Cancer Chemother Pharmacol (2005) 56: 189–198
DOI 10.1007/s00280-004-0984-x

ORIGINAL ARTICLE

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Human cells deficient in transcription-coupled repair show prolonged activation of the Jun N-terminal kinase and increased sensitivity following cisplatin treatment

Received: 5 April 2004 / Accepted: 10 June 2004 / Published online: 20 April 2005

Abstract *Purpose*: The Jun N-terminal kinases (JNKs) are activated by many biological, physical, and chemical stimuli, including the chemotherapeutic agent cisplatin. The primary pathway that repairs cisplatin-DNA adducts is nucleotide excision repair (NER). Xeroderma pigmentosum (XP) cells from complementation group C (XP-C) are competent in the transcription-coupled repair (TCR) pathway of NER but deficient in global genomic repair (GGR), Cockayne's syndrome (CS) cells are deficient in TCR but have normal GGR, and XP-A cells are deficient in both TCR and GGR. We used NER-deficient human fibroblasts to study the role of DNA damage in the activation of JNK and cell death following cisplatin treatment. *Materials and methods*: JNK-1 activity and clonogenic survival were examined in normal and NER-deficient human fibroblasts following cisplatin treatment. *Results*: Cisplatin induced a transient increase in JNK-1 activity of about tenfold in normal and XP-C fibroblasts, which declined to about two- to threefold 24 h after treatment. In contrast, the activation of JNK-1 was persistent in XP-A and CS fibroblasts at 24 h after treatment and CS cells and XP-A cells, but not XP-C cells, were more sensitive to cisplatin than normal cells. *Conclusions*: These results suggest that a deficiency in the TCR pathway of NER results in amplified and prolonged JNK activation due to persistent damage within the transcribed strand of active genes. Further, it is this amplified and prolonged JNK activation that correlates with cisplatin-induced cell death.

Keywords Cisplatin · JNK · XP · CS · DNA damage · Kinase · MAPK

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Introduction

Cisplatin is a very useful drug in the treatment of many types of cancer. Sadly, intrinsic or acquired drug resistance of the malignancy often limits clinical success of cisplatin-based chemotherapy [32]. The cytotoxicity of cisplatin is thought to result from the local DNA distortion caused when cisplatin binds to genomic DNA [26, 41]. This damage induces apoptosis through signaling pathways that are not well understood [14]. In order to devise novel means of overcoming clinical cisplatin resistance, it is essential to understand these pathways.

The mitogen-activated protein kinase (MAPK) family of signaling molecules, that includes p38, ERK and Jun N-terminal kinases (JNKs), has been shown to be important in determining cell fate following cisplatin treatment [11, 14, 39, 55]. Activation of the JNK enzyme cascade affects gene regulation through a set of transcription factors that includes c-Jun, JunD, ATF-2, Elk-1, and Sap-1 [25]. The phosphorylation of these proteins by JNK family members generally enhances their ability to promote transcription of their target genes including many that could directly affect cisplatin resistance, including metallothionein IIa, topoisomerase I, mdr-1, thymidylate synthase, and glutathione-S transferase [46]. JNK also has multiple roles in regulating the activity of the important proapoptotic factor, p53 [19, 20, 48]. It is clear that the JNK pathway contributes in many different ways to determining the fate of cisplatin-treated cells. In this light, it is not surprising that the activity of the JNK pathway has been shown, in different systems, to correlate with both resistance and sensitivity. Mouse cells with no functional c-Jun are resistant to cisplatin [43], while the expression of a dominant negative Jun impedes the repair of cisplatin-damaged DNA and sensitizes human tumor cells [22, 23, 40]. When JNK activity is blocked in RIF-1 cells by the expression of a dominant negative form of its activator, SEK, the resulting cells are more resistant to cisplatin than the parental line [59]. Other workers have produced strong

evidence that persistent JNK activity is associated with the cisplatin-induced apoptosis in human 293 cells [44]. It is clear that the contribution of JNK activity to determining cell fate following cisplatin treatment is not simply to promote *or* prevent cell death. It is likely that the strength and duration of JNK activity within the context of other cell signals determine its effect.

The persistence of JNK activity appears to be important in determining if it will act to promote survival or cell death [42]. The cessation of the JNK signal has been attributed to the activity of the MAPK phosphatase CL100 (CL100/MKP-1) [44]. Despite a lack of direct evidence, the activation and maintenance of JNK signaling is thought to depend on the presence of cisplatin-induced DNA lesions [38, 39, 40].

Nucleotide excision repair (NER) is the enzymatic pathway that recognizes and repairs most of the DNA damage caused by cisplatin [58]. Patients with xeroderma pigmentosum (XP) or Cockayne's syndrome (CS) harbor homozygous, germ-line mutations in genes that are essential to NER (reviewed in reference 13). These genes and their products have been well characterized, and NER has been recreated in vitro from purified proteins [1]. CS cells have a specific defect that blocks the preferential repair of the transcribed strand of active genes (transcription-coupled repair, TCR) and this defect correlates, in some cases, with the reduced activation of JNK following UV irradiation, suggesting that competent TCR can contribute to JNK activation [17]. Others have presented data suggesting that DNA mismatch repair is necessary for recognition of cisplatin-DNA adducts and induction of JNK as well as the tyrosine kinase c-Abl [38], and the loss of mismatch repair is a characteristic of many cisplatin-resistant tumors [2].

