Cancer Immunol Immunother (2000) 49:78–84

ORIGINAL ARTICLE

Timothy S. Collins · Li-Fen Lee · Jenny P.-Y. Ting

Paclitaxel up-regulates interleukin-8 synthesis in human lung carcinoma through an NF-κB- and AP-1-dependent mechanism

Received: 30 July 1998 / Accepted: 7 January 2000

Abstract Lung cancer is a leading cause of cancer-related death in the United States. For this reason we chose to study the specific cellular effects that one chemotherapeutic agent, paclitaxel, has on lung carcinoma. In addition to its known mechanism of action, which is to stabilize microtubules, paclitaxel has been shown to have other interesting and relevant cellular effects. In this report, we demonstrate that a subset of human lung carcinoma cell lines respond to paclitaxel treatment with an up to a fivefold increase in the production of interleukin-8 (IL-8). We demonstrate that this increased production is specific to IL-8 but not to other chemokines, and is both dose- and time-dependent. Increased IL-8 mRNA is seen as early as 45 min with a peak at 4 h after paclitaxel treatment. This increase in mRNA is due to transcriptional activation because actinomycin D treatment blocked the increase. Paclitaxel also activates the mitogen-activated protein kinase family member, JNK1, in dose-dependent fashion. IL-8 enhancement is completely abolished with the use of an inhibitor of NF-κB, the super-repressor IκB. Similar results were obtained upon the inhibition of AP-1 activation with the MEK1/2 inhibitor, U0126. By gaining a better understanding of the differences in cellular response to paclitaxel chemotherapy, these findings might lead to either improved patient selection or to the development of adjuvant therapy targeted at specific-cell signaling proteins.

Key words Lung · Chemokines · Signal transduction · Transcription factors

T. S. Collins
University of North Carolina Lineberger Comprehensive Cancer Center, Department of Surgery, Chapel Hill, NC 27599-7295, USA

L.-F. Lee[1]
University of North Carolina Lineberger Comprehensive Cancer Center, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-7295, USA

J. P.-Y. Ting (✉)
University of North Carolina Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, Campus Box 7295, UNC-Chapel Hill, Chapel Hill, NC 27599-7295, USA

Present address:
[1]University of California at Davis, Davis, USA

Introduction

Lung cancer is the leading cause of cancer-related death in the United States. Estimates in the United States for 1999 of 171 600 new cases and 158 900 deaths illustrate the high case/fatality rate of the disease [18]. Approximately 80% of these cases can be grouped under the heading of non-small-cell lung carcinoma (NSCLC) [19]. Although surgical resection of stage 1 NSCLC can result in a 70% 5-year survival, the majority of cases have more advanced disease [17]. There is clearly a need for both improved detection and adjuvant therapy in the treatment of NSCLC. Several new classes of chemotherapy have been developed and are being studied as potential therapies for NSCLC.

Two members of the taxane family, paclitaxel and docetaxel, are being used either alone or in combination regimens for the treatment of NSCLC. Both agents are known to stabilize microtubules thereby inhibiting normal mitosis [23]. Used alone, paclitaxel has been shown to have response rates of 10%–36% and 1-year survival rates of 33%–42% in patients with NSCLC [9]. A response rate of 32% and a 1-year survival of 39% was achieved in patients receiving paclitaxel in combination with cisplatin [11].

In addition to its effects on microtubules, paclitaxel has other cellular effects. It induces expression of tumor necrosis factor TNF and interleukin-1 (IL-1) in murine macrophages [4, 7, 16] and IL-8 in human ovarian carcinoma [12, 13]. IL-8, an 8-kDa protein, is a known chemokine for neutrophils [2]. In addition, interleukin-8 has other tumor-specific growth effects. Depending on

cell type, IL-8 has been shown to either enhance growth, as in the case of melanoma [24], or inhibit growth, as in a subset of NSCLC [26]. Equally unclear role IL-8 is known to play as an angiogenesis factor in human lung carcinoma [1, 25].

In the work we report, we chose to study what effects paclitaxel has on chemokine production in a group of NSCLC cell lines as a result of the potentially advantageous effects of IL-8 in inhibiting NSCLC tumor growth. We found that paclitaxel up-regulates IL-8 synthesis in a subset of human lung carcinoma. The effect of paclitaxel is specific as it does not affect the synthesis of other chemokines. We also provide evidence that this up-regulation is transcription-dependent. Furthermore, paclitaxel induces c-Jun kinase (JNK1) activation in a dose-dependent manner. In addition, we provide two approaches to inhibit IL-8 production through inhibition of upstream cell signaling proteins involving the NF-κB and mitogen-activated protein kinase (MAPK) pathways.

