Differential Induction of c-Jun NH₂-Terminal Kinase and c-Abl Kinase in DNA Mismatch Repair-proficient and -deficient Cells Exposed to Cisplatin

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ABSTRACT

The c-Abl nonreceptor tyrosine kinase and the c-Jun NH₂-terminal kinase (JNK/stress-activated protein kinase) are activated during the injury response to the DNA-damaging agent cisplatin. Loss of DNA mismatch repair activity results in resistance to cisplatin in human cancer cells, suggesting that the mismatch repair proteins function as a detector for cisplatin DNA adducts. To identify signaling pathways activated by this detector, we investigated the effect of the loss of DNA mismatch repair function on the ability of cisplatin to activate the JNK and c-Abl kinases.

The results demonstrate that cisplatin activates JNK kinase 3.8 ± 0.2-fold more efficiently in DNA mismatch repair-proficient than repair-deficient cells, and that activation of c-Abl is completely absent in the DNA mismatch repair-deficient cells. Furthermore, the results show that cisplatin-induced activation of JNK occurs through a stress-activated protein kinase/extracellular signal-regulated kinase kinase 1-independent mechanism. We conclude that activation of JNK and c-Abl by cisplatin is in part dependent upon the integrity of DNA mismatch repair function, suggesting that these kinases are part of the signal transduction pathway activated when mismatch repair proteins recognize cisplatin adducts in DNA.

INTRODUCTION

Cisplatin is an important chemotherapeutic agent used for the treatment of a variety of human cancers. Unfortunately, the clinical use of cisplatin is limited by its toxicity to the nervous system and kidney and by the frequent development of resistance during treatment (1). DNA is the primary intracellular target of cisplatin, and eukaryotic cells respond to the presence of cisplatin adducts by activating signal transduction pathways that result in cell cycle arrest, an increase in certain types of DNA repair activity, and apoptosis (2). Although the signaling cascades activated as part of the cisplatin-induced injury response are as yet poorly defined, recent studies have demonstrated that the injury response involves activation of the stress-activated protein kinase (also known as JNK) and the nuclear c-Abl protein tyrosine kinase (3). JNK is a serine/threonine kinase that is part of the mitogen-activated protein kinase pathway, which contains, in order, PAKp65, mitogen-activated protein kinase kinase 1 (SEK1) (also called MKK4), and JNK (4–6). JNK kinase phosphorylates the three nuclear transcription factors c-Jun, activator of transcription factor 2, and Elk-1 and stimulates their transcriptional activities (7–9). c-Abl is a nonreceptor tyrosine kinase that is related to SH2 and SH3 protein kinases (10). The finding that c-Abl associates with the retinoblastoma (Rb) protein has supported a role for c-Abl in regulating the cell cycle (11). Other studies have demonstrated that c-Abl phosphorylates the COOH-terminal domain of RNA polymerase II (12) and stimulates transcription (11). Cisplatin-induced activation of the JNK and c-Abl kinases is believed to constitute an initial step in the generation of signals that coordinate the full injury response.

Loss of DNA mismatch repair is associated with hereditary colorectal cancer (13) and is a common finding in a variety of sporadic and inherited human neoplasms, including those for which cisplatin is used routinely (14, 15). Six genes that encode proteins with roles in DNA mismatch repair have been identified in humans (reviewed in Refs. 13 and 16): the MutS homologues hMSH2, hMSH3, and hMSH6 (GTBP/p160); and the MutL homologues hMLH1, hPMS1, and hPMS2. Recently, we and others have demonstrated that loss of DNA mismatch repair due to deficiency of either hMLH1 or hMSH2 confers low level resistance to cisplatin (17, 18) and carboplatin (19). It has been suggested that the DNA mismatch repair proteins serve as a detector system for the presence of DNA damage (20, 21). Indeed, human hMutSα, an heterodimer of hMSH2 and hMSH6 (22), has now been shown to bind to DNA-containing adducts produced by cisplatin, and pure hMSH2 has been reported to bind to platinated DNA in gel shift assays (23).

