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Combined effect of *ERCC1* and *ERCC2* polymorphisms on overall survival in non-squamous non-small-cell lung cancer patients treated with first-line pemetrexed/platinum

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ABSTRACT

Objectives: Polymorphisms of DNA repair genes may affect DNA repair capacity and the sensitivity of platinum doublets chemotherapy in non-small-cell lung cancer (NSCLC). We prospectively evaluated whether single nucleotide polymorphisms (SNPs) of *ERCC1*, *ERCC2*, *XRCC1*, and *XRCC3* were associated with treatment outcome in advanced non-squamous NSCLC patients receiving pemetrexed/platinum as their first-line chemotherapy.

Materials and methods: Genotyping of six SNPs in four DNA repair genes in 58 patients treated with first-line pemetrexed/platinum was performed using TaqMan SNP Genotyping Assays.

Results: The wild-type ERCC1 8092 (C/C) was significantly associated with a better objective response compared to the variant genotypes (C/A + A/A) (48% vs 10%, $P = .005$). In the multivariate Cox proportional hazards model, we found that individuals with a wild-type genotype of *ERCC1* Asn118Asn, *ERCC1* C8092A and *ERCC2* Asp312Asn had significantly better overall survival (OS) than those with a heterozygous or homozygous variant genotype. On the other hand, the heterozygous variant genotype of *ERCC2* Lys751Gln was associated with better OS than that of the wild-type genotype. We further explored the combined effect of SNPs on OS, and found a significant allele/dose-dependent trend toward decreasing OS in patients with an increasing number of unfavorable alleles among four SNPs in *ERCC1* and *ERCC2*. The median OS of patients with two or three unfavorable alleles (30.1 and 30.5 months, respectively) was significantly longer than that of patients with 4 unfavorable alleles (11.8 months, log-rank test for trend, $P = .001$).

Conclusion: A combination of *ERCC1* and *ERCC2* polymorphisms may predict OS among pemetrexed/platinum treated advanced non-squamous NSCLC patients.

1. Introduction

Although molecular therapies targeting epidermal growth factor receptors (EGFR) and anaplastic lymphoma kinase (ALK) using small molecular tyrosine kinase inhibitors are shown to improve patient survival to a certain degree, chemotherapy with platinum doublets remains the main first-line treatment for advanced non-small-cell lung cancer (NSCLC) without EGFR-sensitizing mutations or ALK gene rearrangements [1]. Current clinical guidelines recommend that based on tumor histology, any platinum doublets with a third-generation chemotherapeutic agent can be used in non-squamous cell carcinoma (NSCC) [2]. However, a recent meta-analysis showed that pemetrexed-based platinum doublets provided a significant overall survival (OS) benefit compared with gemcitabine or docetaxel-based platinum doublets in patients with a NSCC histology[3]. Despite the fact that pemetrexed-based platinum doublets improved OS and toxicity profiles compared with gemcitabine or docetaxel-based regimens, the reality is that the response to identical chemotherapeutic agents varies widely among patients with a similar histology and stage[3]. Hence, the use of molecular predictive markers to help identify NSCC patients who may and who may not benefit from first-line pemetrexed-based platinum doubles remains one of the most important areas of study in lung cancer treatment.

Pemetrexed is a multi-targeted antifolate drug that targets the enzymes, including thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyl transferase (GARFT), that participate in both pyrimidine and purine synthesis [4]. Pemetrexed exerts its antitumoral effect mainly by inhibiting TS, and the high TS expression level in squamous cell carcinoma (SCC) of the lung may explain why SCCs are less sensitive to pemetrexed than NSCCs [5]. Several retrospective studies have reported that low TS expression in tumor cells was associated with better progression-free survival (PFS) in

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NSCLC when pemetrexed-based regimes were used [6,7]. However, the association between TS expression, objective response (OR) and OS is still unclear [8,9]. Moreover, the methodology for detection and measurement of TS expression levels varied among studies – some utilized immunohistochemistry and others used quantitative real-time polymerase chain reaction (PCR) [10]. Due to the scarcity of tumor tissue in performing immunohistochemical staining of TS protein or measuring TS mRNA expression in cases of advanced lung cancer, the use of blood cells to detect genetic variations in candidate genes is a reasonable substitute when studying predictive markers of pemetrexed-based chemotherapies.

In addition to TS expression level, genetic alterations of genomic DNA in genes encoding TS, the reduced folate carrier (SCL19A1), methylenetrahydrofolate reductase (MTHFR), folypoly-γ-glutamate synthase (FPGS), and γ-glutamyl hydrolase (GGH) have been explored in several retrospective studies recently and have correlated with the outcome of NSCLC patients receiving pemetrexed-based chemotherapies [11–13]. However, some studies found an inconsistent relationship between the above pemetrexed pathway-associated germline polymorphisms and clinical outcome [14,15], and others failed to detect a significant association with OR and survival [16]. Furthermore, the heterogeneity of patient populations and the different lines of treatment and drug combinations (pemetrexed given as a single agent or in combination with platinum agents) in these studies limited the interpretation of data and clinical application.

