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RESEARCH ARTICLE

Functional Polymorphism in the *MSI1* Gene Promoter Confers a Decreased Risk of Lung Cancer in Chinese by Reducing *MSI1* Expression

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Abstract: ***Background***: *Musashi1* (*MSI1)* is a characteristic stem cell marker that regulates the balance between cell self-renewal and differentiation. Evidence has identified *MSI1* as a pivotal oncogenic regulator in diverse malignancies. However, little evidence uncovers the role of genetic variations of *MSI1* gene in cancer etiology.

Objective: The aim of this study was to investigate the association between genetic variants in the *MSI1* gene and lung cancer risk.

Methods: Based on a two-stage retrospective study with a total of 1559 patients with lung cancer and 1667 healthy controls, we evaluated the relevance between three putative functional SNPs in the *MSI1* promoter (*i.e.*, -2696T>C[rs7959801], -2297T>C[rs3742038] and -1081C>T[rs34570155]) and lung cancer risk.

Results: We found that the SNP rs7959801T>C was significantly associated with lung cancer susceptibility. Compared to those with rs7959801TT wild-genotype, individuals with CT/CC variant genotypes exerted consistently beneficial roles in lung cancer risk in the discovery set (adjusted odd ratios [OR] = 0.67; 95% confidence interval [CI] = 0.57-0.80), and in the validation set (OR=0.69; 95%CI=0.54-0.88). Functional assays indicated that the allele transformation from T to C in rs7959801 of *MSI1* gene arrestingly decreased its transcription activity *in vitro*. Furthermore, the expression levels of *MSI1* were significantly lower in the patients with CT/CC variants than in those who were with TT genotype.

Conclusion: Our findings suggested that the rs7959801T>C polymorphism in the *MSI1* promoter conferred a decreased risk to lung cancer by reducing the expression of *MSI1* and it may be a promising indicator for lung cancer predisposition.

Keywords: Lung cancer, *Musashi1*, Promoter, Genetic variant, Susceptibility, Case-control study.

1. INTRODUCTION

Lung cancer is the most common cancer and ranks the leading cause of cancer-related mortality worldwide [1]. In the past decades, the prevalence of lung cancer is being in rapid rise in China [2, 3]. Although tremendous genetic variants such as Single Nucleotide Polymorphism (SNP) have been reported to be associated with lung cancer risk and progression based on a serial of Genome-Wide Association Studies (GWASs) [4-6], the molecular mechanisms of how these genetic loci participate in cancer initiation and development are still largely unclear. Recent studies indicate that cancer heterogeneity is a result of the hierarchical organization of tumor cells by a portion of cells with stem/progenitor cell features termed as Cancer Stem Cells (CSCs) [7]. Meaningfully, CSCs have been identified to play a critical role in tumor initiation, maintenance and metastasis [8-12], as well as in relapse and drug resistance [13, 14]. Furthermore, evidence reports that genetic variants in key genes of CSCs could influence their biological functions and thus be associated with cancer susceptibility [15]. Above evidence might provide a novel approach to seek especial and functional biomarkers for cancer predisposition.

Musashi RNA binding protein 1(*Musashi1*, also known as *MSI1*, OMIM#603328) is deemed to an important cell

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surface marker of CSCs that functions as a regulator for maintenance of the stem-cell state, differentiation, and tumorigenesis by repressing translation of its downstream targets [16, 17]. Growing evidence reveals that overexpression of *MSI1* results in cell proliferation and apoptosis *via* the translational inhibition of *Numb*, *p21WAF* and *Dickkopf3*, which are well-known factors in cancer stem cell biology and cell cycle regulation [17-19]. In addition, aberrant expression of *MSI1* has been reported to be associated with many malignancies including hepatoma [20], gliomas [21], colorectal adenoma [22] and lung cancer [23]. All these findings suggest that *MSI1* may act as an oncogene.

Human *MSI1* gene locates at chromosome 12q24, contains 15 exons, and encodes a 362-amino acids protein [24]. The correlations between genetic variations in *MSI1* and cancer risk have not yet been established comprehensively. Recently, one study indicated that a SNP in the 3'-UTR of *MSI1* gene was associated with an increased risk of lung cancer in Chinese population [25]. However, no evidence was presented to identify association of polymorphisms in other functional region of *MSI1* gene to human diseases. Because of the fact that the promoter plays an important role in gene's genomic stability, transcriptional efficiency and eventual protein expression; genetic variants in the promoter region may have influence on transcriptional modulation of the gene and thus contribute to disease susceptibility. Herein, we hypothesized that polymorphisms in the promoter of *MSI1* gene were associated with lung cancer risk though affecting gene's expression.

