Tag SNPs in complement receptor-1 contribute to the susceptibility to non-small cell lung cancer

Xinfeng Yu1,2, Juan Rao2, Jia Lin2, Zhi Zhang3, Lei Cao2 and Xuemei Zhang2*

Abstract
Background: Complement receptor 1 (CR1), the receptor for C3b/C4b complement peptides, plays a crucial role in carcinogenesis. However, the association of genetic variants of CR1 with susceptibility to lung cancer remains unexplored.

Methods: This case-control study included 470 non-small cell lung cancer (NSCLC) patients and 470 cancer-free controls. Based on the Chinese population data from HapMap database, we used Haploview 4.2 program to select candidate tag SNPs. Odds ratios (ORs) and 95% confidence intervals (CIs) were computed by logistic regression to evaluate the association of each tag SNP with NSCLC.

Results: Multivariate regression analysis indicated that the rs7525160 CC genotype was associated with an increased risk of developing NSCLC (OR = 1.52, 95% CI = 1.02-2.28; P = 0.028) compared with the GG genotype. When stratified by smoking status, the risk of NSCLC was associated with the rs7525160 C allele carriers in smokers with OR (95% CI) of 1.72 (1.15-2.79), but not in non-smokers with OR (95% CI) of 1.15 (0.81-1.65). When the interaction between smoking status and rs7525160 G > C variant was analyzed with cumulative smoking dose (pack-year). Similarly, GC or CC genotype carriers have increased risk of NSCLC among heavy smokers (pack-year ≥ 25) with OR (95% CI) of 2.01 (1.26-3.20), but not among light smokers (pack-year <25) with OR (95% CI) of 1.32 (0.81-2.16).

Conclusion: CR1 rs7525160 G > C polymorphism was associated with an increased risk of developing NSCLC in Chinese population. The association displays a manner of gene-environmental interaction between CR1 rs7525160 tagSNP and smoking status.

Keywords: CR1, Polymorphism, Tag SNPs, Lung cancer

Background
The complement system plays a critical role in the process of carcinogenesis. Despite of significant research, controversial viewpoints remain on the exact relationship of complement system with cancer. Classically, the complement system fights against cancer by exerting the effects of immunosurveillance in the immunologic microenvironment of tumors [1]. Recently it was found that complement may contribute to tumor growth by a wide variety of mechanisms including dysregulation of mitogenic signaling pathways, sustained cellular proliferation, angiogenesis, insensitivity to apoptosis, invasion and migration, and escape from complement cytotoxicity [2]. This suggested complement, just like a double-edged sword, plays a dual role in carcinogenesis. In particular, component C3 and its receptors have been demonstrated to be a key link between innate and adaptive immunity [3].

Complement receptor type 1 (CR1, CD35) is a multifunctional polymorphic glycoprotein which binds to C3b fragment of C3 and to C4b with lower affinity [4,5]. CR1 belongs to the regulators of complement activation (RCA) family of proteins and is expressed in a wide spectrum of cells and involved in T-cell and B-cell mediated immune regulation [6,7]. CR1 also modulates the complement cascade activation by preventing formation of classical and alternative pathway convertases and by acting as a cofactor for factor I mediated inactivation of C3b and C4b [8,9]. It has been demonstrated that chronic inflammation can predispose to cancer development and spread [10], as a fundamental component of innate immunity, the complement cascade consists of potential proinflammatory...
molecules especially C3 and C5. Moreover, complement activation and abnormal expression in tumor tissues has been demonstrated [11]. Considering the important role of CR1 in complement activation, innate immunity and chronic inflammation, CR1 has emerged as a molecule of immense interest in gaining insight into the susceptibility to cancer.

CR1 gene is located on the Chromosome 1 at the locus 1q32 [12]. Various polymorphisms have been studied including the intronic and exonic density polymorphism for their ability to alter the density of erythrocyte CR1 on the cell membranes [13-15]. There are also the molecular weight variants due to insertion-deletion polymorphisms [16]. Up to now, there have been very few studies on the association of genetic variants of CR1 with susceptibility to autoimmune and inflammatory diseases. It has been proposed that genetic variant at CR1 gene (rs6656401) might influence the susceptibility to late-onset Alzheimer’s disease [17]. CR1 expression in Peripheral Blood Mononuclear Cells (PBMCs) may be a new biomarker for prognosis of nasopharyngeal carcinoma and a potential therapeutic target [18]. Recently, it has been indicated that CR1 A3650G (H1s1208Arg) polymorphism plays a critical role in conferring genetic susceptibility to gallbladder cancer in north Indian population [19]. However, the association of genetic variants of CR1 with risk of lung cancer remains unexplored.

