Pathol. Oncol. Res. (2009) 15:445–450
DOI 10.1007/s12253-009-9155-z

ORIGINAL PAPER

Platinum-Based Chemotherapy in Lung Cancer Affects the Expression of Certain Biomarkers Including ERCC1

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Received: 13 November 2008 / Accepted: 12 February 2009 / Published online: 2 March 2009

Abstract Chemotherapies are widely used in the treatment of lung cancer. However, little is known about their effect in the expression of different tissue markers. Seventeen lung cancer tissue blocks obtained by bronchoscopic biopsies together with their corresponding surgical biopsies after neoadjuvant chemotherapy were studied. They included 9 adenocarcinomas (ADC) and 8 squamous cell carcinomas (SCC). Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues to study the expression of Ki-67, p53, Bcl-2, Bax, Fas-ligand and ERCC1 (excision repair cross-complementation group 1). Out of 17 NSCLC 6 expressed proapoptotic markers and 4 expressed antiapoptotic markers, while in 7 cases the apoptotic markers did not show detectable changes after neoadjuvant chemotherapy. Six of 17 bronchoscopic NSCLC cases expressed increased level of Ki-67 after neoadjuvant treatment. Eight bronchoscopic NSCLC tissues (6 SCC, 2 ADC) expressed ERCC1. All but one ADC became ERCC1 negative after neoadjuvant therapy. There was no newly expressed ERCC1 positive case in the surgical biopsy group. Platinum-based neoadjuvant chemotherapy had no effect on the apoptotic activity of 17 patients' tumor specimen, however, 6 of 17 bronchoscopic NSCLC cases expressed increased level of Ki-67 after neoadjuvant treatment, in 3 cases the level of Ki-67 became decreased, while 8 cases had no detectable change of proliferation activity. The results of the present study suggest that platinum-based chemotherapy probably induces a selection of tumor cells with more aggressive phenotype, and also affects the expression of tissue marker (ERCC1) that could have predictive value.

Keywords Chemotherapy · Immunohistochemistry · Lung cancer · Molecular biology · Neoadjuvant treatment

Abbreviations

NSCLC	non-small cell lung cancer
ADC	adenocarcinoma
SCC	squamous cell carcinoma
BAC	bronchiolo-alveolar carcinoma
ERCC1	excision repair cross-complementation group 1

Introduction

Lung cancer is the leading cause of cancer death, and it is responsible for 20–25% of all cancer deaths. Worldwide, there are more than 1.2 million new cases each year [1]. Patients with lung cancer often present with advanced disease when surgical resection of the tumor is not possible and the treatment is rarely curative. Platinum-based chemotherapies

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are the standard treatment, however, common toxicities often limit their use. In non-small cell lung cancer (NSCLC), accounting for 75% of lung cancer cases, the response rates to chemotherapies still remain between 15–35% despite the rapid development in antitumor treatment options [2]. The 2-year survival of NSCLC patients with advanced disease treated with standard platinum-based chemotherapies is around 20%. The response rates of chemotherapies assessed clinically are widely examined in lung cancer, however, their effects on the expression of certain tissue biomarkers are far less known.

We have studied the expression of different tissue markers in lung cancer patients before and after cisplatin-containing chemotherapy using immunohistochemistry on diagnostic bronchoscopic biopsy materials together with the corresponding surgical tumor tissue samples. The aim was to examine the effect of neoadjuvant chemotherapy on cell proliferation, apoptosis, and the expression level of certain marker involved in DNA repair mechanism that could serve as predictive marker in the treatment of lung cancer.

Patients and Methods

Patients

Seventeen patients with locally advanced primary lung cancer, who were treated with cisplatin-containing chemotherapy were studied. There were 11 men (mean age: 53 years) and 6 women (mean age: 48,7 years). Lung cancer tissue blocks obtained by bronchoscopic biopsies together with their corresponding surgical biopsies after neoadjuvant chemotherapy were analyzed. They included 9 adenocarcinomas (ADC) and 8 squamous cell carcinomas (SCC). All but one patients responded well to induction therapy, therefore, surgical resections were performed. One patient had inoperable adenocarcinoma, and after chemotherapy endobronchial tumor progression was observed, therefore, mechanical tumor extraction was performed during rigid bronchoscopy. The obtained tumor tissue was regarded as the post-chemotherapeutic tumor sample. The patients' clinical and pathological characteristics are summarized in Table 1. Permission for using the archived tissue blocks was obtained from the Regional Ethical Committee (TUKEB N^{o} 7/2006). The tumors were classified histologically according to the criteria of the WHO. Mixed tumors, poorly differentiated as well as anaplastic tumors were excluded from the study.

