Roles of BCL-2 and MDR1 Expression in the Efficacy of Paclitaxel-based Lung Cancer Chemoradiation

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Abstract. *Background: The associations between B-cell lymphoma 2 (BCL-2) and multi-drug resistance associated P-glycoprotein (MDR1) expressions and chemoradiotherapy outcome of patients with non-small cell lung cancer (NSCLC) were analysed. Patients and Methods: Thirty-two NSCLC patients were treated with paclitaxel-based chemoradiotherapy. The tumour expressions of BCL-2 and MDR1 were analysed by means of immunohistochemistry with regard to the clinical response and survival data. Results: Partial remission and stable disease were achieved in 19 (59%) and 10 (31%) cases, respectively. Significant differences in progression-free survival were observed between responders and non-responders (13.7 vs. 6.0 months, p=0.028), and between patients with or without a gross tumour volume (GTV) shrinkage ($GTV_{>50}$ 13.7 vs. 6.0 months, p=0.009). Overexpression of BCL-2 and of MDR1 was observed in 6 (21.4%) cases each. Overexpression of both markers together was associated with poor response (GTV reduction: p=0.005; RECIST: p=0.023) and lower progression-free survival (overexpression of both, low expression of both, mixed: 3.1, 13.4, 4.1 months, respectively, p<0.001). Conclusion: BCL-2 and MDR1 overexpression may predict the inefficacy of paclitaxel-based chemoradiotherapy.*

Lung cancer is the most frequent tumour worldwide. Molecular markers may be of prognostic value, especially in the early stages. The identification of specific genes that may be targeted by new therapies appears to be a potentially rewarding approach.

Individualized combinations of various treatment procedures for locally advanced lung cancer tend to improve local control and survival. Such favourable results may be achieved through the application of increasingly more sophisticated chemotherapeuticals and their combinations with radiotherapy (1, 2). Radiosensitization has been reported to increase therapy efficacy, but it may also increase therapy-induced toxicity (1, 3).

The most frequently applied third-generation chemotherapeuticals are the taxanes, which have been demonstrated in clinical trials to be widely effective in advanced non-small cell lung cancer (NSCLC) (1, 2). The prototype of the taxane family is paclitaxel, an excellent radiosensitizer (1, 2, 4-6). In designing the optimum individual treatment planning, including selection of an effective chemotherapeutical and the anticipation of potential radioresistance, the physician may be aided by predictive markers (7-20).

Multi-drug resistance-associated P-glycoprotein (MDR1, P-gp170, ABCB1) is a well-known plasma membrane drug efflux pump that is associated with resistance to a wide range of anticancer drugs. Paclitaxel is a substrate of this transporter system. The overexpression of MDR1 may play an important role in the paclitaxel-resistance of lung cancer (21-23).

The oncogenic protein B-cell lymphoma 2 (BCL-2), which plays a central role in apoptosis, has been found to correlate with the prognosis of NSCLC. Most studies have suggested a more favourable prognosis in BCL-2-positive cases (7-10), but poorer survival rates of patients with higher BCL-2 expression have also been reported (11-13).

The predictive role of BCL-2 has also been examined in various tumour types. The association between the response to platinum-based chemoradiotherapy and apoptosis-related proteins is unclear. No correlation was found in cancer of the bladder (14), the oesophagus (15) or the rectum (16). The overexpression of BCL-2 predicted a more favourable outcome in head and neck (17) and lung (18) cancer, but an unfavourable effect has been described for oropharyngeal (19) and lung cancer patients (11).

The aim of this study was to analyse the associations between the expressions of BCL-2 and MDR1 with the clinical outcome of paclitaxel-based chemoradiotherapy in patients with NSCLC.

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Key Words: Lung cancer, paclitaxel-based chemoradiotherapy, BCL-2, MDR1, radiosensitivity.

Patients and Methods

The study was conducted in full accordance with the institutional regulations and all the patients gave their written informed consent for participation in the chemotherapy and radiotherapy.