In the current study, we genotyped three potentially functional SNPs in the *MSI1* promoter (*i.e.*, -2696T>C[rs7959 801], -2297T>C[rs3742038] and -1081C>T [rs34570155]), and assessed their associations with lung cancer risk based on a two-stage retrospective study in southern and eastern Chinese populations. For the promising causal SNPs, we further performed a serial of experiments to decode their molecular functions on lung tumorigenesis.

2. MATERIALS AND METHODS

2.1. Study Subjects

The studied populations conducted in southern and eastern China have been described previously [26-28]. In brief, 1056 primary lung cancer cases and 1056 cancer-free controls recruited from Guangzhou city were used as discovery set; 503 patients with lung cancer and 623 healthy controls from Suzhou city were served as validation set. All participants are generally unrelated Han Chinese and none had blood transfusions in the last 6 months. Each subject was asked to provide data on individual's demographic and clinical characteristics, and to donate 5 ml blood after obtaining their written informed consents. The detailed information of subjects' recruitment and definitions of variables was presented previously [26-28]. The study was approved by the Institutional Review Boards of Guangzhou Medical University (Ethics Committee of Guangzhou Medical University: GZMC2007-07-0676) and Soochow University (Ethics Committee of Soochow University: SZUM2008031233).

2.2. SNP Selection and Genotyping

In current study, those SNPs that were located in the promoter region of *MSI1* gene [24] and had Minor Allele Frequency (MAF) >5% in Chinese population were screened based on the dbSNP database (http://www.ncbi.nlm.nih.gov/). Finally, three common SNPs in the *MSI1* promoter (*i.e.*, -2696T>C[rs7959801],-2297T>C[rs3742038] and -1081C>T [rs34570155]) were selected.

The genomic DNA from subjects'peripheral blood was extracted with routine method. The genotypes of the studied SNPs were measured using the TaqMan allelic discrimination assay performed in the ABI PRISM 7500 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA). The primers and probes for the SNPs were purchased from Life Technologies company (Applied Biosystems).

Approximately 10% samples for each SNP were randomly selected to re-genotyping, and the results were in 100% concordance.

2.3. Construction of Reporter Plasmids

Two luciferase reporter plasmids containing rs7959801T or C allele were further constructed to evaluate the effect of this SNP on transcription activity of the *MSI1* (Fig. **1A**). The expatiation of the assay procedures was described elsewhere [29]. In short, a fragment of a total of 2950bp for *MSI1* core promoter (from -2914 to +36 nucleotides relative to the transcription start site) with rs7959801T allele was amplified from subjects with rs7959801TT genotype, using the forward primer 5′-CGGGGTACCTTTACTATGAGGATCAT GAGTTTAC-3′and reverse primer 5′-ACGCGTCGACCC GCTCGAGGGAGGCGAGGCCGGGCTGGG-3′, including *KpnI* and *XhoI* enzymes restriction sites. The amplified product was cleaved with the *KpnI* and *XhoI* enzymes (New England, BioLabs, Ipswich, MA) and then ligated to pGL3 basic vector (Promega, Madison, WI) by T4 DNA ligase (New England BioLabs). The construct with C allele was obtained from wild-type construct by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). All constructs were sequenced to confirm the allele, the orientation and integrity of each insert.

2.4. Luciferase Assays

The luciferase assays were executed as described previously [26, 30]. In brief, two human lung cancer cell lines, A549 and NCI-520 were cultured into 24-well plates and then transfected with 1.5μg reporter plasmids (T or C allele) and 10 ng pRL-TK plasmids (Promega, Madison,WI) using lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA). The activity of each reporter with firefly luciferase and the internal standard with Renilla luciferase was quantified with a Dual-Luciferase Reporter Assay System (Promega).

