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ORIGINAL ARTICLE

A missense variant of MASP2 is associated with increased risk of radiation pneumonitis in lung cancer patients treated with radiation therapy*

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Abstract

Objective In this study, mannan-binding lectin-associated serine protease 2 (*MASP2*) gene variant was evaluated to assess the risk of radiation pneumonitis (RP) in patients with pulmonary malignancies.

Methods A total of 169 lung cancer patients with radiotherapy were included in our prospective study (NCT02490319) and genotyped using the Sanger sequencing method. Multivariate Cox hazards analysis and multiple testing were applied to estimate the hazard ratio (HR) and 95% confidence intervals (CIs) of all factors possibly associated with RP risk.

Results Patients with mean lung disease ≥ 15 Gy and V_{20} ≥ 24% had higher risk of RP ≥ grade 2 compared with their counterparts (HR = 1.888, 95% CI: 1.186–3.004, *P* = 0.007; HR = 2.126, 95% CI: 1.338–3.378, *P* = 0.001, respectively). Importantly, CC + CA genotype of MASP2: rs12711521 was strongly associated with an increased occurrence of RP ≥ grade 2 (HR = 1.949, 95% CI: 1.278–2.971, *P* = 0.002).

Conclusion *MASP2: rs12711521* was found to be significantly associated with RP ≥ grade 2 in our cohort and may thus be one of the important predictors of severe RP before radiotherapy, if further validated in larger population.

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Currently, lung cancer remains as one of the greatest health threats against humans. According to the latest summary of cancer data, in 2020, there will be approximately 228,820 new lung cancer cases and 135,720 deaths in the USA [1]. As a state-of-the-art therapeutic intervention, radiotherapy (RT), with or without the combination of chemotherapy, acts as an effective imaging tool used to treat this deadly disease. However, due to RT-related complications that cause patients intolerant of the RT dosage, the overall efficacy of RT is suppressed.

Pathologically, radiation pneumonitis (RP) generally causes inflammatory response of the lung tissue after irradiation treatment. Subsequently, fibrotic process occurs after acute inflammation is no longer observed. Because of its devastating effects against normal lung tissue and its prevalence in lung cancer patients receiving RT, RP is considered as one of the most common complications and major dose-limiting toxicity factors. Since the radiation dose and size of the irradiated volume have to be adjusted according to the patients' condition, the occurrence of RP hinders the tumor-controlling effects of RT [2–3]. RP results in poor quality of life or life-threatening symptoms in approximately 15%–40% of all patients who are irradiated for lung cancer [4]. Based on the facts mentioned above, to maximize the therapeutic effects and to minimize the adverse effects of RT, identifying reliable biomarkers for RP occurrence is considered significant. Up until now, several patient- and treatment-related factors [5], including Karnofsky Performance Status (KPS), chronic lung disease [6], smoking status, chemotherapy [7–8], dosimetric parameters,

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and plasma values of tumor growth factor (TGF)-β [9–10], have been demonstrated to be associated with RP risk. Additionally, some genetic variants were recently found to be associated with the occurrence and development of RP [11–16].

Complement system is one of the major components of human innate immunity [17]. Complement system generally promotes body clearance of invading microorganisms or damaged cells. There are several pathways that trigger complement activations, including the lectin pathway. The lectin pathway is initiated through the binding of mannan-binding lectin (MBL) with microorganism carbohydrate structures [18], whereas MBL-associated serine protease (MASP), specifically MASP2, acts as a pathway activator by cleaving into C2 and C4 [19]. As MASP2 possesses a crucial role in human defense mechanism, multiple genetic variants of MASP2 have been demonstrated to be associated with increased susceptibility to infection and sepsis [20]. However, up until now, little is known about the impact of variants of MASP2 on radiation-induced damage and tissue fibrosis. Therefore, to identify the clinically significant single-nucleotide polymorphisms (SNPs) on RP occurrence and severity, in this study, we investigated the association between MASP2 SNP *rs12711521* and RP risk in our cohort.

