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Biomarkers in lung oncology

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ABSTRACT

The survival of advanced non-small-cell lung cancer patients is short in spite of advances in new combination chemotherapy regimens. The benefit of adding antiangiogenic drugs and/or EGFR inhibitors is unclear. For the vast majority of patients without EGFR mutations, treatment approaches based on customization should be pursued. BRCA1 is central to the repair of DNA damage and is an important modulator of the differential effect of chemotherapy. Retrospective and prospective data indicate that low BRCA1 mRNA levels predict better response and survival when patients are treated with cisplatin, non-taxane combinations.

For an important subgroup of patients with EGFR mutations, selective treatment with EGFR tyrosine kinase inhibitors is a major advance, with a dramatic impact on clinical outcomes. In a prospective study of customized erlotinib [1], overall response rate was 70% (including 12% complete responses), median progression free survival was 14 months (even longer in women and in patients with del 19), 20% of patients were disease-free at three years, and median survival was 27 months. Nonetheless, these clinical outcomes fall short of curability and continuous treatment with erlotinib or gefitinib is required. It is plausible that several genetically defined subclasses of EGFR mutations could help to improve current clinical outcomes by combining erlotinib or gefitinib with other targeted drugs.

1. Introduction

Metastatic (stage IV) non-small-cell lung cancer (NSCLC) is found in approximately 50% of patients at the time of diagnosis, with short survival times regardless of the type of chemotherapy administered [1]. Among 2714 patients randomized to supportive care or chemotherapy, there was a 9% benefit in one-year survival (from 20% to 29%) and a 1.5-month increase in median survival (from 4.5 to 6 months) in patients receiving chemotherapy [2]. Several platinum-based chemotherapy regimens yield the same outcomes, with median progression free survival (PFS) of four months and median survival of eight months [3].

2. ERCC1 mRNA expression – a biomarker for platinum compounds

Platinum-based chemotherapy is commonly used in the treatment of metastatic NSCLC. A wealth of data indicates that nucleotide excision repair (NER), a highly versatile pathway for DNA damage removal, is often dysfunctional in NSCLC. NER removes numerous types of DNA helix-distorting lesions, including those induced by platinum compounds [4,5]. NER functions by a so-called "cut-and-paste" mechanism in which cisplatin damage recognition, local opening of the DNA helix around the lesion, damage excision, and gap filling occur in successive steps, through the concerted action of various NER factors [4]. The structure-specific endonuclease excision repair cross-complementing 1 (ERCC1), together with its xeroderma pigmentosum group F (XPF) partner, performs an essential late step in the NER process, where it nicks the damaged DNA strand at the 5′ site of the helix-distorting cisplatin lesion. In addition, the ERCC1/XPF structure-specific nuclease also plays a role in the homologous recombination repair of interstrand

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crosslinks [5]. We examined ERCC1 mRNA expression in paraffin-embedded pretreatment tumor specimens from stage IV NSCLC patients treated with cisplatin plus gemcitabine. There were striking differences in survival (15 months for patients with low levels of ERCC1 vs 5 months for those with high levels), and the response rate in tumors with low levels of ERCC1 mRNA was higher (52%) than in those with high levels (36%), although this difference was not significant [6]. Based on these findings, we initiated a phase III trial of customized chemotherapy according to ERCC1 mRNA levels in stage IV NSCLC patients. Patients in the control arm received cisplatin plus docetaxel; in the genotypic arm, patients with low ERCC1 levels received cisplatin plus docetaxel and those with high levels received gemcitabine plus docetaxel [7]. Objective response was attained by 39.3% of patients in the control arm and 50.7% of patients in the genotypic arm. This study showed that assessment of ERCC1 mRNA expression in patient tumor tissue is feasible in the clinical setting and predicts response to docetaxel and cisplatin.

Intriguingly, ERCC1 expression levels were significantly higher in squamous cell carcinoma (SCC) than in adenocarcinoma, both in primary tumors of stage IV NSCLC [6,7] and in early stage NSCLC [8]. Moreover, patients with PS 0 (asymptomatic and fully active) or 1 (symptomatic, fully ambulatory, restricted in physically strenuous activity) had significantly lower median ERCC1 mRNA levels than those with PS 1 [7]. Interestingly, in patients with advanced gastric cancer treated with oxaliplatin-based chemotherapy [9], ERCC1 mRNA levels were also lower in patients with PS 0 and PS 1 than in those with PS 2 (bed-ridden 50% of the time). In 277 (75.7%) patients in the phase III customized ERCC1 trial [7], ERCC1 was measured in the primary tumor sample obtained by bronchial biopsy. In the remaining 89 (24.3%) patients, ERCC1 assessment was performed in biopsies of metastatic sites. Of the biopsies obtained from metastatic sites in the ERCC1 customized chemotherapy trial, more were obtained from peripheral lymph nodes, subcutaneous, skin and pleura metastases in adenocarcinoma than in SCC patients (Table 1). The ERCC1 levels in the primary tumor were significantly higher than those in the metastatic samples (1.76 [range, 0.14–11.10] vs 1.39 [range, 0.32–11.94]) (Rosell R et al., Biomarkers in lung oncology, Pulmonary Pharmacology and Therapeutics (2010)). The ERCC1 levels in the 89 metastatic samples were higher in SCC than in other histologies and higher in liver, bone and adrenal metastases than in lung, although the differences were not significant (Table 2). The let-7 microRNA (miRNA), a master regulator of oncogene expression and cancer pathways, is highly expressed in normal lung tissue and downregulated in NSCLC. Reduced expression of let-7 in early NSCLC was associated with poor survival [10,11]. In cancer cell lines overexpressing let-7, a total of 170 genes, including ERCC1, were downregulated [12]. These findings provide some hints that ERCC1 could be a prognostic marker, since it is overexpressed in target metastatic sites like liver and in patients with poor PS. In addition, in early stage NSCLC, ERCC1 levels are linked to shorter relapse-free survival [8].

