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LncRNA H19 polymorphisms associated with the risk of OSCC in Chinese population

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Abstract. – OBJECTIVE: H19, a maternally expressed imprinted gene transcribing a long noncoding RNA, has previously been reported to be involved in tumorigenesis and cancer progression. The relationship between H19 and susceptibility of multiple tumors is inconsistent, and currently there is a lack of association of H19 with the risk of oral squamous cell carcinoma (OSCC). The objective to design this research is to investigate and discuss the association of H19 with susceptibility to oral squamous cell carcinoma in Chinese population.

PATIENTS AND METHODS: In this research, Illumina Infinium Human Exome BeadChip technology was used for genetic tests in 362 patients who were pathologically diagnosed with new-onset OSCC and 741 healthy controls with matching gender and age, so as to analyze the association of H19 gene polymorphism sites (rs2735971, rs217727, rs2839698 and rs3024270) with susceptibility of OSCC.

RESULTS: The results of this research showed that rs217727 [Additive model: adjusted odds ratio (OR) = 1.22, 95% confidence interval (CI) = 1.02-1.46; AA *vs*. GG: Adjusted OR = 1.20, 95% CI = 1.00-1.44] was related to the susceptibility of oral squamous cell carcinoma. It was not found in the results that the other three sites were associated with the susceptibility of oral squamous cell carcinoma.

CONCLUSIONS: It is indicated in this research that rs217727 is statistically correlated with the susceptibility of OSCC.

Key Words:
LncRNA, H19, Polymorphism, Oral Squamous Cell Carcinoma.

Introduction

Oral Squamous Cell Carcinoma (OSCC) of the Head and Neck (HNSCC) accounts for 8% of malignant tumor among adults; there are nearly 540,000 new cases of the disease in the world every year, of which 271,000 are death, and the death rate reaches up to 50%[1]. Several studies have indicated that smoking, drinking and papillomavirus infection are the most important environmental factors for head and neck carcinoma[2]. However, the susceptibility of head and neck carcinoma in individuals exposed to the same environment is not the same; thus, it can be seen that genetic factors play a significant role in the occurrence of head and neck carcinoma.

Long noncoding RNA (lncRNA) is a class of RNA molecules with a transcript length of more than 200 nucleotides, which does not encode proteins by itself; it is firstly regarded as a by-product of RNA transcription, a transcriptional noise of genome, which has no biological functions. More and more studies have shown that lncRNA plays an important role in transcriptional regulation, post-transcriptional regulation, epigenetic regulation and other aspects and participates in life processes, such as chromosome modification, transcriptional activation and interference, gene modification, chromosome silencing and intranuclear transport[3,4]. It is reported by research that lncRNA has a close correlation with the occurrence and development of tumors; the expressions of lncRNA in atypical hyperplasia tissues and tumors are usually different from those in normal tissues, and some lncRNAs can even be used as marker molecules for tumor prediction[5]. H19 is a maternally expressed gene with a length of 3.0 kb, of which the transcript is an lncRNA with high evolutionary conservation. H19 has the functions of oncogenes[6], and it also has the functions of suppressor genes[7], being closely related to the occurrence of breast cancer, lung cancer and colorectal cancer[8,9]; H19 possesses the functions of both oncogenes and suppressor genes at the same time[6,7]. More and more studies have proven that H19 gene polymorphism is related to the

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occurrence and development of tumors. Some studies reported that rs2839698 in H19 could elevate the risk of gastric cancer[10]. In addition, it was reported by research that rs2839698 in H19 could lower the risk of bladder cancer[11]. According to the latest reports, however, there is no research on association of H19 gene polymorphism with the susceptibility of OSCC. Therefore, in this research it was assumed that H19 gene polymorphism was associated with the susceptibility of OSCC. The case-control study was conducted, which included 362 cases of OSCC and 741 cases of healthy controls with matching age and gender, so as to investigate the association of H19 gene polymorphism with the risk of OSCC.

Patients and Methods

Patients

The case-control study design was utilized in this research, including 362 patients who were histopathologically diagnosed with new-onset OSCC and 741 healthy community controls without history of cancer. All the research objects were the Chinese people of Han nationality who had no kinship with one another. All the cases of OSCC were selected from the First Hospital of Hebei Medical University, without gender and age limit and history of other cancers. Healthy people who participated in screening of chronic diseases in Hebei Province during the same period were randomly selected as the controls, and they were frequency-matched to the cases on basis of age (±5 years old) and gender. Information of the research objects were collected face-to-face. Signed informed consent was obtained from all the research objects, and 5 mL peripheral blood was drawn from them by professional staff at the same time. This study was approved by the Ethics Committee of First Hospital of Hebei Medical University.