2.5. Quantitative Real-time PCR

Total RNA was dissociated from thirty-two lung cancer tissues and their peripheral normal tissues that were collected during the surgical excision from the first hospital, second hospital and the tumor hospital affiliated to Guangzhou

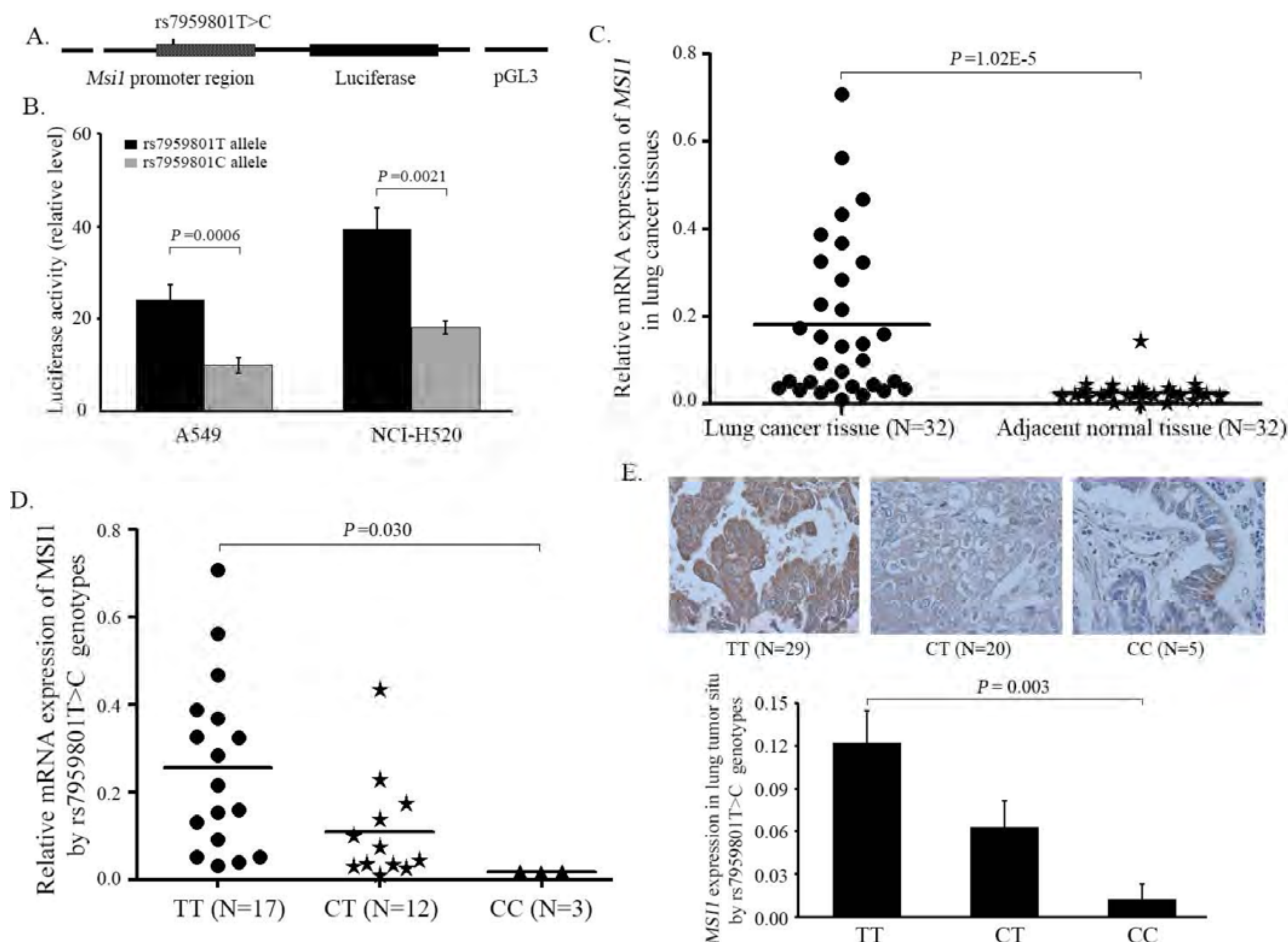

Fig. (1). The effect of rs7959801T>C genotypes on *MSI1* biological phenotypes. (**A**) Schematic diagram of the reporter constructs containing 2950 bp promoter of *MSI1* with rs7959801T or C allele. (**B**) Luciferase activity of the *MSI1* promoter constructs in two lung cancer cell lines (A549 and NCI-H520). The luciferase activity of each construct was normalised against the internal control of renilla luciferase. Columns, mean from three independent experiments; bars, standard deviation; Student's *t*-test was used to compare the expression levels of different constructs. (**C**) mRNA expression of *MSI1* in lung cancer tissues and adjacent normal lung tissues. (**D**) *MSI1* mRNA expression in lung cancer tissues with rs7959801T>C genotypes. (**E**) Protein expressions in lung cancer tissues *in situ* using the Image Pro Plus software to score the *MSI1* expression (magnification ×200). The comparisions of *MSI1* expression in different genotypes were measured with one-way ANOVA test.