Materials and methods

Drugs and cell culture

Four human NSCLC cell lines (squamous cell H157, adenocarcinoma H1437, alveolar cell H358, and adenocarcinoma A549) were used for our experiments. The NSCLC lines were grown in RPMI medium with 10% fetal calf serum, penicillin and streptomycin. Paclitaxel (Sigma) and the MAPK inhibitor, U0126 (Promega), were reconstituted in dimethylsulfoxide (DMSO), which was then used as our negative control. Actinomycin D (Sigma) was purchased and used in its soluble form.

Plasmid constructs

The adenovirus IκB super-repressor was a gift from the laboratory of Dr. Albert Baldwin, at the University of North Carolina.

Paclitaxel treatments

For each IL-8 protein enzyme-linked immunosorbent assay (ELISA), the cells were trypsinized, counted and plated in 12-well plates at a concentration of 2×10^5 cells/well. Following 24–48 h incubation, they were treated for variable amounts of time with either paclitaxel or DMSO. The supernatants were harvested, centrifuged to remove large cellular debris and stored at −70 °C until ELISA was performed. As noted in individual experiments, cells were incubated with 10 μM U0126 for 1 h prior to paclitaxel treatment.

IL-8 ELISA

A matched pair of human IL-8 antibodies (Endogen) was used in a standard sandwich ELISA. Briefly, 96-well microtiter plates were coated overnight with the coating antibody diluted in phosphate-buffered saline (PBS). The following day, each well was blocked for 1 h with 200 μl assay buffer (PBS with 4% bovine serum albumin). The cell supernatants were then diluted and incubated in duplicate wells with the biotinylated antibody for 2 h. A streptavidin–alkaline-phosphatase conjugate was added and incubated for 30 min, followed by the substrate, *P*-nitrophenyl phosphate (Bio-Rad) in carbonate buffer. The plates were read at 405 nM and concentrations determined with the use of an IL-8 standard.

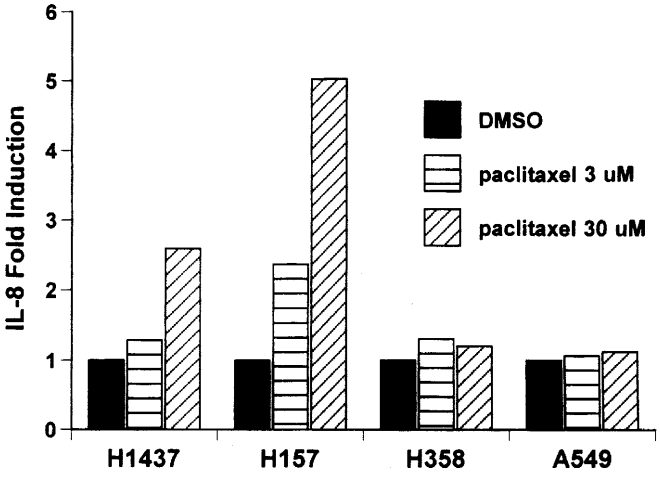

Fig. 1 Screening of four non-small-cell lung cancer (NSCLC) cell lines for interleukin-8 (*IL-8*) induction in response to paclitaxel. Four NSCLC cell lines were screened for their ability to synthesize IL-8 in response to paclitaxel treatment. Each cell line was treated with two different doses of paclitaxel and incubated for 6 h. The supernatant was collected and assayed for IL-8 by enzyme-linked immunosorbent assay (ELISA). The numbers reflect the mean of triplicate wells with duplicate experiments. *DMSO* dimethylsulfoxide

Northern hybridization

RNA was isolated by the guanidinium isothiocyanate and cesium chloride method as previously described [5]. Total RNA (5–10 μg) was electophoresed in a denaturing gel and transferred to a nitrocellulose filter overnight. IL-8 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probes were hybridized to the Northern blots as previously reported [21].

