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ORIGINAL ARTICLE

Association between H19 polymorphisms and NSCLC risk in a Chinese population

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Summary

Purpose: *Long non-coding RNAs (lncRNA) polymorphisms have been reported to associate with the carcinogenicity mechanisms. The association between lncRNA H19 polymorphisms and the risk of non-small cell lung cancer (NSCLC) in Chinese population has not been reported yet. We designed this case-control study to evaluate the effects of H19 polymorphisms on NSCLC susceptibility.*

Methods: *In this case-control study, four single nucleotide polymorphisms (SNPs) (rs2067051, rs217727, rs2839698 and rs4929984) in H19 gene were genotyped in a Chinese population which consisted of 564 NSCLC cases and 1536 controls.*

Results: *rs2067051 was associated with a significantly decreased risk of NSCLC in our population [AA vs. GG: adjusted odds ratio (OR) = 0.75, 95% confidence interval (CI)=0.60-0.93; Additive model: OR=0.79, 95%CI=0.67-0.93)]. rs217727 was related to significantly increased NSCLC susceptibility (TT vs. CC: OR=1.16, 95%CI=1.01-1.33; Additive model: OR=1.16, 95%CI=1.01-1.33). However, no significant association was observed between rs2839698, rs4929984 and NSCLC risk.*

Conclusions: *H19 polymorphism rs2067051 and rs217727 might influence NSCLC susceptibility and the mechanism warrants further exploration.*

Key words: *LncRNA, H19 polymorphism, NSCLC*

Introduction

Lung cancer is one of the most leading causes of cancer-related death worldwide, with a 5-year survival as low as 13% [1]. Non-small cell lung cancer (NSCLC) constitutes 82% of all lung subtypes [2]. The development of lung cancer is a complex and multifactorial process involving a number of etiological factors and multiple genetic and epigenetic alterations [3]. It is biologically possible that host genetic susceptibility is a factor in the development of lung cancer and contributes to the variation in individual cancer risk [4]. Nevertheless, the role of genetic polymorphism and lung cancer susceptibility still remains unknown.

Long non-coding RNAs (lncRNAs) consist of more than 200 nucleotides and do not function as templates for protein synthesis [5]. It is now becoming evident that lncRNAs can regulate gene expression via diverse mechanisms, such as transcription, translation, imprinting, genome rearrangement and chromatin modification [6]. Various lncRNAs have been known to play a role in various diseases, including cancer, via transcriptional and post-transcriptional regulation of the expression of oncogenes or tumor suppressors [7].

LncRNA H19, located on chromosome 11p15.5, has been reported to be associated with carcinogen-

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esis of many cancers. The aberrant expression of lncRNA H19 has been found in cancers including lung, bladder, and osteosarcoma [8-10]. The mechanism on how altered lncRNA H19 expression affect cancer risk is not clear as yet. Studies have shown that lncRNA H19 polymorphisms are associated with cancer susceptibility, including gastric cancer, bladder cancer, colorectal cancer and breast cancer [9-12]. Association between H19 polymorphisms and NSCLC risk remains unclear.

In this study, we conducted a case-control study including 564 NSCLC cases and 1536 controls to examine the effect of 4 most widely studied SNPs in H19 (rs2067051, rs217727, rs2839698, rs4929984) on NSCLC risk in a Chinese population.

Methods

Study population

This study was approved by the ethics committee of Liaocheng Infectious Disease Hospital. A total of 564 NSCLC patients confirmed by two pathologists were consecutively recruited from the Liaocheng Infectious Disease Hospital, Liaocheng, China, from July 2005 to April 2018. Study subjects who had second NSCLC primary tumors or metastasized cancers from other organs were excluded. On the basis of frequency matching for age (±5 years) and gender to the cases, the controls were randomly selected from a pool of 30,000 cancer-free individuals who participated in the community-based screening program for non-infectious diseases conducted in Shandong province. All the subjects were genetically unrelated, ethnic Han Chinese. After signing an informed consent, each subject was interviewed face-to-face to obtain demographic data and information on related risk factors using a structure questionnaire, including tobacco smoking and alcohol consumption. Subsequently, 5 mL venous blood were collected from each subject. Finally, 564 cases and 1536 frequency-matched controls were included in this study. Individuals who smoked one cigarette per day for over 1 year were considered as smokers, and those who had three or more alcohol drinks a week for over 6 months were defined as alcohol drinkers.