Furuta et al. [21] have reported that cells deficient in the TCR pathway of NER have increased sensitivity to cisplatin. More recently, Mansouri et al. [34] have reported that sustained activation of the JNK/p38 MAPK pathways in response to cisplatin leads to induction of the death inducer Fas ligand (FasL) and cell death in ovarian carcinoma cells. JNK and p38 activation by cisplatin was sustained in the cisplatin-sensitive 2008 ovarian carcinoma cells, but transient in their 2008C13 resistant counterparts. In addition, the inhibition of JNK and p38 activation in the sensitive 2008 cells reduced the induction of FasL and blocked cisplatin-induced apoptosis, whereas upregulation of the JNK/p38 pathways in 2008C13 cells led to increased FasL expression and an increase in cisplatin-induced apoptosis. This suggests that the JNK $\rightarrow$ c-Jun $\rightarrow$ FasL $\rightarrow$ Fas pathway plays an important role in mediating cisplatin-induced apoptosis in ovarian cancer cells, and that the duration of JNK activation is critical in determining whether cells survive or undergo apoptosis.

In addition, it has been reported recently that the cisplatin-sensitive 2008 ovarian carcinoma cell line is deficient in expression of FANCF, one of the proteins involved in the Fanconi anemia-BRCA pathway, compared to the 2008C13 cisplatin-resistant derivative of 2008 cells [51]. The Fanconi anemia-BRCA pathway is involved in cell-cycle checkpoint control and DNA repair following cisplatin treatment, and recent results implicate the FA proteins in repair by homologous recombination [12]. Taken together, these results suggest a link between the sustained activation of the JNK/p38 pathway in 2008 cells following cisplatin [34] and the DNA repair deficiency for cisplatin-induced DNA damage reported for these cells [51].

In the study reported here we used human cells with known defects in NER to investigate the role of cisplatin-induced DNA lesions in the induction of JNK and cell death. We present data which suggest that a deficiency in the TCR pathway of NER results in amplified and prolonged JNK activation due to persistent damage within the transcribed strand of active genes. Further, it is this amplified and prolonged JNK activation that correlates with cisplatin-induced cell death. This new information expands our understanding of the clinical cytotoxicity of this drug that may allow the development of new platinum-based treatment strategies.

Materials and methods

Cells

Primary fibroblasts used in these experiments were obtained from National Institute of General Medical Sciences Repository (Camden, N.J.). All cells were grown as monolayers in supplemented α-MEM (FBS 10%, penicillin 100 mg/ml, streptomycin 100 mg/ml, and amphotericin B 250 ng/ml) in a humidified growth chamber under an atmosphere containing 5% CO_2.

Cisplatin treatment

Cells were treated for 1 h with appropriate concentrations of cisplatin. For JNK assays, cisplatin was dissolved in low-serum α-MEM (FBS 0.1%, penicillin 100 mg/ml, streptomycin 100 mg/ml, and amphotericin B 250 ng/ml), and for colony-forming assays cisplatin was dissolved in supplemented α-MEM. Treatment was stopped by removing the cisplatin solution, washing the monolayer and refeeding the cells with fresh low-serum α-MEM. For colony-forming experiments, all cisplatin treatments and washes were performed in supplemented α-MEM.

Colony-forming assays

Cells were seeded at low density (sufficient for approximately 100 colonies per 100-mm dish) in supplemented α-MEM. Triplicate plates were seeded for each cisplatin dose and 4 h after seeding, cells were

treated with cisplatin. Primary fibroblasts were incubated for 10–14 days. Plates were stained and colonies with more than 30 cells were counted as survivors and relative survival was calculated by comparison with mock-treated controls.

In vitro JNK assay

The method used to determine kinase activity was similar to that used by Derijard et al. [15]. Briefly, cells were seeded (in supplemented α-MEM) into 100-mm plates (5×10^5 per plate). The following day, growth medium was replaced with low-serum α-MEM and cisplatin and UV treatments were started 24 h later. At appropriate times after treatment, cells were collected in cold PBS and lysed in NP-40 lysis buffer (NP-40 0.5%, Tris 50 m*M*, pH 8, NaCl 150 m*M*, EDTA 2 m*M*, NaF 100 m*M*, $NaVO_3$ 1 m*M*, and Na pyrophosphate 10 m*M*) with protease inhibitor cocktail (Boehringer Mannheim). Protein concentrations of cleared lysates were determined using the Micro BCA protein assay (Pierce) and identical quantities of total cellular protein (30–150 μg) were added to protein-G Sepharose beads (Pharmacia) and 1 μg monoclonal antibody to human JNK-1 (Pharmingen). Immunoprecipitation was carried out overnight and immune complexes were washed twice with lysis buffer and twice more with kinase buffer (HEPES 20 m*M*, $MgCl_2$ 20 m*M*, *β*-glycerophosphate 10 m*M*, $NaVO_3$ 0.1 m*M*, *p*-nitrophenylphosphate 2 mg/ml, and DTT 2 m*M*). The immune complexes were then incubated in 30 μl kinase buffer with 5 μCi of [γ-^{32}P]ATP and 2 μg GST-c-Jun(1–79) per sample for 20 min at 30°C. The substrate molecules were then separated by SDS-PAGE and kinase activity in the lysates was determined with phosphorimager technology.

