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Original Article

The association between the ERCC1/2 polymorphisms and radiotherapy efficacy in 87 patients with non-small cell lung cancer

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Background: This study sought to investigate the association between the ERCC1/2 single-nucleotide polymorphisms (SNPs) and the efficacy of radiotherapy and prognosis in patients with non-small cell lung cancer (NSCLC).

Methods: We examined 6 SNPs in the ERCC1 and ERCC2 genes in 87 consecutive patients with NSCLC who were treated with definitive radiotherapy. The objective remission rates (ORR), overall survival (OS), and progressive-free survival (PFS) were assessed. A Cox regression analysis was conducted to analyze the independent factors related to death and recurrence.

Result: Patients with the G allele had better OS than patients with the A allele, and there was a statistical difference between the two groups (30.9 *vs.* 16.2 months; P=0.003). Patients with the *AA* genotype had significantly worse OS than patients with the *AG* or *GG* genotypes (6.8 *vs.* 19.8 *vs.* 30.9 months, respectively; P=0.000). The median PFS of the G allele was 18.9 months, which was significantly better than that of the *A* allele (P=0.040). The median PFS of patients with the *GG* genotype, the *AG* genotype, and the *AA* genotype was 18.9, 11.3, and 5.1 months, respectively; the difference among the three groups was statistically significant (P=0.019). Patients with the *G* allele also had better PFS than those with the *A* allele (18.9 *vs.* 11.3 months, P=0.040). The multivariate cox proportional hazard analysis showed that the ERCC1 gene *rs11615* was an independent survival indicator [HR: 1.623, 95% confidence interval (CI): 1.018–2.591, P=0.042] but not an independent recurrence indicator (HR: 1.497, 95% CI: 0.932–2.404, P=0.095).

Conclusions: The ERCC1 *rs11615* SNP may be a potential biomarker for predicting survival prognosis in Chinese NSCLC patients who have undergone definitive radiotherapy. Patients with the *G* allele had better OS than those with the *A* allele.

Keywords: Non-small cell lung cancer (NSCLC), single-nucleotide polymorphisms (SNPs), radiotherapy

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Introduction

Ionizing radiation (IR) acts directly or indirectly to cause various forms of deoxyribonucleic acid (DNA) damage in cells. DNA damage repair is initiated after the cell is genetically damaged (1). Nucleotide-excision repair (NER) is one of the ways of DNA repair. ERCC1-xeroderma pigmentosum group F (XPF) is the complex expression of the ERCC1 and ERCC4 genes. It is a structure-specific endonuclease that cut DNA at single-stranded/double-stranded junctions with a specific polarity. The ERCC2 gene was found to complement the human cell xeroderma pigmentosum group D (XPD), which was identified as an adenosine triphosphate (ATP)-dependent helicase (5'-3' duplex DNA) during NER reaction (2) Single-nucleotide polymorphisms (SNPs) interrupt promoters, codons, or reading frames by altering single nucleotides, which affects the expression of protein amino acid sequences and thus reflects in the phenotype.

Research on the ERCC1/2 gene polymorphisms in non-small cell lung cancer (NSCLC) has mainly focused on disease susceptibility and the efficacy and prognosis of platinum-based chemotherapy (3-7). Numerous studies have shown that the low expression of ERCC1 protein in tumor tissue can improve the sensitivity and survival of platinum-based chemotherapy patients. The *GG* genotype of the ERCC1 gene *rs11615* is the protective factor of NSCLC. The polymorphisms of the ERCC2 gene *rs13181 A>C* in exon 23 leads to the mutation of the codon 751 translation product, which increases the DNA binding force and decreases the DNA repair ability of cells. This genetic polymorphism is associated with the risk of radiation-induced esophagitis (8). To date, very few studies have investigated the association between the genetic polymorphisms of ERCC1/2 and the efficacy and prognosis of radiotherapy among Chinese NSCLC patients. This study sought to explore the correlation between ERCC1/2 SNPs and the radiotherapy efficacy and prognosis and the risk of radiation-induced lung injury (RILI) in NSCLC patients.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/jtd-21-755).

Methods

Patient population

It's a retrospective study. A total of 87 NSCLC patients who received definitive radiotherapy at the Cancer Hospital of University of Chinese Academy of Sciences between August 2014 and December 2017 were evaluable in this study. The eligibility criteria were: (I) have histologically or cytologically confirmed NSCLC; (II) have a lesion that could be measured by an imaging examination; (III) have no secondary primary malignant tumor; (IV) not have undergone any previous anti-cancer treatment; and (V) have a blood sample available. Patients signed informed consent forms before radiotherapy. Information on patient age, gender, smoking history, complications, tumor location, stage, pathology, chemotherapy and radiotherapy technology were collected. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by committee of Zhejiang Cancer Research Institute (Hangzhou, Zhejiang, China).

