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Original Article

Single-nucleotide polymorphisms of *TGFβ1* and *ATM* associated with radiation-induced pneumonitis: a prospective cohort study of thoracic cancer patients in China

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Abstract: Background: We examined the effects of the rs1800469 and rs1800470 single nucleotide polymorphisms (SNPs) of the transforming growth factor-β1 (*TGFβ1*) gene and the rs189037 and rs373759 SNPs of the ataxia telangiectasia mutated (*ATM*) gene on the risk of radiation-induced pneumonitis (RP) in patients who underwent radiotherapy for various thoracic malignancies. Methods: We determined the genotype and allele distributions of rs1800469 (C-509T), rs1800470 (C869T), rs189037 (A-111G), and rs373759 (126713 G>A) in 141 Han Chinese patients who underwent definitive thoracic radiotherapy (50 to 77 Gy, 5 days/wk) for lung cancer (small cell or non-small cell tumors, *n* = 97), esophageal squamous cell carcinoma (ESCC, *n* = 27), or mediastinal cancer (*n* = 17). Clinical variables were evaluated using multivariate logistic regression models to calculate the relative risk of RP associated with the clinical variables, and a Pearson correlation analysis was used to evaluate the relationship between the SNP genotypes and alleles and the incidence of RP for the various risk factors. Results: The T alleles of rs1800470 (CT/TT) and rs1800469 (CT/TT) and the G allele of rs189037 (GA/GG) were associated with the risk of ≥ grade-2 RP in the ESCC patients (*P* = 0.0006, *P* = 0.0127, and *P* = 0.0412, respectively), and that the A alleles of rs189037 (AG/AA) and rs373759 (AG/AA) were associated with the risk of ≥ grade-2 RP in the patients with mediastinal cancer (*P* = 0.0063 and *P* = 0.0003, respectively). None of the SNP genotypes were associated with the risk of RP in lung cancer patients. Conclusion: The T alleles of the rs1800470 (CT/TT) and rs1800469 (CT/TT) SNPs of *TGFβ1* and the G allele of the rs189037 (GA/GG) SNP of *ATM* are independent risk factors for RP in Chinese ESCC patients, and the A alleles of the rs189037 (AG/AA) and rs373759 (AG/AA) SNPs of *ATM* are independent risk factors for RP in Chinese patients with mediastinal cancer. These SNPs might represent useful biomarkers for personalizing radiotherapy regimens for Chinese patients with ESCC or mediastinal cancer to reduce the incidence of RP. Large-cohort studies of these SNPs in thoracic cancer patients are warranted.

Keywords: Radiation pneumonitis, single nucleotide polymorphism, transforming growth factor β1, ataxia telangiectasia mutated gene

Introduction

Thoracic malignancies, including lung, esophagus, and mediastinal cancers, are leading causes of cancer-related morbidity and mortality worldwide. The primary treatments for thoracic cancers include surgical resection, chemotherapy, and radiotherapy. In patients who have medically inoperable or locally advanced thoracic cancer, radiotherapy is an essential therapeutic modality. The effectiveness of radiotherapy can be improved by increasing the radiation dose [1-3]. However, toxicity associated with thoracic radiotherapy can cause radiation-induced lung injury (RILI), which is manifested as acute inflammatory disease, known as radiation-induced pneumonitis (RP), or as chronic scarring of lung tissues, known as radiation-induced pulmonary fibrosis (RPF) [4].