Medical University according to routine method [28, 31]. All tumor samples were histologically confirmed and genotypes of these samples were detected by sequencing. 2 μg total RNA was then reversely transcribed to complementary DNA using random primers and Superscript II (Invitrogen). The mRNA expression of *MSI1* was quantified with the primers (forward: 5′-GAGGGTTCGG GTTTGTCACG-3′ and reverse: 5′-GGCGACATCACCTCCTTTGG-3′) in ABI Prism 7500 sequence detection system based on the SYBR-Green method and β-actin was used as an internal reference gene using the primers: 5′-GGCGGCACCACCAT GTACCCT-3′ and 5′-AGGGGCCGGACTCGTCATACT-3′. Method of $2^{-\Delta ct}$ was used to calculate the relative level of *MSI1*.

2.6. Immunohistochemistry

Immunohistochemistry was performed following standard procedures [31]. In short, sections were dewaxed, and rehydrated by sequential immersion in xylene, graded ethanol and water. The sections were then boiled in 10 mmol/L citrate buffer (pH 6.0) in a microwave oven for antigen retrieval. The slides were treated with 3% hydrogen peroxide in methanol to quench endogenous peroxidase activity, followed by incubation with 1% fish skin gelatin to block the nonspecific staining. Sections were then incubated overnight at 4°C with primary antibody of *MSI1* (Abcam, Cambridge, USA; 1:300 dilution). After washing with Phosphate-Buffered Saline (PBS), the sections was treated with biotinylated goat anti-rabbit IgG for 30 min at room temperature, rinsed with PBS and followed by an avidinbiotin-peroxidase conjugate for 30 min. Reaction products were observed by incubation with diaminobenzidine. Negative controls were treated identically but with the primary antibodies omitted. The immunohistochemical reactions were then visualized under high-power magnification (×200) using an Olympus microscope. Meanwhile, all fifty-four tissue samples were genotyped. Staining intensity score of the *MSI1* expression was calculated using the Image Pro Plus software, and percentage of positive cells examined was scored as 0 (nega-

tive), 1 (<10%), 2 (11-50%), 3 (51-80%) and 4 (>80%). The intensity scores and fraction of positive cell were multiplied to represent the relative level of *MSI1* expression.

2.7. Statistical Analysis

The chi-square test was used to test the distributions of selected characteristics and genotypes between cases and controls. Logistic regression models with or without adjusting for surrounding factors were used to estimate the association between each SNP and lung cancer risk. Breslow-Day test was used to test the homogeneity of the results between stratum-ORs. A multiplicative interaction model was applied to evaluate the possible gene-environment interactions [28]. The statistical power was calculated using the PS Software. The False Positive Report Probability (FPRP) test was applied to detect the false-positive association findings [32]. The Student's *t* test was used to assess the transcriptional levels between different luciferase constructs. The one-way analysis of variance (ANOVA) test and the linear regression model, which has been adjusted for surrounding factors, were used to evaluate the association between *MSI1* genotypes and *MSI1* expression. All tests were two-sided and operated using the SAS software (version 9.3; SAS Institute), and P <0.05 was defined as statistically significant.

3. RESULTS

3.1. Associations Between MSI1 SNPs and Risk of Lung Cancer

The information of demographic characteristics in the studied populations were described previously [28, 31, 33] and presented in Table **S1**. In the discovery set, we only observed a significant correlation between the SNP rs7959801T>C and lung cancer risk. As shown in Table **1**, compared to those with rs7959801TT wild-genotype, the carriers with CT heterozygous genotype had a decreased risk of lung cancer (odds ratio [OR] =0.64, 95% confidence interval [CI] =0.53-0.76; P =1.02E-6).

