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.

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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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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

on July 31, 2009, for the training set and on December 31, 2011, for the validation set.

DNA Extraction and Genotyping

Genomic DNA was extracted from total blood cells using QIAamp Blood MiniKit (QIAGEN Inc., Germany) and subject to SNP study. The primers for multiplex polymerase chain reaction (PCR) and single-base extension primers are designed with Web-based software provided at http://www.autoprimer.com (Beckman Coulter Inc., Fullerton, CA). SNP reactions were performed using the method described previously[27] and analyzed with GenomeLab SNPstream Genotyping System (Beckman Coulter Inc.) at the Microarray core facility of the NTU Center of Genomic Medicine. For multiplex PCR reaction, PCR Buffer, MgCl2 (25mM), dNTP-mix, primer pool (10μM, containing all interested SNPs with same nucleotide transition), AmpliTaq Gold Ò (Applied Biosystems Inc., Foster City, CA), and H2O are mixed to form PCR master mix. Five μg of genomic DNA and 3 μl of PCR master mix are added into each 384-well PCR plate. The plate is sealed tightly with sealing film and spin down and subjected to PCR reaction with the following thermal cycles. (94°C, 1 min; 92°C, 30 sec; 55°C, 30 sec; 72°C, 1 min) for 34 cycles, then stored in 4°C. The reaction mixture is then cleaned up by adding 3 μl clean-up reagent into each well. Reaction at 37C for 30 minutes is followed by reaction at 96°C for 10 minutes. Primer extension is performed by adding 7 μl primer extension mixture (SNP primer Pool [10 μM each] 17.5μl, SNPware Extension Mix 115 μl, SNPware Extension Mix Dilution Buffer 2126 μl, SNPware DNA polymerase 12 μl, H_2O, 1718 μl, total volume 4000 μl) into each well of each plate with the following reaction: 96C, 3 minutes; 94°C, 20 seconds; 40°C, 11 seconds) for 45 cycles, then stored at 4°C. After wash with SNPware Wash Buffer, 8 μl of the hybridization solution (SNPware Hybridazation Solution) is added into each well. After transferring 10 μl of the mix from 384 PCR plate to SNPware tag array plate, hybridization reaction is carried out by incubating the plate at 42°C in the humidified covered container in the oven for 2 hours, then washed by Tag array with SNPware Wash Buffer II, vacuum dried, cleaned, and subjected for imaging by GenomeLab SNPstream array imager. Graphic review and operator adjustment of the genotype clusters can be performed to refine fluorescent cutoff value.

Statistical Analysis

Demographic and clinical information was compared across genotypes using Pearson's ^{2} test. The frequencies of different genotypes were compared between patients with and without treatment response using the Fisher's exact test. Odds ratio (OR) and the 95% confidence intervals (CI) for the response (responder versus nonresponder) were calculated using the logistic regression model. The association between OS and the polymorphisms was estimated by the Kaplan-Meier method. Comparison of survival curves was accessed using log-rank test. Univariate and multivariate hazard ratios (HR) were determined using Cox proportional hazard model. Multivariate analysis was used to adjust for age, histology, disease stage, use of bevacizumab, and type of platinum agent; genotypes were treated as indicator variables. All tests were two-sided and statistical significance was set at $p < 0.05$. Statistical analysis was carried out using SPSS software, version 11.0 (SPSS Inc., Chicago, IL).

RESULTS

Patient Characteristics

Clinical and pathological characteristics of 62 patients of the training set along with their treatment response are listed in Table 1. Median age was 57 years; 56.5% were men; and 84% of the patients had stage-IV disease. All patients received a platinum agent (cisplatin in 59, carboplatin in two and oxaliplatin in one) in addition to gemcitabine with (n = 13) or without (n = 49) bevacizumab in combination as their first-line chemotherapeutic agents. Ten patients, who received bevacizumab as maintenance therapy after completion of the first-lime chemotherapy, were excluded from the progression-free survival analysis of gemcitabine plus platinum chemotherapy. Forty-one of the 62 patients had tumors with known EGFR mutation status; 21 had activating EGFR mutations (deletions in exon 19 or L858R) and 20 had wild-type EGFR. All 62 patients received EGFR tyrosine kinase inhibitors as second- or third-line therapy. Overall objective response rate to chemotherapy was 30.6%; PD rate was 14.6%. The median follow-up time was 22 months