Study population. Patients receiving chemoradiotherapy for primary unresectable or potentially resectable NSCLC at the Department of Oncotherapy in the period between December 2006 and June 2010 were eligible for participation in this study. All tumours were proven by histological verification. The staging procedures were based on the conventional protocol (chest computer tomography (CT), abdominal ultrasound/CT, brain CT, bone scan, bronchoscopy) and on induction chemotherapy. For each patient, the treatment plan was designed by a multidisciplinary team.

Systemic treatment and radiotherapy. During the radiotherapy, all the patients received concomitant taxane-based chemotherapy (weekly paclitaxel 100 mg/m^2 in 4-6 cycles, depending on toxicity). Of the 19 patients (stage IIIB) who completed induction chemotherapy (1 or 2 cycles), 17 (89.5%) received a taxane-based chemotherapy regimen (paclitaxel 175 mg/m^2 with carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2 with cisplatin (CDDP) 75 mg/m^2, at 3-week intervals), while 2 patients received a gemcitabine-based regimen (gemcitabine 1250 mg/m^2 on days 1 and 8, CDDP 70 mg/m^2 on day 1, and then at 3-week intervals) for at least 4 weeks prior to the concomitant chemoradiotherapy. After completion of the chemoradiotherapy, additional consolidation chemotherapy was administered in 18 cases (paclitaxel 175 mg/m^2 with carboplatin 400 mg/m^2 or docetaxel 75 mg/m^2 with CDDP 75 mg/m^2, at 3-week intervals).

CT-based three-dimensional treatment planning and conformal radiotherapy were performed in all cases, with use of an individual immobilization system. The planning target volume encompassed the macroscopic lung cancer, the involved mediastinal and ipsilateral hilar lymph node regions and the safety zone, according to the local protocol. The initial radiation dose was 25×1.8 Gy; after a repeated CT scan, depending on the tumour response, radiotherapy of the reduced volume was continued at an average dose of 22-26 Gy (with the exception of neoadjuvant therapy).

Response analysis. During the treatment, following the administration of a 45 Gy dose, and 4-6 weeks after the completion of the chemoradiotherapy regimen, clinical and diagnostic CT examinations were performed. The CT scans were compared with the pretreatment scans provided for radiotherapy-planning purposes. Response analysis was carried out by means of two methods: the exact values of gross tumor volume (GTV) and GTV1 were determined and the tumour volume reduction was calculated (reductions in the ranges >50%, 50-40% and <40% are referred to as $GTV_{>50}$, GTV_{50-40} and $GTV_{<40}$, respectively), additionally, the CT scans were analysed according to the RECIST criteria system (24).

Immunohistochemistry. Before the chemoradiotherapy, immunohistochemical staining of the biopsy samples was performed to quantify the BCL-2 and MDR1 expressions. Histological samples from 28 out of the 32 evaluated patients were examined prospectively for BCL-2 and MDR1 (clone 494) expressions. (The histological samples from 4 patients were used for K-RAS analysis and the remaining material was not sufficient for further immunohistochemical analysis.) Immunohistochemical studies were carried out on paraffin sections by an indirect peroxidase method. Sections were cut 4-μm-thick. Deparaffinizing, rehydrating and antigen retrieval were performed in a PraeTreatment module (Dako, Glostrup, Denmark) (20 min, 98 °C), using the 3in (pH 6.0) solution produced by LabVision (Fremont, CA, US). The endogenous peroxidase activity was blocked with hydrogen peroxide (3%, 10 min) and a solution of milk powder (in 1% phosphate-buffered saline, 10 min) was used as protein block. The Real-Envision (DAB) kit (Dako) was used as the labelling system. A semiquantitative scoring method was used to rate immunohistochemical staining: positive cell rates in the ranges 0-1%, 2-33%, 34-66% and >66% were scored as 0, 1+, 2+ and 3+, respectively. The intensity staining score was 2+ (moderate) in all cases. Any cytoplasmic staining with BCL-2 was considered positive. BCL-2 and MDR1 expression at a level of 2-3+ was classified as high expression (Figure 1).