Table 1. Selected characteristics in NSCLC cases and controls

Characteristics	*Case*	*Control*	*p* [a]
	n (%)	*n (%)*	
All subjects	564 (100)	1536 (100)	
Age, years			0.534
<60	251 (44.5)	707 (46.0)	
≥60	313 (55.5)	829 (54.0)	
Gender			0.091
Females	208 (36.9)	629 (41.0)	
Males	356 (63.1)	907 (59.0)	
Smoking			
Ever	252 (55.3)	509 (33.1)	<0.001
Never	312 (44.7)	1027 (66.9)	
Drinking			0.260
Ever	256 (45.4)	655 (42.6)	
Never	308 (54.6)	881 (57.4)	

[a] Two-sided chi-square test

Table 2. Primary information and minor allele frequencies of selected single nucleotide polymorphisms

SNPs	*Base change*	*MAF in controls*	*HWE*
rs2067051	G>A	0.283	0.117
rs217727	C>T	0.434	0.328
rs2839698	C>T	0.325	0.602
rs4929984	C>A	0.433	0.868

HWE: Hardy-Weinberg equilibrium, MAF: minor allele frequency, SNPs: single nucleotide polymorphisms

Genotyping

Genotypes were determined using the middle-throughput TaqMan Open-Array Genotyping Platform (Applied Biosystems Inc., Foster City, CA, USA) and genotyping was performed without knowing the case or control status. Two blank controls in each plate were used for quality control. Samples were analyzed with AutoCaller Software (Applied Biosystems Inc., Foster City, CA, USA). Ninety-six samples were also randomly selected from the Open array platform and re-genotyped by using TaqMan allelic assays for the 4 SNPs and the results were all consistent.

Statistics

Chi square (x^2) test was used to evaluate the differences in selected demographic characteristics, smoking and drinking status between cases and controls. Hardy–Weinberg equilibrium was tested by a goodness-of-fit x^2 test to compare the observed genotype frequencies to expected frequencies among the control subjects. Associations between the genotypes and the risk of NSCLC was tested by Cox multivariate logistic regression analysis by computing odds ratios (ORs) and their 95% confidence intervals (CIs) with the adjusted age, gender, drinking and smoking status. The x^2-based *Q* test was applied to evaluate the heterogeneity of associations between subgroups. All the statistical analyses were performed with Statistical Analysis System software (v.9.1 SAS Institute, Cary, NC, USA). The significance was set at $p<0.05$ with two-sided tests.

Results

The characteristics of 564 cases and 1536 controls are shown in Table 1. There was no significant difference in the distributions of age, sex and drinking between the cases and controls (p=0.534, 0.091 and 0.260, respectively). As expected, more

smokers were observed in the case group compared with that in the control group (p<0.001).

Primary information and genotyping results of the 4 selected SNPs are shown in Table 2. The observed genotype frequencies of these variants were in agreement with the Hardy–Weinberg equilibrium among the controls. Minor allele frequency (MAF) of the 4 selected SNPs were not significant (p>0.05).

Multivariate logistic regression analysis revealed that genotype frequency of H19 rs2067051 was significantly different between groups. Subjects carrying H19 rs2067051AA genotype had a significantly decreased NSCLC risk compared to the carriers with GG genotype after adjusted for age, sex, smoking and drinking status (AA *vs.* GG: OR=0.75, 95%CI=0.60-0.93; Additive model: OR=0.79, 95%CI=0.67-0.93). Moreover, individu-

Table 3. Multivariate logistic regression analysis for associations between selected SNPs and risk of NSCLC

