Paclitaxel (Taxol)-induced Gene Expression and Cell Death Are Both Mediated by the Activation of c-Jun NH$_2$-terminal Kinase (JNK/SAPK)*

Li-Fen Lee‡§, Guoxuan Li§, Dennis J. Templeton‡, and Jenny P.-Y. Ting†∗∗

From the Departments of Biology, Microbiology and Immunology, and the Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7295, and the Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106

Paclitaxel (Taxol) is a novel anti-cancer drug that has shown efficacy toward several malignant tumors, particularly ovarian tumors. We reported previously that paclitaxel can induce interleukin (IL)-8 promoter activation in subgroups of ovarian cancer through the activation of both AP-1 and nuclear factor κB. Further analysis of paclitaxel analogs indicates that the degree of IL-8 induction by analysis correlates with the extent of cell death; however, IL-8 itself is not the cause of cell death. This suggests that pathways that lead to IL-8 and cell death may overlap, although IL-8 per se does not kill tumor cells. To decipher the upstream signals for paclitaxel-induced transcriptional activation and cell death, we studied the involvement of protein kinases that lead to the activation of AP-1, specifically the c-Jun NH$_2$-terminal kinase (JNK1), p38, and the extracellular signal-regulated kinase 1 (ERK1). The role of IκB in paclitaxel-induced cell death was also analyzed. Paclitaxel activated JNK, and to a lesser degree p38, but not ERK1. Paclitaxel-induced IL-8 promoter activation was inhibited by dominant-inhibitory mutants of JNK, p38, and the super-repressor form of IκBα, but not by dominant-inhibitory forms of ERK1. Dominant-inhibitory mutants of JNK1 also greatly reduced paclitaxel-induced cell death, and the kinetics of JNK induction was closely followed by DNA fragmentation. These results indicate (i) that paclitaxel activates the JNK signaling pathway and (ii) that JNK activation is a common point of paclitaxel-induced gene induction and cell death.

Paclitaxel (Taxol) is a new generation of chemotherapeutic drug that is effective against malignant non-small cell lung (1, 2), prostate (3, 4), and breast cancer (5), with the most encouraging effects observed in cancer chemotherapy-refractory ovarian cancer (6). Paclitaxel exhibited significant antitumor activity against human ovarian cancer in a nude mouse model (6). Furthermore, paclitaxel significantly inhibited the angiogenic effects observed in cancer chemotherapy-refractory ovarian tumors has been observed previously (18). It is important to determine if the same signals that induce IL-8 gene induction also induce cell killing.

Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases that mediate numerous types of extracellular stimuli (19, 20). The ERK member of MAPK is activated by growth factor via a Ras-dependent signal transduction pathway (21). ERK can phosphorylate and activate various transcription factors, including c-Myc and TCF/Elk1 (22). In contrast, the JNK (JNK1 and JNK2) members of MAPKs, also designated stress-activated protein kinases (SAPKs), can be activated by proinflammatory cytokines (23–25) and environmental stress such as UV light (26, 27), γ-irradiation (28), heat shock, osmotic shock (28), shear stress (29), growth factor withdrawal (30), ceramide (31), and protein synthesis inhibitor (23). The activation of the JNK cascade is also observed in cells stimulated by various mitogenic factors such as growth factors, onoregic Ras, phorbol ester, and T-cell activation signaling (32, 33). Like MAPK, JNK activation requires phosphorylation.

* This work was supported by National Institutes of Health Grant AI 41751 and a grant from the Lineberger Comprehensive Cancer Center. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295. Tel.: 919-966-5538; Fax: 919-966-3015; E-mail: panyun@med.unc.edu.

