DNA Damage and Cellular Stress Responses

Inhibition of the Nedd8 System Sensitizes Cells to DNA Interstrand Cross-linking Agents

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Abstract

The Fanconi anemia pathway is required for repair of DNA interstrand cross-links (ICL). Fanconi anemia pathway--deficient cells are hypersensitive to DNA ICL--inducing drugs such as cisplatin. Conversely, hyperactivation of the Fanconi anemia pathway is a mechanism that may underlie cellular resistance to DNA ICL agents. Modulating FANCD2 monoubiquitination, a key step in the Fanconi anemia pathway, may be an effective therapeutic approach to conferring cellular sensitivity to ICL agents. Here, we show that inhibition of the Nedd8 conjugation system increases cellular sensitivity to DNA ICL--inducing agents. Mechanistically, the Nedd8 inhibition, either by siRNA-mediated knockdown of Nedd8-conjugating enzymes or treatment with a Nedd8-activating enzyme inhibitor MLN4924, suppressed DNA damage--induced FANC D2 monoubiquitination and CHK1 phosphorylation. Our data indicate that inhibition of the Fanconi anemia pathway is largely responsible for the heightened cellular sensitivity to DNA ICLs upon Nedd8 inhibition. These results suggest that a combination of Nedd8 inhibition with ICL-inducing agents may be an effective strategy for sensitizing a subset of drug-resistant cancer cells. Mol Cancer Res; 10(3): 369–77. ©2012 AACR.

Introduction

Cisplatin-based drugs have been used as a primary treatment for many types of cancers for more than 30 years. These drugs cause DNA damage, primarily via formation of interstrand DNA cross-linkages (ICL). ICLs are highly toxic to rapidly dividing cells, and cells that are unable to properly repair the damaged DNA die of apoptosis. However, the effectiveness of the therapy is often compromised largely because cancer cells develop resistance to the drugs (1). Elevated DNA repair pathways are observed in a subset of drug-resistant tumor cells (2, 3). Thus, understanding the cellular response mechanisms that regulate the activation of DNA repair pathways may provide a strategy for sensitizing some drug-resistant tumors. The DNA repair pathways that resolve DNA ICLs, such as nucleotide excision repair and homologous recombination, are coordinated by a DNA damage response pathway termed the Fanconi anemia pathway (4).

Patients with Fanconi anemia, who have a germ line disruption of the Fanconi anemia pathway, exhibit congenital abnormalities, bone marrow failure, and genomic instability leading to cancers (4, 5). Cells from patients with Fanconi anemia display abnormally high sensitivity to DNA ICL--inducing agents, such as cisplatin, mitomycin C (MMC), and melphalan. Fifteen Fanconi anemia genes have been identified to date (FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, O, and P). These act cooperatively in the Fanconi anemia pathway to coordinate the repair of DNA ICLs (6–8). The central regulatory event in the pathway is monoubiquitination of FANC D2, which requires S-phase or DNA damage-induced activation of 8 Fanconi anemia proteins (A, B, C, E, F, G, L, and M) that form a nuclear E3 ubiquitin ligase core complex. The activation of this Fanconi anemia core complex is preceded by a cascade of upstream DNA damage--induced signaling events involving the ATR and CHK1 kinases (4, 9). Monoubiquitinated FANC D2 is required for multiple steps during ICL repair, including the activation of the nucleotide excision repair and translesion synthesis (TLS) steps (4), and the recruitment of homologous recombination repair factors such as BRCA1, BRCA2, RAD51, and FAN1 (4).

Defects in the Fanconi anemia pathway also occur in somatic cells of non--Fanconi anemia individuals, causing diverse types of cancers (5, 10–12). Human tumors with Fanconi anemia gene mutations are particularly sensitive to ICL-inducing agents, such as cisplatin and MMC. Conversely, restoration of a functional Fanconi anemia pathway...
is a mechanism for acquired cellular resistance to DNA ICL agents (10, 13, 14). Interestingly, overexpression of Fanconi anemia genes accounts for drug resistance in melphalan-resistant multiple myeloma (14, 15). For these reasons, the Fanconi anemia pathway may be an effective target for chemosensitization in cancer treatment. Small-molecule inhibitors of the Fanconi anemia pathway have been identified by high-throughput platforms (13, 16), and an inhibitor of HSP90 has been shown to inhibit the Fanconi anemia pathway (17). Recently, the proteasome inhibitor bortezomib, which is used for treating certain types of hematologic tumors, was shown to inhibit the Fanconi anemia pathway, providing a mechanism for its antitumor effect (14, 18).

