Cdc7 Inhibition Reveals a p53-Dependent Replication Checkpoint That Is Defective in Cancer Cells

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ABSTRACT

Cdc7 is an evolutionarily conserved kinase that regulates S phase by promoting replication origin activation. Down-regulation of Cdc7 by small interfering RNA in a variety of tumor cell lines causes an abortive S phase, leading to cell death by either p53-independent apoptosis or aberrant mitosis. Unlike replication fork blockade, Cdc7-depleted tumor cells do not elicit a robust checkpoint response; thus, inhibitory signals preventing additional cell cycle progression are not generated. In normal fibroblasts, however, a p53-dependent pathway actively prevents progression through a lethal S phase in the absence of sufficient Cdc7 kinase. We show that in this experimental system, p53 is required for the lasting maintenance of this checkpoint and for cell viability. With this work we reveal and begin to characterize a novel mechanism that regulates DNA synthesis in human cells, and we suggest that inhibition of Cdc7 kinase represents a promising approach for the development of a new generation of anticancer agents.

INTRODUCTION

Activation of replication origins is a three-step process. First, at the end of mitosis and in G1, a prereplicative complex is formed around origin DNA. This contains the origin recognition complex, Cdc6, Cdt1, and minichromosome maintenance proteins. Once in S phase several components of the prereplicative complex are phosphorylated by at least two kinases, a cyclin-dependent kinase and the Cdc7 kinase. This leads to the unwinding of double-stranded DNA and to the loading of DNA polymerases and accessory factors that participate in the semiconservative synthesis of new DNA strands during chain elongation (1, 2). Cdc7 was first isolated by Hartwell et al. (3, 4) and shown to be required in the cell cycle just before DNA synthesis in budding yeast. Density substitution experiments have demonstrated that Cdc7 is needed for the firing of replication origins but not for ongoing replication forks (5, 6). In mammalian cells, microinjection of neutralizing anti-Cdc7 antibodies was shown to prevent DNA synthesis (7). Because Cdc7-deficient mice are early embryonic lethal, Cdc7 function was additionally characterized in a conditional Cdc7-deficient embryonic stem cell line. In this system, Cdc7 depletion causes an S-phase arrest and p53-dependent cell death (8).

Cdc7 kinase activity is regulated during the cell cycle by binding to an activating subunit (9, 10). Two regulatory subunits for human Cdc7 kinase exist: Dbf4 and Drf1. Both mRNA and protein levels fluctuate during the cell cycle reaching a peak during S phase (7, 11, 12). To date, the reciprocal roles of Dbf4 and Drf1 are not fully understood, and it is plausible that Cdc7, in conjunction with either one of the two subunits, could also have a role in responding to genotoxic stress as suggested in other organisms (13–16).

Chemical inhibitors of DNA replication, such as hydroxyurea and 1-β-D-arabinofuranosylcytosine, that limit deoxynucleotide triphosphate supply or that are incorporated into the nascent strands thus acting as chain terminators have been extensively used in the treatment of malignant hyperproliferative diseases. Others, such as Gemcitabine, are important components of modern chemotherapy regimens (17). Understanding how these drugs affect DNA replication has revealed checkpoint mechanisms that prevent initiation from late firing origins (18, 19), stabilize replication forks (20–22), and prevent chromosome segregation with partially replicated or damaged DNA (23). Recent studies have indicated that single-stranded DNA (ssDNA) is the signaling molecule that triggers a checkpoint response (24). ssDNA is an intermediate in most DNA repair processes (25), and it accumulates when replication forks stall (15, 26). The signal is then amplified through a kinase cascade involving the ATM/ATR and Chk1/2 kinases, thus leading to a coordinated cellular response involving the activation of several proteins involved in DNA repair, cell cycle control, and survival (23). The tumor suppressor p53 is a central player in coordinating cellular responses to different types of stress, but whether the p53 protein and p53-dependent transcription have an important role in initiating and/or maintaining a checkpoint response upon DNA replication blockade is still controversial (27–29).

The need for anticancer drugs acting by novel mechanisms makes it important to characterize the cellular responses of human cells to inhibition of DNA replication origin firing that, in principle, should not generate ssDNA. Among the potential factors involved in the initiation reaction, the Cdc7 kinase is a suitable target for a drug discovery effort, because an enzymatic assay for the screening of inhibitors can be developed.