Results

The NER deficiency in XP-A fibroblasts results in cisplatin sensitivity

Fibroblasts from patients with group-A XP (XP-A) are deficient in an early step in NER and are consequently sensitive to DNA-damaging agents such as cisplatin. Figure 1 and Table 1 show the results obtained for colony survival following cisplatin treatment of XP-A fibroblasts compared to three different normal strains. It can be seen that the XP-A fibroblasts were significantly more sensitive to cisplatin than normal strains.

The activation of JNK-1 following cisplatin exposure is greater in NER-deficient, XP-A fibroblasts

Cisplatin activates JNK-1 in many cell types [29, 39, 42] and has been shown in some situations to correlate with cell death [11, 23, 40, 42]. We studied the role of cisplatin-DNA adducts in the activation of JNK-1 by comparing normal fibroblasts with fibroblasts with no detectable NER. Extracts were collected from XP-A fibroblasts (XP12BE) and repair-proficient fibroblasts (GM9503) 24 h after exposure to different concentrations of cisplatin. JNK-1 was immunoprecipitated from these extracts and kinase activity was assessed. The results are shown in Fig. 2. Relative JNK-1 activity increased to much greater levels in XP-A cells than in normal cells. The increase in JNK-1 activity was dose-dependent in XP-A fibroblasts and increased tenfold in cells exposed to 400 μ*M* cisplatin compared with untreated cells. There

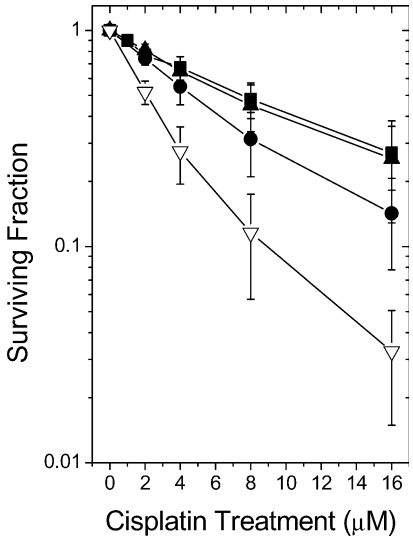

Fig. 1 The colony-forming ability of cisplatin-treated XP-A fibroblasts is greatly reduced compared to normal fibroblasts. The relative survival of an XP-A cell strain (XP12BE, *open triangles*) is shown relative to normal fibroblasts GM9503 (*filled squares*), GM969 (*filled circles*) and GM38A (*filled triangles*). The data presented are the average ± SEM of four experiments each with triplicate plates for each dose point

Table 1 Cisplatin sensitivity of normal and NER deficient human fibroblasts. The cisplatin concentration resulting in 37% survival (D_{37}) was determined for each fibroblast strain in several independent experiments. The mean ± SEM D_{37} values were calculated for each fibroblast strain for the number of independent experiments as indicated in column 3

Fibroblast strain	D_{37} ± SEM (μ*M* cisplatin)	Number of experiments
Normal		
GM9503	9.58 ± 2.12	4
GM38A	7.53 ± 0.71	3
GM969C	7.80 ± 2.14	4
Average	8.37 ± 1.05	11
XP-C		
XP2BE	7.09 ± 1.57	5
XP1PW	7.30 ± 1.53	2
XP1BE	5.48 ± 1.32	4
Average	6.54 ± 0.86	11
XP-A: XP12BE	3.46 ± 0.84*	4
CS-B: CS1AN	2.12 ± 0.23*	4

*$P < 0.05$, vs the average value for the normal strains

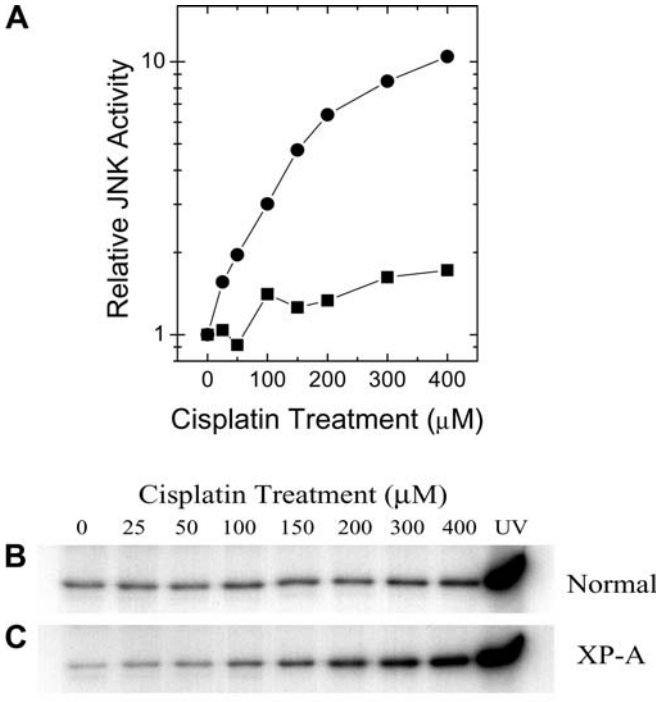

Fig. 2 Activation of JNK-1 is greater following cisplatin exposure in XP-A fibroblasts than in normal fibroblasts. Representative results from a single experiment are shown. **A** Relative activation of JNK-1 24 h following a 1-h treatment at the doses shown in an XP-A fibroblast strain (XP12BE, *circles*) and normal fibroblasts (GM9503, *squares*). Values were derived using phosphorimager analysis of the gels shown in **B** (GM9503) and **C** (XP12BE)

