

Proteomic Identification of a Direct Role for Cyclin D1 in DNA Damage Repair

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Abstract

The human *CCND1* gene, which encodes the cell-cycle protein cyclin D1, is one of the most frequently amplified genes in human cancers. Cyclin D1 activates the cyclin-dependent kinases CDK4 and CDK6 and drives cell proliferation. Beyond the cell-cycle role, the full repertoire of cyclin D1 functions in cancer cells is still unclear. Emerging evidence indicates that cyclin D1 may play a role in DNA damage response. In this review, we discuss observations linking cyclin D1 to DNA damage repair and summarize our recent findings, which show a cyclin D1 function in homologous recombination-mediated DNA repair. *Cancer Res*; 72(17); 4289–93. ©2012 AACR.

Introduction

D-type cyclins are components of the core cell-cycle machinery. The D-cyclin family is composed of 3 proteins (cyclins D1, D2, and D3) that are expressed in proliferating cells (1). The gene encoding cyclin D1 represents the second most frequently amplified locus in the human cancer genome (2). The protein product of this locus, cyclin D1, binds and activates the cyclin-dependent kinases CDK4 and CDK6 (1). During cell-cycle progression, cyclin D1-CDK4 and D1-CDK6 complexes phosphorylate the retinoblastoma protein, pRB, pRB-related p107 and p130 proteins, and Smad3 and FOXM1 transcription factors (1, 3, 4). By far the best-documented function of cyclin D1 is its ability to drive cell-cycle progression through phosphorylation of pRB, p107, and p130. In their hypophosphorylated forms, pRB, p107, and p130 inhibit the transcriptional activity of E2F transcription factors. Phosphorylation of these 3 proteins by cyclin D1-CDK4/6 kinase releases and derepresses E2Fs, thereby allowing G₁→S phase progression (Fig. 1A; ref. 1). In addition to this kinase-dependent function, cyclin D1-CDK4/6 complexes sequester the cell-cycle inhibitors p27^{Kip1} and p21^{Cip1} away from cyclin E-CDK2, thereby contributing to activation of cyclin E-CDK2 kinase (1). Lastly, there is growing evidence that cyclin D1 plays cell-cycle-independent roles that are also independent of CDK4 and CDK6 (5).

Degradation of Cyclin D1 upon DNA Damage

Proliferating cells usually respond to DNA damage by arresting their cell-cycle progression. Several independent reports pointed to downregulation of cyclin D1 as one of the mechanisms that underlie this cell-cycle arrest (6–8). DNA damage was shown to activate GSK3 β , which phosphorylates cyclin D1 on threonine-286. Phosphorylated cyclin D1 is then exported from the nucleus, polyubiquitinated by the SCF^{Fbx4- α Bcrystallin} E3 ubiquitin ligase, and degraded by the proteasome (7, 9). Strikingly, a related cyclin, cyclin D2, does not undergo phosphorylation on the corresponding residue following DNA damage, suggesting that cyclin D1 may play a nonredundant role in transmitting postradiation growth-arresting signals to the core cell-cycle machinery (7, 9). The activity of ATM was shown to be required for cyclin D1 phosphorylation and degradation triggered by double-stranded DNA breaks, whereas the ATR kinase mediates the effect on cyclin D1 following UV irradiation (7, 10, 11). In contrast to these findings implicating F-box protein Fbx4 and cofactor α B crystallin in degradation of cyclin D1, another group postulated that threonine-286-phosphorylated cyclin D1 interacts with and is targeted for degradation by an F-box protein, FBXO31 (8). Moreover, DNA damage was shown to cause proteolysis of cyclin D1 by anaphase-promoting complex/cyclosome (APC/C). This effect is mediated by the destruction box in cyclin D1, and it was shown to be independent of cyclin D1 phosphorylation on threonine-286 (6). It is possible that these different scenarios reflect distinct modes of cyclin D1 degradation in particular cell types. Overall, these reports point to cyclin D1 degradation as an important molecular mechanism that arrests cell proliferation following DNA damage. Persistent high expression of cyclin D1 in cells that have accumulated dsDNA breaks leads to radioresistant DNA synthesis (7). Moreover, it was shown that downregulation of cyclin D1 following UV damage was required for efficient DNA repair, and that forced overexpression of cyclin D1 prevented DNA repair (12).

