Genetic Polymorphism of *XRCC1* Arg399Gln Is Associated With Survival in Non–Small-Cell Lung Cancer Patients Treated With Gemcitabine/Platinum

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Introduction: Elevated DNA-repair capacity has been related to chemoresistance of platinum doublet chemotherapy in non–small-cell lung cancer (NSCLC). We evaluated whether single nucleotide polymorphisms of DN- repair genes excision repair cross-complementing group 1 (*ERCC1*), *ERCC2*, x-ray repair cross-complementing group 1 (*XRCC1*), *XRCC3*, and *RRM1* associate with treatment outcome in NSCLC patients receiving gemcitabine plus platinum as their first-line chemotherapy.

Methods: Genotyping for eight polymorphisms in five DNA-repair genes was performed with the GenomeLab nucleotide polymorphismstream Genotyping System in 62 advanced NSCLC patients in a training set and 45 patients in a validation set treated with gemcitabine/platinum.

Results: In the training set, the wild-type genotype of *XRCC1* Arg399Gln (G/G) was associated with decreased median overall survival (OS) (22 months, 95% confidence interval [CI], 10–34 months versus not reached, log-rank test, $p = 0.005$) than those carrying variant genotypes (G/A+A/A). In addition, there was a statistically significant longer median OS in patients carrying wild-type *ERCC2* Asp312Asn genotype (G/G) (51 months, 95% CI, 19–82 months versus 10 months, log-rank test, $p < 0.001$) than those carrying heterozygous variant genotypes (G/A). In the multivariate Cox model, we found a significant effect of *XRCC1* Arg399Gln (G/A+A/A versus G/G, hazard ratio [HR] 0.290; 95%CI, 0.12–0.705, $p = 0.006$) and *ERCC2* Asp312Asn (G/A versus G/G, HR 14.04; 95% CI, 2.253–87.513, $p = 0.005$) polymorphisms on patients' OS. In the validation set, only *XRCC1* 399 polymorphisms showed significant effect on patients' OS (G/A+A/A vs. G/G, HR 0.474; 95% CI, 0.245–0.915, $p = 0.026$)

Conclusions: Genetic polymorphism of *XRCC1* Arg399Gln may be a candidate for contributing interindividual difference in the OS of gemcitabine/platinum-treated advanced NSCLC patients.

Key Words: Non–small-cell lung cancer, DNA repair, Single nucleotide polymorphism.

(*J Thorac Oncol.* 2012;7: 973–981)

Lung cancer is the leading cause of death with regard to cancer in many countries,[1] including Taiwan. About half of the newly diagnosed non–small-cell lung cancer (NSCLC) cases are already at an advanced stage (stage IIIB and IV), and nearly 90% of these patients die within 2 years.[2] Although molecular therapy targeting epidermal growth factor receptor (EGFR) pathway and vascular endothelial growth factor pathway are recently shown to improve patients' survival to a certain degree,[3] chemotherapy with platinum doublet remains the main treatment modality for advanced lung cancer.[4,5] The reality is that the response to chemotherapy agents varies widely among and within individuals. Hence, the use of molecular predictive markers to help identify who may benefit and who may not remains one of the most exciting new areas of study in oncology.[6]

Recently, the expression of *ERCC1* (excision repair cross-complementing group 1), measured by immunohistochemical staining in surgically resected specimen, was shown to be associated with poor response to platinum-containing adjuvant chemotherapy.[7] *ERCC1* belongs to a group of genes responsible for nucleotide excision repair (NER). Because the cytotoxic effect of platinum drugs is attributed to the formation of bulky platinum-DNA adducts, which block replication and inhibit transcription, removal of these adducts from the genomic DNA is conducted by the NER system. Cisplatin resistance seems to be associated with the increased removal of cisplatin-DNA adducts.[8] Elevated DNA-repair capacity had been related to chemoresistance in NSCLC. *ERCC1* plays a pivotal role in NER, and there is plenty of evidence to show that the level of *ERCC1* (either mRNA or protein expression) is important for the repair of platinum-DNA adducts and the response to platinum-based chemotherapy.[9,10] In addition to measuring protein and mRNA expression, studies addressed to the polymorphism of *ERCC1* (118 *C/T* and *C8092A*) have

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Disclosure:The authors declare no conflict of interest.

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ISSN: 1556-0864/12/0706-0973

also demonstrated its impact on the survival of chemotherapy-treated NSCLC patients.[11,12]

Xeroderma pigmentosum group D/excision repair cross-complementing group 2 (*ERCC2*) is an important NER protein intervening both the transcription-coupled-NER and global genomic-NER subpathways.[6] Populations bearing the genotype Lys751Lys and Asp312Asp are known to have good DNA-repair capacity, whereas those with Gln751Gln and Asn312Asn have suboptimal DNA-repair capacity.[13] X-ray repair cross-complementing group 1 (*XRCC1*) and X-ray repair cross-complementing group 3 (*XRCC3*) are two other proteins involving NER, and the polymorphism of these two proteins (*XRCC1* 399 and *XRCC3* 241) has been recently shown to be a prognostic factor of survival.[14]