Patients with RP present with low-grade fever, dry cough, congestion, painful breathing, dyspnea, and radiological manifestations, such as alveolar infiltrates on chest roentgenogram,

Table 1. Demographic and clinical characteristics of the thoracic cancer patients

Characteristic	*n* (%)
Men	104 (73.8)
Women	37 (26.2)
Age < 54 years	64 (45.4)
Age ≥ 54 years	77 (54.6)
Smoker	77 (54.6)
Nonsmoker	64 (45.4)
Alcohol consumption	52 (36.9)
No alcohol consumption	89 (63.1)
Karnofsky performance status: 80-100	116 (82.3)
Karnofsky performance status: 60-70	25 (17.7)
Lung cancer	97 (68.8)
Esophageal squamous cell carcinoma	27 (19.1)
Mediastinal tumor	17 (12.1)
Surgery	99 (70.2)
No surgery	42 (29.8)
Chronic pneumonic disease	16 (11.3)
No chronic pneumonic disease	125 (88.7)
Chemotherapy	136 (96.5)
No chemotherapy	5 (3.5)
Sequential chemotherapy	118 (83.7)
Concurrent chemotherapy	23 (16.3)
Total radiation dose ≤ 53 Gy	61 (43.3)
Total radiation dose > 53 Gy	80 (56.7)
Mean lung dose < 12 Gy	69 (50.4)
Mean lung dose ≥ 12 Gy	68 (49.6)
V20 ≤ 22%, *n* (%)	70 (50.7)
V20 > 22%, *n* (%)	68 (49.3)

V20, volume of lung exposed to > 20 Gy.

within 1 to 6 months after initiating thoracic radiotherapy [5], whereas RPF develops gradually months to years following radiotherapy [6]. Previous studies have shown that the risk of symptomatic RP (grade-2 to grade-5) ranges from 10% to 45% [5, 7-10], and may be associated with various treatment-related parameters, including the mean lung dose (MLD), the volume of the lung exposed to above-threshold radiation, concurrent chemotherapy, and other clinical factors [7, 11-14]. However, the contribution of these factors to RP is insufficient to explain the level of interpatient variability observed in RP severity [15, 16]. Genetic determinants of radiation toxicity may also contribute to interpatient variation in RP. Treatment strategies that combine conventional dosimetric and clinical determinants of radiation toxicity with the assessment of genetic factors could allow clinicians to maximize the effectiveness of radiotherapy while minimizing the risk of RILI by detecting subclinical RP [17-19].

Previous studies have found that various polymorphisms of the ataxia telangiectasia mutated gene (*ATM*) and the transforming growth factor-β1 gene (*TGFβ1*) were associated with an increased risk of RP [9, 20-24]. A single-nucleotide polymorphism (SNP) of *TGFβ1* has been shown to influence the serum level of TGFβ1 [25], and changes in the plasma level of TGFβ1 during or after radiotherapy have been shown to correlate with the development of symptomatic RP [22, 26-29]. Genetic variants of *TGFβ1* have also been shown to correlate with esophageal radiation toxicity in lung cancer patients [30]. However, the contributions of variation in *ATM* and *TGFβ1* to radiation toxicity may vary based on ethnicity [23]. The majority of studies of the effects of *ATM* and *TGFβ1* SNPs on the development of RP and RPF following thoracic radiotherapy have included patients with lung cancer. Whether variation in *ATM* or *TGFβ1* is associated with RP in patients with other types of thoracic cancer has not been thoroughly investigated.

We hypothesized that a genetic factor that increases the risk of radiation toxicity in lung cancer patients receiving high-dose thoracic radiotherapy might also affect the risk of RP in patients undergoing chest radiotherapy for other types of thoracic cancers. Therefore, we examined the effects of SNPs of *ATM* and *TGFβ1* on the incidence of RP in patients who underwent chest radiotherapy for various thoracic malignancies, including lung cancer, esophageal cancer, and mediastinal cancer. For this analysis, we selected the rs1800470 and rs1800469 SNPs of *TGFβ1*, which have been shown to contribute to the risk of radiation toxicity of the esophagus and lungs in Chinese lung cancer patients [10, 30], and the rs189037 and rs373759 SNPs of *ATM*, which have been shown to contribute to RP in Chinese lung cancer patients [20]. The objective of our study was to perform a broader evaluation of the effects of these SNPs on the risk of RP in Chinese patients than had been previously reported.