The results were further confirmed in the validation set as the individuals with CT variant genotype exerted a 28% decreased risk of lung cancer when compared to the subjects with TT common genotype (OR=0.71, 95%CI=0.55-0.91; P=0.007). The heterogeneity test showed that the above associations in two populations were homogeneous (P =0.783). We then combined the two datasets to increase the study power and found that compared to those with rs7959801TT wild-genotype, the individuals with CT (OR =0.66, 95%CI =0.57-0.77; P =4.23E-8) or CC (OR =0.78, 95%CI =0.61-0.99; P =0.048) variant genotype had a significantly decreased risk for lung cancer, respectively. Also, in the dominant model, the carriers with CT/CC variants had a prominent declining risk of lung cancer compared to the carriers with TT wild-genotype (OR =0.68, 95%CI =0.59-0.79; P =1.18E-7). However, for other SNPs rs3742038T>C and rs34570155C>T, no notable relevance with risk of lung cancer was observed in both two datasets.

3.2. Stratification Analysis of Relationships Between MSI1 rs7959801T>C Genotypes and Lung Cancer Risk

Among all the assumed genetic models, the hereditary effect of rs7959801T>C on lung cancer risk was best in the dominant model as it showed the smallest Akaike's Information Criterion (AIC) value. Hence, we further performed the stratified analysis for relationships between rs7959801T>C and lung cancer risk using dominant model. As shown in Table **2**, although the quantitative effects of rs7959801C variants on cancer risk presented by stratum-ORs were in some difference, the heterogeneity test indicated that no significant deviations occurred in these subgroups (P>0.05 for all). Moreover, no remarkable interaction was found for this SNP with surrounding factors on lung cancer risk (P>0.05 for all).

3.3. Effects of the rs7959801T>C Genotypes on MSI1 Transcriptional Activity

Two luciferase reporter constructs comprised of rs7959801T or C allele were generated and assayed to evaluate the influence of this SNP on *MSI1* transcriptional activity. As shown in Fig. (**1B**), the transcriptional level of the reporter gene integrated the *MSI1* promoter with rs7959801C allele was markedly lower than the construct with T allele both in A549 cell and NCI-520 cell (P value equals to 0.0006 and 0.0021, respectively).

3.4. Correlation Between rs7959801T>C Genotypes and MSI1 Expression

As shown in Fig. (**1C**), we observed that the expression of *MSI1* was significantly higher in lung cancer tissues than in their adjacent normal tissues (paired t test: P =1.02E-5). We further found that the *MSI1* mRNA levels were in negative correlation with the number of rs7959801C variant allele (ANOVA test: P =0.030; lineal regression: P = 0.009; Fig. **1D**). The protein expression of *MSI1* in fifty-four lung cancer tissues *in situ* confirmed the above findings as the Fig. (**1E**) indicated that the tissues with rs7959801C variant genotype (CT or CC) exerted a strikingly decreased *MSI1* expression compared to those with TT wild-genotype (ANOVA test: P =0.003; lineal regression: P =0.0008).

4. DISCUSSION

In this study, we assessed the correlation between genetic variations in *MSI1* promoter and the risk of lung cancer in Chinese populations and found that the SNP rs7959801T>C exerted a rewarding effect on developing lung cancer. Mechanical experiments revealed that the substitute from rs7959801T to C could remarkably decrease the expression of the *MSI1* gene both in mRNA and protein level. To the best of our knowledge, this is the first study to show the relationship between functional SNP in the promoter of *MSI1* gene and lung cancer susceptibility.

Evidence has been considered CSC markers as an important component in the maintenance of self-renewal and resistance to apoptosis pathway activation in these cells [34]. Several studies indicate that CSCs are rich in tumorspheres and give rise to tumors [35]. Notably, recent reports have demonstrated the existence of CSC in lung cancer [36]. As a pivotal molecule for CSC, *MSI1* has been identified to play a critical role in tumorigenesis and tumor progression [37, 38]. Abnormal over-expression of *MSI1* occurred in different types of human malignancies including lung cancer [20, 21, 23]. For example, one study indicated that the expression of

Table 1. The genotype frequencies of studied SNPs in *Musashi-1* promoter and their associations with lung cancer risk.