Radiotherapy

Patients with stage I NSCLC were treated with stereotactic body radiation therapy (SBRT) with a total dose of 50 to 60 Gy (the median biologically effective dose was 100 Gy). Patients with stages II–III NSCLC were treated with intensity modulated radiotherapy (IMRT) with a total dose of 60–70 Gy at 2 Gy/fraction. In relation to the SBRT, the volume of lung that received at least 20 Gy (V20) was kept to less than 10%, V10 was kept to less than 15%, V13.5 was kept less than 1,000 cc, and V12.5 was kept to less than 1,500 cc. Conversely, IMRT required a mean lung dose less than 13 Gy, and V20 was kept to less than 28%, and V30 was kept to less than 20%.

Follow-up examinations

Follow-up examinations were performed using patients' clinical records every 3 months after patient discharge until mortality or withdrawal, or until the follow-up deadline (of 5 years). The objective response (OR) was determined according to the RECIST (version 1.1). RILI was determined according to the Common Terminology Criteria for Adverse Events (version 5.0).

Genotyping methods

Peripheral blood leukocytes were obtained from 2 mL of a whole blood sample and were used for DNA extraction. Genomic DNA was isolated using a Blood Genomic DNA

Table 1 Primary information for the analyzed ERCC1/2 SNPs in our population

Genes	SNPs	Major allele (%)	Minor allele (%)	Genotypes			MAF	χ^2	P
				Homozygous 1	Heterozygote	Homozygous 2			
ERCC1	*rs11615*	*G* (79.3)	*A* (20.7)	*GG* [55]	*GA* [28]	*AA* [4]	0.4117	0.03	0.98
ERCC1	*rs3212961*	*G* (50.0)	*T* (50.0)	*GG* [22]	*GT* [43]	*TT* [22]	0.1447	0.01	0.99
ERCC1	*rs3212986*	*C* (70.7)	*A* (29.3)	*CC* [43]	*CA* [37]	*AA* [7]	0.2568	0.06	0.97
ERCC2	*rs13181*	*T* (93.1)	*G* (6.9)	*TT* [75]	*TG* [12]	*GG* [0]	0.3689	0.48	0.79
ERCC2	*rs238406*	*G* (51.1)	*T* (48.9)	*GG* [24]	*GT* [41]	*TT* [22]	0.4205	0.28	0.87
ERCC2	*rs1799793*	*C* (94.8)	T (5.2)	*CC* [78]	*CT* [9]	*TT* [0]	0.3088	0.26	0.88

SNPs, single-nucleotide polymorphisms; MAF, minor allele frequency.

Kit (Qiagen, Valencia, CA). We genotyped the SNPs of the ERCC1 gene (*rs11615*, *rs3212961*, *and rs3212986*) and ERCC2 gene (*rs13181*, *rs238406*, *and rs1799793*) using the polymerase chain reaction (PCR) restriction fragment-length polymorphism method. PCR-based assays were used to amplify the fragments that contained ERCC1/2 polymorphisms. The primers were designed and provided by the Hangzhou Molecular Detection Biotechnology Co. Ltd. (see Table S1 for further details). The SNPs were analyzed using the illuminaHiSeq2500 platform.

Statistical analysis

IBM SPSS 25.0 was used for the statistical analysis. A Hardy-Weinberg analysis was conducted to analyze the frequencies of the genotypes and alleles. The Chi-square test was used to analyze differences in the selected demographic variables, risk factors, alleles, and genotypes of the 6 SNPs ERCC1/2 between the OR group and non-OR groups, and any association between RILI and clinical or genetic variables in NSCLC patients who underwent radiotherapy. Kaplan-Meier curves were used to estimate the cumulative overall survival (OS) and progressive-free survival (PSF) between different genotype groups. A univariate Cox proportion hazards regression analysis was performed to analyze the risks of OS and PFS. A multivariate Cox hazards regression analysis was performed to adjust for other covariates. The univariate and multivariate logistic regression analyses were used to investigate the association between RILI and ERCC1/2 polymorphisms or clinicopathological factors. All of the tests were two-sided. A P value less than 0.05 was considered statistically significant.

Results

The distribution of the ERCC1/2 SNPs alleles and genotypes in the 87 NSCLC patients

Table 1 sets out details of the genotype frequency and the minor allele frequency (MAF) of each sequence variants of the ERCC1/2 gene in the 87 NSCLC patients. The MAFs of the 6 SNPs of the patients were similar to those of the general population in the National Center for Biotechnology Information (NCBI) dbSNP databases. The distributions of the ERCC1/2 SNPs in the 87 patients were within the parameters of the Hardy-Weinberg equilibrium (P>0.05).