TABLE 1. Clinicopathologic Characteristics of Patients with Non–Small-Cell Lung Cancer

	Training Set	Validation Set
	n = 62	n = 45
Age		
Median (range)	57 (36–78)	63 (43–83)
Gender, n (%)		
Male	35 (56.5)	26 (57.8)
Female	27 (43.5)	19 (42.2)
Cell Type, n (%)		
Adenocarcinoma	52 (83.9)	38 (84.4)
Squamous cell cancer	4 (6.5)	3 (6.7)
Others	6 (9.7)	4
EGFR Mutation Status		
Unknown	21 (34)	N/A
Wild-Type	20 (32)	N/A
Activating Mutation	21 (34)	N/A
Stage, n (%)		
IIIB	10 (16)	10 (22.2)
IV	52 (84)	35 (77.8)
Best Response, n (%)		
PR	19 (30.6)	23 (51.1)
SD	34 (54.8)	9 (20)
PD	9 (14.6)	13 (28.9)

Activating mutation, deletions in exon 19 or L858R;
EGFR, endothelial growth factor receptor; N/A, not available; PR, partial response; SD, stable disease; PD, progressive disease.

(range, 4–65 months). The median progression-free survival time for first-line chemotherapy was 5.6 months (n = 52). There were 23 deaths with a median OS time of 51 months.

Genotype Information and Treatment Response

The genotypic frequencies for each polymorphism are presented in Table 2. Although the A/A genotype has been termed the "variant" by convention, with regard to the *RRM1* G2464A polymorphism, in our study the A/A genotype was found in higher frequencies. *RRM1* A2455G polymorphism was in Hardy-Weinberg disequilibrium among the eight SNPs studied. Genotype frequencies of the other six polymorphisms were consistent with previously reported studies. There was no significant association detected between genotype and sex, age, histology, or disease stage.

Patients were divided into groups based on treatment response as responders or nonresponders (stable disease + PD). No significant correlation was observed between eight SNPs and objective response (Table 2). In multivariate logistic regression analysis, patients receiving bevacizumab in addition to chemotherapy (gemcitabine/platinum) were more likely to be responders compared to those receiving only chemotherapy (OR, 5.53; 95% CI, 1.50–20.35; p = 0.01) after adjusting for polymorphisms, age, histology, EGFR mutation status, disease stage, and type of platinum agent.

Polymorphisms and Survival

There was no significant difference in progression-free survival observed among the eight SNPs (Table 3). Table 4 shows OS analysis data based on the eight polymorphisms examined. The wild-type genotype of *XRCC1* 399 (G/G) was associated with decreased OS in our analysis. The difference in the median survival times between patients carrying the wild-type genotype of *XRCC1* 399 (G/G, 22 months, 95% CI, 10–34 months) and those carrying the heterozygous (A/G, not reached) and homozygous (A/A, not reached) variant genotype was statistically significant (log-rank test, p = 0.005, Fig. 1*A*.). In addition, there was a statistically significant difference in OS associated with *ERCC2* 312 polymorphism. The median survival times of the wild-type genotype *ERCC2* 312 (G/G) and heterozygous variant genotype (A/G) were 51 (95% CI, 19–82) and 10 months, respectively (log-rank test, p < 0.001, Fig. 1*B*.).

In the multivariate Cox proportional hazards model, after adjusting for age, histology, disease stage, EGFR mutation status, use of bevacizumab, and type of platinum agent, and polymorphic genotype as an indicator variable, we found a significant effect of *XRCC1* 399 and *ERCC2* 312 polymorphisms on patients' OS. The HR was significantly lower for patients with *XRCC1* 399 heterozygous variant genotype (A/G) compared with wild-type genotype (G/G) (HR, 0.292; 95% CI, 0.116–0.734, p = 0.009), whereas homozygotes (A/A) showed no significance (HR, 0.276; 95% CI, 0.036–2.134, p = 0.217). Because there were only five

TABLE 2. Genotype Frequencies and Response to Gemcitabine/Platinum Chemotherapy According to Genotype