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One of the effects of paclitaxel is to alter gene expression. In murine macrophages, paclitaxel can induce the expression of a series of lipopolysaccharide-inducible cytokines, such as IL-1α, IL-1β, TNF-α, and interferon-inducible protein 10 (9–12, 16). In human monocytes, paclitaxel also induces cytokine synthesis (17). Most pertinent to this report, paclitaxel can induce IL-8 gene expression at the transcriptional levels in subsets of human ovarian cancer lines (14). This induction is mediated by the activation of NF-κB and AP-1 transcription factors, which bind to cognate sites in the IL-8 promoter. Gel shift assays show that paclitaxel caused a marked increase in protein binding to AP-1 and NF-κB cognate sequences in paclitaxel-responsive cells but not in nonresponsive cells (15). However, little is known about the upstream signaling events that lead to the activation of these transcription factors in response to paclitaxel. The present study identifies upstream signals that are activated by paclitaxel and mediate its effects. Identification of upstream signals involved in IL-8 gene induction may have broad ramifications because a tight correlation between the ability of paclitaxel analogs to induce IL-8 and cell death among ovarian tumors has been observed previously (18). It is important to determine if the same signals that induce IL-8 gene induction also induce cell killing.

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at two conserved residues, threonine and tyrosine, by the MAP kinase kinase SEK1/MKK4/JNK kinase (34). The latter is in turn phosphorylated by upstream MAPK kinase kinase 1 (MEKK1) (32, 35). The JNK kinase cascade was shown to be a common pathway involved in cell proliferation and stress-response signaling. There are at least three major transcription factors that can be phosphorylated by JNK: c-Jun (36), ATF-2 (37), and TCF/E1A1 (38).

In this report we demonstrate that JNK is strongly activated by paclitaxel, a chemotherapeutic agent. The activation of JNK occurs in a dose- and time-dependent manner. The inhibition of JNK activity greatly reduced paclitaxel-induced IL-8 gene expression and cell death, whereas the inhibition of p38 produced a smaller but noticeable effect. These results show the involvement of JNK and p38 in paclitaxel-induced IL-8 transcription activation and in mediating cell death, both of which have anti-tumor affects.

MATERIALS AND METHODS

Cells, Antibodies, and Reagents—The human ovarian cancer cell line OVCA 420 (a gift from Dr. Robert Bast, Jr., M. D. Anderson, Houston, TX) was maintained as monolayer cultures in Dulbecco's modified Eagles's medium supplemented with 10% fetal bovine serum. The derivation of these cells has been described previously (39). Rabbit anti-JNK1, anti-ERK1, and anti-p38 antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The monoclonal antibody M2 was obtained from IBI-Kodak (New Haven, CT). Anti-c-Jun and anti-c-Fos were purchased from Santa Cruz Biotechnology. Myelin basic protein (Bio-Rad) was prepared at a concentration of 5 μg/ml in kinase reaction buffer.

Plasmids and Recombinant Proteins—GST-c-Jun (1–79) linked to Sepharose beads was used as the substrate (40). The pDNA3-FLAG-JNK1(AFP) and pCMV-FLAG-p38(AGF) were kindly provided by Dr. R. J. Davis (University of Massachusetts, Worcester, MA). pCMV Ie30 and control empty vector (pCMV4) were generous gifts from Dr. D. A. Brenner (University of North Carolina, Chapel Hill) (42, 43). pCMVERK1 was a generous gift from Dr. R. J. Davis (University of Massachusetts, Worcester, MA). Transfections were performed in duplicate. 6 h after transfection, the cell medium was replaced with fresh complete medium for 12 h, and then the cells were treated with 30 μM paclitaxel or MeSO. After 24 h of treatment, cells were harvested, washed, and fixed in 0.5% glutaraldehyde in PBS. These cells were washed twice with PBS, resuspended in staining solution containing PBS (pH 7.4), 1 mM MgCl2, 10 mM KFe(CN)6, 10 mM K3Fe(CN)6, and 1 mM X-gal (added just before use) for 1–3 h, and washed twice with PBS. The β-galactosidase-positive cells in each well were counted. Cell survival was determined as the number of blue cells in paclitaxel-treated group/number of blue cells in MeSO-treated group × 100.

