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Original Article

RAD18 polymorphisms are associated with platinum-based chemotherapy toxicity in Chinese patients with non-small cell lung cancer

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Aim: Although targeted therapy is very efficient for lung cancer, traditional platinum-based chemotherapies are still the principal strategy in the absence of positive biomarkers. The aim of the present study is to evaluate the contribution of *RAD18* polymorphisms to platinum-chemotherapy response and its potential side effects in Chinese patients with non-small cell lung cancer (NSCLC).
Methods: A total of 1021 Chinese patients with histological diagnosis of advanced NSCLC were enrolled. Treatment responses were classified into 4 categories (complete response, partial response, stable disease and progressive disease). Gastrointestinal and hematological toxicity incidences were assessed twice a week during the first-line treatment. Ten *RAD18* SNPs were genotyped. A logistic regression model was utilized to analyze the associations between *RAD18* SNPs and treatment response or toxicity.
Results: Among the 10 SNPs tested, none was significantly correlated with the treatment response in a combined cohort. For gastrointestinal toxicity incidences, rs586014 was significantly associated with an increased risk of grade 3 or 4 gastrointestinal toxicity in non-smokers and in the combined cohort; rs654448 and rs618784 were significantly associated with gastrointestinal toxicity in non-smokers; rs6763823 was significantly associated with gastrointestinal toxicity in smokers. For hematological toxicity incidences, rs586014, rs654448 and rs618784 were significantly associated with hematologic toxicity in non-smokers; rs6763823 and rs9880051 were significantly associated with leukocytopenia in smokers.
Conclusion: *RAD18* polymorphisms are correlated with the side effects of platinum-chemotherapy in Chinese patients with advanced NSCLC.

Keywords: NSCLC; platinum-based chemotherapy; *RAD18* polymorphisms; gastrointestinal toxicity; hematological toxicity

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Introduction

The estimated incidence of lung cancer is second to prostate cancer and breast cancer in male and females, respectively[1]. Furthermore, lung cancer is also the leading cause of cancer-related deaths worldwide, of which non-small cell lung cancer (NSCLC) accounts for approximately 80%[2]. The incidence of lung cancer, especially NSCLC, has been increasing rapidly in the last two decades, due to tobacco-use, air pollution, and other cancer-related issues[3]. Previous studies have demonstrated that targeted therapy is efficient and tremendously improves the progress-free survival (PFS) and overall survival (OS) of lung cancer, especially for NSCLC adenocarcinoma[4-6]. Nevertheless, traditional platinum-based chemotherapies are still the principal treatments for NSCLC patients in the absence of positive biomarkers[7, 8].

Platinum inhibits tumor growth by coupling with DNA and terminating DNA replication[9]. In this way, however, the regular reproduction of normal cells will also be suppressed, and normal and functional cells will be inevitably damaged when attempting to suppress tumors.

Previous studies have indicated that DNA repair systems participate in platinum-based chemotherapy resistance[9-12]. DNA inter- or intra-crosslinks caused by platinum chemotherapy can be removed by several DNA repair pathways, including base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR)[10]. Most DNA damage can be successfully repaired by the above error-free DNA repair pathways. However, when error-free DNA repair systems are stalled or saturated, these lesions can be repaired by trans-

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lesion synthesis (TLS), an error-prone DNA repair system. Error-free lesion bypass switches damaged sites to undamaged DNA strands for synthesis past the DNA lesion, while error-prone lesion bypass tolerates DNA distortions to allow synthesis past the lesion[13]. Instead of cutting the mutation base or nucleotide around the lesions and making another copy of the opposite template, the TLS pathway permits continuity of the replication fork by allowing replication through these lesions[14]. First, one of the translesion synthesis polymerases is recruited to the stalled replication fork for replication over the lesion, which is facilitated by DNA damage-induced PCNA monoubiquitination[15, 16]. Second, following incorporation of a nucleotide opposite the damage site, extension polymerase replaces the TLS polymerase and further extends the patch by approximately 18 nucleotides. In this step, the damaged site escaped detection by 3′-5′ exonuclease proofreading. Third, after extension past the DNA lesion, the extension polymerase is switched back to the high fidelity DNA polymerase for resuming DNA replication. Hence, TLS may be regarded as a double-edged sword because translesion synthesis polymerases have a high tendency to introduce mutations at the sites of lesions in the extension step. These mutations might lead to platinum-chemotherapy resistance[17-19] and side effects.