The current hypothesis is that the cisplatin resistance conferred by loss of DNA mismatch repair activity is due to failure of the detector to recognize the presence of the DNA adducts and appropriately activate signal transduction pathways that eventually lead to apoptosis. If this hypothesis is correct, it should be possible to identify the signal transduction pathways with which the detector communicates by comparing pathways differentially activated during the cisplatin-induced cellular injury response in mismatch repair-proficient and -deficient cells. The present studies address the effect of the loss of DNA mismatch repair on the activation of the JNK1 and c-Abl kinases during the injury response. We report here that cisplatin activates JNK1 by a SEK1/MKK4-independent mechanism and that this response is weaker in DNA mismatch repair-deficient cells. Furthermore, the results demonstrate that cisplatin activates c-Abl kinase in the proficient cells but that this response is completely absent in the deficient cells.

MATERIALS AND METHODS

Materials. Cisplatin was obtained from Bristol-Myers Squibb Co. (Princeton, NJ); a stock solution of 1 mg/ml was prepared in 0.9% NaCl.

Cell Lines and Culture. The hMLH1-deficient human colorectal adenocarcinoma cell line HCT116 was obtained from the American Type Culture Collection (ATCC CCL 247; Rockville, MD); a subline into which a wild-type copy of hMLH1 on chromosome 3 had been introduced by microcell fusion (HCT116 + ch3) and a subline that received chromosome 2 as a negative control (HCT116 + ch2) were obtained from Drs. C. R. Boland and M. Koi (24), as were the hMSH2-deficient human endometrial adenocarcinoma cell line...
line HEC9 (25) and a subline complemented with chromosome 2 containing a wild-type copy of hMSH2 (HEC95 + ch2). All cell lines were maintained in Iscove’s modified Dulbecco’s medium (Irvine Scientific, Irvine, CA) supplemented with 100 mM l-glutamine and 10% heat-inactivated fetal bovine serum. The chromosome-complemented lines were grown in medium supplemented with gentamicin (400 μg/ml for HCT116 + ch2 and HCT116 + ch3 and 600 μg/ml for HEC95 + ch2; Life Technologies, Inc., Gaithersburg, MD). Expression of hMLH1 and hMSH2 proteins was documented by Western blot for each of the cell lines (data not shown). Human renal 293 cells were obtained from the American Type Culture Collection (ATCC CRL 1573).

**Immunoprecipitations.** Cells growing in log phase were treated with cisplatin for 1 h at their respective IC50 concentrations (25 μM for the HCT116 cells and 14 μM for the HEC95 cells). At 0.5, 1, 2, 4, and 6 h after the beginning of the cisplatin exposure, cells were lysed in 1 ml of lysis buffer [10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 5 mM DTT, 1 mM sodium vanadate, 0.1 mM phenylmethylsulfonyl fluoride, and 5 mM aminocaproic acid] for 30 min on ice. The insoluble material was removed by centrifugation at 30,000 × g for 20 min at 4°C. Equal amounts of protein were incubated with protein A-Sepharose and anti-JNK1 (2.5 μg; C-17; Santa Cruz Biotechnology, Santa Cruz, CA), anti-Ab1 (2.5 μg; K-12; Santa Cruz Biotechnology), or anti-PAK65 (2.5 μg; C-19; Santa Cruz Biotechnology) for 3 h at 4°C.