Transient Transfection and LacZ Cell Death Assay—OVCA 420 cells were plated 24 h before transfection at a density of 2 × 10⁶ cells/well in a 12-well plate. Cells were cotransfected with the pCMV-βgal (0.1 μg) plasmid, which expresses β-galactosidase, and the expression plasmid for the superpressor Ie30, dominant-inhibitory mutant kinase (1 μg), or a control vector using the SuperFect transfection reagent (Qiagen, Valencia, CA). Transfections were performed in duplicate. 6 h after transfection, the cell medium was replaced with fresh complete medium for 12 h, and then the cells were treated with 30 μM paclitaxel or MeSO. After 24 h of treatment, cells were harvested, washed, and fixed in 0.5% glutaraldehyde in PBS. These cells were washed twice with PBS, resuspended in staining solution containing PBS (pH 7.4), 1 mM MgCl2, 10 mM KFe(CN)6, 10 mM K3Fe(CN)6, and 1 mM X-gal (added just before use) for 1–3 h, and washed twice with PBS. The β-galactosidase-positive cells in each well were counted. Cell survival was determined as the number of blue cells in paclitaxel-treated group/number of blue cells in MeSO-treated group × 100.

Solid Phase and Immunocomplex Kinase Assay—After paclitaxel or MeSO treatment, the cells were washed with ice-cold PBS and lysed with 900 μl of low salt buffer (150 mM NaCl, 20 mM Tris, pH 7.5, 5 mM EDTA, 1% Triton X-100, 50 mM NaF, 10% glycerol, 1 mM Na3VO4, 4 μM aprotinin, 20 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin). The lysate was mixed in 4°C for 15 min and centrifuged at 10,000 × g for 15 min at 4 °C. The supernatant was transferred to a clean tube, and 50 μg of protein was incubated with 10 μg of GST-c-Jun (1–79) beads for 2 h at 4°C. The beads were then washed twice with the lysis buffer and twice with the kinase assay buffer (1× kinase assay buffer: 20 mM HEPES, pH 7.4, 20 mM MgCl2, 20 mM β-glycerophosphate containing 20 mM p-nitrophenyl phosphate, 0.1 mM Na3VO4, 2 mM dithiothreitol). Kinase reactions were initiated by the addition of 5 μl of 10-fold diluted p1850 (100 μg protein) and 3 μl of 100 mM ATP/10 μl (2× ATP) (3,000 Ci/mmol), and H2O2 to a final volume of 35 μl, incubated for 30 min at 37 °C, and then cooled to 0 °C. 5 μl of 3× SDS sample buffer. Samples were boiled for 5 min, centrifuged at 12,500 rpm for 5 min, and then the supernatant was resolved by 10% SDS-polyacrylamide gel electrophoresis. The gel was stained with Coomassie Blue and then dried. The phosphorylation of GST-c-Jun was visualized by autoradiography. Immunocomplex kinase assays were carried out as described (54) with some modifications. MAPKs were precipitated by incubation with protein A-agarose beads (Santa Cruz) and specific antibodies (e.g. anti-JNK1, anti-p38, or anti-ERK1 antibody) in lysis buffer (1% SDS, 0.5% sodium deoxycholate, 0.1% sodium phosphate, 0.1% sodium deoxycholate, 0.1% SDS, 15 mM NaCl, 0.01 mM sodium phosphate, pH 7.2, 2 mM EDTA, 50 mM NaF, 0.2 mM Na3VO4, 100 units/ml aprotinin) for 4 h at 4 °C on a rocket platform. Immunoprecipitates were collected by centrifugation at 2,500 rpm for 5 min at 4 °C. The precipitates were washed twice with lysis buffer and twice with kinase assay buffer (50 mM HEPES, pH 7.4, 1 mM EDTA, 0.01% Brij 35, 0.1 mg/ml bovine serum albumin, 0.1% β-mercaptoethanol, 0.15 mM NaCl) and then mixed with 5 μg of the indicated substrates and 10 μl of ATP mix (930 μM of kinase buffer, 6 μl of 50 mM ATP, 7 μl of 2.0 mM MgCl2, and 3 μl of 2× ATP) at 37 °C for 30 min. The reactions were terminated with SDS sample buffer and boiled for 5 min. The supernatants were resolved by 10% SDS-polyacrylamide gel electrophoresis.