RNase protection assay

Instructions were followed for the RiboQuant multi-probe RNase protection assay system purchased from PharMingen. The human chemokine set hCK-5 was selected because it contained probes for MIP1α, MIP1β, MCP, and IL-8.

c-Jun kinase (JNK) assay

For the kinase experiments, tumor cells were plated and allowed to reach 60%–70% confluence. Following 2 h of paclitaxel treatment, cells were washed twice with ice-cold PBS and lysed with 2 ml lysis buffer (1% Nonidet-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.15 M NaCl, 0.01 M sodium phosphate pH 7.2, 2 mM EDTA, 50 mM sodium fluoride, 0.2 mM sodium vanadate, and 100 U/ml aprotinin). The cell lysates were then aspirated repeatedly through a 21-gauge needle to shear DNA. Following 15 min on an Eppendorf rocker at 4 °C, the lysates were centrifuged at 12,500 rpm for 15 min. The imunocomplex kinase assay was then performed as described in detail previously [14]. Anti-c-Jun antibodies were purchased from Santa Cruz Biotechnology.

Results

Paclitaxel induces IL-8 synthesis in a subset of NSCLC lines

Four NSCLC cell lines were screened for their ability to synthesize IL-8 in response to paclitaxel (Fig. 1). In the

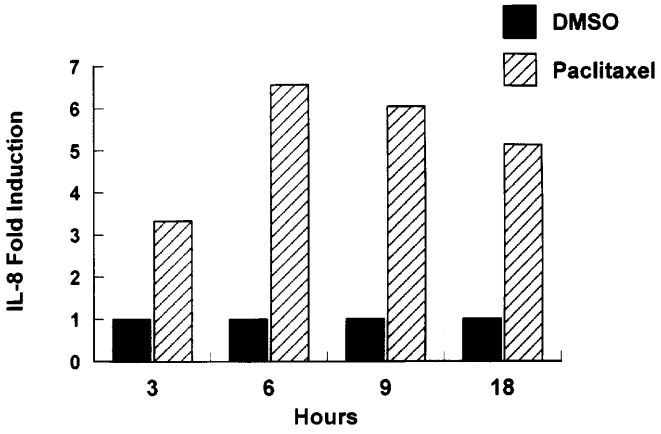

Fig. 2 Time course of IL-8 synthesis. H157 cells were treated with and without 30 μM paclitaxel and the supernatants were assayed by ELISA. Details given in legend to Fig. 1

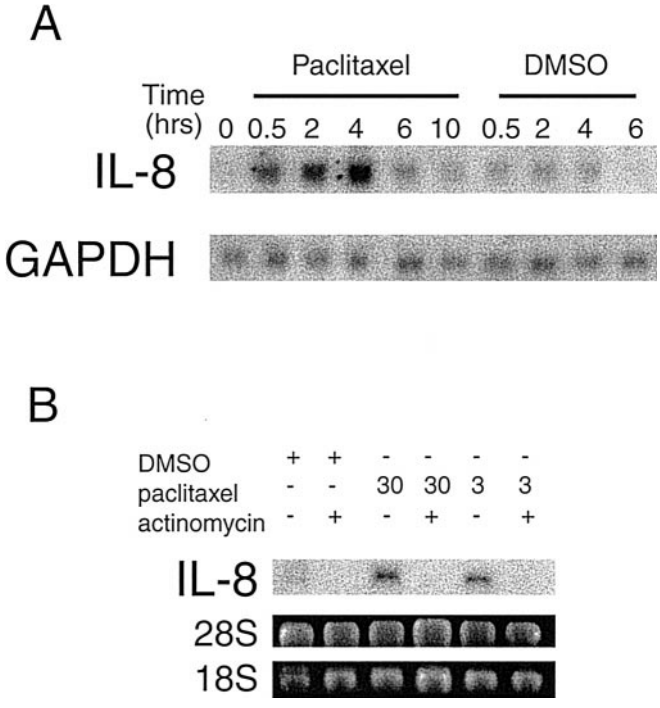

Fig. 3A, B IL-8 mRNA production in response to paclitaxel treatment. **A** Northern blot of H157 cells treated with either 30 μM paclitaxel or DMSO control during a time course. A probe for glyceraldehyde-3-phosphate dehydrogenase was used to confirm equal RNA loading. **B** Northern blot of H157 cells treated with 30 μM paclitaxel for 4 h in the presence or absence of actinomycin D. The ethidium-bromide-stained gel is shown to confirm equal RNA loading