RAD18 is an integral protein with a RING finger domain. Moreover, *RAD18* has ubiquitin-ligating enzyme (E3) activity[20] and is essential for the ubiquitination of proliferating cell nuclear antigen (PCNA). Monoubiquitinated PCNA activates the TLS and recruits translesion synthesis polymerases to the DNA damaged sites[21]. Monoubiquitination of PCNA increases the affinity of translesion synthesis polymerases at damaged sites due to the presence of ubiquitin-binding domains[22]. Although PCNA polyubiquitination has also been reported in response to DNA-damaged sites, the rate is approximately 20-fold lower than PCNA monoubiquitination[23]. Hence, *RAD18* polymorphism might play a key role in the activation of TLS. A previous study demonstrated that *RAD18* knocked out mouse embryonic stem cells were hypersensitive to DNA-damaging agents[24]. *RAD18* participates in the maintenance of genome stability[25]. Furthermore, it has been discovered that the *RAD18* polymorphism is associated with NSCLC risk[26].

In the present study, we hypothesize that platinum-based chemotherapy can increase the global DNA damage level and TLS would be an efficient rescue pathway for both tumor and other functional cells. We used SNP to explore the contribution of the *RAD18* gene to the side-effect toxicity and prognosis of platinum-based chemotherapy.

Materials and methods

Study population

A total of 1021 patients who were recently histologically diagnosed with advanced NSCLC (aNSCLC) were recruited from Shanghai Chest Hospital between Mar 2005 and Jan 2010, as described in our previous study[27]. Patients who accepted at least two treatment cycles and fulfilled the following criteria were included in the study: (1) 18-80 years old; (2) stage III–IV without radical surgery; (3) no history of malignancy except non-melanoma skin cancer, *in situ* carcinoma of the cervix or "cured" malignant tumor (>5-year disease-free survival); (4) no chemotherapy history; (5) Eastern Cooperative Oncology Group 0–2; (6) normal liver and kidney function; (7) no uncontrolled infectious diseases, serious medical or psychological factors or active congestive heart failure; (8) no previous surgical treatment and (9) no relapse. All patients were unrelated ethnic Han Chinese. All patients consented to participate in the study and to allow their biological samples to be genetically analyzed in accordance with the process approved by the Ethical Committee of the Hospital.

Personal information, including age at diagnosis, gender, smoking status and packs per year, family and personal history of disease, was recorded from patients' self-reports. The clinical index involved in the analysis was gathered from clinical laboratory reports and pathological reports.

The patients' responses to treatment were determined by the WHO criteria, which classifies response into four categories: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Term effect was assessed after two cycles of treatment. The gastrointestinal and hematological toxicity incidence was assessed twice a week during first-line treatment, according to the National Cancer Institute Common Toxicity Criteria.

Chemotherapy regimen

All patients involved in this study accepted a platinum-based chemotherapy regimen combined with other medicine. Most patients accepted one of the following treatment regimens: vinorelbine 25 mg/m^2, d 1 and d 8 every 3 weeks in combination with cisplatin (NP) 75 mg/m^2 or carboplatin AUC 5 (NC), both administered on d 1, every 3 weeks; gemcitabine 1250 mg/m^2, d 1 and d 8 every 3 weeks in combination with cisplatin (GP) 75 mg/m^2 or carboplatin AUC 5 (GC), both administered on d 1, every 3 weeks; Taxol 175 mg/m^2, d 1 every 3 weeks in combination with cisplatin (TP) 75 mg/m^2 or carboplatin AUC 5 (TC), both administered on d 1, every 3 weeks; docetaxel 75 mg/m^2, d 1 every 3 weeks in combination with cisplatin (DP) or carboplatin AUC 5 (DC) 75 mg/m^2, also administered on d 1, every 3 weeks. The other patients accepted different regimens with platinum-based combination therapy and other medicines. All patients maintained treatment for at least two cycles and ended up with serious resistance or side effects.

Specimen preparation

Before the patients began their treatment, 2 mL of peripheral blood was collected in EDTA-anticoagulant tubes. Genomic DNA was extracted from the blood, using the QIAamp DNA MAX Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol.