In this work we show that depletion of Cdc7 by small interfering RNA causes a variety of tumor cell lines to progress through a defective S phase leading to p53-independent apoptotic cell death without eliciting a significant checkpoint response, whereas a primary cell line avoids this lethal event by activating a p53-dependent cell cycle checkpoint.

MATERIALS AND METHODS

Cell Lines. HeLa cells (American Type Culture Collection, Manassas, VA) were cultured in modified Eagle’s medium (MEM) supplemented with heat inactivated 10% fetal calf serum (FCS). HCT-116 cells (American Type Culture Collection) were maintained in McCoy’s medium with 10% FCS. H2O2 cells (American Type Culture Collection) were grown in DMEM 10% FCS. Normal human dermal fibroblasts (Promocell, Heidelberg, Germany) were maintained in fibroblast basal medium supplemented with growth factors and with 10% FCS.

HeLa HH2B-green fluorescent protein stable cell line was generated by transfecting HeLa cells with the histone H2B-GFP fusion-expressing plasmid (30).

Immunoblotting. For Western blot analysis, cell extracts were prepared in a buffer containing 50 mmol/L Tris-HCl (pH 6.8) , 1% SDS, and 1 mmol/L DTT and were sonicated for 10 seconds. Protein extract, 15 μg, determined by Bradford assay was loaded in each lane. Antibodies antihistone H3 was from Upstate Biotechnology (Lake Placid, NY); anti-active caspase 3, -Ser 807/811 retinoblastoma protein (Rb), -Ser345 Chk1, -Thr68 Chk2, and -Tyr15 Cdk1 were purchased from Cell Signaling (Beverly, MA); anti-Cyclin E, -Cyclin A,
Differential Cellular Responses to Cdc7 Depletion

Cdc7 depletion causes an S-phase delay and cell death. We observed that three of four oligos, Cdc7-A, Cdc7-B, and Cdc7-C, efficiently reduced Cdc7 mRNA levels (data not shown). Correspondingly, Cdc7 protein levels begin to drop ~24 hours after transfection to become almost undetectable by 48 and 72 hours (Fig. 1A). Fluorescence-activated cell sorter (FACS) analysis of propidium iodide-stained cells indicates that cells accumulate with an S-phase DNA content suggesting a block or slow delay in S-phase progression. Also, a consistent fraction of cells appears to have less than a 2C DNA content (Fig. 1B) suggesting cell death. To confirm a defect in DNA synthesis we repeated the transfection with the Cdc7 and control siRNAs, and cells were labeled with BrdUrd for 1 hour before collection. Cells were then fixed and stained with anti-BrdUrd antibody and propidium iodide. Biparametric FACS analysis, shown in Fig. 1C, indicates that DNA replication is apparently normal in both Cdc7 and control siRNA-treated cells at 24 hours; however, the number of cells incorporating BrdUrd (included in the gated area) drops from 38% to 11% by 72 hours in the Cdc7-depleted population. The intensity of the fluorescence, which directly correlates with the amount of BrdUrd incorporated in the remaining positive cells, is also strongly reduced. Because all of the three active Cdc7 siRNAs caused a decrease in DNA synthesis and accumulation of S-phase and sub-G1 cells, we reasoned that these oligos are equivalent and that these phenotypes directly correlate with Cdc7 depletion and are unlikely to be due to concomitant nonspecific down-regulation of an unknown gene. For all of the other experiments shown, oligo Cdc7-D was used.

To additionally corroborate the notion of a delay in S-phase progression in cells in which Cdc7 levels are decreased, we performed a Nocodazole trapping experiment. Sixty hours after siRNA transfection, Nocodazole, a microtubule polymerization inhibitor that prevents cells from executing mitosis, was added to the medium. DNA content of the cells was measured before and 14 hours after drug treatment. Whereas in control siRNA-treated cells the fraction of cells showing G2 DNA content increases from 9% to 54% in the Cdc7-depleted population, this fraction modestly increases from 14% to 35% indicating that most cells are unable to complete DNA replication (Fig. 1D).

Time lapse microscopy of cells transfected with either control or Cdc7 oligos shows that cell number increases slightly at early time points suggesting that some cells manage to complete one division while Cdc7 protein levels gradually decrease, then they arrest before dying so that by 96 hours there were no remaining live cells (Fig. 2A). These data together with detection of a sub-G1 population by FACS suggested that apoptosis was activated in Cdc7-depleted cells. Indeed, in cell extracts taken at 72 hours after transfection, active caspases 8, 9, and 3 could easily be detected with specific antibodies (data not shown). Furthermore, addition of the pan-caspase inhibitor Z-VAD to the medium rescued cell death (Fig. 2B), cleavage, and activation of caspase 3 (Fig. 2C) and prevented accumulation of sub-G1 cells (data not shown).