SNPs	*Genotype*	*Case*	*Control*	*Adjusted*[a] *OR (95%CI)*	*p value*[a]
rs2067051	GG	324 (57.4)	783 (51.0)	1.00	
	GA	212 (37.6)	631 (41.1)	0.83 (0.67-1.01)	0.067
	AA	27 (4.8)	119 (7.7)	0.75 (0.60-0.93)	0.010
	Additive model			0.79 (0.67-0.93)	0.004
rs217727	CC	162 (28.7)	493 (32.1)	1.00	
	CT	277 (49.1)	751 (48.9)	1.13 (0.90-1.42)	0.295
	TT	125 (22.2)	291 (18.9)	1.16 (1.01-1.33)	0.037
	Additive model			1.16 (1.01-1.33)	0.039
rs2839698	CC	277 (49.1)	712 (46.5)	1.00	
	CT	225 (39.9)	645 (42.1)	0.90 (0.73-1.08)	0.321
	TT	61 (10.8)	175 (11.4)	0.94 (0.80-1.10)	0.442
	Additive model			0.92 (0.80-1.07)	0.286
rs4929984	CC	194 (34.4)	484 (31.5)	1.00	
	CA	277 (49.1)	772 (50.3)	0.89 (0.72-1.11)	0.305
	A	93 (16.5)	278 (18.1)	0.92 (0.76-1.06)	0.262
	Additive model			0.91 (0.79-1.05)	0.194

[a] Multivariate logistic regression analysis with adjustment for age, sex, smoking and drinking

Table 4. Stratified analysis for associations between variant genotypes of rs2067051 and rs217727 and NSCLC risk

Variables	*rs2067051*		*Adjusted*[b] *OR (95%CI)*	*p*[b]	*rs217727*		*Adjusted*[b] *OR(95%CI)*	*p*[b]
	Cases[a]	Controls[a]			Cases[a]	Controls[a]		
	GG/AG/AA	AA/AG/GG			CC/CT/TT	CC/CT/TT		
Age, years								
<60	143/96/11	354/296/55	0.78(0.61-0.99)	0.043	77/116/58	221/350/135	1.12(0.91-1.37)	0.287
≥60	181/116/16	429/335/64	0.79(0.64-0.99)	0.038	85/161/67	272/401/156	1.19(0.99-1.44)	0.062
Gender								
Females	121/72/15	321/265/42	0.84(0.64-1.08)	0.172	48/99/61	189/275/165	1.34(1.08-1.68)	0.004
Males	203/140/12	462/366/77	0.75 (0.61-0.92)	0.006	114/178/64	304/476/126	1.17(0.97-1.41)	0.097
Smoking								
Ever	150/94/8	264/204/41	0.69 (0.53-0.90)	0.006	78/124/50	168/250/90	1.22(0.97-1.54)	0.089
Never	174/118/19	519/427/78	0.83(0.67-1.02)	0.072	84/153/75	325/501/201	1.21(1.01-1.45)	0.039
Drinking								
Ever	153/95/8	336/268/49	0.68 (0.53-0.88)	0.004	77/132/47	214/321/120	1.25(0.99-1.59)	0.058
Never	171/117/19	447/363/70	0.84 (0.68-1.04)	0.105	85/145/78	279/430/171	1.22(1.02-1.47)	0.032

[a] Major homozygote/heterozygote/rare homozygote between cases and controls; [b] Logistic regression with adjustment for age, sex, smoking and drinking.

Table 5. Combined effects of rs2067051 and rs217727 on NSCLC risk

No. of risk allele[a]	*Case*	*Control*	*Adjust*[b] *OR (95%CI)*	*p*[b]
	n (%)	n (%)		
0-1	85 (15.1)	303 (19.7)	1	
2	204 (36.2)	565 (36.8)	1.27 (0.95-1.70)	0.103
3	197 (34.9)	513 (33.4)	1.37 (1.02-1.84)	0.035
4	77 (13.7)	151 (9.8)	1.85 (1.27-2.67)	0.001
Trend			1.20 (1.08-1.33)	0.001
Binary classification				
0-2	289 (51.2)	868 (56.5)	1	
3-4	274 (48.6)	664 (43.2)	1.25 (1.03-1.52)	0.025

[a] The rs2067051 G and rs217727 T allele were assumed as risk alleles based on main effect of individual locus; [b] Adjust by age, sex, smoking status and alcohol status.

als carrying H19 rs217727 TT genotype had a significantly increased NSCLC susceptibility compared to the carriers with CC (TT *vs.* CC: OR=1.16, 95%CI=1.01-1.33; Additive model: OR=1.16, 95% CI=1.01-1.33). No significant association was observed between other two SNPs and NSCLC risk (Table 3).