Single Nucleotide Polymorphism (SNP) Selection

Region of H19 and its promoter regions were chosen based on the database dbSNP, HapMap and UCSC; the minimum allele frequency (MAF) ≥ 0.05, Hardy-Weinberg equilibrium value (Hardy-Weinberg) ≥ 0.05 and linkage disequilibrium value $r^2 < 0.8$ in Chinese population were selected using Haploview software; finally, 4 polymorphic sites (rs2735971, rs217727, rs2839698 and rs3024270) were selected.

Genotyping

In this research, DNA was extracted from all samples using the traditional ammonia-chloroform method. The Illumina Infinium Human Exome BeadChip platform was used for genotyping. χ^2 goodness of fit test was utilized to analyze whether the distribution of genotype at each site in the controls was in line with the Hardy-Weinberg equilibrium. χ^2 test was used to compare the differences in distribution of gender, age, smoking and drinking between the cases and controls. Univariate and multivariate logistic regression models were adopted for analysis and calculation of odds ratio (OR) and corresponding 95% confidence interval (CI), so as to calculate the statistical correlation between the polymorphic sites and the risk of OSCC. Stratified analysis was performed on age, gender, smoking, drinking and other factors.

Statistical Analysis

SPSS19.0 (Version X; IBM, Armonk, NY, USA) software was used for statistical analyses, and two-sided tests were used for all the statistical tests. $p < 0.05$ suggested that the difference was statistically significant.

Results

As shown in Table I, a total of 362 patients who were histopathologically diagnosed with OSCC, and 741 healthy controls with matching age and gender, were enrolled in this work. The differences in age and gender between the cases and the controls were not statistically significant. The proportions of smoking in the cases and the controls were 38.7% and 35.2%, respectively, and there was no remarkably statistical difference between the two groups ($p = 0.263$). There were 37.0% of patients drinking in the cases and 24.2% in the controls, and the difference was statistically significant ($p < 0.001$). Information of selected sites is shown in Table II, of which the MAF of the selected sites in the controls was > 0.05 and conformed to the law of Hardy-Weinberg equilibrium ($p > 0.05$). The analysis on association of the 4 selected sites with the OSCC in Chinese population is shown in Table III. The results of multivariate logistic regression analysis indicated that the risk of OSCC was significantly elevated by rs217727 after the factors gender, age, smoking and drinking were adjusted (AA *vs.* GG: adjusted OR = 1.20, 95% CI = 1.00-1.44; additive

Table I. Selected characteristics in OSCC cases and controls.

Variables	Case N (%)	Control N (%)	p^a
All subjects	362 (100)	741 (100)	
Age			0.223
< 60	104 (53.9)	185 (48.3)	
≥ 60	89 (46.1)	198 (51.7)	
Gender			0.729
Females	173 (47.8)	350 (47.2)	
Males	189 (52.2)	391 (52.8)	
Smoking			0.263
Ever	140 (38.7)	261 (35.2)	
Never	222 (61.3)	480 (64.9)	
Drinking			< 0.001
Ever	134 (37.0)	179 (24.2)	
Never	228 (63.0)	562 (75.8)	

[a]Two-sided χ^2 test.

Table II. Primary information and minor allele frequencies (MAFs) of selected SNPs.

SNP	Base change	MAF in our controls	HWE
rs2735971	C > T	0.316	0.315
rs217727	G > A	0.420	0.342
rs2839698	G > A	0.416	0.202
rs3024270	G > C	0.432	0.321

HWE = Hardy-Weinberg equilibrium. MAF = minor allele frequency.

model: adjusted OR = 1.22, 95% CI = 1.02-1.46). As shown in Table IV, stratified analysis was performed on gender, age, smoking and drinking. The results indicated that the relationship between rs217727 and the susceptibility of OSCC was more distinct among the senior (patients older than 60-yrs) and female (adjusted OR = 1.53, 95% CI = 1.18-1.98, $p = 0.001$; adjusted OR = 1.31, 95% CI = 1.02-1.69, $p = 0.035$); no significantly statistical correlation was found between other three sites and the susceptibility of OSCC through multivariate logistic regression analysis ($p > 0.05$).

Table III. Logistic regression analysis for associations between selected SNPs and risk of OSCC.