SNP pick up and genotyping

A total of 10 tag-SNPs were chosen. Genotype data of the RAD18 gene region (including 2 kb upstream) from the CHB

population were downloaded from phase II the HapMap SNP database (http://www.hapmap.org/), and tag-SNPs were selected by Haploview 4.1 (http://www.broadinstitute.org/haploview), using a minor allele frequency (MAF) cut-off of 0.05 and a correlation coefficient (r^2) threshold of 0.8. Because there was a linkage disequilibrium (LD) in the same gene region, we believed tag-SNPs with r^2>0.8 could represent *RAD18* genetic variants (Table 1). To genotype the SNPs, iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (Sequenom, Inc) was used.

Statistical analysis

For data analysis, CR and PR were combined as responders, and SD and PD were grouped as non-responders. Toxicity outcomes were dichotomized by the presence or absence of grade 3 or 4 toxicity during the first-line treatment.

Testing for Hardy–Weinberg equilibrium among patients was performed using observed genotype frequencies and a nonparametric χ^2 test with one degree of freedom. SNPs with a statistical significance or marginal significance were further examined by a stratified analysis in sub-populations, which were grouped according to sex, age, smoking status or treatment regimens. A logistic regression analysis was used to estimate the odds ratio (ORs) and corresponding 95% confidence interval (95% CI) for the associations between the genotypes with the response to treatment or severe side effects. Progression-free survival (PFS) and overall survival (OS) distributions were analyzed using the Kaplan-Meier method and the log-rank test. Multivariable Cox proportional hazards regression was also used to adjust for gender, age at diagnosis, stage, histological type and smoking status. A P value of <0.05 was

Table 1. Characteristic of the patients and genotype distribution of the selected SNPs.

Patient characteristics	*n* (%)
Total No patients	1021
Median age in years	
≤58 years-old	533 (52.2)
>58 years-old	488 (47.8)
Gender	
Male	718 (70.3)
Female	303 (29.7)
Performance status (PS)	
0–1	943 (93.4)
2	67 (6.6)
TNM stage	
IIIA	81 (7.9)
IIIB	249 (24.4)
IV	689 (67.6)
Histologic type	
Adenocarcinoma	651 (66.8)
Squamous cell	228 (23.4)
Adenosquamocarcinoma	13 (1.3)
Others[a]	83 (8.5)
Chemotherapy regimens	
NP	205 (20.1)
NC	36 (3.5)
GP	198 (19.4)
GC	48 (4.7
TP	77 (7.5)
TC	177 (17.3)
DP	53 (5.2)
DC	28 (2.7)
Others[b]	199 (19.5)
rs373572 (*n*=1004)	
A/A	142 (14.1)
A/G	485 (48.3)
G/G	377 (37.5)
rs615967 (*n*=663)	
A/A	109 (16.4)
A/G	339 (51.1)
G/G	215 (32.4)
rs588232 (*n*=663)	
A/A	214 (32.3)
A/T	340 (51.3)
T/T	109 (16.4)
rs586014 (*n*=997)	
A/A	133 (13.3)
A/G	474 (47.5)
G/G	390 (39.1)
rs654448 (*n*=663)	
A/A	257 (38.8)
A/G	313 (47.2)
G/G	93 (14.0)
rs6763823 (*n*=985)	
A/A	66 (6.7)
A/G	349 (35.4)
G/G	570 (57.9)
rs9880051 (*n*=663)	
A/A	27 (4.1)
A/G	226 (34.1)
G/G	410 (61.8)
rs618784 (*n*=663)	
A/A	262 (39.5)
A/G	312 (47.1)
G/G	89 (13.4)
rs686195 (*n*=663)	
A/A	94 (14.2)
A/G	332 (50.1)
G/G	237 (35.7)
rs669906 (*n*=663)	
A/A	94 (14.2)
A/G	332 (50.1)
G/G	237 (35.7)

[a]Other carcinomas include mixed cell, neuroendocrine carcinoma or undifferentiated carcinoma.
[b]Accepted other platinum-based combination therapy with other medicine.

considered statistically significant. All analyses were performed with R 2.10.0. SHEsis[28, 29] was utilized to determine haplotype blocks and the association between haplotypes and clinical outcomes.

Results

Patients Characteristics

A total of 1021 advanced NSCLC patients were enrolled in this study. Baseline characteristics are summarized in Table 1. See the supplementary data for details.