Elevated DNA repair capacity has been correlated to resistance to platinum-based chemotherapy in NSCLC [17]. The cytotoxic effect of platinum drugs, such as cisplatin and carboplatin, is attributed to the formation of bulky platinum-DNA adducts, which are mainly repaired by the nucleotide excision repair (NER) pathway. *ERCC1* (excision repair cross-complementing group 1) belongs to a group of genes responsible for NER and plays a pivotal role in the recognition of DNA damage and the removal of the damaged nucleotide. The expression of *ERCC1*, measured by immunohistochemical staining, has been recognized to be associated with poor survival in patients receiving platinum containing adjuvant chemotherapy after complete resection of NSCLC [18]. However, in 2013, a validation study using immunohistochemical analysis with the mouse monoclonal antibody 8F1 failed to demonstrate the same predictive effect of ERCC1 [19]. The discordance in the results suggested a change in the specificity of the 8F1 antibody and the expression of a nonfunctional isoform of ERCC1 may lead to potential artifacts. The authors concluded that currently available ERCC1antibodies for immunohistochemical analysis do not have adequate discrimination for use in guiding therapeutic decision-making. A randomized phase III trial was conducted recently to evaluate the clinical significance of prospective testing of ERCC1 as a predictive biomarker using the commercially available antibody 8F1 [20]. The result also showed that prospectively selecting the type of chemotherapy using immunohistochemical analysis for *ERCC1* did not predict OS or PFS for either SCC or NSCC histology; thus, testing of ERCC1 using a commercially available antibody should not be routine practice without further investigation.

In addition to measuring protein expression, studies have found that the polymorphism of *ERCC1* (118*C/T* and *C8092A*) has an impact on the survival of platinum-based chemotherapy-treated NSCLC patients [21,22]. However, only one study reported the usefulness of the *ERCC1* 118*C/T* polymorphism as a molecular predictive marker for first-line pemetrexed and platinum therapy in NSCC patients [23], but the result showed no correlation of polymorphism with OR or survival. Other studies recruited mixed populations of NSCLC patients receiving different combinations of platinum doublets (including gemcitabine, paclitaxel, docetaxel, vinorelbine or pemetrexed) as first-line therapy, and obtained inconsistent results [24–26]. The clinical significance of *ERCC1* polymorphisms in predicting outcomes of non-squamous NSCLC patients receiving first-line pemetrexed-based platinum doublets warrants further investigation.

Besides *ERCC1*, *XPD/ERCC2* (xeroderma pigmentosum group D/excision repair cross-complementing group 2) is an important NER protein intervening in both the TC (transcription-coupled)-NER and GG (global genomic)-NER sub-pathways. Two polymorphisms (*ERCC2* 312 and *ERCC2* 751) that are common and result in an amino acid change have been investigated mainly in relation to risk and clinical outcome of lung cancer [27,28]. *XRCC1* (X-ray repair cross-complementing group 1) and *XRCC3* (X-ray repair cross-complementing group 3) are two other proteins involving base excision repair (BER) and double-stand break repair (DSB), and polymorphisms of these two proteins (*XRCC1* 399 and *XRCC3* 241) have been shown to be prognostic factors for survival of patients with NSCLC [29,30]. Our previous study found that there are survival differences based on the *XRCC1* Arg399Gln polymorphism in NSCLC patients treated with first-line gemcitabine plus platinum chemotherapy [29]. But, with regard to patients treated with first-line pemetrexed-based platinum doublet therapy, which is the current mainstay treatment for non-squamous NSCLC, the predictive value of DNA repair gene polymorphisms is still unknown.

For this study, we prospectively evaluated the associations of six genetic polymorphisms (*ERCC1* Asn118Asn, *ERCC1* C8092A, *ERCC2* Lys751Gln, *ERCC2* Asp312Asn, *XRCC1* Arg399Gln, and *XRCC3* Thr241Met) of four DNA repair genes with treatment response and survival in non-squamous NSCLC patients receiving pemetrexed-based platinum doublets as their first-line chemotherapy.

2. Materials and methods

2.1. Patients

From May 1, 2007 through October 31, 2014, patients with histologically proven (newly diagnosed or recurrent from prior resectable NSCLC) and clinically advanced (stage IV) non-squamous NSCLC at National Taiwan University Hospital (NTUH) were screened for enrollment. All patients were staged with computed tomography of the brain, thorax and abdomen, as well as 99 m Tc-MDP bone scintigraphy. Patients with an Eastern Cooperative Oncology Group performance status ≥ 2 and those who had received previous chemotherapy were excluded. Only those who considered receiving pemetrexed plus platinum (cisplatin or carboplatin) as first-line treatment were eligible for this study. The study was approved by the NTUH Research Ethics Committee and the patients provided written informed consent to participate. After obtaining their consent, 10 ml of each patient's blood was drawn before the initiation of chemotherapy. Before treatment, all patients underwent a complete history and physical examination, including routine hematology and biochemistry analyses. Hematology and biochemistry analyses were repeated before the start of each chemotherapy delivery. Age, gender, histological type, clinical stage, chemotherapy regimen and toxicity were recorded.

2.2. Chemotherapy and clinical response

Patients received pemetrexed 500 mg/m2 on day 1 every three weeks in combination with cisplatin 75 mg/m2 or carboplatin AUC 5. Patients might receive bevacizumab at a dose of 7.5 or 15 mg/kg on day 1, at the discretion of their attending physician. The tumor response to chemotherapy was assessed after three cycles of chemotherapy and every three cycles thereafter, using RECIST 1.1 (Response Evaluation Criteria in Solid Tumors). The best response to chemotherapy was reported as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). OS was calculated from the day of starting pemetrexed plus platinum therapy to the day of the last follow-up (death or clinical visit). For this study, survival data were censored on September 30, 2016.