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# Comparing the Relationship of Taxol-Based Chemotherapy Response with P-glycoprotein and Lung Resistance-Related Protein Expression in Non-Small Cell Lung Cancer

J.-F. Chiou,[1] J.-A. Liang,[2] W.-H. Hsu,[3] J.-J. Wang,[4] S.-T. Ho,[5] and A. Kao[6]

[1]Cancer Center and Department of Radiation Oncology, Taipei Medical University Hospital, Taipei, Taiwan
[2]Department of Radiation Therapy and Oncology, China Medical University Hospital, Taichung, Taiwan
[3]Division of Pulmonary/Critical Care Medicine, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan
[4]Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
[5]Department of Anesthesiology, Tri-Service General Hospital, Taipei, Taiwan
[6]Department of Medical Research, China Medical University Hospital, No. 2, Yuh-Der Road, Taichung 404, Taiwan

**Abstract.** Our aim was to compare Taxol-based chemotherapy response of non-small cell lung cancer (NSCLC) with P-glycoprotein (Pgp) or lung resistance protein expression (LRP). Immunohistochemical analyses were performed on multiple nonconsecutive sections of the biopsy specimens to detect Pgp and LPR expressions in 40 patients with advanced NSCLC before Taxol-based chemotherapy. The chemotherapy response was evaluated by clinical and radiological methods in the third month after completion of treatment. No significant differences of prognostic factors (age, sex, body weight loss, performance status, tumor size, tumor stage, and tumor cell type) were found between the 20 patients with good and the 20 patients with poor responses. The incidence difference of positive Pgp expressions between good and poor responses was significant, however, the difference of LRP expression was not. We concluded that Taxol-based chemotherapy response of patients with NSCLC was related to Pgp but not LPR expression.

**Key words:** Non-small cell lung cancer—Taxol—P-glycoprotein—Lung resistance protein expression

*Correspondence to:* A. Kao; email: albertkaotw@yahoo.com.tw.

## Introduction

There is recent evidence that chemotherapy has a role in nonresectable NSCLC (stage III b or IV) [1, 2]. Recent papers have reported that the multidrug resistance - 1 (MDR1) gene-encoding human multidrug resistance-mediated P-glycoprotein (Pgp) may play an important role in the multidrug resistance of lung cancer [3]. Recently, a new resistance protein called lung resistance-related protein (LRP), has been identified in a lung cancer cell line selected for resistance to doxorubicin [4]. Expression of LRP was found in multidrug resistant cell lines not expressing Pgp [5]. The ideal therapeutic goal in advanced NSCLC is to achieve the highest response with the lowest possible morbidity from the side effects of chemotherapy. Therefore, it has been suggested that the determination of Pgp and LRP expressions at the time of diagnosis may provide valuable information for the design of treatment protocols. To answer whether Taxol-based chemotherapy response is related to Pgp or LRP expression in NSCLC, we compared Taxol-based chemotherapy response with Pgp and LRP expressions.

## Materials and Methods

### *Patients*

Forty patients (aged 40–72 years) with advanced NSCLC (stage IIIb or IV), including 17 epidermoid carcinomas and 23 adenocarcinomas, underwent Taxol-based chemotherapy in this study. Patient enrollment criteria included no prior chemotherapy, radiotherapy, or surgery; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; adequate hematologic function (granulocyte count $\geqq 1500/\mu l$, platelet count $>100000/\mu l$), hepatic (bilirubin $\leqq 1.25 \times$ the upper normal limit), and renal function (serum creatinine $\leqq 1.25 \times$ the upper normal limit); and adequate cardiac function, with no active arrhythmia or congestive heart failure. The patients underwent a complete history and physical examination. All were premedicated with dexamethasone (20 mg), cimetidine (300 mg), and diphenhydramine (50 mg) prior to initiation of the Taxol infusion [6–8]. Taxol 135 mg/m$^2$ was given as a 3-hour infusion on day 1 and cisplatin 75 mg/m$^2$ on day 2. The regimen was repeated every 3 to 4 weeks for up to 6 to 8 cycles unless there was evidence of tumor progression [6–8]. Taxol was well tolerated and none of the patients experienced an allergic reaction. Granulocytopenia was generally mild. The chemotherapy response and criteria were evaluated in the third month after completion of treatment and by clinical and radiological methods [9]. In this study, we just defined complete or partial response as good response in 20 patients, and no response or progressive disease was defined as poor responses in the other 20 patients.