**JNK1 Kinase Assay.** The anti-JNK1 immune complexes were washed twice with lysis buffer and then once with kinase buffer (20 mM HEPEs (pH 7.0), 10 mM MgCl₂, 10 mM MnCl₂, 2 mM DTT, and 0.1 mM sodium vanadate), following which they were resuspended in 30 μl of kinase buffer containing 5 μCi [γ-32P]ATP (3000 Ci/mmol; NEN, Boston, MA), 20 μM cold ATP, and 2.5 μg of GST-c-Jun (1–79) as substrate (Santa Cruz Biotechnology) and incubated for 20 min at 30°C (26). Reactions were terminated by the addition of the SDS sample buffer and boiling for 5 min. The phosphorylated proteins were resolved by 10% SDS-PAGE and visualized by autoradiography. Anti-JNK1 immunoprecipitates were examined for the JNK1 content by immunoblotting with JNK1 antibody (C-17; Santa Cruz Biotechnology). The antigen-antibody complexes were visualized by enhanced chemiluminescence (Amersham Corp., Arlington Heights, IL).

**c-Ab1 Kinase Assay.** The anti-Ab1 immune complexes were washed twice each with lysis buffer/500 mM NaCl, lysis buffer/100 mM NaCl, and lysis buffer alone and then washed twice with kinase buffer (25 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, and 1 mM DTT). The wash was resuspended in 20 μl of kinase buffer with 50 μCi [γ-32P]ATP (3000 Ci/mmol; NEN), 10 μM cold ATP, and 1 μg of GST-CTD as substrate and incubated for 30 min at room temperature (12). Reactions were terminated by the addition of the SDS sample buffer and boiling for 5 min. The phosphorylated proteins were resolved by 15–17.5% SDS-PAGE and visualized by autoradiography. The amounts of c-Ab1 protein in the anti-Ab1 immunoprecipitates were analyzed by immunoblotting with anti-Ab1 (B6F; PharMingen, San Diego, CA). The antigen-antibody complexes were visualized by enhanced chemiluminescence.

**PAK65 Kinase Assay.** The anti-PAK65 immune complexes were washed twice with lysis buffer and then once with kinase buffer [50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM MgCl₂, and 1 mM MnCl₂]. The immune complexes were then resuspended in 40 μl of kinase buffer containing 5 μCi [γ-32P]ATP (3000 Ci/mmol; NEN), 50 μM cold ATP, and 3 μg of MBP as substrate (Sigma Chemical Co., St. Louis, MO) and incubated for 20 min at 30°C (6). Reactions were terminated by the addition of SDS sample buffer and boiling for 5 min. The phosphorylated proteins were resolved by 14% SDS-PAGE and visualized by autoradiography. Immune complexes were analyzed for their PAK65 content by immunoblotting with PAK65 antibody (C-19; Santa Cruz Biotechnology). The antigen-antibody complexes were visualized as described above.

**Western Blot Analysis of SEK1/MKK4 Protein.** HCT116 cells growing in log phase were treated with 25 μM cisplatin for 1 h. At 0.5, 1, and 2 h after the beginning of cisplatin exposure, the cells were lysed in 100 μl of lysis buffer [10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 5 mM DTT, 1 mM sodium vanadate, 0.1 mM phenylmethylsulfonyl fluoride, and 5 mM aminocaproic acid] for 30 min on ice. After centrifugation at 30,000 × g for 20 min at 4°C, samples containing 50 μg of cell lysate in SDS-PAGE loading buffer were separated using a 10% polyacrylamide gel followed by electroblotting onto a polyvinylidene difluoride membrane (Immobilon P; Millipore, Bedford, MA). Immunoblotting was performed using polyclonal anti-phospho-SEK1/MKK4 (New England Biolabs, Inc., Beverly, MA), diluted 1:1000 in 5% bovine serum albumin. After washing the blots, horseradish peroxidase-conjugated anti-rabbit antibody diluted 1:16,000 (Sigma) was added, and the complexes were visualized by enhanced chemiluminescence.