Therefore, we conclude that in HeLa cells, inhibition of Cdc7 kinase causes an impaired S-phase progression leading to apoptotic cell death.

Cdc7 Inhibition Does Not Elicit a Robust Checkpoint Response in Tumor Cell Lines. In striking contrast to an S-phase arrest obtained with Cdc7 depletion, inhibition of DNA polymerases and deoxynucleotide triphosphates depletion causes HeLa and other cell lines to reversibly arrest the cell cycle. This arrest can last for several days without obvious signs of cell death (31). To investigate these differences we studied several cell cycle parameters and markers of checkpoint response in HeLa cells depleted of Cdc7 or treated with hydroxyurea. We used hydroxyurea as an example of a DNA elong-

RESULTS

Cdc7 Depletion Causes an Abortive S-Phase Progression and Cell Death in HeLa Cells. To down-regulate Cdc7 expression we designed four different siRNAs with sequence corresponding to the Cdc7 cDNA. When these were used to transfect p53-deficient HeLa cells, we observed that three of four oligos, Cdc7-A, Cdc7-B, and Cdc7-C, efficiently reduced Cdc7 mRNA levels (data not shown). Correspondingly, Cdc7 protein levels begin to drop ~24 hours after transfection to become almost undetectable by 48 and 72 hours (Fig. 1A). Fluorescence-activated cell sorter (FACS) analysis of propidium iodide-stained cells indicates that cells accumulate with an S-phase DNA content suggesting a block or slow delay in S-phase progression. Also, a consistent fraction of cells appears to have less than a 2C DNA content (Fig. 1B) suggesting cell death. To confirm a defect in DNA synthesis we repeated the transfection with the Cdc7 and control siRNAs, and cells were labeled with BrdUrd for 1 hour before collection. Cells were then fixed and stained with anti-BrdUrd antibody and propidium iodide. Biparametric FACS analysis, shown in Fig. 1C, indicates that DNA replication is apparently normal in both Cdc7 and control siRNA-treated cells at 24 hours; however, the number of cells incorporating BrdUrd (included in the gated area) drops from 38% to 11% by 72 hours in the Cdc7-depleted population. The intensity of the fluorescence, which directly correlates with the amount of BrdUrd incorporated in the remaining positive cells, is also strongly reduced. Because all of the three active Cdc7 siRNAs caused a decrease in DNA synthesis and accumulation of S-phase and sub-G1 cells, we reasoned that these oligos are equivalent and that these phenotypes directly correlate with Cdc7 depletion and are unlikely to be due to concomitant nonspecific down-regulation of an unknown gene. For all of the other experiments shown, oligo Cdc7-D was used.

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First we observed that Mcm2 mobility in polyacrylamide gels was altered in extracts prepared from Cdc7-depleted cells (Fig. 3A). Mcm2 normally runs as a smear of bands corresponding to differentially phosphorylated forms. Alkaline phosphatase treatment demonstrates that faster migrating forms are due to phosphorylation (Supplementary Data). This result is consistent with the predicted role of Cdc7 in regulating minichromosome maintenance activity. Mcm2 mobility was not affected by hydroxyurea. We then observed that p27 levels decreased in hydroxyurea as described previously (31), but did not change in Cdc7-depleted cells and that levels of Cyclin E protein and mRNA (data not shown) decreased upon Cdc7 depletion (Fig. 3A). These observations support the notion of the different nature of an S-phase arrest caused by Cdc7 depletion and by hydroxyurea at the molecular level.