Immunoprecipitation Kinase Assays of p38—The p38 assay was performed according to the manufacturer’s instruction for the p38 MAPK assay (New England BioLabs, Beverly, MA). Briefly, after immunoprecipitation...
RESULTS

Paclitaxel Activates AP-1 Binding Activity—In a previous report, we showed that paclitaxel activates DNA binding through the AP-1 factor in human OVCA 420 cells (15). The results are confirmed as shown in Fig. 1, lanes 1 and 2. Paclitaxel-induced binding was specifically competed by an excess of unlabeled wild type (w) DNA fragment or a mutant (m) DNA fragment. For the supershift study, 1 μl of antibody, either normal unimmunized serum (lane 5), anti-c-Jun (lane 6), or anti-c-Fos (lane 7) was incubated with nuclear extracts and reaction buffer on ice for 1 h before the addition of the radiolabeled probe. The arrow indicates the position of the AP-1 DNA-protein complex.

Precipitation of p38 MAPK with anti-p38 antibody (1:50 to 1:100 dilution), the immunocomplex was incubated with ATF-2 and 200 μM cold ATP in kinase buffer. Kinase reactions were incubated at 30 °C for 15 min and terminated with SDS sample buffer. The products were resolved by 10% SDS-polyacrylamide gel electrophoresis. Samples were analyzed by a Western blot using a phospho-specific ATF-2 antibody (1:1,000 dilution). Protein bands on immunoblots were visualized by enhanced chemical luminescence (Amersham Pharmacia Biotech).

JNK1 Is Activated by Paclitaxel—Because paclitaxel activates c-Jun but JNK1 activates and phosphorylates c-Jun, the effect of paclitaxel on JNK activation is worthy of investigation. A kinetics analysis of kinase activation by paclitaxel was first performed. The activities of JNK, ERK, and p38 in the cell lysates were determined by an immunocomplex kinase assay as described under “Materials and Methods.” As shown in Fig. 2A, the activation of JNK1 activity was observed at the earliest measured time point of 30 min at a concentration of 30 μM paclitaxel. Importantly, this time course is coincidental with the transcriptional activation of the IL-8 gene by paclitaxel (14). p38-MAPK and ERK1 activity increased slightly (<1.5 fold) (Fig. 2A). Others have encountered difficulties in observing p38 activation.2 To exclude the possibility that the absence of a significant p38 activation by paclitaxel was caused by nonfunctional antibody or substrate, an activity assay was performed. A specific antibody to p38 MAPK was used to immunoprecipitate p38 from cell lysates selectively. The resulting immunoprecipitate p38 from cells lysates was resolved and subjected to Western blotting using a phospho-specific ATF-2 (Thr-71) antibody (1:1,000 dilution). Protein bands on immunoblots were visualized by enhanced chemical luminescence (Amersham Pharmacia Biotech).

2 S. E. Earp, personal communications.
to activate JNK is lower than that required to induce IL-8 gene expression (previously observed at 5–30 μM paclitaxel). This suggests that paclitaxel can induce JNK activation efficiently and that the induction of other factors necessary for IL-8 transcription may require a higher concentration of paclitaxel.