Genotypes/Alleles	Discovery Set (Southern Chinese)			-	Validation Set (Eastern Chinese)			-	Merged Set			-
-	Case (n=1056) n(%)	Control [a] (n=1056) n(%)	Adjusted OR (95% CI) [b]	*P* value	Case (n=503) n(%)	Control [a] (n=623) n(%)	Adjusted OR (95% CI) [b]	*P* value	Case (n=1559) n(%)	Control (n=1679) n(%)	Adjusted OR (95% CI) [b]	*P* value
rs7959801T>C	-	-	-	-	-	-	-	-	-	-	-	-
TT	563(53.3)	460(43.6)	1.00 (ref.)	-	278(55.3)	287(46.1)	1.00 (ref.)	-	841(53.9)	747(44.5)	1.00(ref.)	-
CT	385(36.5)	492(46.6)	**0.64(0.53-0.76)**	**1.02E-6**	185(36.8)	270(43.3)	**0.71(0.55-0.91)**	**0.007**	570(36.6)	762(45.4)	**0.66(0.57-0.77)**	**4.23E-8**
CC	108(10.2)	104(9.8)	0.85(0.63-1.15)	0.278	40(7.9)	66(10.6)	**0.63(0.41-0.97)**	**0.031**	148(9.5)	170(10.1)	**0.78(0.61-0.99)**	**0.048**
Group test *P* value	-	-	**7.89E-6**	-	-	-	**0.008**	-	-	-	**2.56E-7**	-
Additive model	-	-	-	-	-	-	-	-	-	-	-	-
CC *vs*. CT *vs*. TT	-	-	**0.81(0.71-0.92)**	**0.001**	-	-	**0.76(0.63-0.91)**	**0.003**	-	-	**0.79(0.71-0.88)**	**1.43E-5**
Dominant model	-	-	-	-	-	-	-	-	-	-	-	-
TT	563(53.3)	460(43.6)	1.00 (ref.)	-	278(55.3)	287(46.1)	1.00 (ref.)	-	841(53.9)	747(44.5)	1.00 (ref.)	-
CT+CC	493(46.7)	596(56.4)	**0.67(0.57-0.80)**	**6.25E-6**	225(44.7)	336(53.9)	**0.69(0.54-0.88)**	**0.002**	718(46.1)	932(55.5)	**0.68(0.59-0.79)**	**1.18E-7**
-	-	-	-	-	-	-	-	-	-	-	-	-
rs3742038T>C	-	-	-	-	-	-	-	-	-	-	-	-
TT	960(90.9)	935(88.5)	1.00 (ref.)	-	440(87.5)	547(87.8)	1.00 (ref.)	-	1400(89.8)	1482(88.3)	1.00(ref.)	-
CT	87(8.2)	114(10.8)	0.74(0.55-1.01)	0.052	57(11.3)	73(11.7)	0.94(0.64-1.36)	0.736	144(9.2)	187(11.1)	0.82(0.65-1.03)	0.081
CC	9(0.9)	7(0.7)	1.25(0.46-3.38)	0.657	6(1.2)	3(0.5)	2.17(0.53-8.83)	0.203	15(1.0)	10(0.6)	1.55(0.69-3.47)	0.259
Group test *P* value	-	-	0.122	-	-	-	0.406	-	-	-	0.107	-
Additive model	-	-	-	-	-	-	-	-	-	-	-	-
CC *vs*. CT *vs*. TT	-	-	0.83(0.64-1.07)	0.141	-	-	1.04(0.75-1.44)	0.824	-	-	0.90(0.74-1.11)	0.338
Dominant model	-	-	-	-	-	-	-	-	-	-	-	-
TT	960(90.9)	935(88.5)	1.00 (ref.)	-	440(87.5)	547(87.8)	1.00 (ref.)	-	1400(89.8)	1482(88.3)	1.00 (ref.)	-
CT+CC	96(9.1)	121(11.5)	0.77(0.58-1.03)	0.076	63(12.5)	76(12.2)	0.99(0.69-1.42)	0.951	159(10.2)	197(11.7)	0.85(0.68-1.07)	0.163
-	-	-	-	-	-	-	-	-	-	-	-	-
rs34570155C>T	-	-	-	-	-	-	-	-	-	-	-	-
CC	510(48.3)	478(45.3)	1.00 (ref.)	-	233(46.3)	293(47.0)	1.00 (ref.)	-	743(47.6)	771(45.9)	1.00(ref.)	-
TC	404(38.3)	446(42.2)	0.85(0.71-1.02)	0.086	196(39.0)	256(41.1)	0.98(0.76-1.28)	0.928	600(38.5)	702(41.8)	0.89(0.77-1.04)	0.113
TT	142(13.4)	132(12.5)	1.02(0.79-1.34)	0.952	74(14.7)	74(11.9)	1.29(0.89-1.87)	0.347	216(13.9)	206(12.3)	1.11(0.89-1.38)	0.442
Group test *P* value	-	-	0.176	-	-	-	0.366	-	-	-	0.116	-
Additive model	-	-	-	-	-	-	-	-	-	-	-	-
TT *vs*. TC *vs*. CC	-	-	0.97(0.85-1.09)	0.571	-	-	1.09(0.92-1.30)	0.306	-	-	1.01(0.91-1.11)	0.950
Dominant model	-	-	-	-	-	-	-	-	-	-	-	-
CC	510(48.3)	478(45.3)	1.00 (ref.)	-	233(46.3)	293(47.0)	1.00 (ref.)	-	1182(75.8)	1343(80.0)	1.00 (ref.)	-
TC+TT	546(51.7)	578(54.7)	0.89(0.75-1.06)	0.188	270(53.7)	330(53.0)	1.06(0.83-1.34)	0.655	377(24.2)	336(20.0)	0.94(0.82-1.08)	0.321