Activation of the DNA damage/replication checkpoint correlates with ATM/ATR-dependent phosphorylation of Chk1 at Ser-345 and Chk2 at Thr-68; these phosphorylation events can be detected with specific anti-phospho antibodies (32, 33). In contrast to hydroxyurea treatment, we observed that upon Cdc7 depletion, Chk1 Ser-345 and Chk2 Thr-68 phosphorylation were below detection limits. Importantly, we observed that the Cdc25A phosphatase, which is targeted for degradation by Chk1 and Chk2 upon S-phase arrest with hydroxyurea (34–36), was present at levels comparable with untreated and control siRNA-treated cells. Finally, we noticed that levels of the inhibitory phosphorylation in the Tyr-15 of Cdk1 mitotic kinase were increased upon hydroxyurea treatment but did not significantly change in the Cdc7-depleted samples (Fig. 3A). These observations suggest that, upon Cdc7 depletion, a DNA replication checkpoint is either not efficiently activated or maintained and that inhibitory signals preventing cells from progressing in the cell cycle might not be generated. In fact, when we combined siRNA experiments with time lapse microscopy, we observed that whereas most of the cells suddenly died by apoptosis, ~3% to 5% of the cells appeared to round-up and be trapped in a mitotic-like state for several hours. We next used HeLa cells expressing a green fluorescent protein-histone H2B fusion protein to follow chromatin dynamics under fluorescence time-lapse microscopy (37). Images were taken every 30 minutes for a 48-hour period of time starting 24 hours after siRNA transfection. Whereas in control cells the processes of chromosome condensation, separation, and decondensation occur within 60 to 90 minutes, we found that in Cdc7 siRNA-transfected samples, cells condensed chromosomes forming an apparently normal metaphase plate but then failed to divide. We also observed that masses of condensed chromatin were temporarily separating from the main plate suggesting that cells were attempting to pull apart tangled chromosomes (Fig. 3B). From this analysis we conclude that HeLa cells upon Cdc7 depletion either die by apoptosis or enter a mitotic catastrophe. Because HeLa cells lack a functional p53 we extended our studies to colon carcinoma HCT-116 cells in which the p53 response is proficient. In these cells, as in HeLa, Cdc7 depletion leads to an accumulation of S-phase cells, to a
decreased uptake of BrdUrd, to caspase activation, and to accumulation of dephosphorylated Mcm2 protein (Fig. 4). Importantly, levels of the p53 and p21 proteins were not increased by Cdc7 depletion, whereas, as reported previously (28), p53 but not p21 induction is seen in HCT-116 treated with hydroxyurea (Fig. 4B).

**Cdc7 Depletion Leads to a p53-Dependent Cell Cycle Arrest in Primary Cells.** To additionally explore potential cell type differences in the response to Cdc7 depletion we performed siRNA experiments in nontransformed, early passage primary normal human dermal fibroblasts. Seventy-two hours after Cdc7 and control siRNA transfection, normal human dermal fibroblasts were harvested and processed for FACS and protein analysis. FACS analysis showed that BrdUrd incorporation was strongly inhibited in Cdc7 siRNA-treated normal human dermal fibroblasts. In contrast to HeLa cells, however, Cdc7-depleted normal human dermal fibroblasts did not show a broad distribution of cells having an S-phase DNA content, but they mostly accumulated within a very sharp peak consistent with either a G1 or early S-phase arrest (Fig. 5A). Cdc7 protein levels were efficiently reduced to undetectable levels correlating with lack of Mcm2 phosphorylation also in this primary cell line. Unlike HCT-116 cells, p53 and p21 protein levels were significantly induced, and phosphorylation of Rb at Ser-807/811, thought to be either Cdk4 or Cdk2 phosphorylation sites, were strongly reduced (Fig. 5, B and C). We reasoned that a checkpoint response causing cell cycle arrest was taking place in Cdc7-depleted fibroblasts, even if p53 Ser-15, Chk1 Ser-345, and Chk2 Thr-68 phosphorylation levels, as well as Chk2 total levels were below detection limits in this experiment (Fig. 5B; data not shown). To test this hypothesis we simultaneously transfected Cdc7 and p53 siRNAs with the rationale that if p53 protein had a role in this checkpoint the effects of Cdc7 depletion would be very different in cells also depleted of p53. Indeed, ablation of both Cdc7 and p53 expression fully prevented p21 accumulation leading to increased levels of Ser-807/811 Rb phosphorylation and lack of accumulation of cells at the G1-S border (Fig. 5C; Table 1). Under these experimental conditions Mcm2 mobility shift was still prevented, consistent with inhibition of Cdc7 kinase also in the absence of p53 (Fig. 5C). These results indicate that p53 mediates cellular responses to Cdc7 depletion in this normal cell line.