		No. of Patients (%)			
Gene	**Genotype**	**Frequencies**	**Responders PR**	**Nonresponders SD + PD**	***p* Value**
ERCC1 Asn118Asn	C/C	36 (58)	12 (33)	24 (67)	0.429
	C/T	22 (35)	7 (32)	15 (68)	
	T/T	4 (7)	0 (0)	4 (100)	
ERCC1 C8092A	C/C	21 (35)	8 (38)	13 (62)	0.720
	C/A	30 (50)	8 (27)	22 (73)	
	A/A	9 (15)	3 (33)	6 (67)	
ERCC2 Lys751Gln	A/A	57 (92)	19 (33)	38 (67)	0.312
	A/C	5 (8)	0 (0)	5 (100)	
ERCC2 Asp312Asn	G/G	60 (97)	18 (30)	42 (70)	0.522
	G/A	2 (3)	1 (50)	1 (50)	
XRCC1 Arg399Gln	G/G	26 (43)	9 (35)	17 (65)	0.919
	G/A	31 (48)	9 (29)	22 (71)	
	A/A	5 (9)	1 (20)	4 (80)	
XRCC3 Thr241Met	C/C	59 (97)	18 (31)	41 (69)	1.000
	C/T	3 (3)	1 (33)	2 (67)	
RRM1 A2455G	A/A	16 (26)	3 (19)	13 (81)	0.335
	A/G	40 (65)	15 (38)	25 (62)	
	G/G	6 (9)	1 (17)	5 (83)	
RRM1 G2464A	G/G	4 (6)	0 (0)	4 (100)	0.469
	A/G	31 (50)	11 (35)	20 (65)	
	A/A	27 (44)	8 (30)	19 (70)	

PR, partial response; SD, stable disease; PD, progressive disease.

TABLE 3. Progression-Free Survival in NSCLC Patients Treated with Gemcitabine/Platinum Chemotherapy According to Genotype

Gene	Genotype	No. of Patients	Median (month) (95% CI)	Log-Rank *p* Value
ERCC1 Asn118Asn	C/C	30	5.1 (3.7–6.5)	0.357
	C/T	18	5.8 (4.9–6.8)	
	T/T	4	3.3 (0–9.3)	
	C/C	16	5.6 (4.4–6.8)	0.752
	C/A	25	5.1 (2.6–7.6)	
ERCC1 C8092A	A/A	8	5.7 (3.7–7.7)	
	A/A	47	5.6 (4.8–6.4)	0.428
ERCC2 Lys751Gln	A/C	5	4.0 (3.2–4.7)	
	G/G	51	5.6 (4.7–6.5)	0.482
ERCC2 Asp312Asn	G/A	1	4.7	
	G/G	26	5.8 (4.2–7.4)	0.672
	G/A	28	5.1 (3.3–7.0)	
XRCC1 Arg399Gln	A/A	5	5.1 (3.1–7.2)	
	C/C	49	5.6 (4.8–6.4)	0.897
XRCC3 Thr241Met	C/T	3	7.4 (0.1–14.7)	
	A/A	15	7.3 (4.0–10.6)	0.251
	A/G	32	5.6 (4.6–6.6)	
RRM1 A2455G	G/G	5	4.3 (4.0–4.6)	
	G/G	4	8.4 (1.5–15.4)	0.388
	A/G	25	5.1 (3.5–6.8)	
RRM1 G2464A	A/A	23	6.6 (5.4–7.8)	

NSCLC, non–small-cell lung cancer.

patients carrying the *XRCC1* 399 homozygous variant genotype, we considered patients with homozygous and heterozygous variant genotypes as individuals carrying at least one A allele (G/A+A/A) of *XRCC1* 399 genotypes. The analysis showed a statistically significant better survival of variant genotypes (G/A+A/A) when compared with the wild-type genotype (G/G) (HR, 0.290; 95% CI, 0.12–0.705, $p = 0.006$). For *ERCC2* 312 polymorphism, the HR was significantly higher for patients with *ERCC2* 312 heterozygous variant genotype (G/A) compared with wild-type genotype (G/G) (HR, 14.04; 95% CI, 2.253–87.513, $p = 0.005$).