The correlation between the ERCC1/2 SNPs and the OR

Among the 87 NSCLC patients, 44 patients had an OR [including a complete response (CR) and a partial response (PR)] and 43 patients had a non-OR [including stable disease (SD) and progressive disease (PD)]. *Table 2* sets out the correlations between the OR and the clinicopathological characteristics of the NSCLC patients who underwent definitive radiotherapy. There was no significant difference between the clinical, pathological, and therapeutic regimen factor and the OR (P>0.05). The correlations between the distribution of the ERCC1/2 gene SNPs and the OR are shown in Table S2. Neither the genotype nor the allele of ERCC1/2 SNPs was significantly correlated with the OR of NSCLC patients who received definitive radiotherapy (all P>0.05).

The Association between the ERCC1/2 SNPs and OS and PFS

The median follow-up time was 24.9 months. The median

Table 2 Comparison of 87 NSCLC patient characteristics between the OR (CR + PR) group and the non-OR (SD + PD) group

Factor	OR (N=44)	Non-OR (N=43)	P
Age (years)			
>65	20	23	0.454
≤65	24	20	
Gender			
Female	6	8	0.528
Male	38	35	
Complication			
Yes	20	21	0.752
No	24	22	
Smoking history (packs-year)			
>30	28	24	0.457
≤30	16	19	
Tumor location			
Peripheral	30	31	0.690
Central	14	12	
Pathology			
Squamous	22	19	0.587
Non-squamous	22	24	
T stage			
1	18	15	0.314
2	16	18	
3	2	6	
4	8	4	
N stage			
0	21	17	0.597
1	3	1	
2	11	13	
3	9	12	
Chemotherapy			
Yes	23	22	0.918
No	21	21	
RT technology			
SBRT	15	12	0.533
IMRT	29	31	
Concurrent CRT			
Yes	15	9	0.128
No	29	34	

NSCLC, non-small cell lung cancer; OR, objective response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; SBRT, stereotactic body radiation therapy; IMRT, intensity modulated radiotherapy; CRT, chemoradiotherapy.

survival time (MST) and PFS of the 87 patients was 24.9 and 16.1 months, respectively. The results of our study indicated that NSCLC patients with different ERCC1 *rs11615* genotypes who received definitive radiotherapy had quite different survival curves (see *Figure 1*). Patients with the *G* allele [30.9 months, 95% confidence interval (CI): 12.832–48.968] had better OS than patients with the *A* allele (16.2 months, 95% CI: 9.102–23.238); the difference between the two groups was statistically significant (P=0.003). Patients with the *AA* genotype had significantly worse OS than that patients with the *AG* or *GG* genotypes (6.8 *vs.* 19.8 *vs.* 30.9 months, respectively, P=0.000). The results also indicated that patients with the ERCC2 *rs3212986* variant *A* allele had better OS than patients with the *C* allele (MST: 30.9 *vs.* 21.5 months); however, the difference was not statistically significant (P=0.086).

A similar association was found between PFS and ERCC1 *rs11615* in our study (see *Figure 2*). The median PFS of patients with the *G* allele was 18.9 months (95% CI: 13.960–24.780), which was significantly higher than that of patients with the *A* allele (11.3 months, 95% CI: 8.655–14.005); the difference between the two groups was statistically significant (P=0.040). The median PFS of patients with the *GG* genotype, the *AG* genotype and the *AA* genotype was 18.9 months (95% CI: 12.556–19.584), 11.3 months (95% CI: 5.755–16.905), and 5.1 months (95% CI: 0–15.126), respectively; the difference among the three groups was statistically significant (P=0.019). *Table 3* shows the association between the ERCC1/2 gene SNPs and prognosis (OS and PFS) for the NSCLC patients who received definitive radiotherapy.