### *Immunohistochemical Analyses*

Formalin-fixed paraffin sections (5-μm) from the biopsy specimens of the NSCLC were deparaffinized in an oven at 50°C for 40 minutes and hydrated with different concentrations of ethanol-water dilutions. Endogenous peroxidase was blocked by 3% hydrogen peroxide for 15 minutes. Antigen retrieval was performed by treatment with enzyme digestion in 0.1% trypsin in PBS for 5 minutes at room temperature and inhibited with 10% skim milk in PBS for 5 minutes. The sections were incubated for 2 hours in a moist chamber at 37°C with primary antibody JSB-1 for Pgp expression or LRP-56 for LRP expression at a 1:50 concentration. After three 5-minute washes in PBS buffer, detection of the primary antibody was performed with a link antibody according to the manufacturer's instructions [8, 10]. All

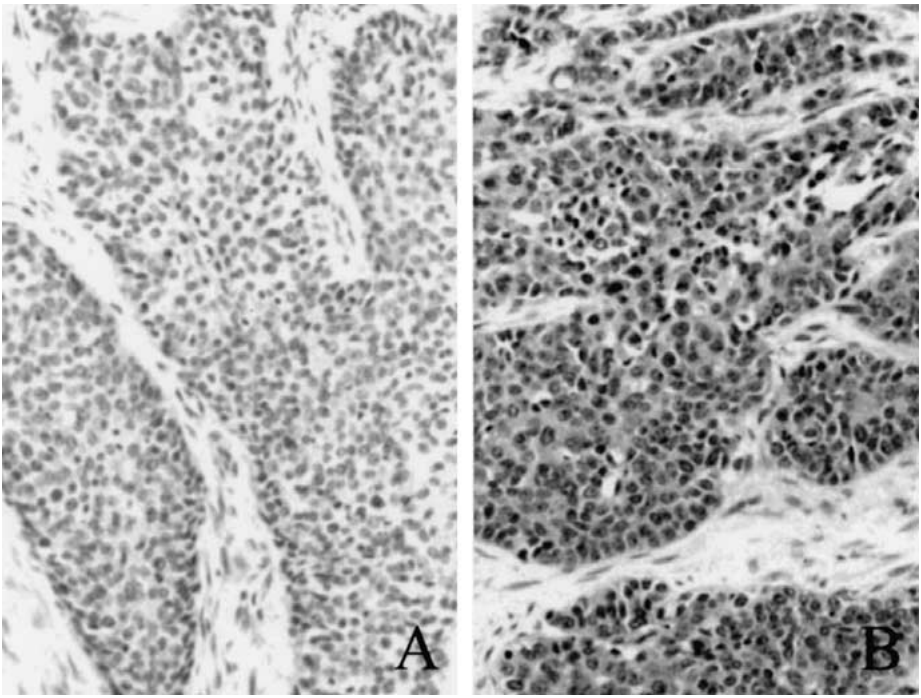


**Fig. 1.** Immunohistochemistry performed on sections of the whole epidermoid carcinoma specimens from two different groups reveals (**A**) negative and (**B**) positive Pgp expression.

specimen evaluations were performed on a Nikon microscope (AFX-DX) using an ocular magnification of ×20 with an eyepiece grid. Positive cells were quantified by evaluating four randomly selected high-power fields (minimum 800 tumor cells). Pgp or LRP expression was interpreted by an experienced pathologist blinded to clinical outcome as follows: negative = (1) when there was a complete absence of staining (−), or (2) scattered (+) or focal (f+) positive cells less than 10% of the specimen with weak staining, and positive = diffuse positive cells equal to or more than 10% of the specimen with weak (++) or strong (+++) staining (Figures 1 and 2).

### *Statistical Analyses*

Chi-square or Fisher exact $p$ tests were used to test the differences of incidences with positive Pgp, and LRP expressions between good versus poor Taxol-based chemotherapy response. If the $p$ value was $<0.05$, the difference was considered significant.

## Results

No significant differences of prognostic factors (age, sex, body weight loss, EGCO performance status, tumor size, tumor stage, and tumor cell type) were found between the 20 patients with good and the other 20 patients with poor responses (Table 1). The incidences of positive Pgp expression in the patients with good