squamous cell carcinoma, H157, treatment with a concentration of 30 μM for 6 h resulted in a 5-fold increase in the amount of IL-8 in the supernatant, as measured by the ELISA. Identical treatment of an adenocarcinoma cell line, H1437, resulted in a smaller but measurably increased amount of IL-8 at the same assay time. In two other cell lines, H358 and A549, there was no measurable increase in IL-8 after 6 h of paclitaxel treatment. Since H157 had the greatest response in the initial screening, subsequent experiments were performed to study the characteristics of paclitaxel's effects. A time course of paclitaxel treatment revealed increased levels of IL-8 in the supernatant as early as 3 h after treatment (Fig. 2), indicating that this is a relatively early response. Longer treatment further enhanced IL-8 induction, with sustained enhancement detectable 18 h after treatment. There was a 3.3-fold increase at 3 h, a 6.6-fold increase at 6 h, a 6.0-fold increase at 9 h and a 5.2-fold increase at 18 h.

To determine the molecular level of IL-8 induction by paclitaxel, Northern hybridization was performed. There were higher levels of IL-8 messenger RNA in the paclitaxel-treated H157 cell line than in the DMSO control (Fig. 3A). The concentration of paclitaxel used for this experiment was 30 μM, which resulted in the highest amount of IL-8 in the supernatant. A notable increase was seen as early as 30 min following treatment, reaching a peak after 4 h. The nitrocellulose filters were stripped of their IL-8 cDNA probe and re-hybridized with a probe for GAPDH as a control for RNA loading.

Paclitaxel causes the de novo synthesis of IL-8 transcripts

The enhanced level of IL-8 could be due to either transcriptional activation of the gene or enhanced message stabilization. Actinomycin D inhibits de novo RNA synthesis and is expected to block paclitaxel-mediated IL-8 induction if the induction is transcriptionally activated. After 4 h of paclitaxel treatment, there were increases in IL-8 mRNA in cells treated with 3 μM or 30 μM paclitaxel (Fig. 3B). Co-treatment with actinomycin D resulted in minimally detectable amounts of IL-8 mRNA with either DMSO or paclitaxel, suggesting that the paclitaxel-induced increases are dependent on de novo RNA synthesis. To demonstrate equal loading of RNA, ethidium bromide staining of 28S and 18S RNA is shown. This indicates that paclitaxel affects IL-8 synthesis at the level of transcriptional activation. As further evidence that paclitaxel treatment stimulates de novo IL-8 synthesis, we measured IL-8 protein levels in the supernatant with and without actinomycin D treatment (Fig. 4). Actinomycin D reduced the levels of IL-8 in the supernatant in the DMSO-treated wells by 50%. While there was a 2.5-fold increase in the paclitaxel-treated wells over the control, co-treatment with actinomycin D abolished this induction and resulted in a decrease in IL-8 levels comparable to those resulting from DMSO and actinomycin D treatment alone.

Paclitaxel induces IL-8 production but does not affect other measured chemokines

To determine if the increase in IL-8 message was a specific effect, we performed an RNase protection assay

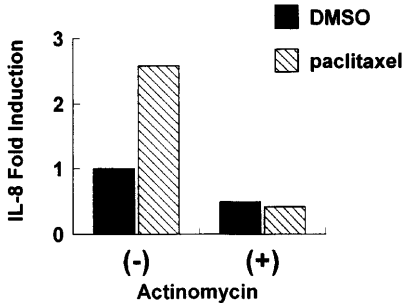

Fig. 4 Actinomycin D blocks paclitaxel-mediated IL-8 induction. H157 cells were treated with either DMSO or 30 μM paclitaxel in the presence or absence of actinomycin D for 6 h. The supernatants were harvested and IL-8 ELISA was performed

