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

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 Æ Chemokines Æ Signal transduction Æ Transcription factors

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 (JNK) 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. The 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.

**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 × 10⁵ 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.

**Northern hybridization**

RNA was isolated by the guanidinium isothiocyanate and cesium chloride method as previously described [5]. Total RNA (5–10 μg) was electrophoresed 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