**RESULTS**

**Differences in Cisplatin Sensitivity.** The mismatch repair-deficient HCT116 and HCT116 + ch2 cells were 2-fold resistant to cisplatin when compared to the repair-proficient subline HCT116 + ch3 that expresses a wild-type copy of hMLH1 (18). Similarly, the HEC59 DNA mismatch repair-deficient cells were 2-fold resistant to cisplatin when compared to the proficient HEC59 + ch2 subline that expresses a wild-type copy of hMSH2. The difference in cisplatin sensitivity was not related to changes in the cellular pharmacology of the drug. The total cellular platinum uptake after a 1-h treatment was 303 ± 58 (SD) fmol/µg protein for the HCT116 + ch2 cells and 289 ± 82 (SD) fmol/µg protein for the HCT116 + ch3 cells. Moreover, the extent of DNA platination was 55 ± 22 (SD) fmol/µg DNA for the HCT116 + ch2 cells and 59 ± 17 (SD) fmol/µg DNA for the HCT116 + ch3 cells (n = 4; P = 0.80, two-sided t test). Moreover, p53 protein is equally induced in both DNA mismatch repair-deficient and -proficient cells after a 1-h treatment with cisplatin (data not shown).

**JNK1 Activation in Response to Cisplatin Injury.** HCT116, HCT116 + ch2, and HCT116 + ch3 cells were exposed to 25 μM cisplatin for 1 h, and lysates prepared at various times after the beginning of drug exposure were precipitated with anti-JNK1 antibody. The immune complexes were then assayed for kinase activity using GST-c-Jun (1–79) as a substrate (26). Using this approach, a low level of GST-Jun phosphorylation was detectable in the anti-JNK1 immunoprecipitates obtained from untreated cells (Fig. 1A). Jun phosphorylation was induced maximally 2 h after exposure to cisplatin. More importantly, cisplatin activated JNK1 kinase more efficiently in the proficient subline HCT116 + ch3 [13.3 ± 3.3 (SD)-fold activation], when compared to the deficient HCT116 cells [2.7 ± 1.5(SD)-fold activation] and HCT116 + ch2 subline [3.5 ± 1.1(SD)-fold activation]. The increase in activity was due to activation of the enzyme because there was no detectable effect of cisplatin on the JNK1 protein level (Fig. 1B).

A second pair of mismatch repair-deficient (HEC59) and -proficient (HEC59 + ch2) cell lines was used to further document the activation of JNK1 by cisplatin. Unfortunately, in this cell system, there was no detectable activation of JNK1 at 2, 4, or 6 h after cisplatin treatment (Fig. 1A). Moreover, cisplatin had no detectable effect on JNK1 levels (Fig. 1B). These results suggested that the response to cisplatin is defective in both deficient and proficient HEC59 cells. The absence of JNK1 activation indicated that either this kinase cannot be activated in these cells or that it is not present at a concentration sufficient to be detected. To distinguish between these possibilities, the cells were treated with UV light, which has been identified as a potent inducer of JNK1 (7, 26). UV light exposure produced activation of JNK1 kinase in both types of HEC59 cells (Fig. 1A), indicating that JNK1 is functionally expressed but not activatable by cisplatin in the HEC95 cells.

c-Ab1 Activation in Response to Cisplatin Injury. Recent studies have demonstrated that cisplatin activates the nuclear c-Ab1 tyrosine kinase.
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Fig. 1. JNK1 activation in response to cisplatin treatment. Cells were exposed to cisplatin (25 μM for the HCT116, HCT116 + ch2, and HCT116 + ch3 cells and 14 μM for the HEC59 and HEC59 + ch2 cells) for 1 h. The cells were harvested at the indicated times after the beginning of exposure. The HEC59 and HEC59 + ch2 cells were also exposed to UV light (40 J/m² UVC) and harvested after 30 min. A, JNK1 activity as determined by an immune complex kinase assay with GST-c-Jun (1-79) as the substrate. The experiment was repeated three times, and similar results were obtained; differences in activity were quantitated using a PhosphorImager (Molecular Dynamics). B, Western blot analysis with anti-JNK1 antibody.