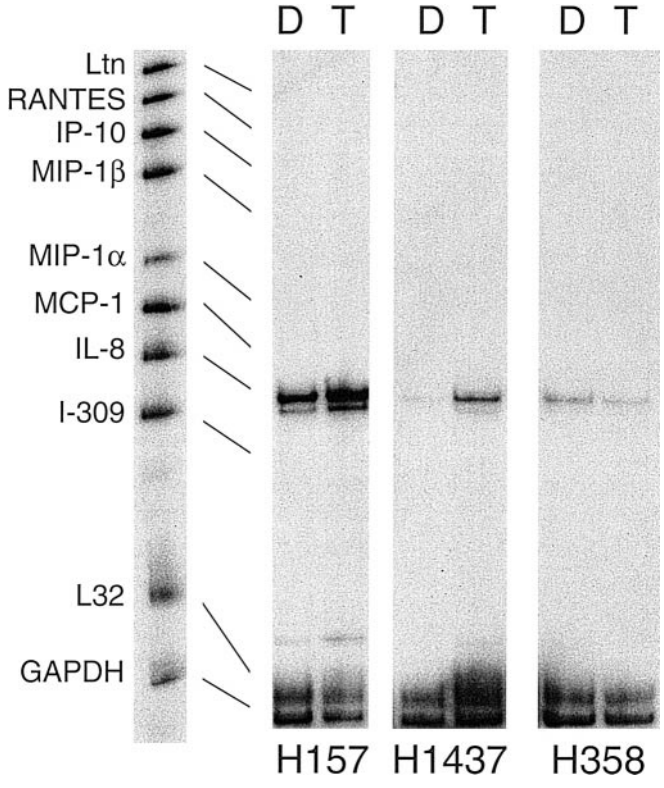

Fig. 5 Paclitaxel-mediated IL-8 induction is a specific effect. RNA was harvested from three NSCLC cell lines that had been treated with either DMSO (*D*) or 30 μM paclitaxel (*T*) for 4 h. An RNase protection assay using the RiboQuant multi-probe RNase protection assay system was then performed

to screen for the presence of mRNA of other chemokines. As expected, an increased level of IL-8 mRNA was observed following 4 h of paclitaxel treatment in both the H157 and H1437 cell lines (Fig. 5). There was no similar increase in the H358 cell line, which agrees with the protein levels measured by ELISA. No detectable RNA was seen for the other chemokines/cytokines, Ltn, RANTES, IP-10, MIP-1α, MIP-1β, MCP-1, or I-309 in all three of the cell lines. Therefore, the effects of paclitaxel are specific to IL-8 and not to several other important chemokines.

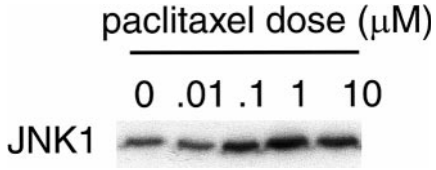

Fig. 6 Paclitaxel activates c-Jun kinase (*JNK1*) in a dose-dependent fashion. H157 cells were treated with varying doses of paclitaxel for 2 h. The cell lysates were harvested and an in vitro immunocomplex kinase assay was performed

JNK1 is activated by paclitaxel

In prior studies, we demonstrated that paclitaxel activated JNK1 in ovarian carcinoma cells [14]. Furthermore, the inhibition of JNK1 with a dominant-negative mutant resulted in decreased IL-8 promoter activity. To determine if paclitaxel was capable of activating JNK1 in a similar fashion in human lung carcinoma, we performed an in vitro immunocomplex kinase assay. Following 2 h of paclitaxel treatment, there was no significant change in JNK activation upon treatment with the lowest dose of paclitaxel (10 nM) as compared to the DMSO control (Fig. 6). However, at the higher doses of 1 μM and 10 μM, there was a significant increase in JNK activation in the H157 cell line.

Paclitaxel induces IL-8 transcription through the NF-kB and AP-1 pathways

Transcriptional induction of the IL-8 gene has been shown to depend on the transcription factors NF-kB and AP-1, although this dependence varies with cell type. Our previous analysis of ovarian carcinoma indicates that NF-kB is induced [14]. Further, the AP-1 protein, activated through the MAPK/JNK pathway is crucial for IL-8 induction in these cells. To address whether NF-kB and MAPK activation enhances IL-8 in NSCLC, we chose to use specific inhibitors of these signaling pathways. An adenoviral construct containing the super-repressor IκB was used to block activation of the transcription factor NF-κB. As a positive control for NF-κB activation, cells were concurrently treated with TNF, a known activator of NF-κB. Forty-eight hours following adenoviral transduction the cells were treated with paclitaxel and TNF (20 ng/ml) for 6 h. Increases in IL-8 protein in the supernatant were 2.5-fold in the paclitaxel-treated wells and 4-fold in the TNF-treated wells (Fig. 7A). In the cells previously transduced with the adenoviral construct of IκB, there was a marked decrease in the amount of IL-8 protein despite either paclitaxel or TNF treatment. Densitometry analysis of the Western blot confirmed a 3-fold increase in the levels of IκBα in the cells transduced with the super-repressor (data not shown).