Prognostic factors for NSCLC patients who received definitive radiotherapy

The analysis results of the associations between the prognosis, clinicopathology, and genetic factors in NSCLC patients are set out *Table 4*. A univariate Cox proportional hazards model was used to analyze the association between the survival or recurrent time and several factors, including gender, age, complication, smoking history, T stage, N stage, chemotherapy, radiation technology, and ERCC1/2 gene polymorphism in NSCLC patients treated with radiotherapy. The multivariate Cox proportional hazard analysis showed that T stage, ERCC1 *rs11615*, and ERCC2 *rs3212986* were independent survival factors for NSCLC patients who received definitive radiotherapy (see *Table 4*). T3–4 stage

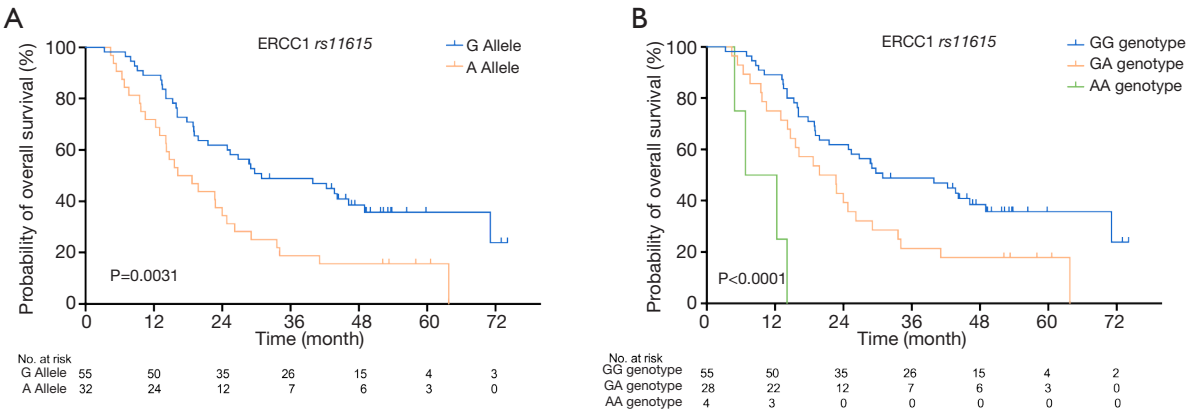

Figure 1 Kaplan-Meier curves of OS of the ERCC1 gene *rs11615* SNP in 87 NSCLC patients who received definitive radiotherapy. (A) OS at allele gene level (P=0.003); (B) OS at genotype level (P=0.000). OS, overall survival; SNP, single-nucleotide polymorphism; NSCLC, non-small cell lung cancer.

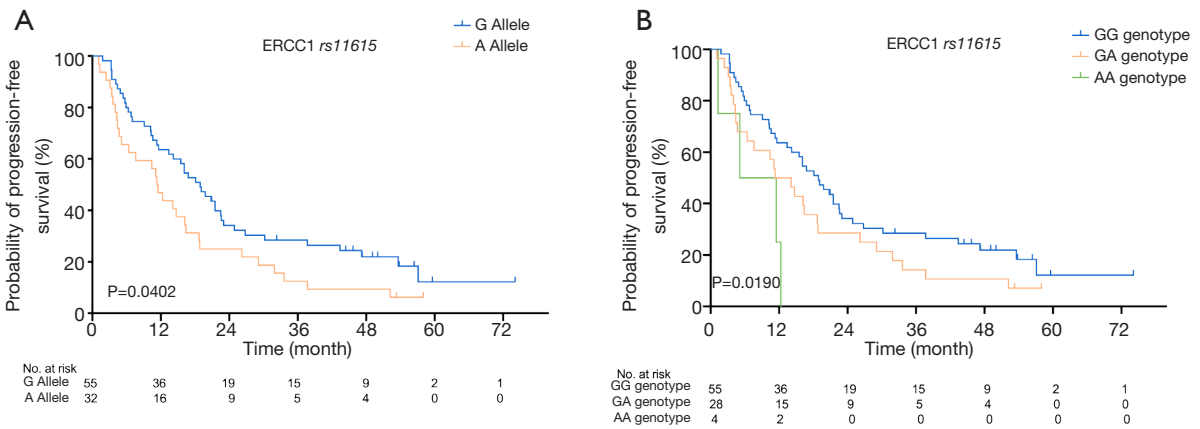

Figure 2 Kaplan-Meier curves of PFS of the ERCC1 gene *rs11615* SNP in NSCLC 87 patients who received definitive radiotherapy. (A) PFS at allele gene level (P=0.040); (B) PFS at genotype level (P=0.019). PFS, progressive-free survival; SNP, single-nucleotide polymorphism; NSCLC, non-small cell lung cancer.