was only a modest increase, of less than twofold, in similarly treated normal fibroblasts (Fig. 2). In order to ensure that these observations were not specific to the two cell strains examined, we went on to study a number of normal and repair-deficient XP-A fibroblast strains. Each of the three primary fibroblast strains from XP-A individuals (XP12BE, XP1WI and XP4LO) demonstrated elevated activation of JNK-1 compared to four different repair-proficient strains (Fig. 3). While the fold increase in JNK-1 activity 24 h after cisplatin treatment varied among the XP-A strains, each of the strains showed significantly greater activation following exposure to 400 μ*M* cisplatin (6- to 13-fold) than each of the repair-proficient strains (1.5- to 2-fold). We conclude that JNK-1 activation following cisplatin treatment is greater in cells that are unable to repair cisplatin-damaged DNA. This strongly suggests that the presence of cisplatin-DNA lesions is an important signal in the induction of JNK-1 activation by cisplatin.

NER deficiency results in persistent JNK-1 activity

We continued our investigation by studying the time-course of JNK-1 activation following cisplatin exposure in primary fibroblasts. We exposed fibroblasts to a 400 μ*M* cisplatin solution for 1 h and assessed JNK-1 activity in lysates collected at several subsequent times.

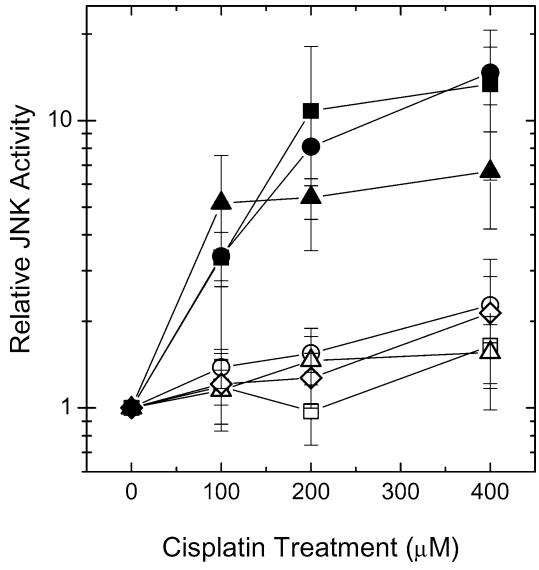

Fig. 3 Activation of JNK-1 in XP-A and normal fibroblasts. Relative activation of JNK-1 24 h following a 1-h treatment at the doses shown in XP-A cells (XP12BE, *filled circles*; XP1WI, *filled squares*; XP4LO, *filled triangles*) and in repair-proficient cells (GM9503, *open squares*; GM969, *open circles*; GM8399, *open diamonds*; GM38A, *open triangles*). The data presented are the means ± SEM of two to seven experiments

JNK-1 activity increased similarly in normal and XP-A fibroblasts for about 5 h after the start of treatment (Fig. 4). After about 5 h, however, JNK-1 activity in repair-proficient fibroblasts begins to decrease while JNK-1 activity in the XP-A strain remained about ten-fold higher for at least 30 h after treatment. These experiments were also performed with other XP-A and normal strains (XP4LO and GM969, respectively) and similar results were found (data not shown). These data

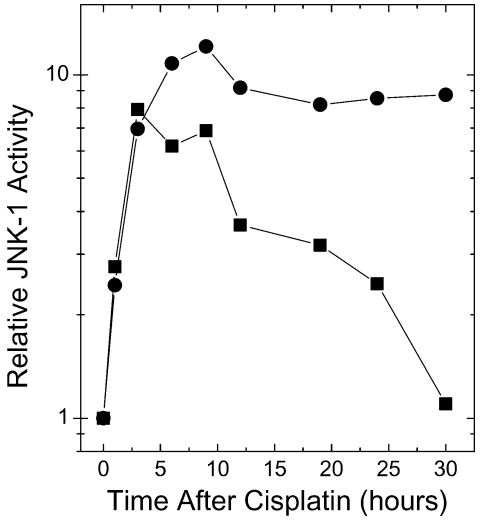

Fig. 4 Activation of JNK-1 is more persistent in XP-A fibroblasts than in repair-proficient fibroblasts. Representative results of a single experiment are shown determining the relative activity of JNK-1 following exposure to 400 μ*M* cisplatin for 1 h in XP-A (XP12BE, *circles*) and normal fibroblasts (GM9503, *squares*)

are consistent with those in Fig. 2 where only a modest increase in JNK-1 activity was detected in normal cells 24 h after treatment. Taken together these data suggest that a deficiency in NER results in amplified and prolonged JNK activation due to persistent unrepaired cisplatin-induced DNA damage.