Materials and methods

Patient population

In this prospective study (NCT02490319), a total of 190 lung cancer patients were included. Patients enrolled received radiation therapy at Tongji Hospital, Huazhong University of Science and Technology (Wuhan, China) between 2009 and 2015. The inclusion criteria were as follows: patients with a radiation dose of at least 45 Gy, patients aged > 18 years, patients with KPS score > 60, and patients with a life expectancy of at least 6 months. Patients with previous thoracic irradiation or severe cardiopulmonary diseases were excluded from our study. Of the 199 patients, 169 [114 with non-small-cell lung cancer (NSCLC) and 55 with small-cell lung cancer (SCLC)] were eventually included for the final genotyping analysis. Samples from 169 patients were initially used to genotype the candidate SNP using the Sanger sequencing method. This study was approved by the Institutional Review Board of Tongji Hospital. Written informed consents were obtained from all patients for the use of their clinical information and for obtaining their blood and DNA samples.

Treatment and follow-up

All patients received RT with 6-MV X-rays from a linear accelerator (Elekta Synergy, Elekta, Sweden). The median total radiation dose was 56 Gy (range, 45 to 66 Gy), with 1.5 to 2 Gy administered per radiation treatment. Intensity-modulated radiation therapy was administered to 46.7% of patients (n = 79). Computed tomography simulation (CT/e, GE, USA) was performed before the RT treatment was planned. The target volumes and critical normal organs were delineated using the three-dimensional planning system (Pinnacle version 9.2). The baseline clinical characteristics and treatment details of the patients are shown in Table 1.

All patients enrolled in this study were examined during and 1 month after RT. Subsequently, the patients were followed up every 3 months for the first year and every 6 months thereafter. At each follow-up visits, all patients were instructed to undergo a chest X-ray or CT, and patients' clinical information, including symptoms, was collected. RP was graded by two radiation oncologists according to the Common Terminology Criteria for Adverse Events version 4.0 as follows: Grade 0, no change; Grade 1, asymptomatic and diagnosed by radiographic findings only; Grade 2, symptomatic, not interfering with daily activities; Grade 3, symptomatic, interfering with daily activities or oxygen required; Grade 4, assisted ventilation required; and Grade 5, fatal.

Genotyping methods

Genomic DNA was extracted using a PureLink Genomic DNA Mini Kit (Invitrogen, K1820-01) from the patients' peripheral blood. Subsequently, the *MASP2: rs12711521* was genotyped by the Sanger sequencing method in the remaining 169 patients. The primer pairs for *MASP2: rs12711521* were F: 5'-GATTCCTCCCTCCCATGCTTC -3' and R: 5'-ATGTACTCCACTCGGCCACT-3'. The polymerase chain reaction products were subsequently subjected to DNA sequencing to detect mutations.

Statistical analyses

The end point for this study was the development of RP ≥ grade 2. The time to the end point was calculated from the start of RT. Patients who did not experience RP ≥ grade 2 within 12 months of RT were censored. The Statistical Package for the Social Sciences (SPSS) version 21.0 statistical software (SPSS, Inc., USA) was used for the statistical analysis. Patients were divided into groups according to their genotypes, and Cox proportional hazards analysis was performed to estimate the hazard ratio (HR) and 95% confidence intervals (CIs) of all factors possibly associated with RP risk. Moreover, multivariate Cox regression analysis was used for the adjustment of covariates. The influences of the genotypes on RP risk were assessed using the Kaplan-Meier analysis and compared using the log-rank tests.

Table 1 Patient characteristics (n = 169)

Characteristics	No. of patients	%
Sex		
Male	125	74.0
Female	44	26.0
Age (years)		
Median	58	
Range	28–78	
Histology		
SCLC	55	32.5
NSCLC	114	67.5
Stage		
I–II	24	10.2
III–IV	145	85.8
KPS		
80–100	123	72.6
< 80	46	27.4
Smoking		
Smoker	106	62.0
Non-smoker	63	38.0
Chemotherapy		
Yes	160	94.7
No	9	5.3
CRT		
Yes	44	26.0
No	125	74.0
Surgery		
Yes	86	50.9
No	83	49.1
IMRT		
Yes	79	46.7
No	90	53.3
Radiation dose (cGy)		
Median	5600	
Range	4500–6600	
MLD (cGy)		
Median	1368	
Range	178–2017	
V_{20}		
Median	24.82	
Range	0–42.00	
COPD		
Yes	19	11.2
No	150	88.8

CRT, concurrent chemoradiation; IMRT, intensity-modulated radiation therapy; V20, volume of normal lung receiving 20 Gy or more radiation; COPD, chronic obstructive pulmonary disease

Results

Patient characteristics and RP

A total of 169 (125, men; 44, women) patients were included in this study. Their characteristics were listed in Table 1. The median age of the patients was 58 years (range, 28–78 years); moreover, 114 and 55 patients had NSCLC and SCLC, respectively. In the study cohort, 85.5% of patients had stage III–IV disease, 50.9% underwent surgery before RT, almost all patients (94.7%) received induction chemotherapy followed by RT, and 26.0% received concurrent chemoradiation. The median radiation dose was 56 Gy (range, 45–66 Gy), the median mean lung disease (MLD) was 13.68 Gy (range, 1.78–20.17 Gy), and the median V_{20} was 24% (range, 0%–42.00%).