The ubiquitin–proteasome system regulates several essential cellular functions, including the cell-cycle and DNA damage responses. Protein ubiquitination is achieved by a cascade of E1 ubiquitin–activating enzymes, E2 ubiquitin–conjugating enzymes, and E3 ubiquitin ligases whereas reversal of ubiquitination is regulated by deubiquitinating enzymes. In addition to the ubiquitin system, eukaryotic cells use “ubiquitin-like modifiers” or Ubls, such as SUMO, Nedd8, and ISG15, which provide additional layers of regulation for protein degradation. Nedd8 shares approximately 60% sequence identity with ubiquitin (19) and is covalently attached to Lys residues on protein substrates in a manner similar to that of the ubiquitin system. The Nedd8 conjugation system consists of a single E1, a heterodimer of UBA3 and NAE1, two E2s, UBE2M (also known as UBC12) and UBE2F (20). The E3 for Nedd8 is not well characterized, and Nedd8 from the E2 can be directly transferred to Nedd8 substrates, including the cullin subunits of CRL complexes with different substrate specificity adaptors for protein degradation. Neddylation (protein modification by Nedd8) of the cullin subunits induces conformational changes within the CRL complexes, resulting in increased catalysis of substrate ubiquitination (20, 22). Therefore, the Nedd8 system affects a wide range of cellular functions that are regulated by the CRL complexes. Recently, a pharmacologic inhibitor of the Nedd8 system, MLN4924, was developed. MLN4924 has potent antitumor activity in cell culture as well as in a xenograft model (23), suggesting that the Nedd8 system may be an effective target for treating cancers.

To better understand the upstream signaling events that regulate FANCD2 monoubiquitination, we undertook a candidate-based siRNA screening that uses FANCD2 Western blot analysis as readouts. We found that knockdown of individual components in the Nedd8 conjugation system decreased DNA damage–induced FANCD2 monoubiquitination and foci formation and CHK1 phosphorylation. The results are phenocopied by treatment of cells with MLN4924, the pharmacologic inhibitor of NAE1. Consistent with these results, treatment with MLN4924, or siRNA-mediated depletion of the Nedd8 system, synergistically elevates cellular sensitivity to DNA ICL–inducing agents in various cell lines. These results suggest that a combination of a Nedd8 inhibitor with DNA ICL agents can be an effective therapeutic strategy against chemoresistant cancer cells.

Materials and Methods

Cell culture and chemicals

HeLa, HCT116, 293T, U2OS, and MCF7 cell lines were cultured in Dulbecco’s Modified Eagle’s Media supplemented with 15% FBS and l-glutamine. These cell lines were obtained from American Type Culture Collection and tested for mycoplasma contamination using Plasmocin (Invigen). We also tested these cell lines for morphology using light microscopy, under low density. Nontransformed LO2 liver cells and WI-38 lung human cell lines were grown in RPMI-1640 media. FA-F cells (2008 cells) corrected with cisplatin (Sigma) was dissolved as 10 mmol/L stock solution in PBS and used as final concentration of 10 μmol/L. Hydroxyurea (Sigma) was dissolved as 2 mol/L stock solution in PBS and used as final concentration of 2 mmol/L. MMC (Sigma) was dissolved in 70% ethanol as 3.3 mmol/L stock concentration. MLN4924 was provided by Millennium Pharmaceuticals Inc. and dissolved in dimethyl sulfoxide (DMSO) as a stock concentration of 10 mmol/L.