Methods

Patients and study design

We evaluated 196 Han Chinese patients with histologically or cytologically confirmed lung

Table 2. Univariate analyses of the risk of ≥ grade-2 radiation-induced pneumonitis (RP)

Risk factor	No RP *n* (%)	RP *n* (%)	*P*-value	OR (95% CI)	β value
Men	59 (29.8)	45 (78.9)			
Women	25 (70.2)	12 (21.1)	0.249	0.63 (0.29-1.39)	0.89
Age < 54 years	43 (51.2)	21 (36.8)			
Age ≥ 54 years	41 (48.8)	36 (63.2)	0.093	1.80 (0.90-3.58)	0.59
Smoker	39 (46.4)	38 (66.7)			
Nonsmoker	45 (53.6)	19 (33.3)	0.018	0.43 (0.22-0.87)	0.84
Alcohol consumption	30 (35.7)	22 (38.6)			
No alcohol consumption	54 (64.3)	35 (61.4)	0.728	1.13 (0.56-2.27)	0.12
KPS: 80-100	70 (83.3)	46 (80.7)			
KPS: 60-70	14 (16.7)	11 (19.3)	0.688	0.84 (0.35-2.00)	-0.18
Lung cancer	49 (58.3)	49 (84.2)	0.003[a]		
ESCC	20 (23.8)	7 (12.3)	0.029[b]	0.36 (0.14-0.92)	-1.03
Mediastinal tumor	15 (17.9)	2 (3.5)	0.004[b]	0.14 (0.03-0.63)	-1.99
Surgical resection	64 (76.2)	35 (61.4)			
No surgery	20 (23.8)	22 (38.6)	0.060	0.50 (0.24-1.03)	-0.69
Chronic pneumonic disease	9 (10.7)	7 (12.3)			
No chronic pneumonic disease	75 (89.3)	50 (87.7)	0.774	1.17 (0.41-3.34)	0.15
Chemotherapy	81 (96.4)	55 (96.5)			
No chemotherapy	3 (3.6)	2 (3.5)	0.678	1.02 (0.17-6.30)	0.02
Sequential chemotherapy	76 (90.5)	42 (73.7)			
Concurrent chemotherapy	8 (9.5)	15 (26.3)	0.008	3.40 (1.33-8.66)	1.22
Total radiation dose ≤ 53 Gy	44 (52.4)	21 (36.8)			
Total radiation dose > 53 Gy	40 (47.6)	36 (63.2)	0.008	2.59 (1.27-5.27)	0.95

OR, odds ratio; CI, confidence interval; KPS, Karnofsky performance status; ESCC, esophageal squamous cell carcinoma; [a]Comparison of all tumor types, [b]Compared with lung cancer cases.

cancer, esophageal cancer, or mediastinal cancer who were treated with definitive radiotherapy at Tongji Hospital Cancer Center (Wuhan, China) between March 2010 and December 2012. Their demographic variables, medical history, and baseline clinical data were recorded at enrollment. Most patients did not undergo pulmonary function tests, but did undergo a comprehensive examination at baseline to identify dyspnea, chronic bronchitis, chronic obstructive pulmonary disease, severe emphysema, asthma, interstitial lung disease, and other respiratory diseases. Patients who had severe a cardiopulmonary disease, a Karnofsky performance status of < 60, an expected survival of < 6 months, or a history of previous thoracic radiotherapy were excluded from our study. Written informed consent was obtained from each patient before participation in our study. Our study protocol was approved by the Ethics Committee of Tongji Medical College.

Blood samples were collected from each patient by venipuncture for the detection of the *TGFβ1* and *ATM* SNPs. Patients requiring surgical tumor resection were treated prior to receiving radiotherapy. Sequential or concurrent chemotherapy was administered as required.