Within 12 months of RT, 99 patients (58.6%) had RP ≥ grade 2. The associations between patient-, tumor- and therapy-related characteristics and RP ≥ grade 2 were listed in Table 2. The univariate and multivariate analyses by Cox regression model revealed that MLD and V_{20} were significantly associated with RP ≥ grade 2. Patients with older age, MLD ≥ 15 Gy, and V_{20} ≥ 24% had higher risk of RP ≥ grade 2 compared with their counterparts (H1.888, 95% CI: 1.186–3.004, P = 0.007; HR = 2.126, 95% CI: 1.338–3.378, P = 0.001, respectively) (Table 2), which were consistent with the results of other publications.

MASP2 single-nucleotide polymorphisms and RP

MASP2: rs12711521 was found to be significantly associated with the occurrence of RP ≥ grade 2 (Table 3). Fig. 1 presented a plot of the RP-free survival percentage for RP ≥ grade 2 for each genotype of *MASP2: rs12711521* determined using the Kaplan-Meier method. Patients with the CC + CA genotype of *MASP2: rs12711521* had significantly higher risks of RP ≥ grade 2 than patients with AA genotype (P = 0.003). Furthermore, multiple Cox proportional hazards analyses with adjustments for all of the characteristics listed in Table 1 revealed that the

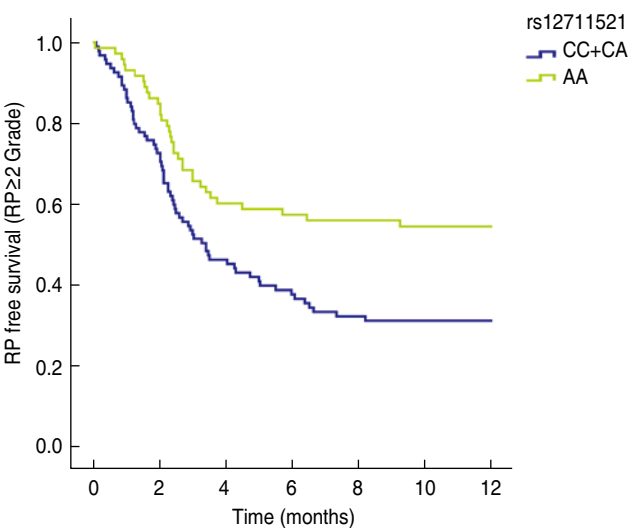

Fig. 1 Kaplan-Meier estimates RP-free survival (RP ≥ grade 2) for each genotype. Patients with CC genotype of *MASP2: rs12711521* had significantly higher risks of RP ≥ grade 2 (P < 0.0001)

Table 2 Association between patient-, tumor-, and therapy-related characteristics and grade ≥ 2 radiation pneumonitis (*n* = 169)

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Sex						
Male	1			1		
Female	1.216	0.773–1.915	0.398	1.379	0.758–2.511	0.292
Age (years)						
< 58	1			1		
≥ 58	1.413	0.951–2.098	0.087	1.541	0.997–2.393	0.052
Histology						
SCLC	1			1		
NSCLC	1.195	0.791–1.804	0.398	1.251	0.727–2.153	0.418
Stage						
I–II	1	1		1		
III–IV	1.100	0.601–2.013	0.758	1.132	0.593–2.163	0.707
KPS						
80–100	1			1		
< 80	1.341	0.877–2.052	0.176	1.566	0.993–2.470	0.054
Smoking						
Smoker	1			1		
Non-smoker	0.926	0.619–1.386	0.708	0.964	0.337–2.192	0.435
Surgery						
Yes	1			1		
No	1.014	0.684–1.504	0.945	0.690	0.390–1.223	0.204
Chemotherapy						
Yes	1	1		1		
No	0.500	0.203–1.233	0.132	0.473	0.189-1.187	0.111
CRT						
Yes	1			1		
No	0.843	0.529–1.344	0.472	0.956	0.575–1.588	0.861
IMRT						
Yes	1			1		
No	1.077	0.726–1.598	0.712	1.098	0.710–1.699	0.675
Radiation dose (cGy)						
< 5600	1			1		
≥ 5600	1.083	0.729–1.610	0.692	1.139	0.633–2.535	0.737
MLD (cGy)						
< 1500	1			1		
≥ 1500	1.510	1.093–2.235	0.045	1.888	1.186–3.004	0.007
V_{20} (%)						
< 24	1			1		
≥ 24	1.730	1.138–2.631	0.010	2.126	1.338–3.378	0.001
COPD						
Yes	1			1		
No	0.639	0.246–1.661	0.358	0.780	0.339–1.998	0.472