siRNAs and antibodies

The siRNAs against UBA3 were siRNA#1 TGTTC-TGGTAGCCTGCGGATGATG and siRNA#2 CGGA-
GCACCTGAAATCTCCATGGTTCT; siRNAs against UBE2M were siRNA#1 GGGCTTCTACAAGAGTGG-
GAAATTT and siRNA#2 (ACTCCATAATTFTATGGCC-
CTCGAGTA); siRNA against NAE1 was AGCACAGTG-
GTATAGTGAAACAAAT; siRNAs against UBE2F were  siRNA#1 CCGAGGGTTTCTGTGAGACAAAT and
siRNA#2 ACTTCCGGATAAAGTGAGATG; siRNA against NAE1 was AGCACAGTG-
GTATAGTGAAACAAAT; siRNA#2 ACTTCCGGATAAAGTGAGATG; and siRNA against FANCM was AAGCTCATAAAGCTCT-
CGGAA. Antibodies used in this study are as follows: anti-
FANCD2, anti-PCNA, and anti-CDC25A antibodies (Santa Cruz Biotechnology); anti-phospho-CHK1 (S317), anti-phospho-SMC1 (S966), anti-claspin, and anti-phospho-CHK2 (T68; Cell Signaling Technology); anti-53BP1 (Bethyl Laboratories); and anti-phospho-nda1 (S343; Oncogene) antibodies.

Immunofluorescence

HeLa cells were pretreated with extraction buffer (0.5% Triton X-100 in PBS buffer) on ice for 3 minutes prior to fixation with 4% paraformaldehyde. Anti-FANCD2, anti-53BP1, anti-PCNA, and anti-γ-H2AX antibodies were used as primary antibodies. Alexa Fluor 488- and 594–conjugated antibodies (Invitrogen) were used as secondary antibodies. For the FANCID2 foci in Fig. 3E, images were collected by a Zeiss Axiosvert microscope equipped with a Yokogawa CSU-22 spinning disk confocal head and a 100 x/1.45NA oil

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to identify upstream factors that affect the damage-inducible FANCD2 monoubiquitination. To identify upstream factors that affect the damage-inducible FANCD2 monoubiquitination (FANCD2-ub), we undertook a candidate-based siRNA screen. Various DNA-damaging agents (UV, hydroxyurea, cisplatin, IR) induce FANCD2 monoubiquitination, and there are few known upstream factors that regulate DNA damage-induced FANCD2 monoubiquitination including the Fanconi anemia core complex members, ATR, and CDK1 (9, 24). We hypothesized that there might be other ubiquitin-mediated signaling events upstream of the monoubiquitination of FANCD2. Depletion of UBA3, an E1 component of the Nedd8 conjugation system, strongly inhibited damage-inducible FANCD2 monoubiquitination (Fig. 1A and B). To further test whether other components in the Nedd8 conjugation system also affect FANCD2 monoubiquitination, we tested knockdown of UBE2M, an E2-conjugating enzyme for Nedd8. UBE2M depletion similarly inhibited FANCD2 monoubiquitination after UV (Fig. 1D and F; HCT116 and HeLa cells, respectively) or after psoralen + UVA (PUVA) treatment (Fig. 1E and G; HCT116 and HeLa cells, respectively). The level of CHK1 phosphorylation (p-CHK1) was also consistently lower, when the Nedd8 conjugation pathway was inhibited. The level of monoubiquitinated PCNA (PCNA-ub) remained similar upon depletion of UBE2M, suggesting that the effect on FANCD2 monoubiquitination is specific. Phosphorylation of other DNA damage response proteins, such as H2AX, BRCA1, and SMC1, was elevated or remained at a similar level upon UBE2M knockdown, suggesting that knockdown of UBE2M caused some level of DNA damage and did not disrupt the overall DNA damage response. Knockdown of UBA3 or UBE2M did not significantly affect cellular proliferation, as measured by bromodeoxyuridine staining (Fig. 1C). Consistent with a previous report (20), knockdown of UBE2M modestly increased the G2–M peak in the cell-cycle distribution analysis (Supplementary Fig. S1).

The monoubiquitinated form of FANCD2 localizes to chromatin and forms damage-inducible foci. Consistent with the reduction of FANCD2 monoubiquitination, foci formation of FANCD2 was also reduced upon UBE2M knockdown (Fig. 2). Interestingly, foci formation of other DNA damage response proteins, such as γ-H2AX, 53BP1, and RPA, were not affected, suggesting that the effect is specific for FANCD2. Because other DNA damage response proteins are not affected, the effect on FANCD2 is not simply due to a disruption in the cell cycle. Knockdown of NAE1, the catalytic subunit of the Nedd8 E1-activating enzyme, also inhibited damage-inducible FANCD2 monoubiquitination and CHK1 phosphorylation, whereas knockdown of UBE2F, a Nedd8 E2 that participates in neddylation of a smaller subset of cullins (20), did not affect the level of FANCD2 monoubiquitination (Supplementary Fig. S2). Depletion of USP1, a deubiquitinating enzyme targeting FANCD2, elevates the level of FANCD2 monoubiquitination (25). Surprisingly, depletion of UBE2M did not reduce the FANCD2 monoubiquitination that is upregulated by USP1 depletion (Supplementary Fig. S3), suggesting that the Nedd8 inhibition specifically abrogates FANCD2 monoubiquitination that is induced by exogenous DNA damage. Altogether, these results suggest that the Nedd8 conjugation system is required for DNA damage–inducible FANCD2 monoubiquitination and CHK1 phosphorylation.
Nedd8 inhibitor MLN4924 inhibits DNA damage–
induced activation of the Fanconi anemia pathway