Dominant-inhibitory Mutants of JNK Blocked IL-8 Promoter Activation by Paclitaxel—To assess directly the involvement of JNK or p38 in IL-8 promoter activation by paclitaxel, dominant-inhibitory mutants were used. OVCA 420 cells were transfected with FLAG-tagged dominant-inhibitory mutants of JNK and p38 (pcDNA3-FLAG-JNK1(APF) and pCMV-Flag-p38(AGF)) (25, 41). Transient transfected cell lysates were immunoprecipitated with mouse monoclonal antibody to the Flag epitope (M2), and then the immunoprecipitates were examined by Western blotting using normal rabbit serum, JNK, and p38 antibody, respectively. The arrows indicate the 46-kDa JNK1 and the 38-kDa mutant p38 protein detected by JNK- and p38-specific antibodies respectively, whereas normal rabbit serum showed no specific bands. The stronger bands at 46 kDa are as in Fig. 2. see Fig. 2 legend. The autoradiogram shown is from one representative experiment; the experiment was repeated three times with comparable results.

The ability of these two mutants to disrupt JNK and p38-MAPK mutants. Cells were transfected with pcDNA3-FLAG-JNK1(APF) or pCMV-Flag-p38(AGF) as indicated. Transient transfected cell lysates were immunoprecipitated by using mouse monoclonal antibody to the Flag epitope (M2), and then the immunoprecipitates were examined by Western blotting using normal rabbit serum, JNK, and p38 antibody, respectively. The arrows indicate the 46-kDa JNK1 and the 38-kDa mutant p38 protein detected by JNK- and p38-specific antibodies respectively, whereas normal rabbit serum showed no specific bands. The stronger bands at 46 kDa are as in Fig. 2. see Fig. 2 legend. The autoradiogram shown is from one representative experiment; the experiment was repeated three times with comparable results.

Paclitaxel-induced Cell Death Is Associated with JNK/SAPK

paclitaxel increased IL-8 promoter activity significantly over the MeSO control; the induction of the empty vector (pcDNA3) did not affect this induction (Fig. 4A, first two lanes from left). Overexpression of a dominant-inhibitory mutant of JNK1 subcloned in the pcDNA3 vector greatly reduced activation of the IL-8 CAT reporter by paclitaxel (third lane). In the second part of the same experiment, cells were transfected with an empty control vector, pCMV, and treated with paclitaxel. This control is necessary because the dominant-inhibitory mutants of ERK1 and p38 were subcloned in the pCMV vector. Again, paclitaxel induced IL-8 promoter activation (fourth and fifth lanes). The cotransfection of the dominant-inhibitory mutants of ERK1 had little effect on IL-8 activation; p38 dominant-inhibitory mutant genes had a more noticeable effect on IL-8 activation. The super-repressor IkBo abolished all induction of the IL-8 CAT construct. This indicates that both the JNK-mediated activation of AP-1 and NF-κB activation by paclitaxel are important in IL-8 gene induction by the drug.

Paclitaxel-induced Activation of JNK Correlates with Cell Death—Previous studies suggest a model in which persistently activated JNK activity is required for inducing apoptosis. To
was used to measure cell viability in cells transfected with a dominant-inhibitory mutant that blocked JNK signaling. OVCA 420 cells were cotransfected with pCMV-βgal and a plasmid containing either a dominant-inhibitory kinase gene or an empty vector. Each transfection was performed in duplicate. The β-galactosidase-positive cells (blue) were stained after a 24-h exposure to paclitaxel and then counted by microscopic examination. 

β-Galactosidase-positive signal was used to measure the number of viable cells capable of producing the gene product. Overexpression of the dominant-inhibitory mutants of JNK1 (JNK1 (APF)) blocked paclitaxel-induced cell death by approximately 80% (Fig. 5, A and B). Dominant-inhibitory forms of MEKK1, which activates the JNK activator SEK1, also decreased paclitaxel-induced cell death (Fig. 5B). Transfection of the dominant-inhibitory mutant p38 ((p38(AGF)) resulted in a small inhibition of cell death induced by paclitaxel. Because NF-κB is activated by paclitaxel (15), it is relevant to assess if molecules in the NF-κB pathway contribute to paclitaxel-induced cell death. Although NF-κB is generally thought of as a survival factor that prevents cell death (42, 58, 59), there is also contrary evidence (60–62). Adenovirus from another laboratory revealed that the super-repressor IκB form did not sensitize cells to paclitaxel-induced death, but it did sensitize cells to TNF-α, a DNA-damaging agent, and another chemotherapeutic (42). Fig. 6 shows that the super-repressor form of IκBα as well as the empty vector also did not suppress paclitaxel-induced cell death. Thus more extensive studies are necessary to clarify the role of NF-κB in paclitaxel-induced cell death.