A specific inhibitor to MEK-1/2, U0126, was then used to determine if an intact MAPK kinase (MEK)/ERK pathway was required for IL-8 synthesis in our cell

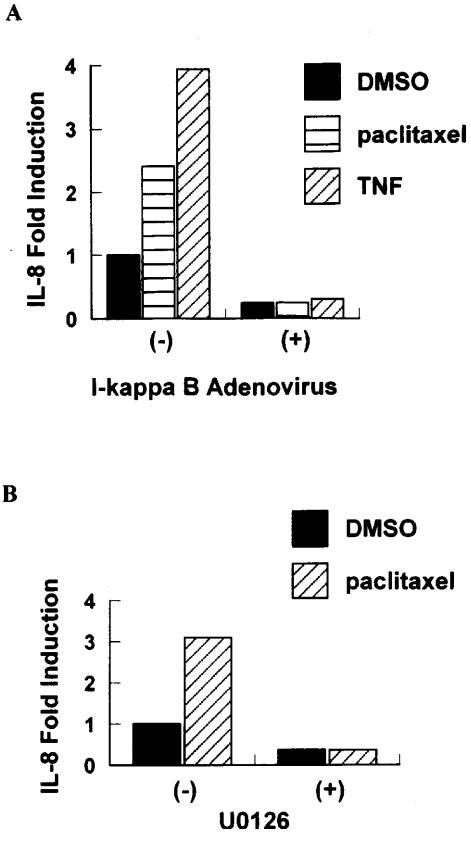

Fig. 7A, B Inhibitors of NF-κB and AP-1 block IL-8 induction in NSCLC. **A** H157 cells were infected with the IκB super-repressor adenovirus; 48 h following infection the cells were treated with either DMSO or 30 mM paclitaxel for 6 h. The cell supernatant was then analyzed by ELISA for IL-8 concentration. **B** H157 cells were pre-treated with either DMSO or 10 μM U0126 for 1 h. Subsequently, cells were treated with either DMSO or 30 μM paclitaxel for 6 h and the supernatant was analyzed as above

line. A 3-fold increase in IL-8 protein was seen in the paclitaxel-treated H157 cells as compared to the control (Fig. 7B). In the wells pre-treated for 1 h with the MEK inhibitor, there was a reduction of IL-8 protein levels of 62% in the DMSO-treated wells. This inhibitor completely eliminated the paclitaxel induction of IL-8. U0126 does not appear to be toxic to the cells at this dose level as growth rates are unaffected by the inhibitor (data not shown).

Discussion

In this report, we show that paclitaxel induces IL-8 synthesis in a subset of human lung carcinoma cell lines. We provide evidence that this increase correlates with increased transcription. Furthermore, by blocking upstream targets of the IL-8 promoter we are able to block de novo production effectively. It will be important to study what effect increased IL-8 production by human lung cancer cells has on their growth potential. In addition, it will be important to know whether the activation of NF-κB or AP-1, seen in response to paclitaxel treatment, has any effects relating to cell survival.

IL-8 is known to have conflicting roles in tumor biology. It was first described as a chemotactic factor for neutrophils. Following overexpression of IL-8 in either Chinese hamster ovarian cells [10] or human ovarian cells [15] there is greatly decreased tumor growth in vivo. This decreased growth has been associated with and thought to be due to increased neutrophilic infiltration at the tumor site. IL-8 is also known to have growth effects. Singh et al. demonstrated that recombinant IL-8 could promote growth of a melanoma cell line which could be blocked in the presence of a polyclonal antibody [24]. In NSCLC, Wang et al. reported decreased proliferation in clones of a commonly studied cell line A549 that expressed higher amounts of IL-8 [26]. In the same report, they showed decreased tumor proliferation in three other NSCLC cell lines treated with recombinant IL-8. Because of the biological roles IL-8 may play in controlling tumor growth, enhancing proinflammatory cell infiltration and in antiogenesis, it is of interest to examine its induction by chemotherapeutic agents.