(hazard ratio, HR): 3.215, 95% CI: 1.792–5.747, P=0.000), ERCC1 *rs11615 A* allele (HR: 2.451, 95% CI: 1.435–4.184, P=0.001) than *G* allele, and ERCC2 *rs3212986 A* allele (HR: 2.538, 95% CI: 1.149–5.618, P=0.021) had higher risks of OS than *C* allele. The univariate and multivariate Cox proportional hazard analyses showed that T stage was also an independent recurrence-related factor in NSCLC patients who received definitive radiotherapy (T3–4 stage, HR: 2.252, 95% CI: 1.300–3.921, P=0.004). ERCC1 *rs11615* was an independent recurrence-related factor in the univariate Cox proportional hazard analysis (HR: 1.623, 95% CI: 1.018–2.591, P=0.042), but there was no significant difference in the multivariate Cox proportional hazard analysis (HR: 1.497, 95% CI: 0.932–2.404, P=0.095).

Association between ERCC1/2 polymorphisms and RILI in NSCLC patients who underwent definitive radiotherapy

The clinical characteristics of patients with grade ≥2 RILI and those with grade <2 RILI are set out in *Table 5*. There were no significant differences in terms of smoking history, complication, tumor location, pathological type, T stage, N stage, radiotherapy technology, and ERCC1/2 polymorphisms variates between the two groups (P>0.05). A logistics regression analysis was conducted to analyze the association between the RILI and ERCC1/2 gene

Table 3 Association between the ERCC1/2 gene polymorphisms and prognosis in 87 NSCLC patients who received definitive radiotherapy

Gene	MST (months)	95% CI	P	Median PFS (months)	95% CI	P
rs11615						
AA	6.77	0–14.05	0.000	5.13	0–15.13	0.019
AG	19.77	11.14–28.40		11.33	5.76–16.91	
GG	30.90	12.83–48.97		18.87	12.56–19.58	
A allele	16.17	9.10–23.24	0.024	11.33	8.66–14.01	0.005
G allele	30.90	12.83–48.97		18.87	13.96–23.78	
rs3212961						
GG	24.77	4.75–44.79	0.735	11.53	6.41–16.66	0.910
GT	23.97	15.75–32.19		18.07	12.67–23.47	
TT	24.93	9.37–40.49		11.60	5.28–19.92	
G allele	24.93	18.95–30.91	0.441	16.17	11.84–20.50	0.897
T allele	24.77	4.75–44.79		11.53	6.41–16.66	
rs3212986						
AA	71.13	0–160.416	0.092	15.53	0–38.01	0.718
AC	29.10	18.92–39.28		18.70	11.91–25.49	
CC	21.53	12.97–30.09		14.67	8.849–20.49	
A allele	30.90	15.68–46.12	0.086	18.01	8.85–20.49	0.423
C allele	21.53	12.97–30.09		14.67	11.89–24.25	
rs13181						
TT	24.93	12.67–36.87	0.692	19.07	9.96–28.19	0.921
TG	24.77	16.19–33.67		16.47	10.17–19.17	
rs238406						
TT	16.17	0–36.26	0686	11.33	8.92–13.74	0.143
TG	28.67	22.72–34.62		21.50	16.86–26.14	
GG	19.80	13.76–25.84		11.30	6.67–15.93	
T allele	26.83	21.44–32.22	0.562	16.83	12.16–21.50	0.097
G allele	19.80	13.76–25.84		11.30	6.67–15.93	
rs1799793						
CC	25.40	18.74–32.05	0.222	15.53	11.79–19.27	0.411
CT	19.07	10.48–27.66		16.13	2.89–29.37	

NSCLC, non-small cell lung cancer; CI, confidence interval; MST, median overall survival time; PFS, progression-free survival.

polymorphism in NSCLC patients who underwent definitive radiotherapy. As *Table 6* shows, no association was observed in relation to the polymorphisms and adverse effects due to radiotherapy.

Discussion

In this study, we assessed the association of prognosis with clinical factors and genetic factors among NSCLC patients who underwent definitive radiotherapy. Our results showed

Table 4 Univariate and multivariate regression analyses of survival and recurrence in patients with NSCLC