**p53 Is Required for the Maintenance of Cell Cycle Arrest and for Cell Viability upon Cdc7 Depletion.** In the previous experiment we could not determine the relative rate of Cdc7 and p53 inhibition and, therefore, we could not discriminate between the possibility that p53 is necessary for the initiation and/or maintenance of the G1-S cell cycle arrest. Thus, we elaborated the experimental design to uncouple the two events. First, normal human dermal fibroblasts were trans-
fected with Cdc7 siRNA oligos to fully deplete Cdc7 kinase and establish the checkpoint response. After 72 hours cells were collected, split into two samples, replated, transfected again with either a mixture of Cdc7 and control siRNAs or with Cdc7 together with p53 siRNAs, and incubated for an additional 72 hours before harvesting. Fig. 6A shows that Cdc7 protein down-regulation can be maintained throughout the duration of the experiment (Fig. 6A, lanes 2 to 4). The cell cycle arrest is also maintained in the sample that was retransfected with Cdc7 and control siRNAs as levels of p53 and p21 remained elevated, Rb remains mostly unphosphorylated and Cyclin A levels were reduced. On the contrary, in the sample that was retransfected with Cdc7 and p53 siRNAs, we observed highly phosphorylated pRb and high levels of Cyclin A. BrdUrd labeling in the last 3 hours of the experiment indicated that ongoing DNA synthesis was detected in ~30% of the cells transfected with the combination of Cdc7/p53 oligos, whereas none of the cells transfected with Cdc7/control oligos incorporated the nucleotide analogue (Fig. 6B). DNA synthesis observed in fibroblasts upon depletion of Cdc7 and p53 is most likely abnormal and did not lead to cell division (data not shown). In fact, consistent with replication stress or with early signs of apoptotic induction (38, 39), we observed that levels of γ-H2AX were elevated in extract prepared from doubly depleted Cdc7/p53 fibroblasts and that up to 65% of the cells were strongly stained with anti-γ-H2AX.

Table 1 DNA content of NHDFs 72 hours after transfection with the indicated siRNAs

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<th>2C</th>
<th>2C &lt; C &lt; 4</th>
<th>4C</th>
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<tr>
<td>Control siRNA</td>
<td>67%</td>
<td>17%</td>
<td>16%</td>
</tr>
<tr>
<td>Cdc7 siRNA</td>
<td>81%</td>
<td>3%</td>
<td>16%</td>
</tr>
<tr>
<td>p53 siRNA</td>
<td>65%</td>
<td>20%</td>
<td>15%</td>
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<tr>
<td>Cdc7+p53 siRNAs</td>
<td>51%</td>
<td>29%</td>
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NOTE. DNA was stained with propidium iodide and measured by FACS. Cell samples correspond to those analyzed in Fig 5C.

Abbreviations: NHDF, normal human dermal fibroblast.

Fig. 4. Cdc7 inhibition does not correlate with p53 induction in HCT-116 cells. A. HCT-116 cells were transfected with either control or Cdc7 siRNAs. After 72 hours DNA content and BrdUrd incorporation were analyzed by FACS. B. Western blot analysis of extracts prepared from HCT-116 colon carcinoma cells 72 hours after transfection with Cdc7 (lane 1) or control (lane 2) siRNAs, untreated (lane 3) or treated for 24 hours with 5 mmol/L hydroxyurea (HU; lane 4). Cdk2 was used as loading control.

Fig. 5. p53-dependent cell cycle arrest in Cdc7-depleted primary fibroblasts. A. Normal human dermal fibroblasts (NHDFs) were transfected with either control or Cdc7 siRNAs. After 72 hours DNA content and BrdUrd incorporation were analyzed by FACS. B. Western blot analysis of extracts prepared from NHDFs after transfection with control (lane 1) or Cdc7-D (lane 2) siRNAs, untreated (lane 3) or treated for 24 hours with 5 mmol/L HU (lane 4). Cdk2 was used as loading control. C. NHDFs were transfected with control, Cdc7, p53, or Cdc7+p53 siRNAs as indicated. After 72 hours cells were collected, and protein extracts prepared and analyzed by Western blot. Actin was used as loading control.
antibodies compared with a weaker staining observed in 32% of cells retransfected with Cdc7/control siRNAs (Fig. 6C). Finally, active caspase 3 and apoptotic cells could be detected in the Cdc7/p53-depleted population (Fig. 6, A and C). Identical results were obtained using a different siRNA that abrogates p53 expression (data not shown).

Therefore, p53 is required to maintain a checkpoint signaling that prevents progression through a lethal S phase under limiting amounts of Cdc7 kinase in normal fibroblasts.