The transcription factor, NF-κB, binds to the promoter region of several cytokines such as TNF, IL-1, IL-6 and IL-8 [3]. It has been previously shown to be active in response to paclitaxel treatment. Paclitaxel treatment results in translocation of NF-κB to the nucleus in murine macrophages [20] and human NSCLC [6]. In addition, our laboratory has previously outlined the specific regions on the IL-8 promoter in an ovarian cell line that are required for activation by paclitaxel, including NF-κB and AP-1 [13].

Perhaps more relevant clinically is that NF-κB activation may play a role in tumor survival in response to chemotherapy in certain cell types. By inhibiting NF-κB with the super-repressor IκB, Wang et al. demonstrated that they could render chemoresistant tumors more susceptible to both TNFα and the chemotherapeutic agent CPT-11 [28]. In our prior work involving ovarian carcinoma, the super-repressor IκB, did not have any effect on the tumor-killing effects of paclitaxel in a paclitaxel-sensitive cell line. Current work is underway to study whether the inhibition of NF-κB activation in NSCLC will lead to improved paclitaxel killing. It is possible that the NF-kB induced by paclitaxel may, in part, explain why, in general, there is a much lower clinical response seen in NSCLC than in other cancers.

It is clear from this work and prior work that paclitaxel treatment results in activation of AP-1 in both ovarian and NSCLC cell lines. In an effort to block AP-1 in this study, we used the MAPK inhibitor, U0126, which was developed to inhibit AP-1 transactivation [8]. In the same work, Favata et al. showed that U0126 acts as a specific inhibitor of MEK 1 and 2. The inhibition of MEK 1 and 2 leads to decreased activation of their downstream kinases ERK 1 and 2 and its downstream substrate, the transcription factor Elk-1. Decreased activity of this transcription factor would lead to decreased induction of the AP-1 subunits, c-Jun and c-Fos. The

decreased IL-8 seen in the U0126 pre-treated cells is likely due to decreased AP-1 activation. We did not, however, study the effects of U0126 on NF-κB activation. A similar finding was made by Sano et al. when they showed that treatment of endothelial cells with ICAM-1 induced the production of IL-8 and RANTES mRNA. They were able to block IL-8 induction with the MEK inhibitor PD98059 [22].

The other relevant aspect of paclitaxel treatment is its known ability to activate the stress-activated protein kinase (SAPK)/JNK MAPK pathway in a variety of tumor cell lines [14, 27]. Prior work in our laboratory showed that paclitaxel activates JNK1 in a dose- and time-dependent manner in ovarian cancer cells. In this work, we extend that observation to include NSCLC. In our work in ovarian carcinoma, we showed that the inhibition of JNK activation with the transient transfection of a dominant-negative mutant resulted in markedly decreased paclitaxel-mediated cell killing [14]. It is possible that tumor-specific differences in the induction of apoptosis may help explain some of the differences seen in clinical response to paclitaxel.

In summary, we report increased IL-8 induction in paclitaxel-treated human lung carcinoma cells. By elucidating the significance of this increased IL-8 synthesis we can better understand how paclitaxel treatment might be influencing tumor growth. In addition, by demonstrating different transcription factors and signal transduction pathways that are activated we may be able to decipher how paclitaxel affects other cellular processes important for proliferation and apoptosis. Furthermore, by understanding under what clinical circumstances IL-8 influences tumor growth, we may be able to modulate IL-8 synthesis with the use of a similar strategy to that used in our current study.

Acknowledgements Supported by a translational grant from the Lineberger Comprehensive Cancer Center and NIH grant AI29564.