Table 1 Patients' characteristics

Number of patients	17
Age (years)	
Median	51
Range	40–65
Gender	
Male	11
Female	6
Stage (TNM at time of surgical diagnosis)	
I / A	2
I / B	4
II / A	2
II / B	3
III / A	4
III / B	1
IV	1
Histology	
ADC (BAC)	9 (1)
SCC	8
Neoadjuvant treatment (≥ 2 cycles)	17
Cisplatin-gemcitabine	14
Cisplatin-etoposide	3

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues to study the expression of Ki-67, p53, Bcl-2, Bax, Fas-ligand and ERCC1 (excision repair cross-complementation group 1). Four μm thick tissue sections were deparaffinized in xylene and rehydrated. Immunohistochemical examination was performed using standard avidin-biotin-peroxidase complex method. Antibody specifications and the conditions of their application in this study are listed in Table 2.

Immunostained sections were examined independently by two pathologists using an empirial scale based on standard criteria for each marker, considering cellular localization and the proportion of tumor cells stained. Biomarkers were evaluated as positive, when the percentage of stained cells was ≥ 5%. The ratio of tumor parenchyma and stroma was determined to make the bronchoscopic and surgical samples comparable (Table 3.).

The low number of cases studied did not make possible to perform statistical analysis, therefore, the evaluation was carried out case by case.

Results

The expression of the different markers in pre-and postoperative NSCLC tissues are summarized in Table 4 and 5.

Expression of p53 in Pre-and Postoperative NSCLC Tissues

P53 was expressed in 7 cases and negative in 10 cases of preoperative NSCLC tissues. In 1 case the expression of

Table 2 Specification of the immunostaining conditions

Biomarker	Source/Clone	Manufacture	Cellular compartment	Dilution	Pre-treatment
p53	Mouse/DO-7	DakoCytomation	N	1.50	Citrate
Bcl-2	Mouse/124	DakoCytomation	C	1:20	Citrate
Bax	Polyclonal	DakoCytomation	C,N	1:100	Citrate
Fas-ligand	APO-1/FAS DX2	DakoCytomation	CM	1.50	Citrate
Ki-67	MIB-1	DakoCytomation	N	1:100	Citrate
ERCC1	Mouse(8F1)	IOZOL	N	1.50	Citrate

Staining pattern: N nuclear, C cytoplasmic, CM cell membrane

p53 became negative, while 4 negative cases turned to be positive. In 12 tumors the neoadjuvant therapy had no effect on the p53 protein expression.

Expression of Fas-ligand in Pre-and Postoperative NSCLC Tissues

In 5 of 17 cases there was no detectable Fas-ligand expression and 12 NSCLC tissue samples expressed this marker in the preoperative tumor specimens. Three of previously negative cases (3/5) became positive in postoperative NSCLC group, however, 3 of Fas-ligand expressing preoperative cases (3/12) did not show Fas-ligand marker after neoadjuvant treatment.

Expression of Bcl-2 in Pre-and Postoperative NSCLC Tissues

Fifteen of 17 preoperative tumor samples did not express Bcl-2. Out of these 15 negative preoperative tissue samples 3 changed to be positive after chemotherapy. From the two Bcl-2 positive cases one became negative after neoadjuvant treatment.

Expression of Bax in Pre-and Postoperative NSCLC Tissues

Only 1 of the 17 preoperative NSCLC tumor tissues was negative for Bax marker. All the others expressed Bax marker almost at the same level of intensity. All of the postoperative NSCLC samples expressed Bax marker.

Expression of Ki-67 in Pre-and Postoperative NSCLC Tissues

Seven of preoperative cases expressed increased level of Ki-67 after neoadjuvant treatment, while 3 of 17 cases indicated decreased expression of the same marker. The rest of tumors (n=7) showed no remarkable change in proliferation marker.