Multivariate analyses were adjusted for all of the factors in this table.*Either MLD or V_{20} was used in the multivariate analyses, but not both

CC + CA genotype of *MASP2: rs12711521* was strongly associated with an increased occurrence of RP ≥ grade 2 (HR = 1.949, 95% CI: 1.278–2.971, *P* = 0.002; Table 3).

MASP2: rs12711521 and dosimetric factors

Patients were divided into four groups based on the dosimetric factors, V_{20} or MLD and *MASP2: rs12711521* genotypes, to evaluate the impact of the *MASP2: rs12711521* genotypes on RP in different dosimetric groups. Patients with CC + CA genotype of *MASP2: rs12711521* and MLD ≥ 15 Gy or V_{20} ≥ 24% had higher risk of RP grade ≥ 2 compared with the other groups (*P* = 0.003 and *P* = 0.002, respectively; Fig. 2a and 2b). Interestingly, patients with *MASP2: rs12711521* AA genotype and

Table 3 Association between genotypes and grade ≥ 2 RP

Polymorphism and Genotype	No.of event	No.of total	Univariate analysis			Multivariate analysis		
			HR	95% CI	*P*	HR	95% CI	*P*
MASP2: rs12711521								
AA	33	73	1			1		
CC+CA	65	95	1.856	1.220–2.824	0.004	1.949	1.278–2.971	0.002

Multiple analyses in this table were adjusted for MLD listed in Table 1. HR, hazard ratio; CI, confidence interval

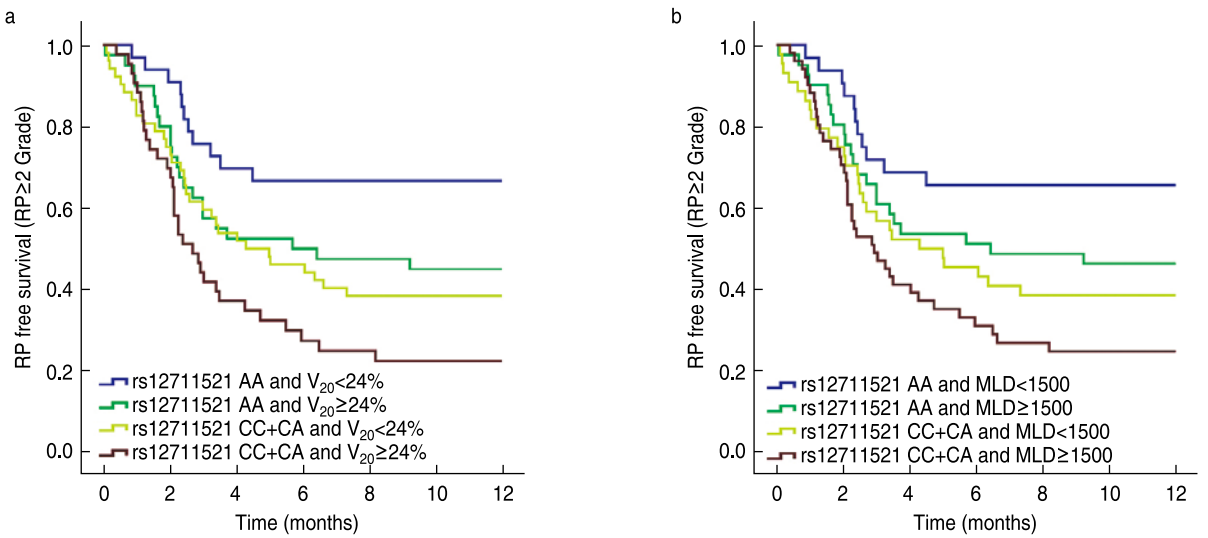

Fig. 2 Kaplan-Meier estimates effect of genotype in *MASP2: rs12711521* and dosimetric parameters on RP-free survival (RP ≥ grade 2). (a) *MASP2: rs12711521* and MLD; (b) *MASP2: rs12711521* and V_{20}

MLD ≥ 15 Gy or V_{20} ≥ 24% had similar incidence of RP ≥ grade 2 with patients with CC/CA genotype who received MLD less than 15 Gy or V_{20} less than 24%, suggesting the dominant and independent role of rs12711521 genotypes in severe RP.