As shown in Table 2, treatment response was evaluated in 966 patients, and 146 (15.1%) were identified as responders, while 820 (84.9%) were non-responders. The incidence of grade 3 or 4 gastrointestinal and homological toxicity is also listed in Table 2.

Table 2. Treatment response and severe toxicity of advanced NSCLC patients.

	n (%)
Response (*n*=966)	
Complete response (CR) or partial response (PR)	146 (15.1)
Progressive disease (PD) or stable disease (SD)	820 (84.9)
Toxicity outcomes	
Any grade 3 or 4 toxicity (*n*=993)	276 (27.8)
Any grade 3 or 4 gastrointestinal toxicity (*n*=972)	63 (6.5)
Any grade 3 or 4 hematologic toxicity (*n*=972)	273 (28.1)
Anemia (*n*=915)	18 (2.0)
Agranulocytosis (*n*=925)	105 (11.4)
Leukocytopenia (*n*=971)	151 (15.6)
Thrombocytopenia (*n*=941)	43 (4.6)

SNP genotyping

Ten SNPs were chosen for genotyping. Table 1 shows the genotype distributions of all SNPs. In the present study, the genotype distributions of all SNPs were consistent with the assumptions of the Hardy-Weinberg equilibrium (P>0.05, Supplementary Table S1). Rs686195 and rs669906 had exactly the same genotype distribution. Hence, rs669906 was omitted in the next analysis. As shown in Figure 1, with a stringent threshold, r^2>0.66, rs686195, rs373572, rs615967 and rs588232 were in high linkage disequilibrium. In addition, rs586014, rs654448 and rs618784, and rs6763823 and rs9880051 were in high linkage disequilibrium.

Association with treatment response

None of the SNPs was significantly correlated with treatment response in a combined cohort. Nevertheless, rs373572 showed a trend toward significance in smokers. Patients carrying the AA genotype of rs373572 were likely to be responders (adjusted P=0.070).

Association with grade 3 or 4 toxicity

The association between *RAD18* polymorphism and side-effect outcomes, including gastrointestinal and hematologic toxicity, were analyzed by logistic regression according to smoking status.

Figure 1. The linkage disequilibrium of *RAD18* polymorphisms in the present study. The parameter of r^2>0.8 was considered as threshold.

As shown in Table 3, we discovered rs586014 was significantly correlated with gastrointestinal toxicity in non-smokers and the combined cohort (adjusted P=0.009, OR 0.52, 95% CI [0.31-0.85] and P=0.003, OR 0.40, 95% CI [0.22-0.75], respectively). In addition, rs654448 and rs618784 were significantly associated with gastrointestinal toxicity in non-smokers (adjusted P=0.018, OR 2.75, 95% CI [1.15-6.25] and P=0.039, OR 1.69, 95% CI [1.03-2.79], respectively), while rs6763823 was significantly associated in smokers (adjusted P=0.022, OR 0.29, 95% CI [0.11-0.90]).

In the present study, we discovered three SNPs that were significantly associated with hematologic toxicity in non-smokers, namely rs586014, rs654448, and rs618784 (Table 4). Because hematologic toxicity consisted of leukocytopenia, anemia, thrombocytopenia or agranulocytosis (Table 2), we discovered rs6763823 and rs9880051 were significantly associated with leukocytopenia in smokers (P<0.01, Table 5). Nevertheless, no association was found in the combined cohort for the same site.

Haplotype analysis

As mentioned above, with a stringent threshold, r^2>0.66, rs686195, rs373572, rs615967 and rs588232 were in high linkage disequilibrium and formed a haplotype block. With a 3% frequency threshold, the haplotypes were AAGA (39.0%), GAGG (3.3%) and GGAG (56.8%) (in the following order: rs373572, rs615967, rs588232 and rs686195). Rs586014, rs654448 and rs618784 formed a haplotype block. With a 3% frequency threshold, the haplotypes were AGG (34.4%) and GAA (61.0%) (in the following order: rs586014, rs654448 and rs618784).

Table 3. Association between RAD18 SNPs and gastrointestinal toxicity in entire population.