References

1. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM (1996) Inhibition of interleukin-8 reduces tumorigenicity of human non-small cell lung cancer in SCID mice. J Clin Invest 97: 2792
2. Baggiolini M, Walz A, Kunkel SL (1989) Neutrophil-activating peptide-I/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 84: 1045
3. Baldwin AS Jr (1996) The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol 14: 649–83: 649
4. Bogdan C, Ding A (1992) Taxol, a microtubule-stabilizing antineoplastic agent, induces expression of tumor necrosis factor alpha and interleukin-1 in macrophages. J Leukoc Biol 52: 119
5. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294
6. Das KC, White CW (1997) Activation of NF-kappaB by antineoplastic agents. Role of protein kinase C J Biol Chem 272: 14914
7. Ding AH, Porteu F, Sanchez E, Nathan CF (1990) Shared actions of endotoxin and taxol on TNF receptors and TNF release. Science 248: 370
8. Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, Van Dyk DE, Pitts WJ, Earl RA, Hobbs F, Copeland RA, Magolda RL, Scherle PA, Trzaskos JM (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J Biol Chem 273: 18623
9. Goldspiel BR (1997) Clinical overview of the taxanes. Pharmacol Ther 5: 110S
10. Hirose K, Hakozaki M, Nyunoya Y, Kobayashi Y (1995) Chemokine gene expression transfection into tumor cells reduced tumorigenicity in nude mice in association with neutrophilic infiltration. Br J Cancer 72: 708
11. Johnson DH, Chang AY, Ettinger DS, Kim KM, Bonomi P (1998) Recent advances with chemotherapy for NSCLC: the ECOG experience. Eastern Cooperative Oncology Group. Oncology (Huntingt) 12: 67
12. Lee L-F, Lofquist AK, Schurer-Maly CC, Day C, Ting JPY, White CM, Martin BK, Haskill JS (1996) Taxol-dependent transcriptional activation of IL-8 expression in a subset of human ovarian cancer. Cancer Res 56: 1303
13. Lee L-F, Haskill JS, Mukaida N, Matsushima K, Ting JPY (1997) Identification of tumor-specific paclitaxel (Taxol)-responsive regulatory elements in the interleukin-8 promoter. Mol Cell Biol 17: 5097
14. Lee LF, Li G, Templeton DJ, Ting JP (1998) Paclitaxel (Taxol)-induced gene expression and cell death are both mediated by the activation of c-Jun NH2-terminal kinase (JNK/SAPK). J Biol Chem 273: 28253
15. Lee L-F, Hellendall RP, Wang Y, Haskill JS, Mukaida N, Matsushima K, Ting JPY (1999) IL-8 reduced tumorigenicity of human ovarian cancer in vivo due to neutrophil infiltration (in press)
16. Manthey CL, Brandes ME, Perera PY, Vogel SN (1992) Taxol increases steady-state levels of lipopolysaccharide-inducible genes and protein-tyrosine phosphorylation in murine macrophages. J Immunol 149: 2459
17. Martini N, Ginsberg RJ (1996) Treatment of stage I and II disease. In Aisner J, Arriagada R, Green MR, Martini N, Perry MC (eds) Comprehensive textbook of thoracic oncology. Williams and Wilkins, Baltimore, p 339
18. Parkin DM, Pisani P, Ferlay J (1999) Global cancer statistics. CA 49: 33
19. Patel AM, Jett JR (1996) Clinical presentation and staging of lung cancer. In: Aisner J, Arriagada R, Green MR, Martini N, Perry MC (eds) Comprehensive textbook of thoracic oncology. Williams and Wilkins, Baltimore, p 293
20. Perera PY, Qureshi N, Vogel SN (1996) Paclitaxel (taxol)-induced NF-kB translocation in murine macrophages. Infect Immun 64: 878
21. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
22. Sano H, Nakagawa N, Chiba R, Kurasawa K, Saito Y, Iwamoto I (1998) Cross-linking of intercellular adhesion molecule-1 induces interleukin- 8 and RANTES production through the activation of MAP kinases in human vascular endothelial cells. Biochem Biophys Res Commun 250: 694
23. Schiff PB, Horwitz S (1980) Taxol stabilizes microtubules in mouse fibroblast cells. Proc Natl Acad Sci USA 77: 1561
24. Singh RK, Gutman M, Radinsky R, Bucana CD, Fidler IJ (1994) Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. Cancer Res 54: 3242
25. Smith DR, Polverini PJ, Kunkel SL, Orringer MB, Whyte RI, Burdick MD, Wilke CA, Strieter RM (1994) Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. J Exp Med 179: 1409
26. Wang C-Y, Mayo MW, Baldwin ASJ (1996) TNF and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. Science 274: 784

27. Wang T-Z, Wang H-S, Ichijo H, Giannakakou P, Foster JS, Fojo T, Wimalasena J (1998) Microtubule-interfering agents activate c-Jun N-terminal kinase/stress-activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. J Biol Chem 273: 4928

28. Wang CY, Cusack JC Jr, Liu R, Baldwin AS Jr (1999) Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. Nat Med 5: 412