Table 3 The tumor parenchyma and tumor stroma ratio was determined from each biopsies to make the bronchoscopic and surgical samples comparable

Case	Histology	Mitotic activity %		Apoptotic activitiy %		Tumor cell / Tumor stroma ratio	
		pre	post	pre	post	pre	post
1	SCC	2	3	1	0	90	60
2	SCC	3	2	1	4	90	70
3	SCC	4	3	0	1	90	75
4	SCC	2	4	1	0	80	70
5	SCC	2	3	1	1	70	85
6	SCC	2	0	0	1	90	80
7	SCC	1	2	1	2	90	70
8	SCC	2	2	0	1	70	60
9	ADC	6	0.5	0	1	80	70
10	ADC	4	0.5	1	1	70	80
11	ADC	3	2	1	2	90	95
12	ADC	2	2	1	0	40	10
13	ADC	4	4	0	0	80	85
14	ACC	2	6	1	2	50	80
15	ADC	3	2	0	1	60	50
16	ADC	2	4	0	3	70	90
17	BAC	1	1	0	1	80	80

Table 4 Biomarkers of apoptosis in bronchoscopic (**pre**operative) and surgical (**post**operative) tumor samples

Case	Histology	p53		FAS		Bcl 2		Bax	
		pre	post	pre	post	pre	post	pre	post
1	SCC	pos	pos	poz	neg	neg	neg	pos	pos
2	SCC	pos	pos	pos	pos	neg	pos	pos	pos
3	SCC	neg	pos	pos	pos	neg	neg	pos	pos
4	SCC	neg	neg	neg	pos	neg	neg	pos	pos
5	SCC	pos	pos	pos	pos	neg	neg	pos	pos
6	SCC	neg	neg	pos	neg	neg	neg	pos	pos
7	SCC	neg	neg	pos	pos	neg	neg	pos	pos
8	SCC	neg	neg	pos	neg	pos	pos	pos	pos
9	ADC	neg	neg	neg	pos	neg	neg	pos	pos
10	ADC	neg	neg	neg	neg	neg	neg	pos	pos
11	ADC	pos	neg	pos	pos	neg	neg	pos	pos
12	ADC	pos	pos	pos	pos	neg	neg	neg	pos
13	ADC	neg	pos	pos	pos	neg	pos	pos	pos
14	ADC	pos	pos	neg	pos	neg	neg	pos	pos
15	ADC	neg	pos	pos	pos	neg	pos	pos	pos
16	ADC	pos	pos	pos	pos	neg	neg	pos	pos
17	BAC	neg	pos	neg	neg	pos	neg	pos	pos

Expression of ERCC1 in Pre-and Postoperative NSCLC Tissues

Eight of preoperative NSCLC tissues (8/17) expressed ERCC1. Six of them were SCC, 2 cases were ADC. All but one ADC of these cases became negative after neoadjuvant therapy (Fig. 1). There was no newly expressed ERCC1 positive case in surgical biopsy group.

Expression of Markers in Different Histologic Types of NSCLC Tumors

Comparing the expression of certain apoptotic markers in different NSCLC histologic subtypes, there was no detectable association related either to SCC or ADC, however, the difference between the expression of ERCC1 in SCC and ADC tissues was remarkable (see above).

Table 5 Expression of ERCC1 marker (+/−) and Ki-67 (%) in pre-and postoperative NSCLC tumors

Case	Histology	ERCC1		Ki-67%	
		bronchoscopic (pre-chemo)	surgical (post-chemo)	bronchoscopic (pre-chemo)	surgical (post-chemo)
1	**SCC**	pos	neg	25	70
2	**SCC**	pos	neg	30	50
3	**SCC**	neg	neg	70	40
4	**SCC**	pos	neg	40	5
5	**SCC**	neg	neg	60	80
6	**SCC**	pos	neg	10	10
7	**SCC**	pos	neg	50	90
8	**SCC**	pos	neg	10	20
9	**ADC**	neg	neg	5	20
10	**ADC**	neg	neg	50	40
11	**ADC**	pos	neg	30	30
12	**ADC**	neg	neg	5	30
13	**ADC**	pos	pos	50	60
14	**ACC**	neg	neg	50	90
15	**ADC**	neg	neg	0	5
16	**ADC**	neg	neg	60	40
17	**BAC**	neg	neg	10	10

Bronchoscopic: preoperative sample before neoadjuvant chemotherapy

Surgical: postoperative sample after neoadjuvant chemotherapy

Discussion

The morphologic regression grade after neoadjuvant chemo-radio-therapy in NSCLC patients has been studied by Junker et al [3]. They observed significantly longer survival times in patients with tumors of high regression grades than those with low ones. They concluded that beyond the achievement of complete tumor resection (R0), a therapy-induced tumor regression of < 10% of vital tumor tissue is pivotal for superior long-term outcomes. However, the presurgical clinical response after patients had received neoadjuvant multimodality therapy had no predictive value in assessing the extent of therapy-induced tumor regression in the resection specimen.