Further stratification analysis was conducted on the association between the above SNPs and NSCLC risk by age, gender, smoking and drinking status. Significant association of rs2067051 with NSCLC risk was found among both younger and older, males, smokers and drinkers, and the effect of rs217727 on NSCLC risk was significant in females, nonsmokers and nondrinkers (Table 4).

The combined effect of the rs 2067051 and rs 217727 on NSCLC risk was evaluated (Table 5). When these two loci were evaluated together by the number (0-4) of putative risk alleles (rs2067051 G and rs217727 T), a significant locus-dosage effect was detected between these groups and risk of NSCLC (p_{trend}=0.001). Compared with the "0-1" group, groups with "3" risk alleles and "4" risk alleles had significantly increased risk of NSCLC with adjusted ORs of 1.37 (95%CI=1.02-1.84) and 1.85 (95%CI=1.27-2.67), respectively .When the groups with ''0-2' risk alleles and ''3-4'' risk alleles were put into one group respectively, the increased risk of NSCLC remained statistically significant for the group with "3-4" risk alleles (adjusted OR=1.25, 95%CI=1.03-1.52).

Discussion

The present a case-control study aimed to detect the association between the H19 polymorphisms and the risk of NSCLC. As shown in this study, rs2067051 and rs217727 were significantly associated with NSCLC susceptibility. To our knowledge, this is the first investigation into the associations between lncRNA H19 polymorphisms and NSCLC risk. Our results demonstrated that rs2067051 was associated with decreased NSCLC risk and rs217727 polymorphism was associated with increased NSCLC risk.

LncRNA H19 imprinted and maternal expressed gene can generate a 2.3-kb non-protein coding molecule and plays key roles in embryogenesis [13]. LncRNA H19 was reported to serve as an oncogene and might promote carcinogenesis by acting as competitive endogenous RNAs (ceRNA) or precursors of microRNAs. A previous study has reported that lncRNA H19 can regulate the expression of YES1 gene by competitively binding miR-17-5p to functioning as ceRNA, resulting in thyroid carcinogenesis [14]. A new study showed that H19 may serve as a ceRNA endogenous RNA to modulate STAT3 by attaching miR-17 in lung cancer and that H19/miR-17/STAT3 axis participated in NSCLC development [15]. Zhang et al. [16] found that H19 was highly expressed in stage III and IV NSCLC and knockout of H19 significantly inhibited NSCLC cell proliferation both *in vitro* and *in vivo*. Luo et al. [17] reported that the expression of H19 was significantly higher in NSCLC tissues and cells and was induced by c-Myc. They also found that the expression of miR-107 increased or decreased according to the expression level of H19.

In the present study we found that H19 polymorphism rs217727 was related to increased NSCLC risk. Insulin-like growth factor 2 (IGF2) is located in close proximity to H19 and can be regulated by H19 [18]. A study has reported that rs217727 variant was associated with increased circulating level of IGF2 [19], thus, we can hypothesize that the inhibitory effect of H19 on IGF2 was interfered by this variant and resulted in increased NSCLC susceptibility. We also found polymor-

phism rs2067051 was associated with decreased risk of NSCLC. The mechanism was not clear. This polymorphism site locates in the flank region of H19, near to the imprinting control regions (ICRs), which are important in regulating H19 expression. This polymorphism may alter the binding ability of ICRs and thus regulate the expression of H19.

Conclusions

H19 polymorphisms rs2067051 and rs217727 were associated with NSCLC risk in a Chinese population. Further relevant studies with diverse ethnic groups and functional characterization are warranted to validate our findings.

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Conflict of interests

The authors declare no conflict of interests.

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