Gemcitabine, a deoxycitidine analogue, in combination with a platinum drug is a standard regimen for the first-line treatment of advanced NSCLC. In addition to being incorporated into DNA after entering the cell and being phosphorylated, gemcitabine exerts its cytotoxic effect by inhibiting the DNA-repair mechanism and ribonucleotide reductase.[15] The synergistic action of gemcitabine and cisplatin is thought to reside in an inhibitory effect of gemcitabine on the repair of the intrastrand adduct and interstrand cross-link, which are induced by cisplatin.[16–18] In addition, gemcitabine inhibits ribonucleotide reductase, and then depletes the deoxynucleotide pools required for DNA repair and replication. As for ribonucleotide reductase, most of the studies have been consistently showing that low ribonucleotide reductase subunit M1 (*RRM1*) mRNA expression is associated with significantly longer overall survival (OS) in gemcitabine-treated patients in NSCLC.[19] In addition, *RRM1* polymorphisms in the promoter region have been correlated with outcome in NSCLC patients treated with gemcitabine[20] and the *RRM1* polymorphisms, 2455 A>G and 2464 G>A, comprise biomarkers of resistance to gemcitabine, and correlate with poor OS in breast cancer patients.[21]

Because of the scarcity of obtaining enough tumor tissue in advanced lung cancer for measuring mRNA expression, or performing immunohistochemical staining of protein, using blood cells is a reasonable substitute for studying predictive markers of chemotherapy response. One solution is to study single nucleotide polymorphisms (SNPs) in blood cells. Recently, SNPs have been confirmed as predictive markers of treatment response, toxicity, and survival of cancer patients.[22,23] As mentioned previously, two common SNPs of *ERCC1*, codon 118 C/T and C8092A, are well recognized. The codon 118 C/T is associated with different mRNA levels, whereas C8092A links to RNA stability. Shorter survival was reported in C/C genotype of C8092A and C/C genotype of codon 118; however, the other studies reported no significant association of genotypes with survival or the opposite results.[22] Prognostic implications of SNPs in other DNA-repair genes *ERCC2*, *XRCC1*, *XRCC3*, and *RRM1* were also inconsistent in various small studies.[11,23–26] The differences in study design, methodology, and reporting of results across studies and ethnic-related differences in allele frequencies may result in the inconsistent associations with SNPs.

According to the above observations, several SNPs of DNA-repair genes may affect the treatment efficacy of platinum agents and gemcitabine, and their synergistic effect when used in combination. For this study, we evaluated the associations of eight genetic polymorphisms (*ERCC1* Asn118Asn, *ERCC1* C8092A, *ERCC2* Lys751Gln, *ERCC2* Asp312Asn, *XRCC1* Arg399Gln, *XRCC3* Thr241Met, *RRM1* A2455G, and *RRM1* G2464A) of five DNA-repair genes with treatment response and OS in NSCLC patients receiving gemcitabine plus platinum as their first-line chemotherapy.

PATIENTS AND METHODS

Patients

For this study, we enrolled 62 patients as the training set. They were histologically diagnosed and staged as clinically advanced (stage IV, or stage IIIB with pleural effusion) NSCLC from 2004 through 2008 in both National Taiwan University Hospital (NTUH) and Taipei Veterans General Hospital (TVGH). All patients were evaluated with computed tomography of the brain, thorax, and abdomen before initiation of therapy. Patients with brain metastasis and Eastern Cooperative Oncology Group performance status more than 2 were not included. Only those who had received or considered receiving chemotherapy as their first-line treatment were eligible for this study. All patients provided written informed consent for participation and for the analysis of genetic polymorphisms in association with clinical findings. After consent, 10 ml of the patient's blood was drawn. Before treatment, all patients underwent a complete history and physical examination, including routine hematology and biochemistry analysis. Hematology and biochemistry analyses are repeated before the start of each chemotherapy delivery. Age, sex, histological type, EGFR mutation status, clinical stage, chemotherapy regimen, and toxicity were recorded. The validation set consisted of 45 NSCLC patients, stage IIIB or IV, from an independent cohort of patients receiving gemcitabine plus cisplatin as their first-line chemotherapy at NTUH or Taichung Veterans General Hospital between 2000 and 2004 with available genomic DNA for analysis. The study was approved by the NTUH Research Ethics Committee, the TVGH Institutional Review Board and Institutional Review Board of the Taichung Veterans General Hospital.

Chemotherapy and Clinical Response

Patients received gemcitabine 1250 mg/m^2 on days 1 and 8 every 3 weeks in combination with cisplatin 75 mg/m^2, carboplatin AUC 5 or oxaliplatin 130 mg/m^2, both administered on day 1 every 3 weeks. Patients might receive bevacizumab at a dose of 7.5 or 15 mg/kg on day 1 as per the decision of their attending physician. The response of tumor to chemotherapy was assessed after three cycles of chemotherapy and every three cycles thereafter, using Response Evaluation Criteria in Solid Tumor criteria. The best response to first-line chemotherapy was reported as complete response, partial response, stable disease, or progressive disease (PD). Progression-free survival was evaluated for the period from the date of treatment initiation to the date when disease progression was first observed or death occurred. OS was calculated from the date of cancer diagnosis to the date of the last follow-up (death or clinical visit). For this study, the survival data were censored