kinase (3). The finding that cells deficient in c-Abl fail to activate JNK in response to cisplatin treatment supports the argument that c-Abl is an upstream signal in this injury response pathway (3). HCT116, HCT116 + ch2, and HCT116 + ch3 cells were treated with 25 μM cisplatin for 1 h, and anti-Abl immunoprecipitates were prepared from cell lysates at 0.5, 1, and 2 h. In vitro kinase assays were performed with the GST-CTD protein as substrate (12). A low level of GST-CTD phosphorylation was detectable in the untreated cells (Fig. 2A), and the extent of GST-CTD phosphorylation increased by a factor of 2.6 ± 0.08 (SD) and 4.0 ± 0.1 (SD)-fold, respectively, at 1 and 2 h after the beginning of drug exposure in the HCT116 + ch3 proficient cells (Fig. 2A). In contrast, there was no induction of c-Abl kinase activity at any of the sampled time points in the mismatch repair-deficient HCT116 and HCT116 + ch2 cells. The finding that cisplatin had no detectable effect on c-Abl protein level indicated an increase in c-Abl activity (Fig. 2B).

The cisplatin-induced c-Abl tyrosine kinase activity was also studied in the HEC59 and HEC59 + ch2 cells. Exposure of the mismatch repair-proficient HEC59 + ch2 cells to cisplatin produced a 3.0 ± 0.1 (SD)-fold stimulation of c-Abl kinase activity at 2 and 4 h after the beginning of drug exposure (Fig. 2A). However, there was no detectable induction of GST-CTD phosphorylation when the HEC59 DNA mismatch repair-deficient cells were exposed to cisplatin. The immunoblot analysis with anti-Abl antibody showed no detectable effect of cisplatin on c-Abl levels in either the HEC59 or HEC59 + ch2 cell lines (Fig. 2B).

Activation of PAKp65 and SEK1/MMK4 in Response to Cisplatin Injury. Having documented that JNK1 was activated during the cisplatin-induced injury response, we sought to determine which of the MAP kinases known to be upstream of JNK1 in the signaling pathway were activated by this type of injury. HCT116 + ch2 and HCT116 + ch3 cells were treated with 25 μM cisplatin for 1 h, and lysates prepared at 0.5, 1, and 2 h were examined for PAKp65 and MKK4 activity. PAKp65 kinase was immunoprecipitated with anti-PAKp65 antibody and assayed using MBP as substrate (6). Fig. 3A shows that cisplatin had no significant effect on PAKp65 activity at

Fig. 2. c-Abl activation in response to cisplatin treatment. Cells were exposed to cisplatin (25 μM for the HCT116, HCT116 + ch2, and HCT116 + ch3 cells and 14 μM for the HEC59 and HEC59 + ch2 cells) for 1 h. The cells were harvested at the indicated times after the beginning of drug exposure. In A, activation of c-Abl was determined by an immune complex kinase assay with GST-CTD as the substrate. The experiment was repeated three times, and similar results were obtained; differences in activity were quantitated using a PhosphorImager (Molecular Dynamics). B, Western blot analysis with anti-Abl antibody.

HCT116

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HEC59 + ch2

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HCT116

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HEC59

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a. Kinase assay

HCT116+ch2  HCT116+ch3

Time(h) 0 0.5 1 2 0 0.5 1 2

<MBP

b. Anti-PAKp65

<PAKp65

Fig. 3. PAKp65 activation in response to cisplatin treatment. Cells were exposed to 25 μM cisplatin for 1 h and were then harvested at the indicated times after the beginning of drug exposure. A, activation of PAKp65 was determined by an immune complex kinase assay with MBP as the substrate. The experiment was repeated three times, and similar results were obtained. B, Western blot analysis with anti-PAKp65 antibody.

any of these time points, and Fig. 3B shows that it had no detectable effect on PAKp65 protein levels either. Lysates were assayed for SEK1/MKK4 kinase by immunoblotting with anti-phospho-SEK1/MKK4 antibody. As a positive control, lysates were prepared from human renal 293 cells exposed to 700 mM NaCl for 0.5 h, a treatment previously documented to activate SEK1/MKK4 kinase in vivo (4, 27). As shown in Fig. 4, there was no detectable phosphorylation of SEK1/MKK4 at 0.5, 1, or 2 h after the beginning of the cisplatin exposure in either the HCT116 + ch2 or the HCT116 + ch3 cells. Taken together, these results indicate that cisplatin activates JNK through a PAKp65- and SEK1/MKK4-independent mechanism in these cells.