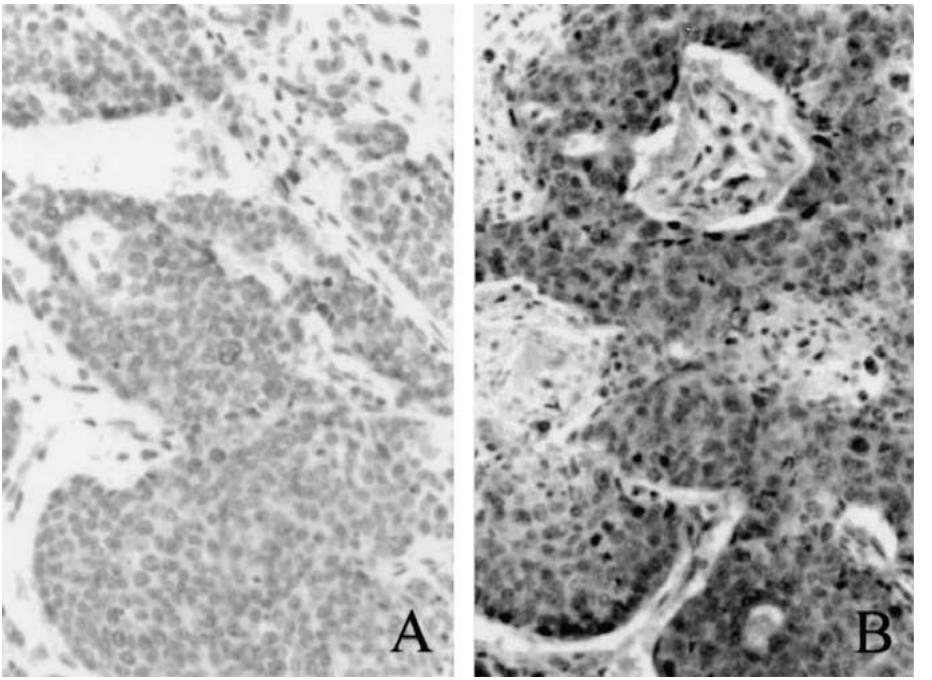


**Fig. 2.** Immunohistochemistry performed on sections of the whole epidermoid carcinoma specimens from two different groups reveals (**A**) negative and (**B**) positive LRP expression.

versus poor Taxol-based chemotherapy response were 0/20 (0%) and 14/20 (70%), respectively. The difference was not significant. The incidences of positive LRP expression in the patients with good versus poor Taxol-based chemotherapy response were 11/20 (55%) and 12 (60%), respectively. The difference was not significant (Table 1).

## Discussion

Taxol, that promotes polymerization of cellular microtubules and prevents mitosis, is the first taxane for treating stage IV NSCLC patients and has had the highest response rates (>20%) for the past 10 years using similar study populations [1, 2]. However, many toxic reactions and drug resistance encountered during the chemotherapy of Taxol in lung cancers [11] will result in an unnecessary waste of the medical insurance budget. Therefore, before initiating Taxol-based chemotherapy, it is important to correctly understand drug resistance expression in NSCLC, to achieve a satisfactory chemotherapy response, decrease unnecessary insurance cost, and avoid lethal side effects.

Pgp is an integral membrane protein belonging to the ATP-binding cassette (ABC) superfamily of transporter protein, which appears to confer resistance by

**Table 1.** Distribution of prognostic factors to Taxol-based chemotherapy response

| Chemo-therapy response | Pgp expression | | | LRP expression | | | Sex | | | Age | | | Body weight loss | | | ECOG | | | Tumor stage | | | Tumor cell type | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posi-tive | Nega-tive | *p* value | Posi-tive | Nega-tive | *p* value | Female | Male | *p* value | Old | Young | *p* value | Severe | Mild | *p* value | Good | Poor | *p* value | IIIb | IV | *p* value | Epider-moid Ca | Adeno Ca | *p* value |
| Good | 0 | 20 | | 11 | 9 | | 8 | 12 | | 9 | 11 | | 5 | 15 | | 9 | 11 | | 8 | 12 | | 9 | 11 | |
| Poor | 14 | 6 | $<0.01$ | 12 | 8 | 0.75 | 7 | 13 | 0.74 | 10 | 10 | 0.75 | 6 | 14 | 0.72 | 7 | 13 | 0.52 | 7 | 13 | 0.74 | 8 | 12 | 0.52 |

Pgp: P-glycoprotein, LRP: lung resistance protein expression, ECOG: Eastern Cooperative Oncology Group, Ca: carcinoma.

decreasing intracellular drug accumulation and enhancing drug efflux [12, 13]. Acquired resistance to Taxol is conferred by the MDR phenotype, and involves the amplification of membrane Pgp and reduced ability to accumulate and retain Taxol due to the energy-dependent Pgp efflux pump, which has a central role in the transport of chemotherapy drugs through the cell membrane [14–16]. In our study, we found that Taxol-based chemotherapy response was correlated to Pgp expression in NSCLC by immunohistochemical staining. In contrast, LRP is not an ABC transporter protein. LRP has recently been identified as a vault protein, which is a typical multisubunit structure involved in nucleocytoplasmic transport [17]. Because of previous findings that increased cytoplasm concentration of the chemotherapy agent increases its contact with the membrane, we believe that efflux of the drug might be enhanced in LRP expression [18]. Although some previous findings indicated that LRP might be resistant to Taxol [19, 20], however, intrinsic and acquired Taxol resistance was primarily mediated by Pgp, but not by LPR expression [21]. Therefore, our results showed that good and poor Taxol-based chemotherapy response in NSCLC were not consistent with negative and positive LRP expressions.

We conclude that Taxol-based chemotherapy response is related to Pgp but not LPR expression detected by immunohistochemical staining in NSCLC. However, further study with more cases is necessary to confirm our findings.

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