TCR-deficient but not GGR-deficient fibroblasts are sensitive to cisplatin

NER-deficient cells that have a specific defect in TCR, the preferential repair of transcriptionally active DNA, can be derived from patients with CS [53]. In vitro studies have shown that there are two complementation groups (CS-A and CS-B) [50]. In contrast, within the heterogeneous disorder, XP, there is a complementation group (XP-C) that is competent in the preferential repair of active genes (TCR) while being deficient in global genomic repair (GGR) and unable to repair lesions throughout inactive regions of the genome [27]. We have taken advantage of these well-characterized cellular phenotypes to study the importance of these two NER sub-pathways in the cellular response to cisplatin. We treated XP-C and CS-B cells with cisplatin and compared the clonogenic survival to that in similarly treated repair-proficient cells (Fig. 5). CS-B cells were sensitive to cisplatin, suggesting that persistent lesions within transcribed DNA result in cell death. In contrast, the XP-C cells showed no marked sensitivity to cisplatin compared to the normal strains.

TCR-deficient but not GGR-deficient fibroblasts show prolonged activation of JNK-1 following cisplatin exposure

Since our results with NER-deficient XP-A compared to NER-proficient normal cells suggested that persistent cisplatin-induced DNA damage is important in the maintenance of JNK activation, we were interested in determining if the location of the DNA damage is important in maintaining JNK activation. We assessed JNK-1 activity following cisplatin treatment in primary fibroblasts from patients with CS and XP-C. JNK-1 activity was elevated in CS-A (CS3BE) and CS-B (CS1AN) fibroblasts 24 h after cisplatin treatment compared to similarly treated repair-proficient fibroblasts (Fig. 6), suggesting that persistent cisplatin lesions within the transcribed strand of active genes causes the maintenance of high levels of JNK-1 activity. However, the fold induction of JNK-1 was less in these CS strains than in the XP-A strains examined. This observation may be attributable to some residual NER in CS cells. That is, CS cells repair damage in the genome without preference for active regions and this slow repair of active sequences may be sufficient to diminish the signal to maintain JNK-1 activity 24 h after exposure.

XP-C cells can efficiently repair strands of DNA actively being transcribed but are unable to recognize lesions in transcriptionally inactive DNA. We used these cells to further examine the role of persistent cisplatin-DNA adducts in the activation of JNK-1. At 24 h after being exposed to cisplatin, the fold increases in JNK-1 activity for the two XP-C strains (XP1BE and XP1PW) were similar to the increases seen in normal fibroblasts (Fig. 7). While these data suggest that the removal of cisplatin-DNA lesions in the bulk of the genome does not affect the activation of JNK-1, it is also possible that the defect in XP-C precludes JNK-1 signaling. That is, the inability to recognize lesions in the bulk of the genome may inhibit the mechanism that triggers JNK-1 activity. To ensure that cisplatin does induce JNK-1 in XP-C fibroblasts we exposed XP1PW fibroblasts to 400 μM and measured JNK-1 activation at various times (Fig. 8). Kinase activity reached a maximum of

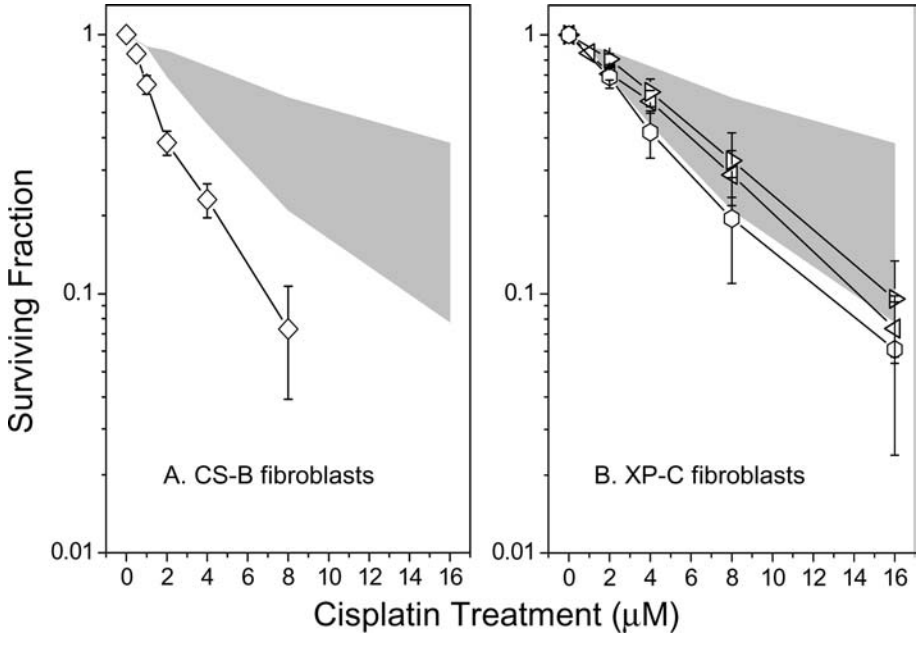

Fig. 5 Cells with defective TCR are sensitive to cisplatin while cells deficient in GGR show cisplatin sensitivity similar to normal cells. The relative clonogenic survival of CS-B fibroblasts (*panel A*: CS1AN, *diamonds*) and XP-C fibroblasts (*panel B*: XP2BE, *right triangles*; XP1PW, *left triangles*; XP1BE, *open circles*) are shown. The data presented are the means ± SEM of two to five experiments of three replicates as indicated in Table 1. Some error bars lie within the symbols. Shaded areas represent the range in sensitivity for the three normal strains as shown in Fig. 1