DISCUSSION

In this study we have investigated the effects of Cdc7 kinase depletion in several human cell lines. First, in HeLa and other tumor cell lines, Cdc7 depletion causes an obvious S-phase defect. This phenotype, together with the observation that the Mcm2 protein becomes underphosphorylated, reinforces the idea that in human cells Cdc7 kinase regulates origin firing and S-phase progression by phosphorylating minichromosome maintenance proteins as thoroughly demonstrated in other organisms (2, 40). Upon Cdc7 depletion, these tumor cells do not stop at the G1-S transition, but they cease DNA polymerization while they are scattered throughout S phase. The most plausible explanation is that only a limited amount of origins of replication fire, thus only a limited number of replication forks are established in these cells. Because forks can pause and eventually collapse (21), DNA synthesis arrest occurs stochastically throughout S phase. During this abnormal S-phase progression with low or no Cdc7 activity, cells enter p53-independent apoptosis. The few cells that temporarily escape the apoptotic program attempt to execute mitotic division; however, they fail to separate their chromosomes that appear to be strictly connected to each other. This could be either because they are only partially replicated or because Cdc7 kinase might influence either directly or indirectly some other aspects of chromosome dynamics such as DNA topology or cohesion as demonstrated recently in Schizosaccharomyces pombe (41).

The second finding is that unlike the stress caused by most inhibitors of DNA elongation and DNA damaging agents, the stress caused by Cdc7 depletion does not generate a sustained checkpoint response in tumor cells. To date we cannot exclude that checkpoint kinases play a role in the response to Cdc7 inhibition, although we can speculate that in Cdc7-depleted cells arresting in S phase, ssDNA is not accumulated in sufficient amounts to trigger their activation. Another possibility is that human Cdc7 kinase might also be required for the establishment or the maintenance of a replication checkpoint. Nevertheless, the most relevant finding is that in primary human somatic cells a cell cycle checkpoint mechanism that responds to Cdc7 depletion exists. Cdc7 depletion causes a cell cycle arrest with mostly unreplicated DNA, elevated p53, and cyclin-dependent kinase inhibitor p21 induction and hypo-phosphorylated Rb. The phenotypes observed upon Cdc7 inhibition in human cells are different from the one described in murine embryonic stem cells (8) where Cdc7 depletion causes a p53-dependent apoptosis but are strongly reminiscent of the ones caused by inhibition of the licensing reaction (42, 43). Shreeeram et al. (42) suggested that primary fibroblasts might have a G1 checkpoint sensing the lack of licensed origins. It is possible that to a second round of transfection with either a combination of Cdc7 and control siRNAs (lane 3) or a combination of Cdc7 and p53 siRNAs (lane 4). Cells were collected after an additional 72 hours of incubation. B. An aliquot of the same cells analyzed in A, lanes 1 and 2, respectively. In lanes 3 and 4, NHDFs that were first treated with Cdc7 siRNAs for 72 hours were replated and the following day subjected to a second round of transfection with either a combination of Cdc7 and control siRNAs (lane 3) or a combination of Cdc7 and p53 siRNAs (lane 4). Cells were collected after an additional 72 hours of incubation. B. An aliquot of the same cells analyzed in A, lanes 1 and 2, respectively. In lanes 3 and 4, NHDFs that were first treated with Cdc7 siRNAs for 72 hours were replated and the following day subjected
the licensing and the Cdc7 depletion-induced checkpoints share the
same components: it can be speculated that depletion of Cdc7 itself,
that in human cells binds to chromatin before initiation of DNA
synthesis (44), might impair the stability of prereplicative complexes.
Alternatively, the checkpoint may respond to a signal produced from
a few replication forks established from those origins that have
escaped the block and that, therefore, should be considered as intra-S
checkpoint.

Finally, we demonstrate that in normal fibroblasts the Cdc7 deple-
tion-induced checkpoint is an active mechanism. By down-regulating
p53 in cells arrested previously by Cdc7 depletion, the cell cycle
blockade can be completely bypassed. Most notably DNA synthesis
resumes in the absence of detectable Cdc7 kinase, and cells progress
through an aberrant S phase leading to apoptosis. Because the main
role of Cdc7 is to activate replication origins, we can speculate that
DNA synthesis most likely resumes from either previously established
replication forks or from those origins in which the Cdc7 kinase had
already executed its function before the enforcement of the check-
point.

Cdc7-induced checkpoint appears to be altered in cancer cells at the
level of p53 but also upstream of p53, because this is not induced in
some p53-proficient tumor cell lines. Finally, the observation that
upon Cdc7 inhibition, the tumor cells analyzed enter a p53-independent
apoptosis whereas in normal fibroblasts, p53 and Rb proteins are
directly involved in a mechanism that protects from Cdc7 inhibition,
lays the groundwork for testing Cdc7 as a potential therapeutic target.

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