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Original articles

Pharmacogenetics of the DNA repair pathways in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy

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ABSTRACT

Genetic variants in DNA repair genes may play a role in the effectiveness of platinum-based chemotherapy in non-small cell lung cancer (NSCLC). We analyzed 17 SNPs in eight genes (*ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, XPA, XRCC1* and *XRCC2*) involved in DNA repair mechanisms and its association with outcome in NSCLC. This prospective study included patients with stages III and IV treated with platinum-based chemotherapy. All patients ($n = 161$) received cisplatin or carboplatin plus a third-generation drug. Additionally, stage IIIA and IIIB patients ($n = 74$) received concomitant or sequential radiotherapy. Germline polymorphisms were analyzed using the BioMark system in blood DNA samples. We found that in stage III patients, response was significantly associated with SNPs in *ERCC1* and in *ERCC3* genes, while radiotherapy-derived toxicity correlated with SNPs in the *ERCC2* gene. In stage IV patients, response was associated with a genetic variant in the *ERCC4* gene and survival with a SNP in the *XRCC1* gene. The complexity of the DNA repair mechanisms along with the heterogeneity in the treatment of lung cancer could explain the role of multiple genes as putative biomarkers of patient outcome.

Introduction

Lung cancer is the leading cause of cancer mortality worldwide [1] and its incidence is increasing, particularly in industrialized countries. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all cases and about two-thirds of patients are diagnosed at an advanced stage [2]. Less than 30% of patients respond to the standard treatment that consists in platinum-based doublet chemotherapy [3]. Although the most important prognostic factor is the clinical stage, survival varies significantly among patients, with an overall survival of approximately 10% in 5 years.

Platinum compounds such as cisplatin and carboplatin are antineoplastic agents whose cytotoxic effect is attributed to the formation of bulky platinum-DNA adducts. These structures result in destabilization of the double helix that blocks replication and inhibits transcription [4]. The repair of the damaged DNA molecules is mainly performed by two repair pathways, nucleotide excision repair (NER) and base excision repair (BER). The activity of these DNA repair mechanisms plays a central role in determining platinum compound sensitivity, and a high DNA repair capacity has been related to chemoresistance in NSCLC.

NER, a highly versatile and sophisticated DNA damage removal pathway, initiates with the recognition of the DNA lesion [5]. The main steps in this process are the separation of the double helix at the DNA lesion site, the excision of the lesion-containing the single stranded DNA fragment, the DNA repair synthesis to replace the gap, and the ligation of the remaining single stranded nick. In mammalian cells, a number of major proteins are involved in this pathway and the study of six of the genes that code for these proteins (ERCC1, ERCC2/XPD, ERCC3/XPB, ERCC4/XPF, ERCC5/XPG and XPA) is the goal of the present work. All these genes play a pivotal role in the NER pathway, and previous studies have demonstrated that their level of expression is important for the repair of platinum-DNA adducts and the response to platinum-based chemotherapy [6,7]. Numerous studies on the impact of genetic polymorphisms in the genes mentioned earlier on the outcome in chemotherapy-treated NSCLC patients have been published and reviewed [8,9].

Base excision repair (BER) is the cellular mechanism responsible for removing small, non-helix-distorting base lesions from the genome, including those caused by platinum-based drugs and radiotherapy. XRCC1 and XRCC2 are the two most studied proteins

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participating in the BER pathway, and different studies have analyzed the association between genetic polymorphisms in their coding genes and response to platinum-based chemotherapy in patients with advanced NSCLC [8,9].

It is often difficult to perform expression and immunohistochemical studies obtaining sufficient tumor tissue in advanced lung cancer. Germline gene polymorphisms are easy to measure and constant over time. They are therefore an ideal tool for developing markers of clinical outcome in patients with advanced NSCLC. Two comprehensive reviews related to this topic have recently been published. Hildebrandt et al. [10] reviewed the associations between genetic variation and clinical outcomes in advanced NSCLC treated with platinum-based chemotherapy. They included in the review those pathways involved in drug influx and efflux, metabolism and detoxification, DNA damage repair and other downstream cellular processes that modulate the effect of platinum-based therapy. The authors concluded that the effects of each individual SNP on clinical outcomes are modest and suggest a more comprehensive approach that incorporates polygenetic, phenotypic, epidemiologic and clinical variables. Bonanno et al. [11] reviewed the platinum drugs and DNA repair mechanisms in lung cancer. They pointed out that the main pathways involved in these mechanisms are NER and homologous recombination that includes the most studied potential predictive markers *ERCC1* and *BRCA1*. The authors also remarked the interest of the potential clinical usefulness of integrated analysis of multiple DNA repair components. To help introduce new pharmacogenetic markers in clinical decision making, we evaluated the association of 17 single nucleotide polymorphisms (SNPs) in eight DNA repair genes with response rate, survival and toxicity in NSCLC patients treated with platinum-based chemotherapy.