Validation Set

The characteristics of the 45 NSCLC patients in the validation set are listed in Table 1. Median age was 63 years; 57.8% were men; and 77.8% of patients had stage-IV disease. Overall objective response rate to chemotherapy was 50.1%; PD rate was 28.9%. They also received EGFR tyrosine kinase inhibitors as second- or third-line therapy. The median follow-up time was 34 months (range, 2–127 months). The median progression-free survival time for first-line chemotherapy was 7 months. There were 40 deaths with a median OS time of 34 months. We tested only *XRCC1* 399 and *ERCC2* 312 genotyping for this validation set. No significant correlation was observed between these two SNPs and objective response, and there was no significant difference in progression-free survival observed according to these two SNPs. The wild-type genotype of *XRCC1* 399 (G/G) was associated with decreased

TABLE 4. Overall Survival in NSCLC Patients Treated with Gemcitabine/Platinum Chemotherapy According to Genotype: Univariate Survival Analysis

Gene	Genotype	No. of Patients	Median (m) (95%CI)	Log-Rank *p* Value	Univariate Hazard Ratio (95%CI)	*p* Value
ERCC1 Asn118Asn	C/C	36	51 (18–83)	0.682	0.617 (0.174–2.183)	0.453
	C/T	22	NR		0.554 (0.142–2.164)	0.396
	T/T	4	22 (4–39)		1.0 (Reference)	0.688
ERCC1 C8092A	C/C	21	51 (13–88)	0.384	1.0 (Reference)	0.408
	C/A	30	NR		0.659 (0.253–1.718)	0.394
	A/A	9	35 (9–56)		1.590 (0.525–4.810)	0.412
ERCC2 Lys751Gln	A/A	57	51 (19–82)	0.901	1.0 (Reference)	
	A/C	5	NR		0.912 (0.213–3.907)	0.901
ERCC2 Asp312Asn	G/G	60	51 (19–82)	<0.001	1.0 (Reference)	
	G/A	2	10		24.964 (4.087–152.48)	<0.001
XRCC1 Arg399Gln	G/G	26	22 (10–34)	0.005	1.0 (Reference)	0.003
	G/A	31	NR		0.263 (0.107–0.644)	0.003
	A/A	5	NR		0.246 (0.032–1.879)	0.176
XRCC3 Thr241Met	C/C	59	51 (19–82)	0.257	22.260 (0.006–80659)	0.458
	C/T	3	NR		1.0 (Reference)	
RRM1 A2455G	A/A	16	51 (20–81)	0.739	0.716 (0.137–3.751)	0.692
	A/G	40	32		1.064 (0.242–4.676)	0.935
	G/G	6	29		1.0 (Reference)	0.742
RRM1 G2464A	G/G	4	NR	0.685	1.0 (Reference)	0.701
	A/G	31	32		2.394 (0.310–18.462)	0.402
	A/A	27	51		2.345 (0.294–18.676)	0.421

CI, confidence interval; NR, not reached.