Recently, a pharmacologic inhibitor of the Nedd8 system
was described (23). This investigational agent, MLN4924,
targets the NAE1 component of the Nedd8 E1 enzyme,
stabilizes substrates of CRL E3 ligases such as CDT1,
induces DNA rereplication and checkpoint activation,
and triggers apoptosis (23, 26, 27). We tested whether
MLN4924 phenocopies the siRNA results described above.
We pretreated cells with MLN4924 and then exposed them
to UV or PUVA for variable times (Fig. 3A and B; HCT116
and HeLa cells, respectively; and Supplementary Fig. S4 for

Figure 1. Knockdown of Nedd8 conjugation system disrupts
FANCD2 monoubiquitination and CHK1 phosphorylation.
A, representative Western blot analysis for the candidate siRNA
screens. HeLa cells were treated with siRNAs against various E3
ligase components. Forty-eight to 60 hours later, cells were treated
with UV (30 J/m^2), then the cells were harvested and lysed for the
Western blot analysis. B, HeLa cells were treated with UBA3
siRNA#1, followed by UV treatment. Cells were harvested at indicated time points. C, HeLa cells
were transfected with the siRNAs, and 48 hours later, cells were
incubated with 10 μmol/L bromodeoxyuridine (BrdUrd) for 40
minutes. Integrated BrdUrd was visualized using fluorescein
isothiocyanate (FITC)-conjugated BrdUrd antibody and quantified by
fluorescence-activated cell-sorting analysis. D, HCT116 cells
were treated with 2 different UBE2M siRNAs, followed by UV
(30 J/m²) treatment before being harvested for Western blot
analysis. E, HCT116 cells were treated with UBE2M#1 siRNA,
followed by PUVA treatment, then harvested at indicated time points
for Western blot analysis. F and G, HeLa cells were treated
with UBE2M siRNAs followed by UV (30 J/m²) and PUVA treatment,
respectively, for Western blot analysis. Cul1, culin 1; PI, propidium iodide.
HeLa treated with UV). Consistent with previous reports, phosphorylation of BRCA1, SMC1, CHK1, CHK2, γ-H2AX, and monoubiquitination of FANCD2 and PCNA were induced by the compound treatment (compare 0 time points between DMSO and MLN4924). When combined with DNA-damaging agents, the compound specifically inhibited the sustained activation of p-CHK1 and FANCD2-ub whereas activation of other DNA repair proteins remained unaffected. These effects were observed in other cell types, such as U2OS (Supplementary Fig. S5), MCF7 (Supplementary Fig. S6), and Fanconi anemia–deficient ovarian tumor line 2008. Longer treatment of MLN4924 was required to observe the reduced level of p-CHK1 and FANCD2-ub at the lower inhibitor dosage (Supplementary Fig. S7). In a reverse experiment, when MLN4924 was added after exogenous DNA damage, p-CHK1 and FANCD2-ub levels were suppressed, whereas p-CHK2, PCNA-ub, and γ-H2AX remained unaffected (Fig. 3C). The effect of MLN4924 was dose-dependent, as shown in Fig. 3D. One discrepancy between the siRNA and MLN4924 results is that the spontaneous activation of DNA checkpoints was not observed following Nedd8 knockdown by siRNA. Perhaps longer treatment of cells with the siRNAs (60 hours) may elicit secondary effects that suppress activation of DNA checkpoint completely.