SNP	Genotype	Case (%)	Control (%)	Adjusted OR[a] (95% CI)	p-value[a]
rs2735971	CC	191 (52.9)	351 (47.5)	1.00	
	CT	141 (39.1)	308 (41.7)	0.86 (0.66-1.13)	0.272
	TT	129 (8.0)	80 (10.8)	0.81 (0.64-1.02)	0.076
	Additive model			0.83 (0.68-1.01)	0.063
rs217727	GG	101 (27.9)	255 (34.5)	1.00	
	GA	181 (50.0)	348 (47.0)	1.31 (0.97-1.76)	0.078
	AA	80 (22.1)	137 (18.5)	1.20 (1.00-1.44)	0.050
	Additive model			1.22 (1.02-1.46)	0.031
rs2839698	GG	133 (36.7)	244 (32.9)	1.00	
	GA	171 (47.2)	377 (50.9)	0.83 (0.61-1.07)	0.127
	AA	58 (16.0)	120 (16.2)	0.92 (0.76-1.12)	0.423
	Additive model			0.89 (0.74-1.08)	0.230
rs3024270	GG	104 (28.7)	245 (33.1)	1.00	
	GC	183 (50.6)	350 (47.2)	1.23 (0.92-1.66)	0.164
	CC	75 (20.7)	145 (19.6)	1.10 (0.91-1.32)	0.330
	Additive model			1.11 (0.93-1.34)	0.238

[a]Logistic regression with adjustment for age, sex, smoking and drinking. Significant values ($p < 0.05$) are in bold.

Table IV. Stratified analysis for associations between variant genotype of rs217727 and OSCC risk.

Variables	rs217727		Adjusted[b] OR (95% CI)	p^b
	Cases[a] GG/AG/AA	Controls[a] AA/AG/GG		
Age, yr				
< 60	150/242/50	105/106/87	1.05 (0.79-1.38)	0.748
≥ 60	57/94/51	44/87/29	1.53 (1.18-1.98)	0.001
Gender				
Females	120/198/73	135/150/64	1.31 (1.02-1.69)	0.035
Males	51/99/39	50/82/41	1.12 (0.86-1.44)	0.410
Smoking				
Ever	35/75/30	81/131/49	1.32 (0.95-1.81)	0.094
Never	66/106/50	174/217/88	1.23 (0.98-1.53)	0.071
Drinking				
Ever	33/70/31	55/91/33	1.33 (0.95-1.86)	0.098
Never	68/111/149	200/257/104	1.21 (0.97-21.50)	0.089

[a]Major homozygote/heterozygote/rare homozygote between cases and controls; [b]Logistic regression with adjustment for age, sex, smoking and drinking. Significant values ($p < 0.05$) are in bold.

Discussion

In this case-control study, the statistical association of H19 polymorphism sites with the susceptibility of OSCC in Chinese population was investigated and discussed. We showed that rs217727 was statistically correlated with the risk of OSCC. Results of far-reaching significance were obtained in this research, which confirmed for the first time that genetic variation of H19 might be closely related to the occurrence of OSC. H19 is located on human chromosome 11p15.5. Some studies reported that H19 played an important role in the processes of proliferation, apoptosis, infiltration and metastasis of tumor cells[12]. For instance, the expression of lncRNA H19 in gastric cancer was up-regulate, which was related to its poor prognosis[13]. LncRNA H19 was highly expressed in pancreatic cancer tissues and could promote the metastasis of pancreatic cancer tissues[14]. The expression of H19 was high in hepatocellular carcinoma cell lines, and inhibiting the expression could enhance the invasion and migration ability of hepatocellular carcinoma cells[15]. It was also reported that lncRNA SNPs could influence the expression and function of genes as well as the occurrence of tumors[15]. Changes in lncRNA polymorphic site may affect its stability and expression level, thus influencing the occurrence and development of tumors. Some studies reported that compared with A base, rs11752942G base could significantly increase the expression level of lincRNA-uc003opf by conjugating with micro-RNA-149, thus activating the proliferation of esophageal squamous cell carcinoma cells[16]. For example, the polymorphic site rs7958904 on HOX transcript antisense RNA (HOTAIR) could reduce the risk of colon cancer[17], while rs920778 could raise the risk of gastric cancer[18]. LincRNA-ENST00000515084 rs12325489 polymorphic site could increase the risk of breast cancer[19]. It was observed by research that the polymorphic site rs217727 on H19 was related to the risk of breast cancer (OR = 0.79; 95% CI = 0.55-0.97)[20]. Another study reported that the polymorphic site rs217727 on H19 could elevate the risk of bladder cancer (OR = 1.31, 95% CI = 1.03-1.67)[11]. In this case-control study, it was found that rs217727 could increase the risk of OSCC in Chinese population (additive model: adjusted OR = 1.22, 95% CI = 1.02-1.46; AA *vs.* GG: adjusted OR = 1.20, 95% CI = 1.00-1.44), of which the mechanism might be that the change of rs217727 G > A lowered the stability of H19, thus affecting the expression and raising the probability of OSCC. However, further function tests are required to verify its exact mechanism.

Conclusions

The results of this research discussed the association of H19 gene polymorphisms with the susceptibility of OSCC in Chinese population for the first time. However, it needs a larger sample size to verify the results, and function tests should be performed to explore the specific mechanism.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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