Radiotherapy

Patients were irradiated with 6-MV X-rays from an Elekta Precise or an Elekta Synergy linear accelerator (Elekta AB, Stockholm, Sweden). A total dose ranging from 50 to 77 Gy was administered at 2 Gy per fraction for 5 days per week. Tumor volume included only proximal lymph node involvement. Lung volume was calculated based on both lungs in the exhaled state, excluding the tumor volume. No corrections were made based on tissue heterogeneity. The lung volume receiving ≥ 20 Gy of radiation (V20) and the MLD were minimized based on the size and location of the tumor. Each diagnosis of RP was confirmed by three radiation oncologists, and the grade of RP was evaluated based on the Common Terminology Criteria for Adverse Events, version 4.0. Each patient underwent a weekly follow-up evaluation via telephone or in person until radiotherapy was completed, and monthly follow-up evaluations were performed until ≥ grade-2 RP was observed. Patients who received a total radiation dose of < 50 Gy, and those who died during the follow-up period were not included in our study. The time to ≥ grade-2 RP development was calculated from the start of radiotherapy.

Table 3. Multivariate analyses of the risk of ≥ grade-2 radiation-induced pneumonitis (RP)

Risk factor	No RP *n* (%)	RP *n* (%)	*P*-value	OR (95% CI)	β value
Smoker	39 (46.4)	38 (66.7)			
Nonsmoker	45 (53.6)	19 (33.3)	0.028	0.42 (0.20-0.91)	-0.86
Lung cancer	49 (58.3)	49 (84.2)			
ESCC	20 (23.8)	7 (12.3)	0.067[a]	0.37 (0.13-1.07)	-0.97
Mediastinal tumor	15 (17.9)	2 (3.5)	0.015[a]	0.14 (0.03-0.63)	-1.95
Sequential chemotherapy	76 (90.5)	42 (73.7)			
Concurrent chemotherapy	8 (9.5)	15 (26.3)	0.037	2.97 (1.07-8.26)	1.09
Total radiation dose ≤ 53 Gy	44 (52.4)	21 (36.8)			
Total radiation dose > 53 Gy	40 (47.6)	36 (63.2)	0.027	2.44 (1.11-5.35)	0.89

OR, odds ratio; CI, confidence interval; ESCC, esophageal squamous cell carcinoma; [a]Compared with lung cancer cases.

SNP genotyping

Genomic DNA was isolated from peripheral blood leukocytes by using the QuickGene DNA Whole Blood Kit S (Fuji Film, Tokyo, Japan), according to the manufacturer's instructions, and stored at -80°C. The rs1800470 (C869T) and rs1800469 (C-509T) SNPs of *TGFβ1* (HGVSnames:NC_000019.10:g.41353016G>A and NC_000019.10:g.41354391A>G, respectively) and the rs189037 (A-111G) and rs373759 (126713 G>A) SNPs of *ATM* (HGVS names: NM_002519.2:c.-570C>T and NC_000011.10:g.108349930C>T, respectively) were genotyped using a TaqMan SNP genotyping assay and an ABI Prism 7900 HT Sequence Detection System (Life Technologies, Carlsbad, CA, USA). For each SNP, the call rate was > 98%. To verify the accuracy of the TaqMan genotyping results, 10% of the samples were randomly selected, and genotyped again to confirm concordance ≥ 99%.

Statistical analysis

The statistical analyses were performed using the SPSS, version 16.0, software (IBM, Armonk, NY, USA). A chi-squared analysis was used to compare differences in the distributions of the SNP genotypes. The threshold for significant deviation from Hardy-Weinberg equilibrium (HWE) was set as $P = 0.05$. Logistic regression models were used to calculate the odds ratio (OR) and 95% confidence interval (CI) of the risk of RP. Significant risk factors identified in univariate analyses were subjected to a multivariate analysis with adjustment for the relevant covariates. A Pearson correlation analysis was used to evaluate the relationship between the SNP genotypes and the clinical risk factors identified in the multivariate analysis. All of the *P*-values were two-sided, and the results of comparisons with $P < 0.05$ were considered to represent statistically significant differences.