Consistent with the siRNA results, MLN4924 effectively suppressed DNA damage–induced foci formation of FANCD2, whereas foci formation of γ-H2AX and 53BP1 was not affected (Fig. 3E). Treatment with MLN4924 without additional damage caused foci formation of these DNA damage response proteins to varying degrees, suggesting that the drug itself triggers DNA damage. Altogether, MLN4924 phenocopied the siRNA results in which the inhibition of the Nedd8 conjugation system suppresses DNA damage–induced FANCD2 monoubiquitination and CHK1 phosphorylation. Therefore, Nedd8-targeting inhibitors, such as MLN4924, may sensitize cancer cells to DNA-damaging agents, particularly to agents that generate DNA ICLs, which require the functional Fanconi anemia pathway for repair.

We previously reported that the FANCM subunit of the Fanconi anemia core complex is ubiquitinated and degraded during mitosis, mediated by the CRL3β-TrCP E3 ubiquitin ligase. FANCM degradation appears to be one mechanism for cell-cycle–specific activation of the Fanconi anemia pathway (28). The failure of the mitosis-specific degradation of FANCM leads to improper chromatin loading of the Fanconi anemia core complex and heightened cellular sensitivity to MMC. Because the degradation is mediated by a CRL complex, we reasoned that inhibition of Nedd8 conjugation may disrupt the timed degradation of FANCM during mitosis. FANCM was phosphorylated and degraded when cells were released into the mitotic phase of the cell cycle (Fig. 3F, lanes 1–4), and treatment (3–4 hours) of proteasome inhibitor MG132 inhibited the degradation of FANCM (lanes 5–8). Treatment with MLN4924 similarly stabilized FANCM (lanes 9–12), suggesting that the CRL complex activity is inhibited by the Nedd8 inhibitor. From these results, we suggest that the perturbed degradation of FANCM during mitosis by inhibition of Nedd8 is one mechanism reflected by heightened cellular sensitivity to the Nedd8 inhibition.

**Inhibition of the Nedd8 conjugation system hypersensitizes cancer cells to DNA ICL agents**

We next tested whether inhibition of the Nedd8 system causes hypersensitization of cells to DNA ICL agents. Knockdown of UBE2M in HeLa cells significantly reduced cell growth after MMC treatment, as shown by clonogenic assay (Fig. 4A). Knockdown of FANCM, a Fanconi anemia gene shown to be required for resistance to MMC (29), was tested as a positive control. Treatment of HCT116 colon cancer cell lines with MLN4924 significantly reduced cell survival in combination with the cisplatin (Fig. 4B; 2 independent experiments). These results show that the Nedd8 system is required for cellular resistance to the DNA cross-linking agents. The degree of sensitization appears to be preferential to the transformed cell lines, as 2 normal human diploid cells, LO2 and WI-38 cells, were not significantly sensitized to cisplatin by the Nedd8 inhibition (Supplementary Fig. S8). The MLN4924-mediated sensitization of cells to DNA ICL agents resulted, at least partially, from inhibition of the Fanconi anemia pathway, as FANCF-deficient and ICL-sensitive ovarian tumor cells (2008 + F) showed enhanced sensitivity to MLN4924, compared with FANCF-deficient and ICL-sensitive counterpart 2008 cells (Fig. 4C). The slight sensitization of the Fanconi anemia–deficient tumor cells 2008 by MLN4924 to cisplatin...
suggests that MLN4924 disrupt targets other than the Fanconi anemia pathway, including the proteins of the ATR/CHK1 signaling pathway. To directly show the reduced DNA repair capacity upon Nedd8 inhibition, we conducted cytogenetic analysis of 293T cells. We observed synergistic effects of depleting UBE2M (Fig. 4D), or treating MLN4924 (Fig. 4E), with MMC treatment in inducing chromosomal aberrations, suggesting that Nedd8 inhibition...
compromised cellular DNA repair capacity upon DNA ICLs. Altogether, these results show that inhibition of Nedd8 conjugation system disrupts cellular resistance to DNA ICLs.

Discussion

We have shown that inhibition of the Nedd8 conjugation system sensitizes various cancer cells to DNA ICL–inducing agents. Mechanistically, Nedd8 inhibition abrogated DNA damage–inducible activation of CHK1 phosphorylation and FANCD2 monoubiquitination. Previous reports have shown that treatment with MLN4924 activates DNA damage responses, such as CHK1 phosphorylation (23, 26). While our study is consistent with this result, it further shows that damage-induced activation of CHK1 and FANCD2 is suppressed upon Nedd8 inhibition. Thus, the Nedd8 conjugation system is required for sustained activation of CHK1 and FANCD2.