Factor	Overall survival						Progression-free survival					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Gender (male *vs.* female)	1.177	0.580–2.388	0.652				1.145	0.616–2.127	0.669			
Age (>65 *vs.* ≤65 years)	1.320	0.797–2.184	0.281				1.155	0.731–1.826	0.537			
Complication (yea *vs.* no)	1.188	0.724–1.946	0.497				1.093	0.692–1.754	0.705			
Smoking (>30 *vs.* ≤30 pack-year)	1.406	0.833–2.375	0.201				1.125	0.703–1.799	0.624			
T stage (T3–4 *vs.* T1–2)	2.976	1.689–5.263	0.000	3.215	1.792–5.747	0.000	2.392	1.389–4.115	0.002	2.252	1.300–3.921	0.004
N stage (N2–3 *vs.* N0–1)	1.721	1.034–2.865	0.037				1.616	1.016–2.564	0.042			
Chemotherapy (no *vs.* yes)	0.578	0.346–0.964	0.036				1.587	0.998–2.525	0.051			
Concurrent chemoradiotherapy (no *vs.* yes)	1.227	0.707–2.128	0.468				1.336	0.824–2.262	0.226			
Radiotherapy technology (IMRT *vs.* SBRT)	1.756	0.988–3.123	0.055				1.773	1.059–2.968	0.029			
Target therapy (no *vs.* yes)	1.047	0.475–2.304	0.910				1.114	0.532–2.326	0.776			
ERCC1 *rs11615 A vs. G*	2.105	1.271–3.484	0.004	2.451	1.435–4.184	0.001	1.623	1.018–2.591	0.042	1.497	0.932–2.404	0.095
ERCC1 *rs3212961 T vs. G*	1.276	0.686–2.370	0.442				1.035	0.610–1.756	0.897			
ERCC1 *rs3212986 A vs. C*	1.554	0.936–2.581	0.088	2.538	1.149–5.618	0.021	1.206	0.763–1.905	0.423			
ERCC2 *rs13181 G vs. T*	1.153	0.568–2.342	0.692				1.033	0.542–1.969	0.921			
ERCC2 *rs238406 T vs. G*	1.177	0.678–2.043	0.562				1.516	0.924–2.488	0.100			
ERCC2 *rs1799793 T vs. C*	1.585	0.752–3.333	0.226				1.342	0.664–2.717	0.413			

NSCLC, non-small cell lung cancer. CI, confidence interval; IMRT, intensity modulated radiotherapy; SBRT, stereotactic body radiation therapy.

that a genetic polymorphic site (i.e., *rs11615*), which is a functional variant of the NER pathway that repairs the DNA gene ERCC1, may be an independent indicator of survival in NSCLC patients who undergo definitive radiotherapy. As the key enzymes in the NER pathway, the proteins expressed by the ERCC1 and ERCC2/XPD genes play an important role in this pathway. The protein expressed by ERCC1 is the rate-limiting enzyme of the NER pathway. By forming a tight heterodimer with specific 5' terminal endonuclease function by removing a complementary gene F (XPF) with the DNA repair enzyme, the damaged DNA can be identified and removed to ensure the fidelity of the gene (9). The protein expressed by ERCC2 is an ATP-dependent DNA helicase, which recognizes and opens the damaged DNA double strand and then assists the endonuclease to remove the damaged DNA (10).

The SNPs of ERCC1 and ERCC2 were associated with platinum-based chemosensitivity and prognosis in patients with NSCLC, but the results that followed were heterogeneous. The *A* allele of ERCC1 *rs11615* was associated with poor OS. In relation to ERCC2, the *T* allele of r*s1799793* was linked to an unfavorable OS, and the *G* allele of *rs13181* had a worse OS and PFS in NSCLC patients who underwent platinum-based chemotherapy (10). Tan *et al.* (11) found that the ERCC1 *rs11615 A* allele was associated with poor OS in advanced NSCLC patients who received platinum chemotherapy. Conversely, Ren *et al.* (12) found that the presence of the *rs11615* polymorphism (*CT* or *TT*) was related to better survival than *CC*. Additionally, some research has shown that the ERCC1 SNPs, especially *rs11615*, are associated with chemoradiotherapy rather than chemotherapy. A meta-analysis showed that ERCC1 had no significant correlation with platinum-based chemotherapy, but there was a significant correlation in platinum-based radiochemotherapy and prognosis (13); however, it should be noted that the meta-analysis focused on the Caucasian race. A study by Sullivan *et al.* (14) also showed that ERCC1 *rs11615* was correlated with the efficacy of radiochemotherapy in NSCLC patients, but not in chemotherapy. Similarly, in esophageal cancer, *rs11615*

Table 5 Comparison of 87 NSCLC patient characteristics between the grade ≥2 RILI group and the grade <2 RILI group

Factors	Grade ≥2 RILI (N=16)	Grade <2 RILI (N=71)	P value
Gender			0.045
Male	16	57	
Female	0	14	
Age (years)			0.372
>65	7	37	
≤65	9	34	
Complication			0.129
Yes	5	36	
No	11	35	
Smoking history (packs-year)			0.137
>30	12	40	
≤30	4	31	
Tumor location			1.000
Peripheral	11	50	
Central	5	21	
Pathology			0.139
Squamous	6	40	
Non-squamous	10	31	
T stage			0.531
T1–2	12	55	
T3–4	4	16	
N stage			0.548
N0–1	8	34	
N2-3	8	37	
Radiotherapy technology			0.600
SBRT	5	22	
IMRT	11	49	
Chemotherapy			0.372
Yes	7	37	
No	9	34	
Concurrent CRT			0.533
Yes	4	20	
No	12	51	
Target therapy			0.523
Yes	2	7	
No	14	64	
rs11615			0.581
G allele	10	45	
A allele	6	26	
rs3212961			0.376
G allele	3	19	
T allele	13	52	
rs3212986			0.372
C allele	9	34	
A allele	7	37	
rs13181			0.616
T allele	14	61	
G allele	2	10	
rs238406			0.533
G allele	4	20	
T allele	12	51	
rs1799793			0.523
C allele	14	64	
T allele	2	7	