Bold type: statistically significant, $P < 0.05$.

[a] The observed genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium ($p^2+2pq+q^2 = 1$) in the control subjects of both sets ($P > 0.05$ for all).

[b] Adjusted in a logistic regression model that included age, sex, smoking status, alcohol use and family history of cancer.

Table 2. Stratification analysis of the *Musashi-1* rs7959801T>C genotypes by selected variables in lung cancer patients and controls.

-	Cases (n = 1559)		-	Controls (n = 1679)		Adjusted OR (95% CI) [a]	P_{homo} [b]	P_{inter} [c]
	CT+CC n (%)	TT n (%)	-	CT+CC n (%)	TT n (%)	CT+CC *vs.* TT	-	-
Age (years)	-	-	-	-	-	-	0.099	0.271
≤ 60	367(45.4)	442(54.6)	-	505(57.6)	372(42.4)	**0.61(0.51-0.75)**	-	-
> 60	351(46.8)	399(53.2)	-	427(53.2)	375(46.8)	**0.75(0.62-0.92)**	-	-
Sex	-	-	-	-	-	-	0.909	0.945
Male	505(46.3)	586(53.7)	-	662(55.9)	523(44.1)	**0.68(0.58-0.80)**	-	-
Female	213(45.5)	255(54.5)	-	270(54.7)	224(45.3)	**0.69(0.53-0.89)**	-	-
Smoking status	-	-	-	-	-	-	0.439	0.374
Ever	373(45.3)	451(54.7)	-	429(56.1)	336(43.9)	**0.65(0.53-0.79)**	-	-
Never	345(46.9)	390(53.1)	-	503(55.0)	411(45.0)	**0.72(0.59-0.88)**	-	-
Drinking status	-	-	-	-	-	-	0.250	0.268
Ever	123(42.0)	170(58.0)	-	190(55.6)	152(44.4)	**0.58(0.42-0.80)**	-	-
Never	595(47.0)	671(53.0)	-	742(55.5)	595(44.5)	**0.71(0.61-0.83)**	-	-
Family history of cancer	-	-	-	-	-	-	0.945	0.882
Yes	54(41.9)	75(58.1)	-	76(51.7)	71(48.3)	0.64(0.39-1.05)	-	-
No	664(46.4)	766(53.6)	-	856(55.9)	676(44.1)	**0.69(0.59-0.79)**	-	-
Family history of lung cancer	-	-	-	-	-	-	0.430	0.677
Yes	20(38.5)	32(61.5)	-	24(55.8)	19(44.2)	**0.36(0.14-0.96)**	-	-
No	698(46.3)	809(53.7)	-	908(55.5)	728(44.5)	**0.69(0.60-0.79)**	-	-
Histological types	-	-	-			-	0.324	-
Adenocarcinoma	284(46.2)	331(53.8)				**0.68(0.57-0.82)**	-	-
Squamous cell carcinoma	240(45.5)	287(54.5)				**0.67(0.55-0.82)**	-	-
Large cell carcinoma	34(51.5)	32(48.5)		932(55.5)	747(44.5)	0.84(0.52-1.38)	-	-
Small cell lung cancer	85(44.0)	108(56.0)				**0.62(0.46-0.84)**	-	-
Other carcinomas [c]	75(47.5)	83(52.5)				0.72(0.52-1.00)	-	-
Stages	-	-				-	0.459	-
I	97(48.5)	103(51.5)				0.75(0.56-1.01)	-	-
II	58(39.5)	89(60.5)		932(55.5)	747(44.5)	**0.51(0.36-0.73)**	-	-
III	231(47.1)	259(52.9)				**0.71(0.58-0.87)**	-	-
IV	332(46.0)	390(54.0)				**0.68(0.57-0.81)**	-	-

Bold type: statistically significant, $P < 0.05$.