Statistical analysis. All analyses were carried out using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The main outcome measures were the tumour response and the time to progression. The associations between the molecular marker expressions and clinical factors, tumour response, local relapse and presence of distant metastasis within 6 months were evaluated with the chi-square test, while those with age were assessed with the one-way ANOVA test. In terms of both progression-free (PFS) and overall survival (OS), the outcome was analysed by Kaplan-Meier analysis (pairwise comparisons – Breslow test).

Results

Patient characteristics, response and survival. Thirty-two patients received paclitaxel-based chemoradiotherapy, at a mean dose of 64.0 (45.0-70.0) Gy, in combination with a mean of 5 (4-6) cycles of chemotherapy. The mean age (±SD) of the patients was 58.9 (±6.2) years; 21 (66%) were men. Most of the patients had stage IIIB cancer (75%). Neoadjuvant treatment was administered to 5 patients with stage IIIA and 3 with stage IIB sulcus superior (Pancoast) tumours, respectively. The performance status of the patients was good (ECOG 0 and 1, 44% and 56%). The histological type was adenocarcinoma in 20 (62.5%) and squamous cell carcinoma in 12 cases (37.5%).

After the chemoradiotherapy, surgical treatment was possible in 10 cases (31%) and 18 patients (56%) received consolidation chemotherapy. At the time of the last follow-up (median 17 months), 14 (44%) patients had died, 12 due to lung cancer, 1 following the surgical procedure due to pulmonary embolization, and another after the chemoradiotherapy due to pneumonitis, all in stage IIIB. Fifteen patients (47%) developed local or distant recurrence.

Of the 32 lung cancer patients, 19 (59%) exhibited partial remission (PR), while 10 (31%) had stable disease (SD). The condition of all three patients (10%) with progressive disease (PD; 2 locoregional and 1 distant metastasis) worsened during the treatment. There was a significant difference in the duration of PFS between the responders (PR) and the non-responders (SD+PD) (13.7 *vs*. 6.0 months, p=0.028), but

