. Unlike CHK1, PIF1-depleted cells show an increased level of subG1 cells in the absence of thymidine and this is also evident in the codepleted cultures.

Figure 5. Apoptosis in PIF1-depleted cells is caspase-3 dependent. A, Western blot analysis of cleaved caspase-3 (cleaved c3) in extracts obtained from HCT116 cells transfected with control or PIF1 siRNAs and treated or not treated with 2 mmol/L thymidine for indicated times. β-Actin levels are presented as loading controls. B, HCT116 cells transfected with control or PIF1 siRNAs were harvested and analyzed for cleaved caspase-3 or DNA content by flow cytometry. Top panels present scatter plots of cleaved caspase-3 and DNA content. Bottom panels present DNA content of all cells analyzed in the experiment. C, average percentages of cells having activated caspase-3 in indicated conditions from 3 independent experiments as presented in B. D, HCT116 cells transfected with control or PIF1 siRNAs treated or not treated with thymidine were exposed to caspase-3 inhibitor and analyzed for DNA content by flow cytometry. Average percentages of cells showing subG1 DNA contents in the presence or absence of caspase-3 inhibitor from these assays are presented. Values represent the means obtained from 3 experiments $\pm$ SDs.
Discussion

DNA helicases have been shown to play key roles in the protection of cells from DNA replication stress and DNA damage (4). Consistent with a role for PIF1 in the maintenance of replication fork integrity, we show that PIF1 depletion in human tumor cells can prevent entry into S-phase and slow S-phase progression upon release from thymidine-induced replication arrest. CHK1 activation following replication stress is suppressed in PIF1-depleted tumor cells. Cell viability is reduced and there is an increased level of apoptosis in late G1/early S-phase that is enhanced by treatment with replication inhibitors (Fig. 6C). PIF1-depleted cells arrested at G1/S have elevated levels of cyclin E as well as p21, suggesting that they are prevented from entering S-phase and, to some extent, may be protected from death. Consistent with this conclusion, p21−/− cells that lack this G1/S checkpoint show increased levels of cell death.

Yeast Pif1 helicases are involved in a wide range of DNA transactions, including the regulation of telomere length and the resolution of G-quadruplex DNA that are important for the maintenance of genomic stability. Studies of the single PIF1 helicase in mammalian cells have not produced consistent evidence for similar roles. Knockouts of the mouse gene do not lead to genetic instability and mPif1−/− mice have no obvious phenotype (13). Thus, it was proposed that PIF1 function is redundant in mammalian cells. Nevertheless, in vitro assays of PIF1 binding and unwinding on artificial substrates resembling replication forks (14) suggested that PIF1 could play a role in the generation of ssDNA that triggers activation of CHK1. Consistent with this argument, CHK1 activation, which is dependent upon ssDNA formation (23), was lower in PIF1-depleted cells exposed to thymidine. Other 5′-3′ helicases from human cells have been characterized and these may share overlapping functions with PIF1. In particular, it has recently been reported that BACH1/FANCJ is required for ATR-dependent phosphorylation events triggered by DNA replication stress (31) and shares an affinity for G-quadruplex structures (32). Thus, tumor cells may show varying dependence upon helicases determined by the level of expression or genetic status of the various helicases and the level or nature of the replication stress they encounter.

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the induction of apoptosis in CHK1 and PIF1-depleted cells. Both types of cells commit to apoptosis in late G$_1$/early S-phase by a caspase-3-dependent mechanism in response to replication inhibitors. Furthermore, codepletion of PIF1 and CHK1 show levels of apoptosis similar to those found in cells depleted of the 2 proteins alone. On the other hand, the induction of death in PIF1-depleted cells did not strictly require treatment with replication inhibitors. Further work is needed to investigate the relationship between PIF1, CHK1 activation, and the induction of cell death.

These findings also have potential therapeutic applications. Therapies that specifically induce apoptosis in tumor cells, but not nontumor cells, obviously have distinct advantages for the treatment of cancer patients. Although mouse knockouts indicate that mPIF1 is not essential for development or tumor suppression, our initial data suggest that tumor cell lines are particularly responsive to the apoptotic effects of PIF1 depletion. Recently, an inhibitor of the WRN helicase was identified and shown to have growth inhibitory and proapoptotic activities in a number of tumor cell lines (34). Our findings indicate that searches for inhibitors of other helicases with roles in replication and/or repair may be warranted.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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Suppression of Apoptosis by PIF1 Helicase in Human Tumor Cells

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