NSCLC, non-small cell lung cancer; RILI, radiation-induced lung injury; CRT, chemoradiotherapy.

was found to be significantly associated with the short-term therapeutic efficacy (the CR rate) and survival time of ESCC patients who received docetaxel plus cisplatin regimen-based concurrent radiochemotherapy (15).

The results on the effects of SNPs of ERCC1/2 in relation to the efficacy of radiotherapy were conflicting. ERCC1/2 was identified and considered a potential biomarker in response to radiation (16). However, Jin *et al.* (17) developed an OS model and a methodology to identify a biomarker for radiosensitivity in ERCC1/2, and found the ERCC1 *rs11615 GG* genotype and the ERCC2 *rs238406 CC* genotype were correlated with radioresistance, and those patients could benefit from higher dose of radiation. However, it should be noted that the main subjects in

Table 6 Logistic regression analysis of the relationship between the ERCC1/2 polymorphisms and RILI

SNP	Allele	OR	95% CI	P value
rs11615	*G allele*	1	Ref.	0.925
	A allele	1.063	0.299–3.774	
rs3212961	*C allele*	1	Ref.	0.454
	A allele	1.739	0.408–7.411	
rs3212986	*C allele*	1	Ref.	0.555
	A allele	1.432	0.430–4.739	
rs13181	*A allele*	1	Ref.	0.755
	C allele	1.331	0.220–8.065	
rs238406	*G allele*	1	Ref.	0.887
	T allele	1.105	0.278–4.393	
rs1799793	*C allele*	1	Ref.	0.261
	T allele	1.445	0.247–8.439	

RILI, radiation-induced lung injury; SNP, single-nucleotide polymorphism.

Spring *et al.*'s study were Caucasians.

The distribution of ERCC1 *rs11615* genotype frequency is highly dependent upon race. Africans have the highest ERCC1 *rs11615 GG* genotype frequency (more than 90%), while the *GG* genotype frequency in Asians is 20–60%, which is higher than that in Europeans (7–20%) (17). In the present study, which was the first to focus on Chinese NSCLC patients who underwent radiotherapy, we found that the OS and PFS of patients with the ERCC1 *rs11615 G* allele were significantly better than those with the *A* allele (P<0.05). The univariate and multivariate Cox proportional hazard model analyses also showed that *rs11615* was an independent factor related to survival, but not to PFS (P=0.095). Similar results were found for the subgroup of patients who underwent IMRT; thus, *rs11615* may be a potential biomarker that predicts survival outcomes in NSCLC patients, and patients with *A* allele or the *AA* genotype will have worse OS than those with the *G* allele or the *GG/GA* genotype. The subgroup of patients who received SBRT had similar results to those who received IMRT. There was a trend towards poorer clinical outcomes for patients with the *A* allele; however, it should be noted that these results were not significant (this may be attribute to the study's small sample size). In relation to ERCC2, only one study has shown that the *rs13181 T* allele significantly increased tumor recurrence risk in gastric cancer, and it should be noted that some studies have found opposite results (16). In relation to *rs1799793*, which is described as Asp312Asn in some studies, several pre-clinical studies reported that the Asn/Asn genotype had a sub-optimal DNA repair capacity relative to the Asp-containing genotypes. In the present study, no association with clinical outcomes was found for ERCC2 SNP.

The cell-death mechanism related to ionizing radiation is mainly lethal DNA damage, which leads to cell-cycle arrest and apoptosis. Theoretically, as they are involved in DNA damage repair, the ERCC1/2 SNPs genes could be associated with the sensitivity, efficacy, and prognosis of radiotherapy. The SNPs of ERCC1 could affect micro-ribonucleic acid (mRNA) expression, and have attracted increasing interest as potential predictors of cancer therapy outcomes and patient prognosis indicators for lung cancer patients. *rs11615 A>G*, which is described as *T>C* in some articles, is a synonymous mutation and aspartic acid in transcription and translation (the 'AAT' and 'AAC' codons, respectively). As the usage of 'AAT' codon increases, the expression and protein level of mRNA increases (by about 50%), which improves the DNA damage repair ability of tumor cells and improves tumor resistance to radiotherapy and platinum-based chemotherapy (1,14).