[a] ORs were adjusted for age, sex, and smoking status, and alcohol use, family history of cancer in a logistic regression model.

[b] P value of homogeneity test between strata for the related ORs of rs7959801T>C (rs7959801 CT+CC *vs.* TT genotypes).

[c] P value of test for the multiplicative interaction between rs7959801T>C genotypes and selected variables on cancer risk in logistic regression models.

MSI1 was enriched in lung cancer cells and tissue specimens. Silencing of *MSI1* gene by shRNA lentivirus-mediated could reduce spheroid colony proliferation and inhibited cell growth *via* the reduction of nuclear localization of β-catenin and inhibition of the processing of intracellular Notch [39]. In addition, another study showed a close link of increased

MSI1 expression to the development of lung cancer [40]. In the current study, we consistently found that the expression of *MSI1* was higher in lung cancer tissues than in their adjacent normal tissues. All above results suggested that the *MSI1* might act as oncogene in lung tumorigenesis.

Emerging studies have identified that genetic variants in the potentially functional area of genes could influence gene's structure, function or expression and thus cause unexpected consequence [31, 41]. And also, evidence reported that several SNPs in *MSI1* was associated with various human diseases [42]. However, up to now, few studies have interpreted the potential effects of genetic variants in *MSI1* gene on cancer susceptibility. There was only one study that reported a SNP in the 3'-UTR of *MSI1* associated with an increased risk of lung cancer [25]. Nevertheless, no further evidence shows how this SNP contributes to lung cancer development. Herein, based on the candidate gene approach that is economical and has rather high statistical power than GWAS and Quantitative Trait Locus (QTL) approaches, we tested the association between three putative functional SNPs in the promoter of *MSI1* gene and lung cancer risk in discovery set and then validated in other independent population. We found that the SNP rs7959801T>C was significantly associated with a decreased risk of lung cancer. The alteration from rs7959801T to C could decreased the expression of *MSI1*, which might decrease the number or undermine the biological function of lung CSCs and thus exert a decreased risk for lung cancer. All these findings indicated that the SNP rs7959801T>C of *MSI1* may be a useful biomarker for lung cancer susceptibility in Chinese.

Several deficiencies and limitations should be concerned in the present study. Because of that there were two hospital-based retrospective studies, restricted Chinese Han populations; some selection biases were unavoidable in this study. In fact, the genotype frequencies of all selected SNPs among controls fitting the Hardy-Weinberg equilibrium law indicate the randomness of subjects selection. Also, we have obtained concordant results of the association between rs7959801T>C and lung cancer risk both in two populations accompanied by a 99.9% statistically study power. In addition, it yielded a value of 0.000 with a 0.001 prior probability lower than the preset FPRP-level criterion of 0.20, suggesting that this finding is noteworthy. Furthermore, the association between the SNP rs7959801T>C and risk of lung cancer had been supported by a serial of functional assays, further proving that our results were not achieved by chance. Even so, to further better reveal the effect of rs7959801 T>C in the promoter of *MSI1* gene on lung carcinogenesis, *in vivo* experiment such as xenograft models should be performed.

CONCLUSION

In summary, our findings provide the first evidence that the SNP rs7959801T>C in the promoter of *MSI1* gene confers a rewarding role in lung cancer risk by reducing *MSI1* expression, which may be a functional biomarker for the risk of lung cancer. Further studies with larger population-based studies in different ethnic groups and *in vivo* animal experiments are needed to validate our findings.

LIST OF ABBREVIATIONS

CSCs	=	Cancer Stem Cells
GWASs	=	Genome-Wide Association Studies
Musashi1	=	Musashi RNA binding protein 1
OR	=	Odd Ratio
SNPs	=	Single Nucleotide Polymorphisms

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Boards of Guangzhou Medical University (Ethics Committee of Guangzhou Medical University: GZMC2007-07-0676) and Soochow University (Ethics Committee of Soochow University: SZUM2008031233).

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All human research procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION

Written informed consent was obtained from each individual. After given a written informed consent, each participant was scheduled for an interview with a structured questionnaire to provide data on smoking status, alcohol use and other factors including family history of cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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