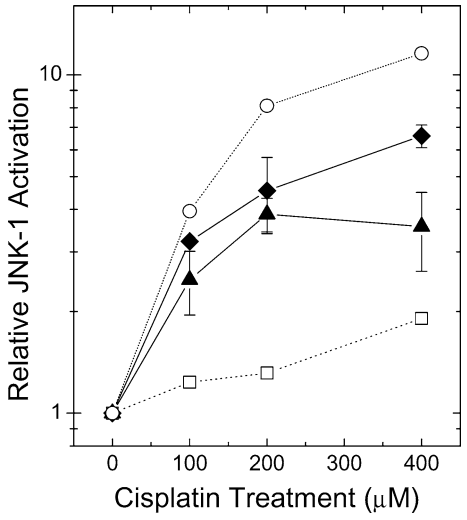

Fig. 6 Activation of JNK-1 in CS and normal fibroblasts. Relative activation of JNK-1 24 h following a 1-h treatment at the doses shown in CS-A fibroblasts (CS3BE, *filled diamonds*) and CS-B fibroblasts (CS1AN, *filled triangles*). The data presented are the means ± SEM of two experiments. For comparison, the mean results from all XP-A (*open circles*) and normal strains (*open squares*) are included

about tenfold around 6 h after exposure and gradually decreased to about threefold 24 h after exposure, similar to that found for normal fibroblast strains. The time period of this decrease corresponds to the removal of cisplatin-DNA lesions [3, 38] and further supports our suggestion that it is the persistence of adducts in the transcribed strand of active genes that maintains JNK activity.

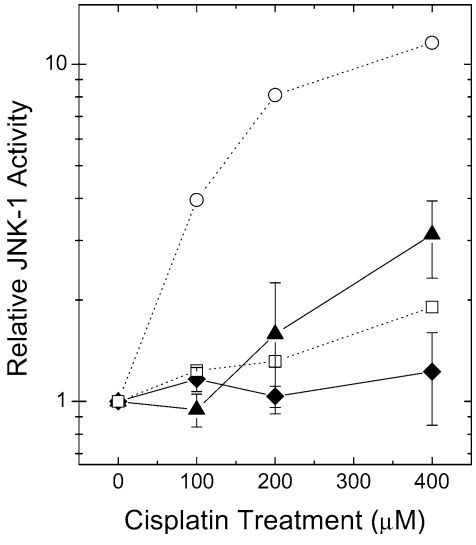

Fig. 7 Cisplatin-induced activation of JNK-1 in XP-C fibroblasts is similar to that in normal fibroblasts. Relative JNK-1 activity in lysates collected 24 h after exposing XP-C fibroblasts (XP1PW, *filled triangles*; XP1BE, *filled diamonds*) to the indicated doses of cisplatin. The data presented are the means ± SEM of two experiments. For comparison, the mean results from all XP-A (*open circles*) and normal strains (*open squares*) are included

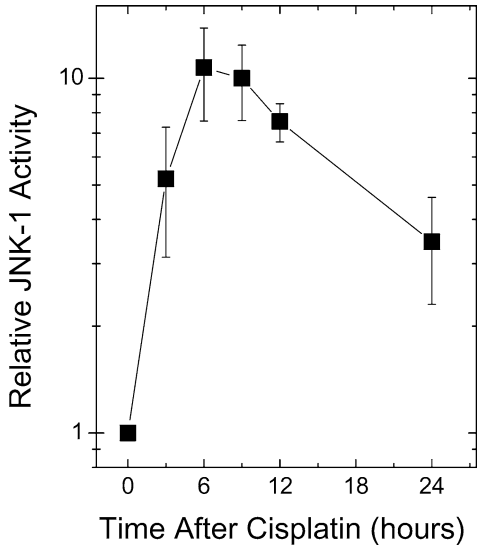

Fig. 8 JNK-1 activity reaches a maximum 6 h after cisplatin treatment in XP-C fibroblasts. JNK-1 activity is shown in cisplatin-treated cells relative to untreated cells. XP-C fibroblasts (XP1PW) were exposed to 400 μ*M* cisplatin for 1 h and lysates were collected at the times shown and assayed for JNK-1 activity. The data presented are the means ± SEM of three experiments