**DISCUSSION**

A previous report from our group indicates that paclitaxel specifically induces the transcription of the IL-8 gene via activation of its promoter (14). This occurs in approximately half of ovarian tumor cell lines and, significantly, in half of freshly explanted tumors. The induction is by the transcriptional activation of IL-8 promoters. The paclitaxel-responsive regulatory elements in the IL-8 promoter consist of the AP-1 and NF-κB cognate binding sites (15). Further analysis shows that both the AP-1 and NF-κB transcription factor are activated by paclitaxel. Gel shift analysis shows that paclitaxel treatment of ovarian tumors causes increased binding to an AP-1 cis-acting regulatory element in the IL-8 promoter (15). This is correlated with enhanced activation of the IL-8 promoter as well as with the activation of a promoter with the canonical AP-1 binding site. These findings provide a venue to examine intracellular events that lie upstream of AP-1 which are activated by paclitaxel. Once these intracellular events are identified, it is then feasible to determine if they contribute to the therapeutic effects of paclitaxel in controlling tumor growth and proliferation. The activation of these intracellular events in subsets of tumor cells may also provide a prognostic marker that can predict the efficacy of paclitaxel in controlling tumors in vivo.

In this report, we assess whether MAPK, ERK1, JNK1, and p38 constitute upstream signaling events that lead to the transcriptional induction of IL-8 by paclitaxel. In vitro kinase assays show that JNK can be activated by paclitaxel. The activation of JNK by paclitaxel suggests its potential involvement in IL-8 induction because activated JNK is known to phosphorylate several transcription factors, including c-Jun, ATF2, and c-Fos (32, 37, 38). Indeed, the JNK1 dominant-inhibitory mutant blocked paclitaxel-induced IL-8 promoter activity (Fig. 4). These results support the conclusion that JNK-mediated activation of c-Jun leads to IL-8 gene activation. The induction of IL-8 has important biologic consequences in controlling tu-

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**C.-Y. Wang, personal observation.**
mor growth, as another study from our group shows that the introduction of IL-8 into human ovarian tumors resulted in massive granulocytic and monocytic infiltration and the subsequent reduction or elimination of tumor growth.\(^4\)

Activation of the JNK signaling pathway is increasingly found to play a major role in differentiation and cell death. It is known that JNK1 is activated predominantly in response to stress-activating signals such as growth factor withdrawal, heat shock, osmotic, UV light, protein synthesis inhibitors, and proinflammatory cytokines (24, 25). On the other hand, activation of the JNK pathway can also induce apoptosis or cell death. For example, DAXX, a recently identified Fas-binding protein, can induce apoptosis via activation of the JNK pathway (64). The apoptosis signal-regulated kinase (ASK), is also an activating kinase of the JNK pathway. In response to TNF-\(\alpha\), ASK is sufficient to induce apoptosis and is required for TNF-\(\alpha\)-induced apoptosis (65). Several reports have also demonstrated that the duration of JNK activation is a determining factor for cell proliferation or death (26). Transient JNK induction, such as signaling from anti-CD28 plus phorbol 12-myristate 13-acetate (PMA) or PMA plus ionomycin, causes a rapid course of JNK1 activation leading to cell proliferation. More sustained JNK activation (e.g. UV light, \(\gamma\)-irradiation, DNA damage, drug treatment) causes cell apoptosis (26, 55, 66). Our results are consistent with the observations reported by Chen and co-workers because paclitaxel, like other stress agents, causes a more sustained pattern of JNK activation (Fig. 5), and this prolonged activation of JNK is directly linked to cell death based on the use of dominant-inhibitory JNK mutants. A worthy line of investigation is to determine if JNK activation occurs in response to microtubule stabilization by paclitaxel or if it is independent of microtubule stabilization (66). It will be intriguing to differentiate these molecular mechanisms of paclitaxel’s action. One way to approach this may be the use of paclitaxel analogs. Upon the examination of 12 structurally related paclitaxel analogs, Watson et al. (18) reported a tight correlation between paclitaxel-induced gene expression and cell death. In their hands, paclitaxel analogs that most dramatically up-regulated IL-8 expression were the most effective in inhibiting cell survival. They also found that IL-8 production was not directly responsible for cell killing because treatment with anti-IL-8 antibodies failed to block cell death (18). Taken together, all of these studies suggest that JNK activation is required for both IL-8 gene transcription and cell killing.