There are increasing data on the predictive values of molecular markers, such as apoptosis and cell cycle regulator proteins for neoadjuvant chemotherapy response. Morero et al have recently evaluated p53, bcl-2, p21WAF1/CIP1, p27Kip1, and Ki-67 immunohistochemical expression and apoptotic index in mediastinal lymph node metastases from NSCLC patients before platinum-based neoadjuvant chemotherapy [4]. They found that a high level of p21WAF1 expression is associated with a poor outcome.

Ikuta et al have used heterogeneously apoptosis-sensitive NSCLC cell lines in order to analyze the effect of cisplatin on various apoptotic pathways [5]. They concluded, that anti-apoptotic Bcl-xL and pro-apoptotic p53 are necessary but not sufficient for resistance to cisplatin-induced apoptosis in NSCLC cells.

Filipits et al have conducted a large study in order to determine whether cell cycle regulators are of prognostic and/or predictive value [6]. They concluded that NSCLC patients with p27Kip1-negative tumors benefit from adjuvant cisplatin-based chemotherapy after complete tumor resection.

Similarly, it has been reported that the absence of immunohistochemical evidence of the excision repair cross-complementation group 1 (ERCC1) protein in tumors was associated with survival benefit in NSCLC patients, who received cisplatin-based adjuvant chemotherapy [7].

The management of patients with advanced NSCLC present challenges for clinicians. Numerous studies have analyzed the clinical effects of chemotherapies either in inoperable non-small cell lung cancer cases or in neo-adjuvant and adjuvant settings [8–13]. However, the molecular changes developed within the tumor tissue due to cytotoxic treatment are far less known.

We have studied the effect of cisplatin-containing chemotherapy on the lung cancer tumor tissue comparing pre-chemotherapeutic bronchoscopic biopsies and post-chemotherapeutic surgical resections. The reliability of diagnostic biopsies as compared to the corresponding surgically resected tumors regarding the expression of different markers was studied by Meert et al [14]. When evaluated immunohistochemically the expression of p53, EGFR, c-erbB-2 and Ki-67 in 28 lung cancer patients, they found concordant results in 85% concluding that biopsies may provide reliable information and could help to elaborate a therapeutic strategy.

In our work, increased Ki-67 expression after therapy was observed in one third of cases. It is, however, mostly in opposite with the findings of Lang et al [15]. They have found that drug-induced effects, i.e. reduced proliferative capacity indicated by decreased Ki-67 expression and activation of caspase-3 as indicator of late phase apoptosis, were more pronounced in ADCs as compared to SCCs.

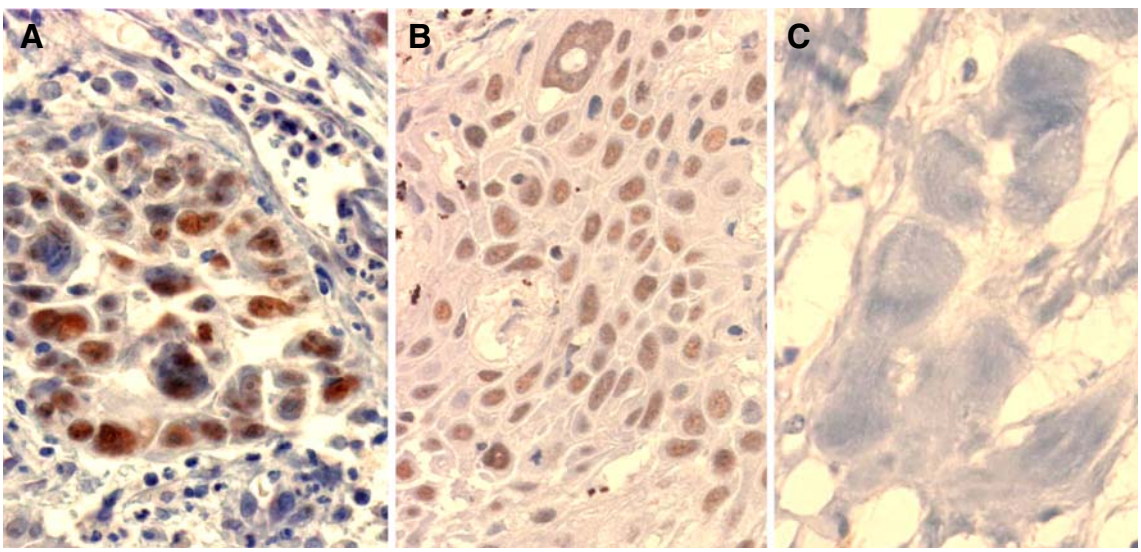

Fig. 1 ERCC1 nuclear staining in **a** adenocarcinoma (×200) and **b** squamous cell carcinoma (×200) of bronchoscopic samples. **c** ERCC1 nuclear staining was not detectable in the corresponding surgical sample after neoadjuvant chemotherapy of case **b** (×400)