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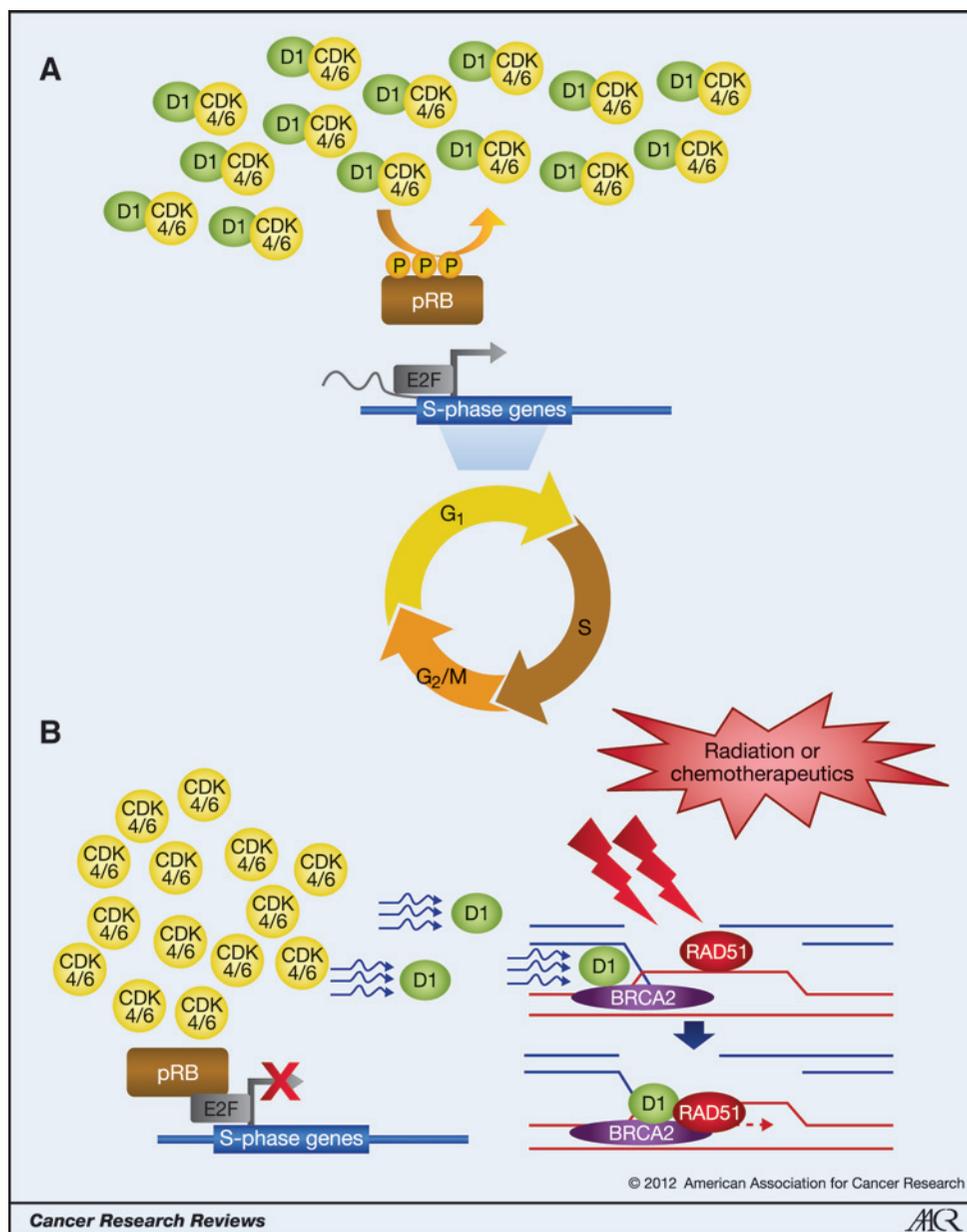