DISCUSSION

Previous work has shown that the loss of DNA mismatch repair results in constitutive resistance to cisplatin in human cancer cells (17, 18) and carboplatin (19). The current paradigm is that the DNA mismatch repair system serves as a detector for DNA damage; resistance is thought to result from failure of the cell to recognize the cisplatin adducts and to activate signaling pathways that trigger apoptosis (20, 21). If this paradigm is correct, then the detector must be able to initiate activation of signaling pathways. The present studies identify JNK1 and c-Abl as members of two of the pathways activated by the detector function of the mismatch repair proteins.

In the hMLH1-defective HCT116 colon cell system used in these experiments, cisplatin caused a 3.8-fold greater increase in JNK1 activity in the mismatch repair-proficient than in the -deficient cells. Unfortunately, the drug failed to activate JNK1 in either the repair-proficient or -deficient HEC59 endometrial cells; therefore, a differential effect could not be further documented in a second mismatch repair-proficient and -deficient cell pair. Nevertheless, the data from the HCT116-proficient and -deficient cells argue cogently that DNA mismatch repair activity is required for maximal activation of JNK1 in response to cisplatin-induced injury. In the case of c-Abl, cisplatin produced a 4.0- and 3.0-fold increase in activity in the proficient HCT116 + ch3 and HEC59 + ch2 cells, respectively, that was completely ablated by loss of DNA mismatch repair function in the HCT116, HCT116 + ch2, and HEC59 cells. The failure of cisplatin to activate JNK at concentrations that produced an increase in c-Abl activity in the HEC59 cell system could be due to several different things: (a) c-Abl and JNK may not function in the same signaling pathway in response to cisplatin injury; (b) JNK activation may not be a universal response to this type of damage. This is consistent with recent observations of Liu et al. (28), who showed that activation of c-Abl and JNK represent distinct signaling responses to DNA damage and that JNK was moderately stimulated by mitomycin C and cisplatin at high doses but was not activated by ionizing radiation in mouse 3T3 fibroblasts; and (c) because the HEC59 cell line exhibits microsatellite instability (25), it is possible that there has been mutational inactivation of some proteins upstream of JNK in the signaling response to cisplatin. Although the biochemical events that link DNA mismatch repair proteins to the activation of JNK and c-Abl pathways have yet to be identified, the fact that both of these proteins have established nuclear functions places these molecules in a position where they can provide such coupling independent of the activation status of cytoplasmic mitogen-activated protein kinase (9, 29).

Cisplatin kills cells by activating apoptosis (30); however, the fact that cisplatin failed to activate JNK at concentrations that produced apoptosis in either the HEC59 or HEC59 + ch2 cells suggests that JNK activation is not essential to successful initiation of apoptosis by cisplatin. This is consistent with recent evidence, indicating that JNK is not on the critical pathway by which apoptosis is activated in human breast cancer cells exposed to tumor necrosis factor (31), although contradictory results have been obtained in different laboratories (32, 33). No other information is currently available on the role that JNK activation plays in cisplatin-induced apoptosis.

The mechanism by which the putative detector function of the DNA mismatch repair proteins activates JNK1 also remains to be elucidated. Our results show that in the HCT116 colon carcinoma cells, cisplatin activates JNK1 without any significant effect on the activities of the PAKp65 and SEK1 kinases, suggesting that PAKp65 and SEK1-independent mechanisms for JNK activation exist. This is consistent with the observations of Sánchez et al. (4), who showed that activation of JNK by epidermal growth factor was not accompanied by SEK1 activation in human renal 293 cells. One potential mechanism may be cisplatin-induced dissociation of JNK from inhibitory regulatory proteins such as p21WAF1/CIP1, which acts as a non-enzymatic inhibitor of JNK kinase (34). A second possibility might be cisplatin-induced inhibition of JNK phosphatases, such as MKP-1, which serves as an important regulator of JNK activity in response to the DNA-damaging agents, UVC and methyl methanesulfonate (35).