Some studies have shown an association between ERCC1/2 polymorphisms and radiation-induced adverse effects in malignant tumors. One study reported that patients bearing the *rs11615 T* allele (*TT* or *CT* genotypes) variant had a significantly reduced risk of developing RILI compared to those carrying the *CC* genotype in lung cancer (18).

In addition, research has shown that ERCC2 *rs13181* is related to acute radiation-induced esophageal toxicity in lung cancer patients (19). The results of the present study showed that the ERCC1/2 polymorphisms had no statistically significant effect on RILI risk. This supports the results of a previous study on *rs13181*, but does not support the results of a previous study on *rs11615*.

In addition to *rs11615*, we also found that *rs3212986* was associated with OS in NSCLC patients who received radiotherapy, and that the *A* allele patients had better OS than the *C* allele patients; however, the difference was not statistically significant. In the Cox regression analysis, *rs3212986* was found to be an independent predictor of prognosis. It has been hypothesized that this is related to reduced ERCC1 synthesis due to the mRNA instability of the *A* allele. This may lead to an accumulation of DNA damage, which may make tumors more immunogenic and more responsive to immunotherapy (20).

This study had a number of limitations. First, the sample size of the study was small, which may have caused selection bias and affected the results. Second, this was a retrospective study, and the radiotherapy technology received by patients was not consistent; thus, the correlation between the radiophysical parameters and ERCC1/2 SNP could not be systematically analyzed. Finally, this study only analyzed the ERCC1/2 SNP, but the DNA damage mediated by ionizing radiation is the result of multiple pathway mechanisms and multiple genes. It is difficult to predict efficacy by relying on a specific biomarker. In the future, we intend to investigate more potential biomarkers for different pathways, and develop prediction models by combining multiple biomarkers.

In conclusion, the study provided the first clinical evidence that the ERCC1 *rs11615* SNP may be a potential biomarker for predicting the survival prognosis of Chinese NSCLC patients who have undergone definitive radiotherapy. Patients with the *G* allele had better OS than those with the *A* allele. Further investigations need to be conducted to confirm these findings.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by committee of Zhejiang Cancer Research Institute (Hangzhou, Zhejiang, China) and informed consent was taken from all the patients.

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Supplementary

Table S1 Primer information of the ERCC1/2 gene of SNPs

Gene	Strand	Primer information
ERCC1		
rs11615	Upstream	*TCGGGAATTACGTCGCCAAATT*
	Downstream	*GTCCAGGGTTAGGAGGAGAGAGAAG*
rs3212961	Upstream	*CATGTCATCTCAGATGTGAAAAACGT*
	Downstream	*TGAAGAGACTGAGACCTCTCAACTC*
rs3212986	Upstream	*GACTGAGCCAATTCAGCCACTA*
	Downstream	*GCACCTTCAGCTTTCTTTAGTTCCT*
ERCC2		
rs13181	Upstream	*GGAGTCACCAGGAACCGTTTAT*
	Downstream	*GCTCTGGATTATACGGACATCTCCA*
rs238406	Upstream	*GGGCATCAAATTCCTGGGACAA*
	Downstream	*TTTGAAGAGTGGTTGGGTTTCCA*
rs1799793	Upstream	*CTCAGGAAGCCCAGGAAATGCT*
	Downstream	*CTCATCTCTCCGCAGGATCAAAG*

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Table S2 ERCC1/2 SNPs of 87 patients with NSCLC in the CR + PR group and the SD + PD group

Factor	OR (N=44)	Non-OR (N=43)	P
rs11615			
AA	0	4	0.130
AG	14	14	
GG	30	25	
A allele	14	18	0.331
G allele	30	25	
rs3212961			
GG	8	14	0.194
GT	22	21	
TT	14	8	
G allele	8	14	0.123
T allele	36	29	
rs3212986			
CC	21	22	0.806
CA	20	17	
AA	3	4	
C allele	21	22	0.749
A allele	23	21	
rs13181			
TT	39	36	0.549
TG	5	7	
GG	0	0	
T allele	39	36	0.549
G allele	5	7	
rs238406			
TT	10	12	0.790
TG	22	19	
GG	12	12	
T allele	32	31	0.947
G allele	12	12	
rs1799793			
CC	39	39	0.668
CT	5	4	
TT	0	0	
C allele	39	39	1.000
T allele	5	4	

CR, complete response; PR, partial response; SD stable disease; PD, progressive disease

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