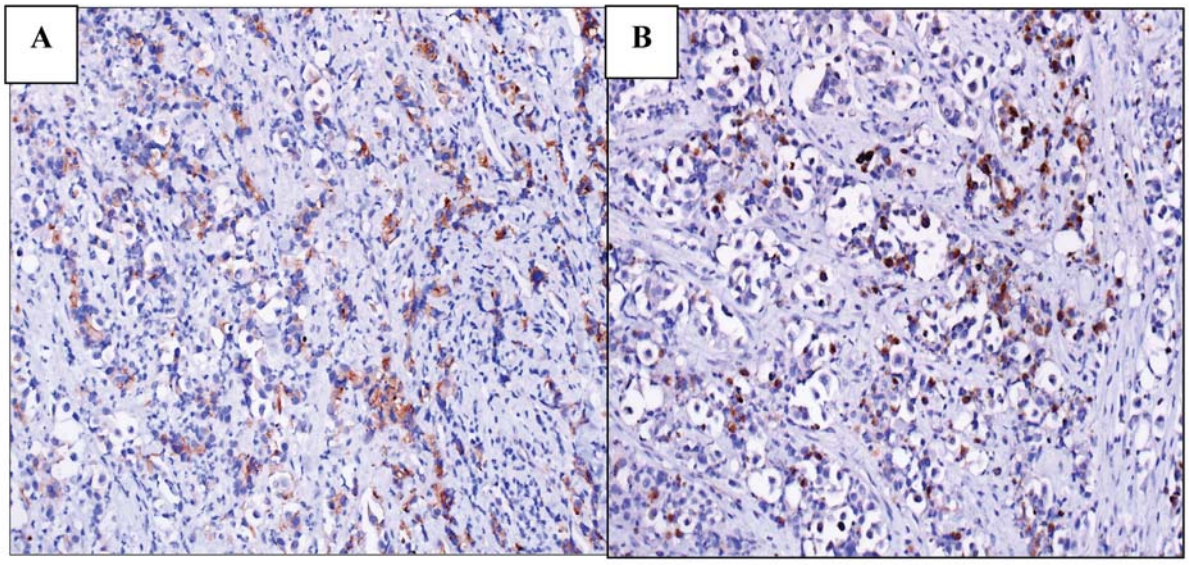

Figure 1. *Immunohistochemistry of biopsy specimens. A: Moderate cytoplasmic positivity for MDR1 (original magnification ×224); B: moderate nuclear and cytoplasmic positivity for BCL-2 (original magnification ×224).*

Table I. *Distribution of BCL-2 and multi-drug resistance associated P-glycoprotein (MDR1) expression.*

n=28	BCL-2			MDR1			BCL-2 and MDR1		
	Negative	1+	2+/3+	Negative	1+	2+/3+	Both low/neg	Mixed expression	Both 2+/3+
n	16	6	6	19	3	6	19	6	3
%	57.2	21.4	21.4	67.8	10.7	21.4	67.9	21.4	10.7

there was no significant difference in the OS duration (29.1 *vs*. 15.7 months, p=0.256). Our analysis of the relationship between PFS and OS and the tumour volume shrinkage ($GTV_{>50}$, GTV_{50-40} and $GTV_{40>}$) indicated that the PFS results were more favourable in patients with than in those without tumour shrinkage ($GTV_{>50}$ 13.7 *vs*. 6.0 months, p=0.009; GTV_{50-40} 13.43 *vs*. 4.8 months, p=0.008; $GTV_{<40}$ 13.4 *vs*. 4.8 months, p=0.008), but no association was found between the OS and the shrinkage ($GTV_{>50}$ 29.1 *vs*. 26.6 months, p=0.979; GTV_{50-40} 26.6 *vs*. 22.0 months, p=0.656; $GTV_{<40}$ 26.7 *vs*. 29.1 months, p=0.846). Of the 10 patients who underwent surgery, 5 (50%) exhibited pathologically complete remission and the other 5 a partial pathological response. The difference between the surgical and non-surgical populations was not significant from the point of view of their survival (29.1 *vs*. 22.1 months, p=0.119), but there was a more favourable trend in the outcome after surgery. The OS rate correlated significantly only with consolidation chemotherapy (survival with or without chemotherapy, 13.4 *vs*. 4.8 months, p<0.001), and the duration of PFS was also longer (29.4 *vs*. 11.3 months, p<0.001).

Association of expression of drug resistance- and apoptosis-related proteins with clinicopathological characteristics. There were 16 (57.2%) BCL-2-negative and 19 (67.8%) MDR1-negative cases. A low expression of BCL-2 and of MDR1 was observed in 6 (21.4%) and 3 (10.7%) cases, respectively. A high expression of BCL-2 and of MDR1 was observed in 6 (21.4%) cases each. The tumour in three (10.7%) patients displayed high expression of both markers (Table I). There were no significant correlations between a high expression of BCL-2 or MDR1 and other characteristics of the patients (age, gender, stage or histology).

Association of expression of drug resistance- and apoptosis-related proteins with response and outcome of patients. A high expression of both markers simultaneously was significantly associated with a poor response to paclitaxel-based chemoradiotherapy, when evaluated against either a GTV reduction during therapy (p=0.019), or a tumour response according to RECIST (p=0.005) (Table II). A high expression of BCL-2 or of MDR1 was significantly associated with a lower duration of PFS (BCL-2 high *vs*. low/negative, 4.2 *vs*. 13.4 months p=0.025; MDR1 high *vs*.