The suppression of CHK1 phosphorylation and FANCD2 monoubiquitination was not due to alteration in cell-cycle progression. Other DNA damage responses that occur during S–G2 phases, such as proliferating cell nuclear antigen (PCNA) monoubiquitination, remain unchanged upon Nedd8 inhibition. Furthermore, phosphorylation of ATM substrates, such as CHK2, 53BP1, NBS1, BRCA1, and γ-H2AX, remained unaffected or even elevated under the same conditions. We therefore conclude that a Nedd8-mediated protein modification event is required for damage-inducible ATR/CHK1 activation and for subsequent FANCD2 monoubiquitination (Fig. 5).

How Nedd8 inhibition suppresses CHK1 phosphorylation and FANCD2 activation requires further investigation. Increasing evidence supports a strong interaction between ATR/CHK1 signaling and the Fanconi anemia pathway. ATR and CHK1 function is required for induction of FANCD2 monoubiquitination (9, 30), and CHK1–mediated phosphorylation of FANCE is required for cellular
neddylation events, most likely the activation of specific CRL E3 ligases, is required for damage-inducible CHK1 phosphorylation and FANCD2 monoubiquitination. We suggest that disruption in the CRL-mediated destruction of FANCM is one mechanism leading to the abrogation of the Fanconi anemia pathway.

The primary role of Nedd8 is to activate CRL complex destruction, resulting in inhibition of its activity (36). The effect of Nedd8 inhibition in suppressing the DNA damage response is unlikely to be mediated via p53 modification, as our results were observed in several cell types regardless of p53 status (Fig. 3 and Supplementary Figs. S5 and S6). We previously reported that a CRL complex containing β-TRCP mediates mitotic degradation of FANCM (28). The perturbation of timed degradation leads to disruption of the Fanconi anemia core complex recruitment cycle and increase in the cellular sensitization to MMC. On the basis of our evidence that MLN4924 disrupts the mitotic degradation of FANCM (Fig. 3F), we propose that Nedd8 inhibition disrupts Fanconi anemia pathway, at least in part, through perturbation of mitotic FANCM degradation. However, we cannot rule out the possibility that there may be additional CRL complexes and substrates that regulate the Fanconi anemia pathway.

Because various cellular signaling pathways affect cellular sensitivity to DNA ICL agents, a pharmacologic inhibitor of such pathways may improve current chemotherapeutic regimens. Previous reports have shown that proteasome inhibitors sensitize cells to DNA-damaging agents, suggesting that these agents may be synergistic in combination therapy (37, 38). Proteasome function is required for activation of DNA damage responses including the Fanconi anemia pathway (14, 18), providing a basis for the proteasome-mediated sensitization of cells to DNA-damaging agents. The Nedd8–CRL complexes account for approximately 20% of proteasome-mediated protein degradation in cells (23). Furthermore, inhibition of proteasome, but not of the Nedd8 conjugation, causes overaccumulation of overall ubiquitin conjugates in cells (not shown), suggesting more selectivity of the Nedd8 inhibition. Thus, inhibition of the Nedd8 system, in combination with DNA-damaging agents, and more specifically ICL agents might offer an alternative strategy which could provide more specificity in targeting the DNA damage response. Furthermore, our work suggests that the proteasome-mediated suppression of the Fanconi anemia pathway could be largely due to the suppression of the Nedd8 and CRL-mediated protein degradation. Further investigation of the mechanism involved in this pathway will provide better insight into DNA damage signaling and may provide a new avenue for the design of more specific anticancer drugs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 5. A protein neddylation event is required upstream of the Fanconi anemia pathway. A schematic model for the Nedd8-mediated activation of the DNA damage-inducible FANCD2 monoubiquitination. While interruption of the Nedd8 conjugation system suppresses the ATM-mediated signaling pathways, particularly CHK1 phosphorylation and FANCD2 monoubiquitination, ATM-mediated pathways or PCNA ubiquitination remain unaffected. We hypothesize that specific protein neddylation events, most likely the activation of specific CRL E3 ligases, is required for damage-inducible CHK1 phosphorylation and FANCD2 monoubiquitination. We suggest that disruption in the CRL-mediated destruction of FANCM is one mechanism leading to the abrogation of the Fanconi anemia pathway.

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