	Smoker				Non-smoker				Combined			
RAD18 genotype	Any grade 0–2 gastrointestinal toxicity, *n* (%)	Any grade 3 or 4 gastrointestinal toxicity, *n* (%)	OR (95% CI)[a]	*P*-value	Any grade 0–2 gastrointestinal toxicity, *n* (%)	Any grade 3 or 4 gastrointestinal toxicity, *n* (%)	OR (95% CI)[a]	*P*-value	Any grade 0–2 gastrointestinal toxicity, *n* (%)	Any grade 3 or 4 gastrointestinal toxicity, *n* (%)	OR (95% CI)[a]	*P*-value
rs586014												
A/A	66 (12.7)	6 (23.1)	0.94 (0.52–1.70)	0.823	44 (12.1)	10 (27.0)	0.52 (0.31–0.85)	0.009	110 (12.4)	16 (25.4)	0.67 (0.46–0.98)	0.036
A/G	252 (48.4)	8 (30.8)			170 (46.7)	17 (45.9)			424 (47.7)	25 (39.7)		
G/G	203 (39.0)	12 (46.2)			150 (41.2)	10 (27.0)			355 (39.9)	22 (34.9)		
A/G+G/G	455 (87.3)	20 (76.9)	0.46 (0.19–1.31)	0.112	320 (87.9)	27 (73.0)	0.35 (0.16–0.80)	0.009	779 (87.6)	47 (74.6)	0.40 (0.22–0.75)	0.003
rs654448												
G/G	130 (37.2)	10 (45.5)	0.84 (0.42–1.59)	0.595	98 (41.9)	11 (30.6)	1.76 (1.06–2.93)	0.028	229 (39.2)	21 (36.2)	1.30 (0.88–1.91)	0.187
A/G	171 (49.0)	9 (40.9)			106 (45.3)	15 (41.7)			277 (47.4)	24 (41.4)		
A/A	48 (13.8)	3 (13.6)			30 (12.8)	10 (27.8)			78 (13.4)	13 (22.4)		
A/A+A/G	219 (62.8)	12 (54.5)	1.03 (0.23–3.19)	0.965	136 (58.1)	25 (69.4)	2.75 (1.15–6.25)	0.018	355 (60.8)	37 (63.8)	1.90 (0.94–3.60)	0.059
rs6763823												
A/A	37 (7.2)	5 (19.2)	0.78 (0.44–1.44)	0.400	20 (5.6)	0 (0)	1.87 (1.00–3.78)	0.062	57 (6.5)	5 (7.9)	1.18 (0.78–1.86)	0.449
A/G	168 (32.6)	5 (19.2)			147 (40.9)	12 (32.4)			318 (36.2)	17 (27.0)		
G/G	310 (60.2)	16 (61.5)			192 (53.5)	25 (67.6)			503 (57.3)	41 (65.1)		
A/G+G/G	478 (92.8)	21 (80.8)	0.29 (0.11–0.90)	0.022	339 (94.4)	37 (100)	-	-	821 (93.5)	58 (92.1.)	0.26 (0.77–2.05)	0.351
rs618784												
G/G	130 (37.2)	11 (50.0)	0.76 (0.37–1.46)	0.416	102 (43.6)	11 (30.6)	1.69 (1.03–2.79)	0.039	233 (39.9)	22 (37.9)	1.24 (0.83–1.83)	0.283
A/G	175 (50.1)	8 (36.4)			101 (43.2)	16 (44.4)			276 (47.3)	24 (41.4)		
A/A	44 (12.6)	3 (13.6)			31 (13.2)	9 (25.0)			75 (12.8)	12 (20.7)		
A/A+A/G	219 (62.8)	11 (50.0)	1.13 (0.26–3.53)	0.846	132 (56.4)	25 (69.4)	2.29 (0.93–5.29)	0.058	351 (60.1)	36 (62.1)	1.79 (0.87–3.45)	0.095

[a] Data were calculated by unconditional logistic regression and adjusted for performance status and type of treatment regimen.

Table 4. Association between RAD18 SNPs and hematologic toxicity in non-smokers.