Table II. *Association of molecular marker expression (BCL-2, MDR1) with clinical outcome.*

n=28	Response (RECIST)				Volume change (%)				PFS	
Expression	PR	SD	PD	*p*-Value*	>50	50-40	<40	*p*-Value*	Months (median±S.D.)	*p*-Value**
BCL-2										
Neg/low	16	5	1	0.082	13	3	6	0.151	13.4±2.5	0.025
High	2	2	2		1	1	4		4.2±0.6	
MDR1										
Neg/low	17	4	1	0.016	11	4	4	0.001	13.4±2.2	<0.001
High	1	3	2		3	-	-		1.63±1.1	
Both neg/low	15	3	1	0.005	13	3	3	0.019	13.4±0.2	<0.001
Mixed	3	3	-		1	1	4		4.1±1.9	
Both high	0	1	2		0	0	3		3.1±1.4	

*Chi-square test; **Kaplan-Meier; PR, partial remission; SD, stable disease; PD, progressive disease; PFS, progression-free survival.

low/negative, 1.63 *vs*. 13.4 months, respectively, $p<0.001$), whether evaluated separately or together (BCL-2 and MDR1 both high, both low/negative or mixed expression, 3.1, 13.4 and 4.1 months, respectively, $p<0.001$). PFS was shorter in cases with MDR1-positive tumours than in those with MDR1-negative ones (3.1 *vs*. 13.4 months $p=0.003$). In patients with pathologically complete remission, both markers were negative. No association was found between OS, the appearance of early metastases (within 6 months) and the expression of BCL-2 or MDR1. Local recurrence within 6 months was more frequent in patients with overexpression of BCL-2 ($p=0.0023$) or of MDR1 ($p=0.007$).

Discussion

The present study demonstrated that overexpression of the evaluated anti-apoptotic and cell membrane proteins can help predict the efficacy of paclitaxel-based chemoradiotherapy. We observed a strong association between the concurrent overexpression of BCL-2 and MDR1, and the tumour response and PFS in NSCLC patients. The novelty of our study lies in the analysis of the efficacy of paclitaxel and concomitant radiotherapy in relation to the expressions of the above markers.

We mainly used paclitaxel with radiotherapy or paclitaxel in combination with carboplatin in the course of induction and consolidation therapy. Paclitaxel stabilizes microtubules, which blocks cell cycle progression in the most radiosensitive G_2-M phase, and also induces BCL-2 hyperphosphorylation, resulting in its inactivation, thereby facilitating apoptosis (25, 26). In our cohort, those patients demonstrating no response to paclitaxel-based chemoradiotherapy had a significantly more unfavourable PFS, and a worse (although not significantly) OS, which highlights the importance of predicting the potentially non-responsive patient population.

We observed a significant association between the tumour response, the reduced PFS and the overexpression of MDR1. Preclinical data demonstrated that the increased activity of the signalling pathway in paclitaxel-resistant cell lines was directly attributable to the overexpression of MDR1. It was reported that MDR1 may contribute to the multidrug resistance of lung cancer (21-23).

The prognostic value of BCL-2 positivity has been widely studied. Several papers have confirmed a more favourable disease progression in patients with BCL-2-positive tumours with either surgically resected (7) or locally advanced (10) NSCLC, or even irrespective of the stage (8, 9). However, some studies did not find a significant association between BCL-2 expression and survival (28, 29), or even reported a worse survival in the event of high BCL-2 expression (11-13).

Considerably fewer studies have examined the interaction between the expression of BCL-2 and the outcome of oncological treatment, *i.e*. the role of BCL-2 in predicting the tumour response, and yielded controversial results (14-19). Jeong *et al*. treated NSCLC patients with cisplatin-based chemoradiotherapy and observed that a high expression of BCL-2 was significantly associated with a longer survival and a better response to the treatment (18). The findings of Fokkema *et al*. indicated a more favourable PFS of patients with an overexpression of BCL-2 following radiotherapy with or without carboplatin-based chemo-radiotherapy, although they did not analyse the radiotherapy and chemoradiotherapy cohorts separately (10). Hwang *et al*. reported that BCL-2 expression predicted a poor outcome for radiation-treated NSCLC patients (11). Our own results revealed that, as compared with patients with a negative or low BCL-2 expression, patients with an overexpression of BCL-2 demonstrated a significantly worse RECIST tumour response and a poorer PFS after