Discussion

The JNK-1 molecule responds to many genotoxic stresses and many of its upstream activators have been well characterized [47]. What remains unknown are the events that translate these physical or chemical stresses into biological signals. Cisplatin, like UV light, causes a spectrum of DNA lesions that block transcription and DNA replication and most of this DNA damage is repaired by NER. The activation of the JNK pathway by UV light is, at least primarily, the result of cytosolic events involving molecules at the plasma membrane and appears to be independent of DNA damage [16]. In contrast, increased JNK activity following cisplatin treatment is thought to result from DNA damage [39, 40]. Until now, the importance of cisplatin-DNA adducts in activating JNK has been inferred from comparison with the less cytotoxic stereoisomer, transplatin. Transplatin does not detectably activate JNK and this is thought to be because transplatin-DNA adducts are quickly repaired [10, 39, 40]. However, if cisplatin-induced JNK activation is mediated by interactions with other biomolecules such as lipid or protein, then transplatin could also be expected to interact differently with these other targets, failing to activate JNK. We have tested this possibility by comparing JNK activation in primary fibroblasts with differing capacities to repair DNA. XP-A fibroblasts are sensitive to cisplatin and have a substantial deficiency in NER. In these NER-deficient cells, cisplatin adducts persist in the genome much longer than in repair-proficient cells.

JNK activity in the fibroblasts tested reached a maximum 4–6 h after cisplatin treatment. This coincides

with the completion of the two-step cisplatin-DNA reaction [49]. This is much slower than UV-induced activation that reaches a maximum only a few minutes after exposure in all cells tested (our unpublished results; [15]). There was a greater increase in JNK activity 24 h after cisplatin exposure in XP-A compared with normal fibroblasts. This difference was evident because there was a decrease in JNK activity in normal fibroblasts that began 6 h after treatment and this time period is that over which most cisplatin adducts are repaired in normal cells [3, 18]. Taken together, these data strongly support the notion that the DNA adducts per se promote JNK function.

XP is a heterogeneous disorder that encompasses seven known complementation groups that have defects in some aspect of NER. XP-C cells retain the ability to efficiently repair the transcribed strand of active genes (TCR) but are unable to recognize and repair DNA damage to the rest of the genome (GGR) [54]. Cisplatin exposure caused only a transient activation of JNK in XP-C fibroblasts that was similar to the activation seen in repair-proficient cells. Since the cisplatin lesions in untranscribed regions of the genome remained unrepaired in XP-C fibroblasts, we conclude that these lesions are not important in the maintenance of the JNK signal. These data are consistent with a number of results that have shown the importance of intragenic DNA damage in the activation of cell signals [56]. XP-A and CS fibroblasts have been shown to have a lower threshold for UV-induced accumulation of several genes than TCR-proficient XP-C or normal fibroblasts [4, 35]. Interestingly, a number of these gene products (for example, p53 and metallothionein IIa) are affected by JNK activity directly through post-translational modification, gene promotion, or both.

p53 is a commonly altered gene in human malignancy [24], and the protein affects cell physiology through a barrage of functions. p53 mediates apoptosis, cell cycle progression, and DNA repair, and each of these is thought to be important in cisplatin resistance [14, 32, 36]. Activity of the MAPK family is essential to regulating p53 in the stress response. A number of amino acid residues within the p53 gene are targets for phosphorylation by MAPKs and both p38 and JNK have been shown to be important regulators of p53. In mouse cells, JNK (through c-Jun) represses the p53 gene [48], can stabilize and activate p53 in response to stress [9, 19], and targets p53 for ubiquitin-mediated degradation in nonstressed cells [20]. Buschmann et al. [9] have recently shown that cisplatin fails to activate JNK in normal human fibroblasts 1 h after exposure to 100 μM. This is consistent with our results showing that JNK activation in human fibroblasts only reaches a maximum 4–6 h after treatment. It remains to be shown that cisplatin-induced JNK activation causes the specific phosphorylation (Thr-81) that is essential to the stabilization and activation of p53 following UV irradiation and other cell stress.

Kinase activity of p38 has been shown to promote cisplatin- and UV-induced activation of p53 and apoptosis [7, 45]. Other workers, however, have shown that p38 is not activated by cisplatin in human tumor cells [39]. Since tertiary conformation appears to affect the ability of p53 to accept phosphorylation [7], it is likely that the order and duration of the different MAPK signals that converge at p53 determine its role in response to genotoxic stress. This complexity may explain how p53 activation, despite being a predominantly proapoptotic factor, has been shown in some systems to be associated with cisplatin resistance [6, 57]. Clearly, p53 is an important effector of JNK and the elucidation of this interaction will be essential in understanding the role of JNK in clinical cisplatin resistance.

Expression of metallothionein confers resistance to cisplatin by binding to the drug, reducing the effective intracellular concentration [28]. Expression of the human metallothionein IIa gene (hMTIIa) is controlled, in part, by JNK activity through the AP-1 sequence encoded in its promoter [46]. Some cisplatin-resistant cancer cells have been shown recently to have greater expression of a gene that is driven by the MTIIa promoter than a cisplatin-sensitive counterpart [52]. It may be that MTIIa expression as part of an early response is important in the protective actions of JNK within the early hours after cisplatin exposure.

One group has reported a defect in the UV-induced activation of the JNK pathway in CS-B cells suggesting that the CSB gene product is required for the detection of DNA lesions [17]. We have found no evidence of subnormal JNK induction following exposure to cisplatin or UV radiation in CS-B fibroblasts (data not shown). It may be that some defect in the CS cells is accountable for lower levels of induced kinase activity compared with the XP-A cells studied. However, we think this difference is more likely caused by the nonpreferential NER in CS cells that would be much slower than the intact TCR in XP-C or normal fibroblasts

The nature of the signal that is initiated at the transcription-blocking lesions is not well understood but is thought to involve the stalled RNA polymerase II (polII) [4, 31]. Clearly, the initiation of these cell signals does not require the ubiquitination or the degradation of polII since these actions are defective in CS cells while the ability to activate the JNK cascade remains intact [5, 37].