Worldwide, lung cancer is the most common cancer in terms of both incidence and mortality [20]. NSCLC is the most common subtype of lung cancer and less aggressive and metastatic than SCLC. Although cigarette smoking is the predominant risk factor for lung cancer, inherited genetic characteristics are presumed to account in part for this interindividual variation in lung cancer susceptibility. Recently, several genome-wide association studies have demonstrated the common genetic variations associated with susceptibility to lung cancer [21-24]. Given the involvement of the complement system in coordinating innate immunity and inflammatory response [25], further examination of the potential association between genetic variation of CR1 genes and lung cancer is warranted.

In the current study, we conducted a case-control study to investigate the association of tag SNPs in CR1 gene with the risk of NSCLC and effect of the interaction of gene-environment on the risk of NSCLC.

**Results**

**Subject characteristics**

The frequency distributions of select characteristics in cases and control subjects were shown in Table 1. The mean age (±SD) was 59.6 ± 10.5 years for the cancer patients and 57.2 ± 13.3 years for the controls. No significant difference was found in the mean age between cases and controls (\(P = 0.470\)). There was no significant difference in proportion of sex and smoking status between cases and controls (\(P = 0.832\) and \(P = 0.321\), respectively). However, there was significant difference between cases and controls when compared by pack-year smoked (\(P = 0.001\)). The heavy smokers (\(\geq 25\) pack-year) accounted for 61.5% in cases but only 45.5% in controls, which suggested that cigarette smoking was a prominent contributor to the risk of lung cancer. Of the 470 case patients, 178 (37.9%) were diagnosed as adenocarcinoma, 238 (50.6%) as squamous cell carcinoma, and 100 (%) as other types, including large cell carcinoma (\(n = 49\)) and mixed cell carcinoma (\(n = 5\)).

**Association of CR1 tag SNP with NSCLC risk**

Total 13 selected tag SNPs of CR1 in HapMap database among Chinese population were analyzed. Except for rs9429782 polymorphism, the genotype distributions of other SNPs in controls were consistent to Hardy-Weinberg equilibrium. Therefore, we excluded the rs9429782 from further analysis. In order to screen the genetic variants that confer the susceptibility to lung cancer, 12 candidate tag5SNPs were genotyped in a case-control study consisting of 470 lung cancer patients and 470 cancer-free controls as shown in Table 2. Importantly, genotype frequency of one intronic SNP (rs7525160 G > C) in cases was found to be significantly different from those of controls (\(\chi^2 = 6.339, P = 0.042\)). Further multivariate regression model with adjustment for age, gender, and smoking status was used to assess the association between rs7525160 G > C polymorphism

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**Table 1 Distributions of select characteristics in cases and control subjects**

| Variables        | Cases (n = 470) | Controls (n = 470) | \(P^*\) |
|------------------|----------------|-------------------|---------|
| Sex              |                |                   |         |
| Male             | 324            | 328               | 0.832   |
| Female           | 146            | 142               |         |
| Age              |                |                   | 0.470   |
| <50              | 84             | 96                |         |
| 50-59            | 177            | 187               |         |
| 60-69            | 129            | 111               |         |
| ≥70              | 80             | 76                |         |
| Smoking status   |                |                   | 0.321   |
| Non-smoker       | 265            | 281               |         |
| Smoker           | 205            | 189               |         |
| Pack-year smoked |                |                   | 0.001   |
| <25              | 75             | 96                |         |
| ≥25              | 130            | 93                |         |

* Two-sided \(\chi^2\) test.
and the risk of NSCLC. The results indicated that the rs7525160 CC genotype was associated with an increased risk of developing NSCLC with OR (95% CI) of 1.52 (1.02-2.28) compared with the GG genotype. Other tagSNPs of CR1 were not significantly associated with the risk of NSCLC in our study population (P > 0.05).

Generalized Multifactor Dimensionality Reduction (GMDR) was used to evaluate gene-gene interaction. The summary of gene-gene interaction models is listed in Table 3. The SNP rs7525160 in CR1 had the highest testing balanced accuracy among 12 SNPs. The three-way interaction model among rs4844600, rs10494885 and rs7525160 showed high testing balance accuracy and cross validation consistency, but the testing balanced accuracy was lower than the two-way gene-gene interaction in NSCLC. For each model, the interaction was not significant (P > 0.05).