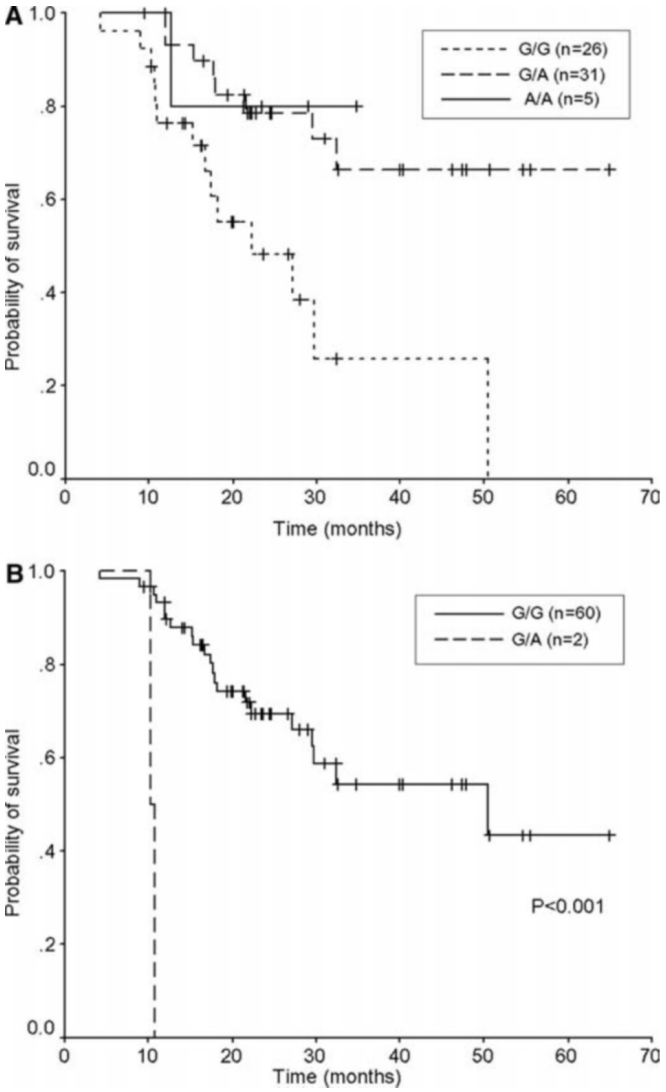

FIGURE 1. *A,* Kaplan-Meier estimates of the overall survival of 62 NSCLC patients in the training set according to XRCC1 399 polymorphism ($p = 0.005$, log-rank test). *B,* Kaplan-Meier estimates of the overall survival of 62 NSCLC patients in the training set according to ERCC2 312 polymorphism ($p < 0.001$, log-rank test). NSCLC, non–small-cell lung cancer; ERCC2, excision repair cross-complementing group 2.

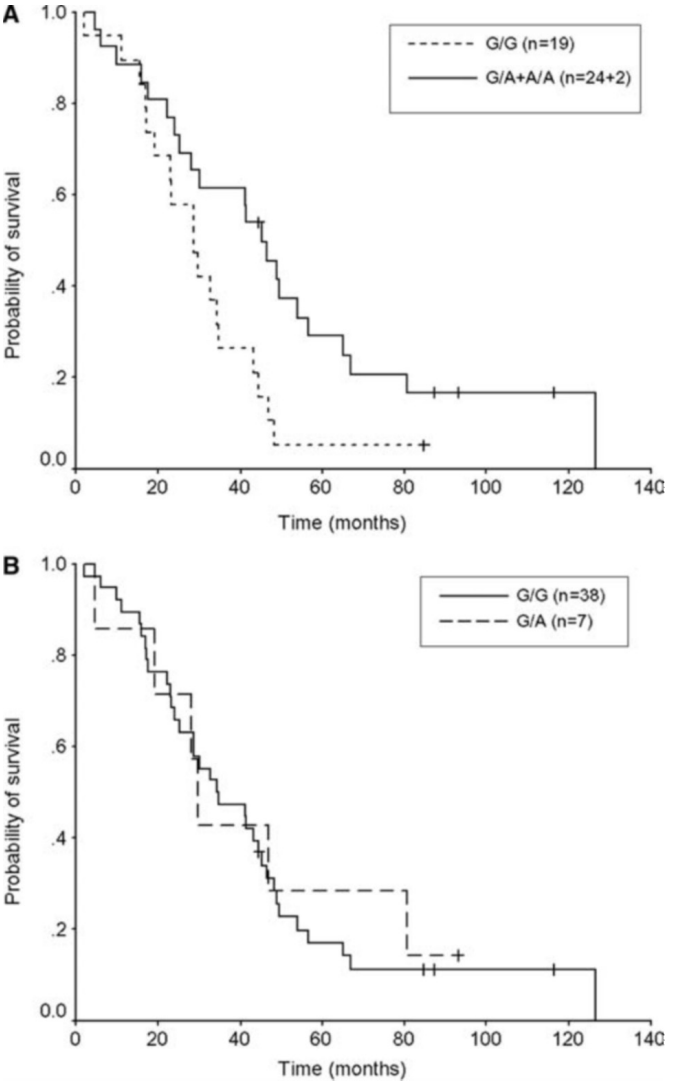

FIGURE 2. *A,* Kaplan-Meier estimates of the overall survival of 45 NSCLC patients in the validation set according to XRCC1 399 polymorphism ($p = 0.023$, log-rank test). *B,* Kaplan-Meier estimates of the overall survival of 45 NSCLC patients in the validation set according to ERCC2 312 polymorphism ($p = 0.787$, Log-rank test). NSCLC, non–small-cell lung cancer; ERCC2, excision repair cross-complementing group 2.