Results

Demographic and clinical characteristics of the thoracic cancer patients

The patients' demographic and clinical characteristics are summarized in **Table 1**. Of the 196 patients who underwent definitive thoracic radiotherapy, 141 patients completed our study (55 patients lost to follow up), among whom 104 were men and 37 were women. The median age was 63 years (range: 35-83 years). The thoracic cancer cohort consisted of 97 cases (68.8%) of lung cancer, 27 cases (19.1%) of esophageal squamous cell carcinoma (ESCC), and 17 cases (12.1%) of mediastinal cancer. Sixty-five of the lung cancer patients had non-small cell lung carcinoma (NSCLC), and 32 lung cancer patients had small cell lung carcinoma (SCLC). Patients with mediastinal malignancies included 11 thymoma cases, four thymic carcinoma cases, and two mediastinal squamous cell carcinoma cases.

Ninety-nine of the patients (70.2%) underwent surgical tumor resection before radiotherapy, which consisted of radical surgery and cytoreductive surgery for R1 or R2 resection. Platinum- or taxane-based sequential or concurrent chemotherapy was administered to 136 (96.5%) of the patients. The median total radiation dose (Dt) was 56 Gy (range: 50-77 Gy; $n = 141$). The median MLD was 12.7 Gy (range: 5.97-18.6 Gy) for patients who developed ≥ grade-2 RP and 11.9 Gy (range: 1.78-19.8 Gy; $n = 137$; $P = 0.187$) for those who did not. The median V20 was 23% (range: 10%-38%) for patients who developed ≥ grade-2 RP and 22%

Table 4. Polymorphism genotype distributions in the thoracic cancer patients based on ≥ grade-2 radiation-induced pneumonitis (RP)

	TGF-β1: rs1800470 genotype			*TGF-β1*: rs1800469 genotype			*ATM*: rs189037 genotype			*ATM*: rs373759 genotype		
	CC	CT	TT	CC	CT	TT	AA	AG	GG	AA	AG	GG
Lung cancer (*n* = 97)												
No RP	0.184	0.612	0.204	0.224	0.612	0.163	0.265	0.449	0.286	0.122	0.49	0.388
RP	0.104	0.604	0.292	0.333	0.5	0.167	0.229	0.458	0.312	0.146	0.479	0.375
HWE (*P*)		0.02			0.21			0.37			0.75	
Esophageal squamous cell carcinoma (*n* = 27)												
No RP	0.35	0.35	0.3	0.45	3.5	0.2	0.2	0.6	0.2	0.15	0.45	0.4
RP	0	0.571	0.429	0.714	0.143	0.143	0.143	0.429	0.429	0.143	0.429	0.429
HWE (*P*)		0.34			0.08			0.54			0.81	
Mediastinal cancer (*n* = 17)												
No RP	0.267	0.6	0.133	0.067	0.6	0.333	0.267	0.6	0.133	0.267	0.467	0.267
RP	0.5	0	0.5	0.5	0	0.5	0.5	0.5	0	0.5	0.5	0
HWE (*P*)		0.76			0.62			0.38			0.82	

HWE, Hardy-Weinberg equilibrium.

(range: 1.0%-38%; n = 138; P = 0.384) for those who did not. The median follow-up period was 13 months.

Clinical risk factors for RP in the thoracic cancer patients

Of the 141 patients who completed the study, 57 (40.4%) of the patients developed ≥ grade-2 RP, among whom 51 patients developed grade-2 RP and six patients developed grade-3 RP. The median time to RP was 45 days (range: 8-210 days). The univariate analyses showed that nonsmokers had a significantly lower risk of developing ≥ grade-2 RP (OR = 0.43, P = 0.018), compared with patients with a history of smoking (**Table 2**). Patients with ESCC (OR = 0.36, P = 0.029) and those with mediastinal cancer (OR = 0.14, P = 0.004) had a significantly lower risk of ≥ grade-2 RP than lung cancer patients, and patients who underwent concurrent chemotherapy (OR = 3.40, P = 0.008) or were treated with an Dt > 53 Gy (OR = 2.59, P = 0.008) had a significantly higher risk of ≥ grade-2 RP, compared with patients who underwent sequential chemotherapy or were treated with and Dt ≤ 53 Gy (**Table 2**).