Discussion

In this study, *MASP2: rs12711521* was found for the first time to be significantly associated with the occurrence of RP ≥ grade 2. Patients with the CC + CA genotype of *MASP2: rs12711521* had a significantly increased risk of RP after RT for lung cancer. We also discovered that the association between *MASP2: rs12711521* and RP grade≥ 2 was independent of MLD and V_{20}.

In this study, the incidence rate of RP ≥ grade 2 was 58.6%, which was similar to the incidence rates reported in previous studies. Due to the prospective nature of our study, the incidence rate of RP was relatively higher than the incidence rates in some retrospective studies. We also confirmed that age, MLD, and V_{20} were closely associated with RP risk. In our cohort, patients with MLD ≥ 15 Gy and V_{20} ≥ 24% had a greater risk of developing RP grade ≥ 2 than their counterparts, which verified the associations between the radiation dosimetric-related factors and the occurrence of RP.

Up until now, the exact role of MASP2 in tissue inflammation and fibrosis is largely unknown. However, since MASP2 functions as a critical molecule in the complement pathway activation, investigating the influences of complement pathway on pulmonary fibrosis was considered significant. A study utilizing complement C5-deficient bleomycin-induced pulmonary fibrosis mice model indicated that C5-deficient mice exhibited increased inflammatory response during the acute phase of bleomycin treatment. However, in chronic phase of bleomycin injury, C5-deficient mice showed lower tissue fibrosis compared with the C5-sufficient group. Further study indicated that such pro-fibrotic effects of C5 were possibly associated with the expression of TGF-β1 and matrix metalloproteinase-3 [21]. Additionally, another genome-wide and linkage region-specific association study utilizing bleomycin treatment mice model indicated that MASP2 E384Q and V172I polymorphisms were significantly associated with bleomycin-induced lung fibrosis [22]. Our study presented additional evidence of MASP2 participation in radiation-induced lung fibrosis.

Moreover, as a missense variant, with *rs12711521* being located in the coding region of MASP2, *in silico* prediction using PolyPhen (http://genetics.bwh.harvard.edu/pph2/) demonstrated possibly damaging impact (0.51), which indicated that *rs12711521* might be associated with MASP2 normal protein structure or expression value. Detailed molecular and biological effects of *rs12711521* require further functional analysis.

Moreover, we demonstrated for the first time the prevalence and clinical value of *MASP2: rs12711521* on RP in independent Chinese Han cohort, and based on the result of our study, *MASP2: rs12711521* may be one of the important predictors of severe RP before RT in addition to radiation dosimetric factors. Patients with RP susceptibility genotypes will significantly benefit from the early prediction and prevention of RP by genotyping before the initiation of RT. Furthermore, this study is considered beneficial in selecting patients without RP susceptibility genotypes and increasing their radiation dose appropriately for a better tumor control. Specifically for patients with favorable genotypes, increased MLD and V_{20} will not increase their incidence of severe RP, which could assist the oncologist in individually adjusting patients' radiation dose. On the contrary, our results still require further validation in expanded cohorts from different races since the substantial ethnic variation exists in SNP frequencies.

Furthermore, our findings suggest the possible role of MASP2 in the pathogenesis of RP. According to the latest report, MASP2 monoclonal antibody (narsoplimab, OMS721) has been developed, and its recent clinical trial demonstrated promising result in the treatment of thrombotic microangiopathy in patients undergoing hematopoietic stem cell transplantation [23]. If MASP2 biological function was confirmed in RP, targeted inhibition of MASP2 via narsoplimab might provide novel therapeutic value in future RP treatment.

In summary, this is the first study to present the associations between RP risk and MASP2: rs12711521 and thus indicated that in addition to radiation dosimetric factors, MASP2 SNP can be used as a useful predictive biomarker of RP risk before RT. Thus, patients will significantly benefit from the early prediction and prevention of RP by genotyping before the initiation of RT. Moreover, this study will benefit lung cancer patients receiving RT since appropriately tailored radiation dose might result in better control of their diseases and lower occurrence and severity of RP.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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