OS in the validation set. The difference in the median survival times between patients carrying the wild-type genotype of *XRCC1* 399 (G/G, 29 months, 95% CI, 20–38 months) and those carrying at least one A allele (G/A+A/A) of *XRCC1* 399 genotypes (45 months, 95% CI, 36–54 months) was statistically significant (log-rank test, $p = 0.023$, Fig. 2*A*.). However, there was no statistically significant difference in OS associated with *ERCC2* 312 polymorphism in the validation set (log-rank test, $p = 0.787$, Fig. 2*B*.). In the multivariate Cox proportional hazards model, after adjusting for age, histology, disease stage, and polymorphic genotype as an indicator variable, we only found one significant effect of *XRCC1* 399 polymorphisms on patients' OS in the validation set. The analysis showed a statistically significant better survival of variant genotypes (G/A+A/A) when compared with the wild-type genotype (G/G) (HR, 0.474; 95% CI, 0.245–0.915, $p = 0.026$).

DISCUSSION

The study of SNP provides opportunity in seeking for suitable markers to predict treatment outcome with cytotoxic chemotherapy.[6] In this study, we evaluated the role of eight DNA-repair gene polymorphisms in the treatment outcome of gemcitabine/platinum-treated advanced NSCLC patients. Our

data showed that there are survival differences according to the *XRCC1* Arg399Gln polymorphism in the training set and the validation set. Patients with wild-type *XRCC1* 399 (G/G) had significantly shorter OS than those harboring non–wild-type *XRCC1* 399 (A/G+A/A). However, patients with wild-type *ERCC2* 312 (G/G) had significantly longer OS than those harboring non–wild-type *ERCC2* 312 (A/G) only in the training set. The other polymorphisms in our analysis showed no significant association with patient survival.

For individualized therapy, it is important to develop reliable biomarkers to select treatments for patients most likely to obtain benefit. Biomarkers predictive for survival benefit from a treatment are far more useful for guiding management than those that simply portend a favorable or unfavorable prognosis, independent of treatment. To further clarify whether the *XRCC1* Arg399Gln polymorphism is of predictive or prognostic value on survival outcomes, a cohort of 47 patients with advanced NSCLC receiving non–gemcitabine-containing platinum doublets as their first-line chemotherapy at NTUH and TVGH were recruited for *XRCC1* 399 genotyping (Supplemental Fig., Supplemental Digital Content 1, http://links.lww.com/JTO/A283). There was no statistically significant difference in OS according to the *XRCC1* 399 polymorphism in this cohort ($p = 0.155$, log-rank test). The results suggested that the *XRCC1* 399 polymorphism could predict survival outcomes from gemcitabine/platinum doublet treatment in advanced NSCLC patients, but lacks prognostic significance in non-gemcitabine platinum doublets treated patients.

The *XRCC1* is a scaffold protein essential to the repair of base excision repair (BER) and single-strand breaks pathways.[28] Impaired DNA-repair mechanisms resulting in the decreased removal of platinum-DNA adducts may improve survival in patients already diagnosed with cancer, when treated with a platinum agent. On the contrary, decreased DNA repair may increase the risk of developing cancer.[8] Polymorphisms in DNA-repair genes may consequently contribute to interindividual diversity in DNA-repair capacity; however, the results from several studies have been generally inconsistent.[29] More than 60 SNPs have been identified in the *XRCC1* gene with the most extensively investigated coding region SNPs being Arg399Gln on exon 10 because of its location within the region of the *BRCT1* binding domain. The effect of *XRCC1* genotype on the ability of a cell to repair DNA damage has been evaluated most commonly involving human lymphocytes challenged with a DNA-damaging agent with the repair ability compared across individuals having different genotypes.[28] There are various lines of evidence showing that the variant genotype of *XRCC1* 399 can decrease repair capability using the above measurement. For example, removal of DNA adducts from two lymphoblast cell lines exposed to vinyl chloride metabolite had been evaluated and showed that the efficiency of repair of DNA adducts in the *XRCC1* 399 homozygous wild-type (G/G) cells was four times greater than the efficiency of repair in the homozygous variant (A/A) cells.[30] In addition, Slyskova et al.[31] evaluated the removal of oxidative DNA damage after light irradiation and found a 3.4-fold deficit of homozygous variant (A/A) lymphocytes in DNA-repair capacity.