The activation of c-Abl is not unique to cisplatin-induced cellular injury. Treatment with certain other agents that damage DNA, such as ionizing radiation, mitomycin C, and cytarabine, is also associated with JNK and c-Abl activation (3, 36). Furthermore, recent studies have demonstrated that c-Abl kinase directly contributes to the regulation of growth arrest induced by ionizing radiation (37). Because each of these agents produces a different type of DNA damage, it appears that c-Abl and JNK are involved in the injury response initiated by disparate types of damage that are putatively recognized by different detectors. However, the fact that c-Abl is activated by such a wide variety of cytotoxic agents suggests that it may serve to couple detectors of cellular injury to the apoptotic response.

Very little is known of the role that c-Abl kinase plays in triggering apoptosis. The overexpression of c-Abl inhibits growth by causing cell cycle arrest, and this growth-suppressive activity is functionally similar to that of tumor suppressor genes such as p53 and Rb (38, 39). Other studies have demonstrated that ionizing radiation stimulates

HCT116+ch2  HCT116+ch3  293

Time(h) 0 0.5 1 2 0 0.5 1 2

<+

Anti-phospho-SEK1/MKK4

Fig. 4. SEK1/MKK4 activation in response to cisplatin treatment. Cells were exposed to 25 μM cisplatin for 1 h and were then harvested at the indicated times after the beginning of drug exposure. After lysis, 50 μg of cell protein were electrophoresed in a 10% SDS-polyacrylamide gel, transferred to polyvinylidene difluoride membranes, and analyzed for phospho-SEK1/MKK4 protein. The human 293 kidney cells treated (+) or untreated (−) with 700 mM NaCl and harvested after 30 min were used as a positive control.
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tyrosine-specific protein kinases in human B-lymphocyte precursors, triggering apoptosis and clonogenic cell death (40). Because the c-Abl tyrosine kinase negatively regulates cell growth, its activation in response to radiation-induced injury may provide such coupling between detectors of cellular injury to apoptosis.

This laboratory has previously reported data suggesting that only quite small degrees of resistance (<2-fold) are sufficient for a tumor to lose in vivo responsiveness to cisplatin (41). Using the HCT116 cell system, we have demonstrated recently that in vitro exposure to cisplatin results in rapid enrichment for DNA mismatch repair-deficient cells (42). In addition, MS2H2^-/- and MS2H2^+/- embryonic stem cells grown as xenografts were responsive to treatment with a single LD10 dosage of cisplatin, whereas isogenic MS2H2^-/- tumors were not. These results demonstrate that the degree of cisplatin resistance conferred by loss of DNA mismatch repair is sufficient to produce both enrichment of mismatch repair-deficient cells during treatment in vitro and a large difference in clinical responsiveness in vivo.

Although the results we report here identify the pathways that activate JNK and c-Abl as possible targets for the putative detector function of the DNA mismatch repair proteins, it is important to emphasize that it has not been established that a complex of DNA mismatch repair proteins can actually assemble on cisplatin adducts in vitro, and the concept that such a complex serves as a detector of DNA damage is supported, at the present time, only by the observation that a heterodimer of hMSH2 and hMSH6 can bind to platinated DNA in gel shift assays and that loss of DNA mismatch repair defects in drug resistance rather than hypersensitivity.

REFERENCES


Differential Induction of c-Jun NH$_2$-Terminal Kinase and c-Abl Kinase in DNA Mismatch Repair-proficient and -deficient Cells Exposed to Cisplatin

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