Figure 1. Model of cyclin D1's function in cell proliferation and DNA repair. A, during normal cell-cycle progression, cyclin D1 (D1) forms complexes with CDK4 or CDK6 to regulate G₁-phase progression by phosphorylating pRB, p107, and p130 proteins. pRB phosphorylation releases E2F transcription factors and allows E2F-dependent transcription of S-phase genes. B, after DNA is damaged by radiation or chemotherapeutic agents, cyclin D1 protein levels are significantly reduced and CDK4/6 kinase activity is diminished. Hypophosphorylated pRB inhibits E2F's ability to transactivate genes, thereby contributing to cell-cycle arrest. Part of the remaining pool of cyclin D1 protein is recruited to DNA damage sites, in a BRCA2-dependent manner, to facilitate RAD51 localization or to stabilize RAD51 at the DNA damage sites, thereby assisting the homologous recombination-mediated DNA repair. Note that it is unclear whether CDK4 or CDK6 is recruited to DNA damage sites along with cyclin D1.

To complicate this picture, some reports showed an increase of cyclin D1 levels following DNA damage (13–16). These findings hint that cyclin D1 may play an active role in DNA damage repair.

Interaction of Cyclin D1 with DNA Damage Proteins

The first indications that cyclin D1 might play a direct role in DNA damage repair came from observations that functionally

linked cyclin D1 with proteins involved in DNA repair. Li and colleagues (17) showed that cyclin D1 tethered to chromatin can recruit RAD51, a protein that plays an essential role in the homologous recombination process. Intriguingly, recruitment of RAD51 by cyclin D1 took place after DNA damage, but not in naïve cells, suggesting a functional relevance of this interaction for DNA repair (17). The same group showed a link between cyclin D1 and another DNA repair protein, BRCA1, by

demonstrating that cyclin D1 antagonizes BRCA1-mediated repression of estrogen receptor α transcriptional activity (18). The effect was mediated through the ability of cyclin D1 to compete with BRCA1 for estrogen receptor α binding (18). A direct link between cyclin D1 and BRCA1 was provided by the observation that cyclin D1-CDK4 kinase phosphorylates BRCA1 on serine-632. This event inhibited recruitment of BRCA1 to target promoters (19). BRCA1 and the BRCA1 splice variant BRCA1-IRIS were found to transcriptionally upregulate cyclin D1 (20, 21), whereas cyclin D1 was shown to induce BRCA1 expression through the pRB→E2F pathway (22).

A possible functional link between cyclin D1 and another DNA repair protein, BRCA2, was suggested by the observation that cyclin D1, BRCA2, and RAD51 physically interact with Sp1 transcription factor (23, 24).

Cyclin D1 was also demonstrated to physically interact with 2 other proteins involved in DNA repair, PCNA (25, 26) and replication factor C [RFC (27)]. Both PCNA and RFC functionally interact with BRCA1 during repair of UV-induced DNA damage (28). Collectively, all of these observations pointed to multiple functional links between cyclin D1 and DNA repair proteins, and they suggested that cyclin D1 may play a role in DNA damage repair.

Consistent with this view, several studies indicated that elevated levels of cyclin D1 confer relative radiation resistance to cancer cells (29–33). A correlation was noted between high levels of cyclin D1 and unfavorable response to radiotherapy (34, 35). Conversely, knockdown of cyclin D1 was shown to sensitize cancer cells to radiation or the DNA-damaging agent cisplatin (33, 36). Moreover, antisense-mediated downregulation of cyclin D1 expression increased the sensitivity of zebrafish embryos to radiation (37).