Cisplatin exposure mediates the kinase activity of JNK through at least two separate biochemical signals [44]. The first is the signal to activate JNK and this signal appears to be dependent on the known upstream activators, SEK1 and MEKK1 [43]. The second signal is mediated by the MAPK phosphatase, CL100/MKP-1, that has been shown to decrease cisplatin-induced JNK activity [44]. Our results indicate that this phosphatase-mediated down-regulation of JNK is forestalled by the presence of cisplatin-DNA adducts.

The results presented here show a correlation between sustained activation of the JNK pathway and cisplatin sensitivity. This is consistent with the recent report of Mansouri et al. [34] who report a sustained

activation of the JNK/p38 MAPK pathway in the cisplatin-sensitive 2008 ovarian carcinoma cells compared to a transient activation of the JNK/p38 MAPK pathway in their 2008C13 resistant counterparts. The sustained activation of the JNK/p38 MAPK pathways in response to cisplatin leads to induction of the death-inducer FasL and cell death in ovarian carcinoma cells [34]. In addition, the inhibition of JNK and p38 activation in the sensitive 2008 cells reduced the induction of FasL and blocked cisplatin-induced apoptosis, whereas upregulation of the JNK/p38 pathways in 2008C13 cells led to increased FasL expression and an increase in cisplatin-induced apoptosis. This suggests that the JNK → c-Jun → FasL → Fas pathway plays an important role in mediating cisplatin-induced apoptosis in ovarian cancer cells, and that the duration of JNK activation is critical in determining whether cells survive or undergo apoptosis.

The role of JNK in determining clinical success with cisplatin has not been defined. The in vitro technique for assessing cell survival that is most predictive of clinical sensitivity, at least for ionizing radiation, is the colony-forming assay [33]. We employed this assay here to investigate the effect of different DNA repair defects on the survival of cisplatin-treated cells. The sensitivity of CS-B fibroblasts compared with XP-C and normal fibroblasts suggests that, in addition to maintaining JNK activity, persistent DNA lesions result in cell death. This correlation is consistent with the conclusions of others who have reported that persistent JNK activation promotes apoptosis in human cells [34, 42]. It may be transient JNK activation, on the other hand, that is important in the induction of a number of protective mechanisms such as cell cycle arrest, thiol expression, or DNA repair. These multiple roles for JNK, within a complex response to cisplatin, could explain some seemingly contradictory observations. For example, the expression of a dominant interfering c-Jun construct in human tumor cells has been shown to enhance p53-mediated apoptosis, impede NER, and cause cisplatin sensitivity [22, 23, 40]. In contrast, NIH 3T3 cells with a targeted deletion of c-Jun, are resistant to cisplatin [43]. This deletion precludes some downstream signaling, presumably without affecting the immediate early (IE) functions of JNK, suggesting that the IE actions of JNK are protective. This model is consistent with a recent report that the phosphatase activity of CL100/MKP-1 protects human 293T cells from apoptosis by dephosphorylation of JNK [44].

Cisplatin is a synthetic compound that produces a unique spectrum of DNA lesions. For this reason, the cellular response to the drug is surely an incidental combination of mechanisms that have been evolutionarily designed for other purposes. This is evident in the slow repair and relative cytotoxicity of cisplatin-DNA adducts [18, 58] compared with UV-induced DNA lesions. Within this context, it is not surprising that many different cell mechanisms have apparently contradictory roles in cisplatin resistance. The results of the present study indicate an increased cisplatin sensitivity as measured by clonogenic survival in TCR-deficient XP-A and CS fibroblasts compared to TCR-proficient XP-C and normal fibroblasts. A similar result has been reported using a 72-h cell proliferation assay as a measure of cisplatin sensitivity [21]. The normal cisplatin sensitivity of the GGR-deficient XP-C cells is surprising since we have shown that XP-C cells have a deficiency in the repair of cisplatin-damaged DNA [8]. This suggests that it is the signal of persistent damage in active genes and the persistent activation of JNK that leads to cell death in TCR-deficient CS and XP cells, whereas the transient expression of JNK in TCR-proficient XP-C cells results in cell survival even in the presence of unrepaired DNA lesions in transcriptionally inactive DNA.

The results presented here suggest a role for lesions within transcriptionally active genomic DNA in the prolonged activation of the MAPK, JNK-1. This prolonged activation is associated with reduced survival of fibroblasts that are compromised in their ability to perform TCR. While the mechanisms are not well understood, it is clear that JNK is an important part of the elaborate mechanism that governs the cellular "decision" to live or die in response to cisplatin exposure. These mechanisms must be understood if the clinical use of cisplatin is to be modified to maximize the efficacy while minimizing the morbidity of platinum-based chemotherapy.

Acknowledgements This work was supported by the National Cancer Institute of Canada with funds from the Canadian Cancer Society.

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