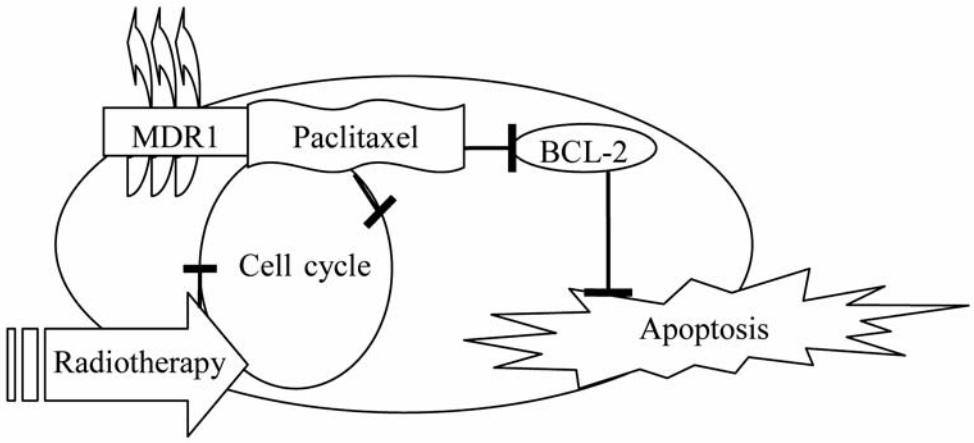

Figure 2. *The role of BCL-2 and MDR1 in paclitaxel-based chemoradioresistance. MDR1 expression indicates enhanced drug efflux activity. BCL-2, as an anti-apoptosis factor, may inhibit radiotherapy-induced programmed cell death.*

paclitaxel-based chemoradiotherapy, and in the event of the overexpression of both MDR1 and BCL-2, the tumour response was significantly poorer.

BCL-2 inhibits programmed cell death, and can be associated with a more aggressive tumour cell phenotype, with conseqvent resistance to treatment protocols based on microtubule damage. The efficacy of radiotherapy-induced apoptosis, and therefore the whole of the treatment, may be reduced in tumours exhibiting an overexpression of BCL-2 (11-13). The predictive value of BCL-2, especially for radiotherapy combined with third-generation chemotherapy, a prevalent advanced treatment procedure, is still unclear.

In our study, the overexpression of both biomarkers was found in the patients with the poorest tumour response and PFS. We hypothesize that a high MDR1 expression indicates an enhanced drug efflux activity, leaving the cell without an adequate amount of chemotherapeutic agent. A high BCL-2 expression, as an antiapoptotic mechanism, may inhibit radiotherapy-induced programmed cell death (Figure 2). This hypothesis is supported by the finding that both markers were negative in tumours from patients demonstrating complete pathological remission.

The overexpression of MDR1 in our cohort was associated with a poorer tumour response. However, for patients with an overexpression of BCL-2, but not of MDR1, there was a slightly, although not significantly better therapeutic efficacy as compared with patients displaying an overexpression of both markers. We assume that paclitaxel terminated the inhibition of apoptosis by hyperphosphorylating and inactivating BCL-2, and thereby partially restoring radiosensitivity (25, 27) (Figure 2).

Our findings demonstrate that the concomitant application of paclitaxel and radiotherapy is potentially ineffective in the treatment of NSCLC, leading to a shorter PFS and more frequent local remission if the tumour indicates the overexpression of both MDR1 and BCL-2. The findings suggest that paclitaxel-based chemoradiotherapy is questionable in this group of patients, who may benefit more from the combination of other drugs with radiotherapy, which may be favourable even in the case of an overexpression of BCL-2 and since platinum derivatives are not substrates of the multidrug efflux pump (18, 30).

In conclusion, the present study has revealed that the overexpression of BCL-2 and MDR1 is of potential predictive value as regards the inefficacy of paclitaxel-based chemoradiotherapy in NSCLC.

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