Interaction of CR1 SNP with smoking
Cigarette smoking is a well-known risk for lung cancer, so stratification by smoking status was performed to investigate the association of rs7525160 G>C variant with the risk of NSCLC. The results indicated that the rs7525160 CC genotype was associated with an increased risk of developing NSCLC with OR (95% CI) of 1.52 (1.02-2.28) compared with the GG genotype. Other tagSNPs of CR1 were not significantly associated with the risk of NSCLC in our study population (P > 0.05).

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with OR (95% CI) of 1.32 (0.81-2.16). The P value for heterogeneity of the stratification analysis by smoking status is 0.015. However, the P value for interaction between rs7525160 polymorphism and smoking is 0.172 and the power for the interaction is 0.49.

**Discussion**

The chronic airway inflammation and dysfunctional immune system might promote pulmonary carcinogenesis. Implicated in the immune and inflammatory responses, the complement cascade plays a pivotal role in the development of cancer. Thus, it is likely that the genetic variants of CR1 in the complement system confer the susceptibility to lung cancer. In this study, we have for the first time demonstrated that one intronic SNP (rs7525160 G > C) out of 13 tag SNPs of CR1 was associated with the risk of NSCLC in Chinese population. Notably, the rs7525160 CC genotype was associated with an increased risk of developing NSCLC (OR = 1.52, 95% CI = 1.02-2.28; \( P = 0.028 \)) compared with the GG genotype. MDR analysis also showed that there was no gene-gene interaction among 12 tag SNPs in CR1 gene. Moreover, the risk of NSCLC was associated with the rs7525160 C allele carriers in smokers with OR (95% CI) of 1.72 (1.15-2.59), but not in non-smokers with OR (95% CI) of 1.15 (0.81-1.65), indicating this SNP is a smoking-modifying risk factor for susceptibility to NSCLC. To the best of our knowledge, this study shed new insight into the interplay of genetic variation of CR1 with lung cancer risk. More importantly, it highlights the potential gene-environmental interaction influences the susceptibility to lung cancer.

The complement system has been proposed to get involved in innate immunity with the ability to “complement” antibody-mediated elimination of immune complex and foreign pathogens [26]. Upon complement activation, the biologically active peptides C5a and C3a elicit a lot of pro-inflammatory effects and could be closely associated with tumorigenesis [27]. Complement proteins play a dual role in the tumor microenvironment. On one hand, they exert a defensive effect against tumor through complement or antibody-dependent cytotoxicity [1,28]. On the other hand, they may escape from immunosurveillance and facilitate carcinogenesis [2]. Specifically, a number of experimental evidence has suggested an association between complement activation and tumor growth [29,30], which provides a strong biologically link between the abnormal expression and activity of complement cascade and carcinogenesis.

Till now, a few studies have been carried out to demonstrate the association of genetic variants in complement proteins with susceptibility to cancer. A significant association of CR2 SNP (rs3813946) with the development of nasopharyngeal carcinoma was indicated in Cantonese population [31], and the genetic variations of complement system genes C5 and C9 plays a potential role in susceptibility to non-Hodgkin lymphoma (NHL) [32]. Recently, it has been shown that complement factor H Y402H polymorphism interact with cigarette smoking to confer the susceptibility to lung cancer [33]. Furthermore, it has been indicated that CR1 A3650G (His1208Arg) polymorphism plays a critical role in conferring genetic susceptibility to gallbladder cancer in north Indian population [19]. However, whether the genetic variants of CR1 are related to the risk of lung cancer remains unknown. In this case-control study, we found an intronic SNP (rs7525160 G > C) with CC genotype was significantly associated with an increased risk of NSCLC. Consistently, our results were in accordance with the study that genetic polymorphisms in innate immunity genes may play a role in the carcinogenesis of lung cancer [34]. It is likely that some genetic variations in strong link disequilibrium with this intronic SNP (rs7525160 G > C) are functional, which provides a new insight into the hallmarks in susceptibility to lung cancer and further

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### Table 3 Summary of MDR gene-gene interaction results

| Models | Training bal. acc. (%) | Testing bal. acc. (%) | P value | Cross-validation consistency |
|--------|------------------------|-----------------------|---------|-----------------------------|
| rs7525160 | 54.03 | 50.53 | 0.828 | 7/10 |
| rs4844600, rs10494885 | 55.45 | 49.32 | 0.989 | 3/10 |
| rs4844600, rs10494885, rs7525160 | 57.60 | 48.48 | 0.623 | 6/10 |