RAD18 genotype	Non-smoker			
	Any grade 0–2 hematologic toxicity, *n* (%)	Any grade 3 or 4 hematologic toxicity, *n* (%)	OR (95% CI)[a]	*P*-value
rs586014				
A/A	33 (11.4)	22 (21.4)	0.68 (0.49–0.94)	0.019
A/G	135 (46.6)	47 (45.6)		
G/G	122 (42.1)	34 (33.0)		
A/G+G/G	257 (88.6)	81 (78.6)	0.48 (0.26–0.88)	0.015
rs654448				
G/G	85 (43.4)	25 (33.8)	1.51 (1.03–2.23)	0.035
A/G	87 (44.4)	32 (43.2)		
A/A	24 (12.2)	17 (23.0)		
A/A+A/G	111 (56.6)	49 (66.2)	2.13 (1.05–4.27)	0.033
rs9880051				
G/G	5 (2.6)	2 (2.7)	1.74 (1.04–2.99)	0.039
A/G	87 (44.4)	21 (28.4)		
A/A	104 (53.1)	51 (68.9)		
A/A+A/G	191 (97.4)	72 (97.3)	1.95 (1.11–3.49)	0.021
rs618784				
G/G	89 (45.4)	26 (35.1)	1.46 (1.00–2.15)	0.049
A/G	82 (41.8)	32 (43.2)		
A/A	25 (12.8)	16 (21.6)		
A/A+A/G	107 (54.6)	48 (64.9)	1.88 (0.92–3.77)	0.079

[a]Data were calculated by unconditional logistic regression and adjusted for performance status and type of treatment regimen.

Table 5. Association between RAD18 SNPs and leukocytopenia toxicity in smokers.

RAD18 genotype	Smoker			
	Any grade 0–2 leukocytopenia toxicity, *n* (%)	Any grade 3 or 4 leukocytopenia toxicity, *n* (%)	OR (95% CI)[a]	*P*-value
rs6763823				
G/G	293 (62.5)	41 (50.6)	0.77 (0.54-1.10)	0.147
A/G	139 (29.6)	34 (42.0)		
A/A	37 (7.9)	6 (7.4)		
A/A+A/G	176 (37.5)	40 (49.4)	0.60 (0.38-0.95)	0.031
rs9880051				
G/G	16 (5.0)	4 (7.1)	0.54 (0.35-0.84)	0.006
A/G	84 (26.2)	26 (46.4)		
A/A	221 (68.8)	26 (46.4)		
A/A+A/G	305 (95.0)	52 (92.9)	0.39 (0.22-0.70)	0.002

[a]Data were calculated by unconditional logistic regression and adjusted for performance status and type of treatment regimen.

Rs6763823 and rs9880051 formed a haplotype block. With a 3% frequency threshold, the haplotypes were AA (20.9%) and GG (76.7%) (in the following order: rs6763823 and rs9880051).

There was no significant association between any haplotype and treatment response in the combined cohort or the subgroups, which were grouped by smoking status (P>0.05).

There was no significant association between any haplotypes and gastrointestinal toxicity in the combined cohort (P>0.05). However, the AGG haplotype of rs586014-rs654448-rs618784 had an increased risk of gastrointestinal toxicity in nonsmokers (P=0.018 and P_{sim}=0.056 after 10 000 times permutation), while GAA showed the opposite effect of AGG (P=0.018 and P_{sim}=0.056 after 10 000 times permutation).

There was no significant association between any haplotypes and hematologic toxicity in the combined cohort (P>0.05). Nevertheless, the AGG haplotype of rs586014-rs654448-

rs618784 had an increased risk of hematologic toxicity in non-smokers (P=0.023 and P_{sim}=0.028 after 10 000 times permutation), while GAA showed the opposite effect of AGG (P=0.023 and P_{sim}=0.028 after 10 000 times permutation).

Association with progression-free survival (PFS) or overall survival (OS)

Utilizing the multivariable Cox proportional hazards model, we analyzed the relationship between *RAD18* polymorphism and PFS or OS. However, none of the SNPs was found to be associated with PFS or OS.

Discussion

In the present study, we investigated the potential association between *RAD18* polymorphisms, treatment responses and the increased toxicity of platinum-based chemotherapy treatment for NSCLC. An allele of rs586014 was significantly associated with an increased risk of grade 3 or 4 gastrointestinal toxicity in non-smokers and in the combined cohort. Moreover, rs654448 and rs618784 were significantly associated with gastrointestinal toxicity in non-smokers, while rs6763823 was significantly associated with gastrointestinal toxicity in smokers. We also discovered three SNPs that were significantly associated with hematologic toxicity in non-smokers. Furthermore, rs6763823 and rs9880051 were significantly associated with leukocytopenia in smokers. We found that the AGG haplotype of rs586014-rs654448-rs618784 had an increased risk of gastrointestinal and hematologic toxicity in nonsmokers.