Epidemiological studies of polymorphisms in DNA-repair genes can provide insight into the in vivo relationship between DNA-repair genes and lung cancer risk[32] and treatment outcome among lung cancer patients.[11,12] Although lung cancer risk in the presence of the variant *XRCC1* 399 allele has not yielded significant associations in several studies, Kiyohara et al.[33] found that the *XRCC1* 399 homozygous variant genotype (A/A) was associated with an increased risk of lung cancer among Asians (OR = 1.34, 95% CI = 1.16–1.54), but not of whites (OR = 0.94, 95% CI = 0.80–1.11) in a meta-analysis. Many studies have shown the association between the *XRCC1* 399 polymorphism and the clinical outcome of NSCLC patients including treatment response and OS; however, the results have been inconsistent among studies. One study of platinum-treated patients found a positive effect of variant *XRCC1* allele on survival outcome.[34] On the contrary, Gurubhagavatula et al.[23] reported that individuals with *XRCC1* 399 variant genotype were associated with shorter OS. Another study conducted by Sun et al.[35] did not find a considerable association between *XRCC1* 399 polymorphism and treatment response in Chinese population. In the present study, we demonstrated that patients carrying at least one variant *XRCC1* 399 allele had better OS than those with wild-type *XRCC1* 399. Our result was in agreement with the observation that impaired DNA-repair mechanisms may improve survival of NSCLC patients receiving platinum agents; because most of the studies indicated that a variant genotype of *XRCC1* 399 can decrease DNA-repair capability.

Although the NER pathway is the major repair mechanisms for cisplatin-DNA adducts, the biologic effect of the *XRCC1* protein on cisplatin drug action is indirect and may be through repair of other types of cisplatin-induce damage, such as double-strand breaks (DSBs)[36] and ICL.[37] *XRCC1*-DNA ligase III complex, which is involved in the BER pathway, had been proposed by Audebert et al.[36] as a component of an alternative nonhomologous end-joining route of DNA DSBs. In addition, Zhu et al.[38] found that poly (ADP-ribose) polymerase-1 , together with its partners *XRCC1* and DNA ligase III, binds to a DNA duplex containing a platinum ICL. The above observation implies that *XRCC1*, a coordinator or BER and single-stand breaks repair, may be important for platinum DSBs and ICL repair.

The excision repair cross-complementing rodent repair deficiency, group 2 (*ERCC2*), also named the xeroderma pigmentosum group D, belongs to the *TFIIH* complex and involves in NER and transcription. Two polymorphisms (*ERCC2* 312 and *ERCC2* 751), which are common and result in an amino-acid change, have mainly been investigated in relation to risk and clinical outcome of lung cancer.[23,25,39] It has been reported that *ERCC2* 312 variant alleles may induce higher level of DNA adducts, which is interpreted as a lower repair efficiency for the *ERCC2* 312 variant allele.[40] However, these findings remain a subject of controversy. Clarkson et al.[41] used evolutional analysis and reported that polymorphisms of *ERCC2* 312 and *ERCC2* 751 are not well-conserved among different organisms, and they strongly predicted that both polymorphisms are benign. Laine et al.[42] also demonstrated that polymorphisms in the *ERCC2* gene using the baculovirus expression system to reconstitute recombinant *TFIIH* complexes in which the *ERCC2* variants were introduced do not generate significant variations in NER efficiency.

In the present study, we demonstrated that patients with wild-type ERCC2 312 (G/G) had significant longer OS than those harboring *ERCC2* 312 heterozygous variant genotype (G/A) in the training set. But there was no statistically significant difference in OS associated with *ERCC2* 312 polymorphism in the validation set. The above results suggested that no convincing evidence supported the functional significance of *ERCC2* 312 polymorphism on DNA repair and clinical outcomes of NSCLC patients.

Because of the relatively small patient population analyzed in the present study, its main limitation is that it cannot be generalized in NSCLC patients. However, our study included a highly homogeneous cohort of gemcitabine/platinum-treated advanced NSCLC patients among the Chinese population in Taiwan that might contribute to the relevant association of polymorphisms with clinical outcome.

In conclusion, our study, based on the analysis of eight polymorphisms in five DNA-repair genes, identified the *XRCC1* 399 polymorphism as a candidate for contributing interindividual difference in the OS of gemcitabine/platinum-treated advanced NSCLC patients. Our results suggest that polymorphisms of DNA-repair genes may play an important role in the outcome of advanced NSCLC patients, and imply the utility of polymorphism as predictive markers for treatment outcome of chemotherapy.

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