### Table 4 Risk of CR1 genotypes with NSCLC by smoking status

| Smoking status | GG\(^1\) & OR (95% CI)\(^5\) & P value & CG + CC\(^2\) & OR (95% CI)\(^5\) & P value |
|----------------|--------|------------------|---------|----------------|------------------|---------|
| Non-smoker     | 84/99  | 1.00 (reference) |         | 181/182        | 1.15 (0.81-1.65) | 0.440   |
| Smoker         | 55/77  | 0.86 (0.54-1.38) | 0.528   | 150/112        | 1.72 (1.15-2.59) | 0.009   |
| <25 pack-years | 19/41  | 0.59 (0.31-1.10) | 0.099   | 56/55          | 1.32 (0.81-2.61) | 0.266   |
| ≥25 pack-years | 36/36  | 1.18 (0.67-2.08) | 0.562   | 94/57          | 2.01 (1.26-3.20) | 0.003   |

\(^1\)Number of cases/number of controls.
\(^5\)Data were calculated by logistic regression and adjusted for age and gender.
functional experiments are warranted to address the proposal.

Functionally, human CR1 exists on the surface of almost all peripheral blood cells and plays a key role in immune complex clearance and complement inhibition at the cell surface by binding to activated products C3b and C4b [4,35]. CR1 also possesses cofactor activity for the serum protease factor I and is thus involved in the generation of further fragments of C3/4b with the activation of complement cascade and the cellular immune response [4]. In our study, the association of CR1 polymorphism with lung cancer is biologically plausible in that the intronic polymorphism could affect the density of CR1 molecules on the cell surface, thereby contributing to autoimmune disorders and neoplasm.

Tobacco smoking is an established risk factor for susceptibility to lung cancer. However, not all people who suffer from lung cancer are smokers. Lung cancer in non-smokers can be induced by second hand smoke, air pollutants and diesel exhaust [36-39]. Our present data showed significant difference of pack-year smoked, but not smoking status between NSCLC cases and controls, which suggested the important role of other environmental factors in the development of NSCLC. Tobacco could induce chronic and sustained inflammation in lung microenvironment, contributing to pulmonary carcinogenesis in smokers [40]. Support also comes from the epidemiologic data regarding inflammation and lung cancer [41]. CR1, an important molecule implicated in immunity and inflammation, could protect the host from invasion of exogenous chemicals derived from cigarette smoking. Genetic variant of CR1 could alter gene function and result in deregulation of the inflammatory and immune responses, thereby, modulating the susceptibility to lung cancer. More importantly, we observed a potential interaction of this SNP (rs7525160 G > C) with smoking status, suggesting the gene-environmental interaction plays a prominent role in the susceptibility to lung cancer. Our present study has its limitation. Our patients may not be representative of total NSCLC patients at large because they were recruited from only one hospital. In addition, due to the relatively small sample size, further case-control studies are still needed to replicate and extend our findings.

Conclusion

We conducted a case-control study in Chinese subjects and found an intronic SNP (rs7525160 G > C) of CR1 was significantly associated with lung cancer risk. To the best of our knowledge, this study provides the first evidence that genetic variant of CR1 (rs7525160 G > C) was a smoking-modifying contributor to the development of lung cancer.

Methods

Study subjects

This case-control study consisted of 470 patients with histopathologically confirmed NSCLC and 470 cancer-free controls. All subjects were genetic unrelated ethnic Han Chinese. Patients were recruited between January 2008 and December 2012 at Tangshan Gongren Hospital (Tangshan, China). There were no age, gender or stage restrictions, however, patients with previous malignancy or metastasized cancer from other organs were excluded. The response rate for patients was 94%. The controls were randomly selected from a pool of a cancer-free population from a nutritional survey conducted in the same region. The selection criteria for control subjects included: i) no individual history of cancer; ii) frequency

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**Table 5 Primers used in this study**