Although many studies have demonstrated the relationship between *RAD18* and cancer development[26, 30–32], this is the first known study to focus on the relationship between *RAD18* polymorphism and platinum-based chemotherapy response or severe toxicity in NSCLC patients. *RAD18* is a single-strand DNA binding protein that forms a complex with *RAD6* and is essential for carrying out TLS[33]. Compared with other repair pathways, TLS has a high tendency to introduce incorrect bases during translesion DNA synthesis. For example, a previous study demonstrated that *RAD18* might accumulate at blocked replication forks and initiate the signal to recruit Pol ι[34]. Pol ι has a very low accuracy in DNA synthesis and tends to incorporate G or T opposite template T during DNA synthesis[35]. Such low fidelity translesion DNA synthesis might increase spontaneous mutagenesis, therefore resulting in platinum-chemotherapy tolerance and toxicity within normal cells.

Previous studies have demonstrated that rs373572 is associated with the risk of NSCLS and colorectal cancer[26, 31, 36]. Moreover, rs373572 was found to be a unique SNP located in the coding-region of *RAD18* in the present study. *RAD18* has several functional domains, including RING-finger motif[37], zinc-finger motif[38] and E3 ubiquitin-ligase domain[39]. Because rs373572 is located in the E3 ubiquitin-ligase domain, it might affect the E3 ubiquitin-ligase activity of *RAD18* and further influence the ubiquitination of PCNA and activation of TLS.

A previous study found a significantly higher *RAD18* expression level in esophageal carcinomas[40]. Another recent study indicated that *RAD18* overexpression might confer resistance to ionizing radiation in human glioma cells[41]. In the present study, we discovered that rs586014 was remarkably correlated with gastrointestinal and hematologic toxicity in non-smokers (P<0.01). Considering that rs586014 is located within 2 kb upstream of *RAD18*, the potential impact of rs586014 on the expression level of *RAD18* and the side effects of platinum-base chemotherapy may be an interesting study direction.

Rs6763823 and rs9880051 were located in the intron region of *RAD18*. Although the association between these two SNPs and leukocytopenia toxicity in smokers was significant, additional studies are needed to confirm this finding because the number of patients with leukocytopenia in this study was small.

Considering *RAD18* is essential in the activation of PCNA[21], *RAD18* polymorphism might exhibit an epistasis effect in the TLS pathway rather than a direct influence. Additionally, we discovered that the most significant associations with toxicities were observed in non-smokers. We speculated that smoking might result in somatic mutations in translesion polymerases, such as *POLK* and *POLI*, and further conceal the association between *RAD18* and chemotherapy resistance and side effects in smokers. Ultimately, all of the polymorphisms in the present study were non-functional sites; therefore, the mechanism by which they influence *RAD18* remains unknown. Some polymorphisms might be linked with gain of function sites, while the others might be linked with loss of function sites, which might be the reason why different polymorphisms had different effects relative to smoking status. Nevertheless, further study is required to confirm our hypothesis.

Platinum compounds, including cisplatin and carboplatin, are widely utilized in the treatment of tumors[42]. Platinum compounds react with DNA and lead to DNA lesions, including intrastrand crosslinks (Pt-d[CpG], Pt-d [ApG] and Pt-d [GpNgG]), interstrand crosslinks and single nucleotide damage involving guanine[43]. Hence, TLS that allows bypass of the intrastrand crosslinks and constitutes a critical initial step in interstrand crosslink repair[44] is advantageous to tumor cells' survival[45-48]. These crosslink bypasses can result in resistance against platinum-based chemotherapy[47, 49]. TLS plays a similar role in normal cells in the face of damaging lesions and influences the side effects of platinum-based compounds.

In summary, we discovered several *RAD18* SNPs that were associated with platinum-based chemotherapy toxicity. The present study provides reference for the future study of platinum-based chemotherapy response and severe toxicity. However, due to the limitations of the present study, further *in vivo* functional studies are needed to elucidate the biological basis of these findings.

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Author contribution

Prof Bao-hui HAN and Min-hua SHAO designed the research; Tian-qing CHU performed the research; Rong LI analyzed the data; Tian-qing CHU, Rong LI and Jun-yi YE wrote the paper.

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