Patients and methods

Patients

Eligible patients were diagnosed cytologically or histologically of NSCLC and classified in stage III (A/B) or IV according to the TNM [12]. Patients with an Eastern Cooperative Oncology Group (ECOG) >2 were not included. All patients were evaluated with computed tomography of the thorax and upper abdomen and underwent a complete history and physical examination, including hematology and biochemistry analyses before starting treatment. Age at diagnosis, gender and smoking status were recorded. This study was approved by the institutional ethics committee and all patients signed informed consent for blood collection and analysis.

All patients ($n = 161$) received platinum-based doublets consisting of cisplatin or carboplatin plus a third-generation drug (Table 1). Patients with stages IIIA and IIIB ($n = 74$) also received concomitant or sequential radiotherapy.

Response to treatment was assessed using Response Evaluation Criteria in Solid Tumors (RECIST). This tool classifies response into four categories: complete response (CR), partial response (PR), stable disease (SD), and progression disease (PD) [13]. Progression-free survival (PFS) was defined from the date of first treatment until disease progression or death from any cause. Overall survival (OS) was calculated from the date of diagnosis until death or last clinical follow-up. For analysis, we grouped CR and PR as "responders" and SD and PD as "non-responders".

Toxicity associated to chemotherapy was graded according to the Common Terminology Criteria for Adverse Events Scale [14]. Toxicity associated to radiotherapy was recorded in all 74 stage III patients, following the RTOG/EORTC Acute Radiation Morbidity Scoring Scheme [15].

Table 1
Baseline patient characteristics.

	No.	%
Mean age, years	63.7	
Range	(36 – 85)	
Gender		
Male	125	77.6
Female	36	22.4
ECOG		
0	28	17.4
1	120	74.5
2	13	8.1
Smoking status		
Current smokers	74	46.0
Former smokers	70	43.4
Non-smokers	17	10.6
Histological type		
Adenocarcinoma	78	48.4
Squamous cell carcinoma	61	37.9
Large cell carcinoma	22	13.7
Disease stage		
IIIA	34	21.1
IIIB	40	24.9
IV	87	54.0
Platinum based chemotherapy plus:		
Gemcitabine	54	33.5
Vinorelbine	48	29.9
Taxane	31	19.2
Pemetrexed	28	17.4
Response		
Responders (CR + PR)	89	55.3
Non-responders (SD + PD)	72	44.7

ECOG, Eastern Cooperative Oncology Group; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Genetic studies

Eight genes involved in the DNA repair pathways were analyzed. Six of them (*ERCC1, ERCC2/XPD, ERCC3/XPB, ERCC4/XPF, ERCC5/XPG* and *XPA*) belong to the NER pathway, and two (*XRCC1, XRCC2*) belong to the BER pathway. Seventeen SNPs were selected for the study. All SNPs were selected using public literature resources (NCBI-PubMed), except those of the *ERCC3/XPB* gene that were selected using the HapMap genome browser (http://hapmap.ncbi.nlm.nih.gov) and the Haploview software v4.2 (an r^2 threshold of 0.8 and a minor allele frequency (MAF) ≥10% in Caucasians). Table 2 shows the characteristics of the studied polymorphisms.

Table 2
Primary information on the analyzed polymorphisms.

Gene (chromosome)	dbSNP	Nucleotide change	AA change	MAF[a]
ERCC1 (Chr 19)	rs11615	c.354T > C	p.Asn118Asn	0.339
	rs3212948	c.321 + 74C > G	–	0.301
	rs3212986	c.*197G > T C8092A	p.Gln504Lys	0.224
ERCC2/XPD (Chr 19)	rs1799793	c.934G > A	p.Asp312Asn	0.298
	rs13181	c.2251A > C	p.Lys751Gln	0.304
ERCC3/XPB (Chr 2)	rs4150454	c.1343-337A > G	–	0.360
	rs4150402	c.471 + 52A > G	–	0.248
	rs3738948	c.2064 + 741A > G	–	0.273
ERCC4/XPF (Chr 16)	rs1799801	c.2505T > C	p.Ser835Ser	0.326
ERCC5/XPG (Chr 13)	rs1047768	c.138T > C	p.His46His	0.422
	rs17655	c.3310G > C	p.Asp1104His	0.239
XPA (Chr 9)	rs1800975	c.-4A > G (c.23A > G)	–	0.304
XRCC1 (Chr 19)	rs25487	c.1196A > G	p.Arg399Gln	0.391
	rs25489	c.839G > A	p.Arg280His	0.040
	rs1799782	c.580C > T	p.Arg194Trp	0.068
	rs3213239	c.-1450_ -1449insGGCC	–	0.342
XRCC2 (Chr 7)	rs3218536	c.563G > A	p.Arg188His	0.096

[a] MAF, minor allele frequency.