It should be noted, however, that many authors reached the opposite conclusion. For example, Coco Martin and colleagues (38) showed that ectopic overexpression of cyclin D1 sensitized cancer cells to irradiation by rendering the cells more susceptible to radiation-induced apoptosis. A similar conclusion was reached by Trent and colleagues (39), who proposed that the cyclin D1-driven enhancement of radiation sensitivity is CDK independent and is mediated, at least in part, through the ability of cyclin D1 to transcriptionally induce expression of a heat-shock protein, HSPB8. In some studies, cyclin D1 overexpression was shown to correlate with increased radiosensitivity and a favorable response to radio- or chemotherapy in squamous cell carcinoma of the head and neck (40), oral squamous cell carcinoma (41), and early-stage laryngeal cancer (42). It is possible that these conflicting observations reflect the different types of tumors studied. Alternatively, these findings might point to a dual role for cyclin D1 in DNA damage response: Following acute DNA damage, a decrease in cyclin D1 levels would trigger cell-cycle arrest, while the remaining pool of cyclin D1 would play an important, positive function in promoting DNA damage repair. Hence, strong overexpression of cyclin D1 to levels that cannot be efficiently reduced following DNA damage would compromise cell-cycle checkpoints and impair cell survival, whereas moderate levels of cyclin D1 after DNA damage-induced cell-cycle arrest could augment DNA repair. Following this logic, a complete knock-

down that cyclin D1 might compromise DNA repair by depleting a protein that is needed for DNA repair.

Direct Role of Cyclin D1 in Homologous Recombination-Based DNA Repair

In a recent study (43) we used immunoaffinity purification of cyclin D1-containing complexes followed by high-throughput shotgun mass spectrometry to identify cyclin D1-interacting proteins (the cyclin D1 interactome) in several human cancer cell lines. Among the cyclin D1 interactors, we observed a strong enrichment for proteins belonging to the DNA damage repair category.

One of these proteins was RAD51, an essential DNA recombinase that mediates homologous recombination-based DNA repair (44). An interaction between endogenous RAD51 and cyclin D1 was verified by immunoprecipitation–Western blotting in a wide panel of human cancer cell lines, indicating that the interaction is not cell-type specific. Of importance, the interaction is strongly enhanced following irradiation of cells, suggesting that DNA damage may induce posttranslational modification of cyclin D1 and/or RAD51, which then stabilizes cyclin D1–RAD51 binding (43).

Surprisingly, we detected cyclin D1 at the sites of dsDNA breaks where it colocalized with RAD51, suggesting that cyclin D1 may directly participate in the repair of damaged DNA. We also observed that RAD51 recruitment to DNA damage sites was significantly reduced in cells depleted of cyclin D1. Moreover, knockdown of cyclin D1 reduced the rate of homologous recombination-mediated DNA repair (43). These findings are consistent with and fully support the observations of Li and colleagues (17), who showed that cyclin D1, when targeted to chromatin, can recruit RAD51 following DNA damage. Collectively, these findings suggest a model in which cyclin D1 localizes to dsDNA breaks following DNA damage, and helps to recruit RAD51 (Fig. 1B).

How then is cyclin D1 recruited to broken DNA? Among the cyclin D1-interacting proteins detected in our screen, we observed BRCA2. Like RAD51, BRCA2 is recruited to DNA damage sites. BRCA2 localization at the DNA damage foci represents a crucial step for homologous recombination, and it occurs prior to the recruitment of RAD51 (45). BRCA2 displaces the single-stranded DNA binding protein RPA from single-stranded regions generated by end resection, and it facilitates loading of RAD51 onto single-stranded DNA (45). We observed that depletion of cyclin D1 had no effect on BRCA2 recruitment to DNA damage foci. However, knockdown of BRCA2 decreased loading of cyclin D1, suggesting that BRCA2 is responsible for recruiting cyclin D1 to DNA damage sites. Consistent with the observed association between BRCA2 and cyclin D1 in cancer cells, we found in an *in vitro* binding assay that purified recombinant cyclin D1 binds to purified BRCA2 fragments.

We propose that cyclin D1 is recruited to dsDNA breaks via BRCA2. Cyclin D1 then helps to either recruit RAD51 or stabilize RAD51 on the repair foci, thereby contributing to the homologous recombination process (Fig. 1B).