The multivariate analyses showed that nonsmokers had a significantly lower risk odeveloping ≥ grade-2 RP (OR = 0.42, P = 0.028), compared with patients with a history of smoking (**Table 3**). Patients with mediastinal cancer (OR = 0.14, P = 0.015) had a significantly lower risk of ≥ grade-2 RP than patients with lung cancer, but the risk of ≥ grade-2 RP among lung cancer patients was not significantly different than that of ESCC patients (P = 0.067; **Table 3**). Patients who underwent concurrent chemotherapy (OR = 2.97, P = 0.037) or were treated with an Dt > 53 Gy (OR = 2.44, P = 0.027) had a significantly higher risk of ≥ grade-2 RP, compared with patients who underwent sequential chemotherapy or were treated with an Dt ≤ 53 Gy (**Table 3**).

Contribution of genetic factors to the risk of RP in thoracic cancer patients

We examined whether the clinical risk factors for RP identified in the multivariate analyses correlated with the genotype distributions and allelic frequencies for rs1800470, rs1800469, rs189037, and rs373759 in thoracic cancer patients (**Table 4**). With the exception of rs1800470 in lung cancer patients (P = 0.02), which has been previously shown to exist in the Chinese population at genotype frequencies in agreement with Hardy-Weinberg equilibrium [31], the genotype distributions of the four SNPs in our thoracic cancer cohort did not depart significantly from the expectations of HWE (P > 0.05).

The logistic regression analyses showed that none of the genotypes of the rs1800470, rs1800469, rs189037, and rs373759 SNPs were significantly associated with ≥ grade-2 RP in lung cancer patients (**Table 5**). However, in ESCC patients, the T alleles of the rs1800470 (CT/TT) and rs1800469 (CT/TT) SNPs of *TGFβ1* and the G allele of the rs189037 (GA/GG) SNP of *ATM* were significantly associated with the risk of ≥ grade-2 RP (P = 0.0006, P = 0.0127, and P = 0.0412, respectively). In addition, the A alleles of the rs189037 (AG/AA) and rs373759 (AG/AA) SNPs of *ATM* were significantly associated with the risk of ≥ grade-2 RP in patients with mediastinal cancers (P = 0.0063 and P = 0.0003, respectively; **Table 5**). No significant correlation was observed between the SNP genotypes and smoking status, concurrent chemotherapy, or Dt > 53 Gy (P > 0.05 for all, data not shown).

Discussion

In our current study, we investigated risk factors for RP in Chinese patients with various types of thoracic cancer. Our analysis of whether SNPs of *TGFβ1* and *ATM* influence the risk of RP found that, although none of the genotypes of the rs1800470 (formerly rs1982073) and rs1800469 SNPs of *TGFβ1* or those of the rs189037 and rs373759 SNPs of *ATM* were associated with RP in lung cancer patients, the T alleles of rs1800470 (CT/TT) and rs1800469 (CT/TT) and the G allele of rs189037 (GA/GG) were independent risk factors for RP in ESCC patients, and the A alleles of rs189037 (AG/AA) and rs373759 (AG/AA) were independent risk factors for RP in patients with mediastinal cancer. The contribution of these SNPs to the risk of RP in ESCC and mediastinal cancer patients has not been previously reported.

The TGFβ1 protein can function as a proinflammatory cytokine. The rs1800469 SNP (C-509T) is located in the promoter region of *TGFβ1*, and differences in the rs1800469 genotype are associated with differences in the plasma level

Table 5. Associations between the polymorphisms and ≥ grade-2 radiation-induced pneumonitis (RP) in thoracic cancer patients