Our present work revealed differences between ADC and SCC regarding the changes in marker expression after chemotherapy. In SCC group pronounced changes were found regarding the post-treatment decrease of ERCC1 expression, however, in ADC tumors there was no change detectable. It has recently been shown that ERCC1 immunnegativity was significantly related to tumor responsiveness to neoadjuvant chemotherapy with cisplatin, however, such a status had no clear prognostic value of cisplatin-based neoadjuvant therapy in NSCLC patients [16]. According to our results, we can speculate that although chemotherapy increases resectability of the tumor in certain cases, survival is not necessarily improved in operated patients as the treatment could have long-term tumor cell selective effects as well.

The results of the present study suggest that platinum-based chemotherapy may induce the selection of tumor cells with more aggressive phenotype as it was detectable in the increased expression of Ki-67, and also affects the expression of tissue markers that could have predictive value. This knowledge might be of importance when designing treatment protocols for NSCLC patients.

Acknowledgements The authors thank László Kopper MD PhD DSc for his professional advice, Katalin Vajda MD for her diagnostic work and Anna Tamási for her excellent technical assistance.

References

1. U.S. Cancer Statistics Working Group. United States Cancer Statistics: 2002–2004 Incidence and Mortality Web-based Report Version. Atlanta (GA): Department of Health and Human Services, Centers for Disease Control and Prevention, and National Cancer Institute; 2007
2. Noble J, Ellis PM, Mackay JA et al (2006) Second-line or subsequent systemic therapy for recurrent or progressive non-small cell lung cancer: a systematic review and practice guideline. J Thorac Oncol 9:1042–58
3. Junker K, Langner K, Klinke F et al (2001) Grading of tumor regression in non-small cell lung cancer: morphology and prognosis. Chest. 120(5):1584–91
4. Morero JL, Poleri C, Martin C et al (2007) Influence of apoptosis and cell cycle regulator proteins on chemotherapy response and survival in stage IIIA/IIIB NSCLC patients. J Thorac Oncol. 4:293–8
5. Ikuta K, Takemura K, Kihara M et al (2005) Defects in apoptotic signal transduction in cisplatin-resistant non-small cell lung cancer cells. Oncol Rep. 13(6):1229–34
6. Filipits M, Pirker R, Dunant A et al (2007) Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program. J Clin Oncol. 25 (19):2735–40
7. Olaussen KA, Dunant A, Fouret P et al (2006) DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 355:983–91
8. Rigas JR, Kelly K (2007) Current treatment paradigms for locally advanced non-small cell lung cancer. J Thorac Oncol. 2(Suppl 2): S77–85
9. Gilligan D, Nicolson M, Smith I et al (2007) Preoperative chemotherapy in patients with resectable non-small cell lung cancer: results of the MRC LU22/NVALT 2/EORTC 08012 multicentre randomised trial and update of systematic review. Lancet. 369(9577):1929–37
10. de Marinis F, Tedesco B, Treggiari S et al (2007) Role of induction chemotherapy in resectable N2 non-small cell lung cancer. J Thorac Oncol. 5(Suppl 5):S31–4
11. Abratt RP, Lee JS, Han JY et al (2006) Phase II trial of gemcitabine-carboplatin-paclitaxel as neoadjuvant chemotherapy for operable non-small cell lung cancer. J Thorac Oncol. 2:135–40
12. Stinchcombe TE, Socinski MA (2007) The role of induction therapy for resectable non-small cell lung cancer. Drugs. 67 (3):321–32
13. De Pauw R, van Meerbeeck JP (2007) Neoadjuvant chemotherapy in the treatment of nonsmall-cell lung cancer. Curr Opin Oncol. 19 (2):92–7
14. Meert AP, Martin B, Verdebout JM et al (2004) Correlation of different markers (p53, EGF-R, c-erbB-2, Ki-67) expression in the diagnostic biopsies and the corresponding resected tumors in non-small cell lung cancer. Lung Cancer. 44(3):295–301
15. Lang DS, Droemann D, Schultz H et al (2007) A novel human ex vivo model for the analysis of molecular events during lung cancer chemotherapy. Respir Res. 14;8:43
16. Fujii T, Toyooka S, Ichimura K et al (2008) ERCC1 protein expression predicts the response of cisplatin-based neoadjuvant chemotherapy in non-small-cell lung cancer. Lung Cancer 59 (3):377–84