Of note, we showed that the function of cyclin D1 in DNA repair is independent of its role in cell-cycle progression. All of

our DNA repair analyses were performed in pRB-negative cancer cells, which do not require cyclin D1 for proliferation (46, 47). Moreover, the DNA repair role of cyclin D1 is independent of cyclin D1's ability to activate CDK4 and CDK6.

Possible Implications for Cancer Treatment

Inhibition of cyclin D-associated kinase activity is currently being considered as an attractive strategy for treatment of several cancer types (48), and inhibitors of cyclin D-CDK4 and D-CDK6 kinases are currently in clinical trials. The demonstration that cyclin D1 plays a CDK-independent function in DNA damage repair suggests that cyclin D1 protein (rather than cyclin D1-associated kinase) may represent a more effective anticancer target, because inhibition of cyclin D1 is expected to impair both cell proliferation and DNA repair.

Our study also suggests a function of cyclin D1 in pRB-negative cancer cells. It is very well documented that cancer cells that have lost pRB no longer require D-cyclins for proliferation (46, 47). Consequently, depletion of cyclin D1 or inhibition of CDK4/6 kinase activity has no impact on the proliferation of pRB-negative cancer cells. However, our study raises the possibility that targeting cyclin D1 might also have a therapeutic value in pRB-negative tumors, where it is expected to decrease the efficiency of DNA damage repair.

Unresolved Questions

A direct role for cyclin D1 in DNA damage repair was detected in a screen that used human cancer cells. It remains to be seen whether cyclin D1 plays a role in DNA repair in normal cells as well. Consistent with this possibility, we observed that immortalized mouse embryonic fibroblasts lacking all 3 D-cyclins are more susceptible to radiation-induced DNA damage compared with their wild-type counterparts.

Another unresolved issue is whether other D-type cyclins (D2 and D3) play a similar role in DNA repair. It will be interesting to determine whether these proteins also interact with RAD51 and BRCA2 and are recruited to DNA damage sites.

A more fundamental question is whether D-cyclins or other components of mammalian core cell-cycle machinery affect the choice of DNA repair pathway. Mammalian cells can repair dsDNA breaks through error-prone nonhomologous end joining or through relatively faithful homologous recombination.

The identity of molecules/signaling pathways that determine the choice of DNA repair pathway is not fully understood. Several reports showed that *bona fide* cell-cycle CDKs can phosphorylate DNA repair proteins and may determine the choice of DNA repair pathway. In budding yeast, it was shown that homologous recombination is controlled at an early step called DNA-end resection by an activity of Cdc28 [a yeast homolog of mammalian CDKs (49)]. Cdc28 phosphorylates Sae2 and the nuclease Dna2 (both of which are key proteins in DNA resection), thereby allowing progression of the DNA end-resection process, which is a prerequisite for homologous recombination (50, 51). Conversely, inhibition of yeast CDK activity during DNA damage causes yeast to employ nonhomologous end joining to repair DNA (51). A similar regulation was observed in human cells (52). However, the processes that govern DNA repair pathways in mammalian cells seem to be more complex. For instance, cyclin A-CDK2 (and possibly cyclin B-CDK1) kinase was reported to block interaction between the C-terminus of BRCA2 and RAD51, and to likely inhibit homologous recombination by phosphorylating serine-3291 on BRCA2 (53). It is clear that the cell-cycle and DNA-repair machineries intersect at several points, and more work is needed to fully understand the functional interplay between these pathways.

Disclosure of Potential Conflicts of Interest

D.M. Livingston and P. Sicinski received a commercial research grant from Novartis and are members of the consultant/advisory board of Novartis. No other potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Jirawatnotai, P. Sicinski

Development of methodology: D.M. Livingston

Acquisition of data: D.M. Livingston

Analysis and interpretation of data: D.M. Livingston

Writing, review, and/or revision of the manuscript: S. Jirawatnotai, Y. Hu, D. M. Livingston, P. Sicinski

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