SNP	Lung cancer (*n* = 97)				Esophageal squamous cell carcinoma (*n* = 27)				Mediastinal cancer (*n* = 17)			
	No RP	RP	*P*-value	OR (95% CI)	No RP	RP	*P*-value	OR (95% CI)	No RP	RP	*P*-value	OR (95% CI)
TGF-β1: rs1800470												
TT	10 (20.4)	14 (29.2)	0.403		6 (30.0)	3 (42.9)	0.081		2 (13.3)	1 (50.0)	0.174	
TC	30 (61.2)	29 (60.4)		0.69 (0.27-1.80)	7 (35.0)	4 (57.1)		1.14 (0.18-7.28)	9 (60.0)	0 (0)		NC
CC	9 (18.4)	5 (10.4)		0.40 (0.10-1.55)	7 (35.0)	0 (0)		NC	4 (26.7)	1 (50.0)		0.50 (0.02-12.90)
T	51.0	59.4	0.2349		47.5	71.4	0.0006		43.3	50.0	0.3447	
C	49.0	40.6			52.5	28.6			56.7	50.0		
TGF-β1: rs1800469												
TT	11 (22.4)	16 (33.3)	0.453		9 (45.0)	5 (71.4)	0.444		1 (6.7)	1 (50.0)	0.126	
CT	30 (61.2)	24 (50.0)		0.55 (0.22-1.40)	7 (350)	1 (14.3)		0.26 (0.02-2.73)	9 (60.0)	0 (0)		NC
CC	8 (16.3)	8 (16.7)		0.69 (0.20-2.39)	4 (20.0)	1 (14.3)		0.45 (0.04-5.21)	5 (33.3)	1 (50.0)		0.20 (0.01-0.67)
T	53.1	58.3	0.453		62.5	78.6	0.0127		36.7	50.0	0.0571	
C	46.9	41.7			37.5	21.4			63.3	50.0		
ATM: rs189037												
GG	14 (28.6)	15 (31.2)	0.909		4 (20.0)	3 (42.9)	0.515		2 (13.3)	0 (0)	0.667	
GA	22 (44.9)	22 (45.8)		0.89 (0.37-2.38)	12 (60.0)	3 (42.9)		0.33 (0.05-2.37)	9 (60.0)	1 (50.0)		NC
AA	13 (26.5)	11 (22.9)		0.79 (0.27-2.34)	4 (20.0)	1 (14.3)		0.33 (0.02-4.74)	4 (26.7)	1 (50.0)		NC
G	51.0	54.2	0.6559		50.0	64.3	0.0412		43.3	25.0	0.0063	
A	49.0	45.8			50.0	35.7			56.7	75.0		
ATM: rs373759												
GG	19 (38.8)	18 (37.5)	0.944		8 (40.0)	3 (42.9)	0.991		4 (26.7)	0 (0)	0.527	
GA	24 (49.0)	23 (47.9)		1.01 (0.43-2.40)	9 (45.0)	3 (42.9)		0.89 (0.14-5.72)	7 (46.7)	1 (50.0)		NC
AA	6 (12.2)	7 (14.6)		1.23 (0.35-4.37)	3 (15.0)	1 (14.3)		0.89 (0.06-12.25)	4 (26.7)	1 (50.0)		NC
G	63.3	61.5	0.792		62.5	64.3	0.7932		50.0	25.0	0.0003	
A	36.7	38.5			37.5	35.7			50.0	75.0		

SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; NC, not calculated. Multivariate analyses in this table were adjusted for smoking status, chronic pneumonic disease, total radiation dose and the type of chemotherapy.

of TGFβ1 [25, 26]. The nonsynonymous polymorphism at rs1800470 in *TGFβ1* (C869T) has also been shown to influence the serum level of TGFβ1 [32]. Patients with elevated serum TGFβ1 following radiotherapy have a significantly higher risk of RILI [33, 34], and polymorphisms at rs1800469 and rs1800470 are associated with radiation sensitivity in patients with lung cancer [30, 35].