Table 1
Metastatic site of biopsies obtained for ERCC1 mRNA analysis according to histology in a customized trial [7].

Metastatic Site	Histology		p
	Squamous Cell Carcinoma	Non-Squamous Cell Carcinoma	0.22[a]
Peripheral lymph nodes	7 (15.9)	37 (84.1)	<0.0001
Brain	1 (25)	3 (75)	0.32
Liver	1 (50)	1 (50)	0.99
Bone	0	7 (100)	–
Subcutaneous	1 (12.5)	7 (87.5)	0.03
Skin	0	9 (100)	–
Pleura	1 (12.5)	7 (87.5)	0.03
Lung	1 (33.3)	2 (66.7)	0.56
Kidney	1 (100)	0	–
Adrenal	1 (33.3)	2 (66.7)	0.56

[a] There was no significant difference in the number of metastatic sites according to histology.

Table 2
ERCC1 mRNA levels in samples obtained from biopsies of metastatic sites in stage IV NSCLC patients in a customized trial [7].

	N (%)	ERCC1 mRNA levels Median (range)	p
Total	89 (100)	1.39 (0.32–11.94)	
Histology			0.01
Squamous	14 (13.9)	2.00 (0.19–13.40)	
Non-squamous	75 (29.3)	1.62 (0.14–11.94)	
ECOG PS			0.06
0	35 (26.1)	1.26 (0.32–6.36)	
1	54 (24.2)	1.63 (0.34–11.94)	
Metastatic site			0.77
Peripheral lymph nodes	44 (49.4)	1.36 (0.32–6.17)	
Brain	4 (4.5)	0.76 (0.48–11.94)	
Liver	2 (2.2)	2.69 (0.90–4.48)	
Bone	7 (7.9)	2.02 (1–5.51)	
Subcutaneous	8 (9)	1.34 (0.44–3.04)	
Skin	9 (10.1)	1.41 (0.47–6.36)	
Pleura	8 (9)	1.97 (1.21–3.47)	
Lung	3 (3.4)	1.62 (1.13–1.80)	
Kidney	1 (1.1)	1.17	
Adrenal	3 (3.4)	2.63 (1.24–3.76)	

2.1. Clinical studies testing platinum outcome according to ERCC1 and RRM1

In recent years, ERCC1 and other components of DNA damage-processing systems have been examined in the clinical setting (reviewed in Rosell et al. [4,13]). High tumor tissue levels of ERCC1 mRNA in ovarian and gastric cancer patients have been associated with cisplatin resistance [14,15]. When intratumoral ERCC1 mRNA derived from paraffin-embedded tumor specimens was measured by real-time reverse transcriptase quantitative polymerase chain reaction (RT-QPCR) in metastatic colon cancer patients treated with oxaliplatin and 5-fluorouracil (5-FU), high levels of ERCC1 significantly correlated with poor response and shorter survival [16]. Significant differences were also observed according to ERCC1 mRNA levels in advanced gastric cancer patients treated with oxaliplatin plus 5-FU (median survival, 15.8 vs 6.2 months; $P < 0.001$) [9].

Several studies in stage IV gemcitabine/cisplatin-treated NSCLC show that patients with low ERCC1 or ribonucleotide reductase subunit M1 (RRM1) mRNA levels have a median survival up to 15 months [17–19]. However, the predictive value of low ERCC1 mRNA levels found in our original study [6] was not sustained either in a second study, where low ERCC1 levels showed a non-significant trend towards better survival [17], or in the phase III ERCC1 customized trial [7], where there was a trend towards longer PFS in the genotypic arm (5.1 months in the control arm vs 6.7 months in patients with low ERCC1 in the genotypic arm) but not towards improved survival [7]. Another study also found no benefit associated with ERCC1 mRNA expression in stage IV NSCLC patients receiving docetaxel plus carboplatin or other combinations, including vinblastine [20]. Since ERCC1 can predict survival to gemcitabine plus cisplatin but not to docetaxel plus cisplatin, antimicrotubule drugs may not be the best partner for cisplatin in the presence of low ERCC1 levels. A close correlation has been observed between expression levels of ERCC1, RRM1 and BRCA1 in several studies [8,21,22]. BRCA1 expression confers differential chemosensitivity in cancer cell lines [23,24]; low levels of BRCA1 expression confer sensitivity to cisplatin and resistance to paclitaxel and docetaxel, while high levels lead to resistance to cisplatin