The p38 kinase pathway that is activated by many stimuli common to the JNK/SAPK pathway is activated to a lesser degree in paclitaxel-treated human ovarian cancer cells (25, 67, 68).
Transfection of the dominant-inhibitory mutant p38 (p38(AGF)) resulted in a small inhibition of IL-8 promoter CAT activity and cell death induced by paclitaxel. In composite, these data point to a role for p38 in all of these assays. During the preparation of this manuscript, another report showed that paclitaxel primarily affected JNK activation but not p38-MAPK activation in B lymphoblasts (57). Thus the effect of paclitaxel on p38 is likely slight.

An earlier study also showed that IL-8 induction by paclitaxel requires activation of the NF-κB transcription factor (15). Recent work from several laboratories has demonstrated that the Rel/NF-κB transcription factor regulates apoptosis by serving as a survival signal in many cell types (42, 58, 59). Furthermore, Wang et al. (42) have shown that cells transfected with the super-repressor IκBα, which inactivates NF-κB, are more prone to cytotoxicity in response to pro-apoptotics including the inflammatory cytokine TNF-α and the DNA-damaging agents, ionizing radiation and daunorubicin.

Like many other activators of the cell stress response, paclitaxel activates both AP-1 and NF-κB signaling pathways and induces cell death (15). Similar divergent signaling via the TNF-α receptor is well characterized, involving separate activation of cell death pathways (through the TNF-α receptor-associated factor Traf1) and cell survival pathways through the NF-κB-activating factor Traf2 (69). Inhibition of Traf2-dependent signaling potentiates TNF-α-induced apoptosis (70). Similarly, in addition to its role in AP-1 signaling, the JNK activator MEKK1 is implicated in activation of NF-κB (71–73), although the mechanism of NF-κB activation by MEKK1 remains unclear. Thus, stress signaling involving MEKK1 and TNF-α (and likely paclitaxel and other stress stimulators) appears to involve a balance of death and survival pathways, the final outcome of which may depend on quantitative, combinatorial, or temporal considerations. In support of this, activation of caspases during Fas-stimulated apoptosis blocks subsequent TNF-α-mediated destruction of IκBα and is coincident with complete cleavage of MEKK1 by caspase(s) to a cytosoluble, pro-apoptotic form (63). The dual activation of NF-κB and JNK by paclitaxel may similarly involve a balance between these two signals with likely opposite effects on cell survival. On the other hand, NF-κB-mediated signals that regulate the process of apoptosis are likely complex. For example, under some circumstances, activation of NF-κB may also promote apoptosis (60–62). In one study (61), apoptotic death induced by serum withdrawal was shown to be accompanied by NF-κB activation.

NF-κB activation is an important survival factor in many cell types (42, 58, 59). Furthermore, Wang et al. (42) have shown that cells transfected with the super-repressor IκBα, which inactivates NF-κB, are more prone to cytotoxicity in response to pro-apoptotics including the inflammatory cytokine TNF-α and the DNA-damaging agents, ionizing radiation and daunorubicin.

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