The ATM protein plays a key role in the repair of radiation-damaged DNA [36, 37]. The rs189037 (A-111G) SNP is located in the core promoter of *ATM*, and the rs373759 (126713 G>A) SNP is located in an intron in the 3' region of the gene [38]. The A alleles of rs189037 (GA/AA) and rs373759 (GA/AA) are associated with a higher level of ATM mRNA expression in the peritumoral tissues of lung cancer patients than that observed in peritumoral tissues with the GG genotype [20]. The rs189037 and rs373759 SNPs are also associated with radiation sensitivity in human fibroblast cell lines [39]. Our results suggest that the T alleles of rs1800469 and rs1800470, and the A alleles of rs189037 and rs373759 contributed to the risk of RP in our ESCC and mediastinal cancer patients by affecting the serum levels of TGFβ1 and ATM, respectively. However, our analysis was primarily descriptive, and we did not measure serum levels of TGFβ1 and ATM in our thoracic cancer patients.

The findings of previous studies of the effects of *TGFβ1* SNPs in lung cancer patients have been inconsistent. Although Kelsey et al. [35] found that the T allele (C/T, T/T) of rs1800469 was a risk factor for RP and the T allele of rs1800470 was not, Yuan et al. [10] reported that the T allele (C/T, T/T) of rs1800470 was a risk factor for RP and the T allele of rs1800469 was not, with both studies being performed using lung cancer cohorts consisting primarily of white patients. Our findings are consistent with those of multiple studies that have failed to demonstrate correlations between rs1800469 and rs1800470 and the risk of RP in both white and Chinese lung cancer patients [23, 40, 41]. Although the findings of Wang and Bi [40] demonstrate that ethnicity contributes to variation in the effects of the *TGFβ1* SNPs on RP in lung cancer patients, inconsistencies between the findings of studies that used lung cancer patients with similar genetic backgrounds, such as those of Kelsey et al. [35] and Yuan et al. [10], suggest that differences in radiotherapy regimens, the assessment of RP, other clinical variables, or environmental factors may also be involved.

Our findings regarding the *ATM* SNPs in our lung cancer group are inconsistent with those of a previous study by Zhang et al. [20] of the effects of rs189037 and rs373759 on the risk of radiation toxicity in Chinese patients with NSCLC or SCLC. We also included both NSCLC and SCLC cases in our thoracic cancer cohort. However, the incidence of ≥ grade-2 RP in our study (40.4%) was substantially higher than that reported by Zhang et al. (17.4%). We did not consider heterogeneity in the target volume or the treatment area for 70.2% (99/141) of our patients who underwent surgical resection before radiotherapy. It is possible that differences in radiotherapy regimens may have contributed to the differences between our findings and those of other studies. However, the rate of RP in our study was similar to that of other studies of RP in both white and Chinese lung cancer patients (40.0%-45.4%) [10, 42].

Certain limitations to our findings should also be considered. Significant deviation from HWE was observed for the rs1800470 SNP of *TGFβ1* in our lung cancer group, which may have contributed to differences between our findings and those of Yuan et al. [10]. In addition, our sample size was relatively small, especially with regard to the number of ESCC and mediastinal cancer patients, limiting the statistical power of our study design. Thus, our findings regarding the association between the SNPs tested and RP should be considered as preliminary. Future studies with larger cohorts of NSCLC, SCLC, ESCC, and mediastinal cancer patients in which the levels of *TGFβ1* and *ATM* in serum or peritumoral tissues are examined are warranted to confirm our findings. Furthermore, our multivariate analyses indicated that smoking, lung cancer, concurrent chemotherapy, and an Dt > 53 Gy were risk factors for RP. Therefore, a lack of statistical power might also have affected our analysis of associations between the various SNPs and the clinical variables.

Conclusion

The results of our current study showed that the T alleles of the rs1800470 (CT/TT) and rs1800469 (CT/TT) SNPs of *TGFβ1* and the G allele of the rs189037 (GA/GG) SNP of *ATM* are independent risk factors for RP in Chinese

ESCC patients, and that the A alleles of rs189037 (AG/AA) and rs373759 (AG/AA) are independent risk factors for RP in Chinese patients with mediastinal cancer. These SNPs may represent useful biomarkers for personalizing radiotherapy regimens for patients with ESCC or mediastinal cancer by identifying those who are at high risk of RP.

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Disclosure of conflict of interest

None.

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