| SNP ID   | Alleles | 1st-PCR primer sequences | 2nd-PCR primer sequences | UEP sequences                      |
|----------|---------|--------------------------|--------------------------|-----------------------------------|
| rs7525160 | G/C     | ACGTTGGATGCAAATCAAGGGTTAAAGTC | ACGTTGGATGTCCTGACATGTACTGCCCTGCT | CCGTGTGGCTGGTTTTTCTT |
| rs3886100 | G/A     | ACGTTGGATGGCCTCAAGCTCTGCTCAAAATC | ACGTGGATGTCCTGACATGTACTGCCCTGCT | GAGCAAGAGGACACTTAG |
| rs11118167 | T/C     | ACGTTGGATGATGGGACAGATGTACTCTAGCC | ACGTTGGATGATAATTGGAGTTTAAAGGGC | CAATGATATAATGACTCTGCTTCTATC |
| rs9429782 | G/T     | ACGTTGGATGCAGCCCGGAGGCCTTCTAACGGAA | ACGTTGGATGAACTCTACATCCAGGAGGAG | GGTGACGACGACGAG |
| rs1049885 | C/T     | ACGTTGGATGATGGACAGAGCCTCTGGCACAGACATG | ACGTTGGATGACAGAGCCTCTGGCACAGACATG | CTTGACCTTTCTCTGCTGTTAC |
| rs7542544 | C/A     | ACGTTGGATGCCGCGAAACGCGACACAGAGGAG | ACGTTGGATGAACTCTACATCCAGGAGGAG | CCAATGACCCCAAGAC |
| rs6991117 | A/G     | ACGTTGGATGACCGCAGCCGACACAGAGGAG | ACGTTGGATGAACTCTACATCCAGGAGGAG | CCGGCGCTGACATCAAATCTCA |
| rs6556401 | G/A     | ACGTTGGATGAAAGGACACAGACACAGAGGAG | ACGTTGGATGACCGCAGCCGACACAGAGGAG | CCGGCGCTGACATCAAATCTCA |
| rs2296160 | C/T     | ACGTTGGATGCCGAAATCTCTCAGAAGAAC | ACGTTGGATGACCGCAGCCGACACAGAGGAG | CTTGACCTTTCTCTGCTGTTAC |
| rs9429704 | T/C     | ACGTTGGATGCAGCCCGGAGGCCTTCTAACGGAA | ACGTTGGATGAACTCTACATCCAGGAGGAG | GGAAGTTGGACGACATGAT |
| rs4844600 | G/A     | ACGTTGGATGAAAGGACACAGACACAGAGGAG | ACGTTGGATGACCGCAGCCGACACAGAGGAG | CCGGCGCTGACATCAAATCTCA |
| rs38188361 | C/T     | ACGTTGGATGAAAGGACACAGACACAGAGGAG | ACGTTGGATGACCGCAGCCGACACAGAGGAG | CCGGCGCTGACATCAAATCTCA |
| rs17048010 | T/C     | ACGTTGGATGAAAGGACACAGACACAGAGGAG | ACGTTGGATGACCGCAGCCGACACAGAGGAG | CCGGCGCTGACATCAAATCTCA |
matched to cases according to gender, age (±5 years); iii) the residential region; and iv) the time period for blood sample collection. At recruitment, informed consent was obtained from each subject, and each participant was then interviewed to collect detailed information on demographic characteristics. This study was approved by the institutional review board of Hebei United University.

Tag SNPs selection and genotyping
Based on the Chinese population data from HapMap database, we used Haploview 4.2 program to select candidate tag SNPs with an r² threshold of 0.80 and minor allele frequency (MAF) greater than 1%. Furthermore, we also added two potential functional polymorphisms, rs9429942 and rs6691117 [42,43]. Therefore, we included 13 SNPs in our study, which represents common genetic variants in Chinese population.

Genotyping was performed at Bomiao Tech (Beijing, China) using iPLEX Gold Genotyping Assay and Sequenom MassArray (Sequenom, San Diego, CA, USA). Sequenom’s MassArray Designer was used to design PCR and extension primers for each SNP. Primer information for selected tag SNPs was listed in Table 5.

Statistical analysis
We used Chi-square test to examine the differences in the distributions of demographic characteristics, and genotype frequencies between cases and controls. The NSCLC risk associated with CRI tag SNPs was estimated as odds ratios (OR) and 95% confidence intervals (CI) computed by logistic regression model adjusted for age, gender, and smoking status where it was appropriate. Smokers were considered current smokers if they smoked up to 1 year before the date of cancer diagnosis for NSCLC patients or before the date of the interview for controls. The number of pack-years smoked was determined as an indication of cumulative cigarette-dose level [pack-year = (cigarettes per day/20) × (years smoked)]. Light and heavy smokers were categorized by using the 50th percentile pack-year value of the controls as the cut points (i.e., ≤25 and >25 pack-years). All statistical tests were 2 sided with P < 0.05 as the significant level. Statistical analyses were done using SPSS (version 16.0, SPSS Inc, Chicago, IL). Gene-gene and gene-smoking interactions were analyzed by open-resource GMDR software package (version 0.9) and Quanto (http://www.hydra.usc.edu/gxe) [44,45].

Abbreviations
CRI: Complement receptor 1; OR: Odds ratio; CI: Confidence interval; SNP: Single nucleotide polymorphism.

Competing interests
The authors declare no competing financial interest.

Authors’ contributions
XY drafted the article. XY and JR conducted genotyping of CRI. JL, ZZ and LC collected clinical data and analyzed the data. XZ contributed the research plan and approved the data. All authors read and approved the final manuscript.

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