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Association on polymorphisms in LncRNA HOTAIR and susceptibility to HNSCC in Chinese population

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Abstract. – OBJECTIVE: More and more evidence has shown that the critical functions of long non-coding RNA (lncRNA) polymorphism in the carcinogenicity mechanism of a variety of cancers. The association between lncRNA HOX transcript antisense intergenic RNA (HOTAIR) polymorphism and the risk of head and neck squamous cell carcinoma (HNSCC) in Chinese population has not been reported. To investigated the effects of HOTAIR polymorphism on cancer susceptibility, the influence of HOTAIR variants on the risk of HNSCC was analyzed in this study.

PATIENTS AND METHODS: In this case-control study, the tagging SNPs (rs874945, rs4759314, and rs7958904) in HOTAIR gene were genotyped in Chinese population consisting of 366HNSCC cases and 732 controls.

RESULTS: It was found that rs4759314 was associated with a significantly increased risk of HNSCC in Chinese population [GG vs. AA: adjusted odds ratio (OR) = 1.23, 95% confidence interval (CI) = 1.01-1.50; additive model: OR = 1.21, 95%CI = 1.01-1.46]. However, there were no significant associations of rs874945 and rs7958904 with HNSCC risk.

CONCLUSIONS: HOTAIR rs4759314 may influence HNSCC susceptibility and serve as a diagnostic biomarker.

Key Words
LncRNA, HOTAIR, Polymorphism, HNSCC.

Introduction

Head and neck cancer (HNC), predominantly head and neck squamous cell carcinoma (HNSCC), includes cancers of oral cavity, oropharynx, hypopharynx, and larynx. It is the sixth most common cancer in the world[1]. 45,780 new individuals will be diagnosed with cancer of the oral cavity and the pharynx. 8,650 deaths of these diseases will occur in 2015 in the United States according to the most recent available estimates[2]. Applications of tobacco and alcohol have been widely considered to be major risk factors of HNSCC[3]. However, not all individuals with such risk factors ultimately develop HNSCC, suggesting that genetic susceptibility may play an important role in the development of HNSCC.

Non-coding RNAs longer than 200 nucleotides are defined as long noncoding RNAs (lncRNAs)[4]. The ENCODE project suggests that lncRNAs play roles in 76% of the human genome transcription[5]. Growing evidence indicates that lncRNAs could interact with DNA, RNA, and protein at a transcriptional and post-transcriptional level so as to regulate the expression of protein-coding genes, and affect the range of biological processes, such as cell growth, survival, migration, and invasion[4]. The HOX transcript antisense intergenic RNA (HOTAIR) is a member of the lncRNAs generating from the HOXC locus locating on the 12q13.13 of human chromosome. HOTAIR participates in the development and progression of a variety of malignancies including breast cancer[6], colorectal cancer[7], and gastric cancer[8]. A systematic review and meta-analysis[9] revealed that HOTAIR represents a potential biomarker of prognosis in patients with HNSCC. Considering the implication of HOTAIR in cancers and the function of single-nucleotide polymorphisms (SNPs) within lncRNA genes on the expression or structure of lncRNAs, increasing studies were conducted to explore the associations between SNPs within HOTAIR and cancer risks. Association between HOTAIR SNPs and cancers were found[10,11]. However, there is no study on the relationship between HOTAIR polymorphism and HNSCC risk.

Thus, this investigation, a case-control study including 366 HNSCC cases and 732 controls, was conducted to examine the effect of tagSNPs in HOTAIR on HNSCC risk in Chinese population.

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Patients and Methods

Ethics Statement

The institutional Review Board of Beijing Luhe Hospital Capital Medical University approved this case-control study. The design and performance involving human subjects were clearly described in a research protocol. All participants were voluntary and signed the informed consent before this research.

Study Patients

A total of 366 HNSCC patients confirmed by two pathologists were consecutively recruited from Beijing Luhe Hospital Capital Medical University from January 2009 to May 2013. Subjects with second oral cavity cancer primary tumors, primary tumors of the nasopharynx or sinonasal tract, metastasized cancer from other organs, or any histopathologic diagnosis other than oral cavity cancer were excluded. 732 healthy controls were randomly selected from a cohort of more than 12,000 participants in a community-based screening program for non-infectious diseases in Beijing, China. Control subjects were matched to the cases by age (±5 years old) and sex. All participants were genetically unrelated, ethnic Han Chinese population. Data on demographic characteristics (e.g., age and gender) and environmental exposure (e.g., smoking and drinking status) were collected in a face-to-face interview. Each participant donated 5 mL of venous blood and provided a written informed consent.

SNPs Selection and Genotyping

TagSNPs in the HOTAIR genes were selected on the basis of the HapMap database and literature review. We used Haploview software to search tagSNPsMAF (minor allele frequency) >0.05. A linkage disequilibrium value of $r^2<0.8$ was taken into account for SNP selection. Ultimately, three candidate SNPs were chosen: 874945, rs4759314 and rs7958904 in the HOTAIR gene.

Genomic DNA was extracted from a leukocyte pellet by proteinase K digestion, followed by phenol-chloroform extraction and ethanol precipitation. TaqMan allelic discrimination assay on the platform of 7900HT Real-time polymerase chain reaction (PCR) system was performed to distinguish the SNP genotype. Two negative controls (no DNA) were included in each 384-well reaction plate, and the genotyping results were determined by using SDS2.3 Allelic Discrimination Software (Applied Biosystems, Waltham, MA, USA).

Statistical Analysis

The goodness of fit χ^2 was used to test Hardy-Weinberg equilibrium of SNPs among the control subjects. Two-sided χ^2-tests were used to evaluate differences in the distributions of demographic characteristics, selected variables, and genotypes between the cases and controls. The associations of SNPs with HNSCC risks were estimated by computing the crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) using logistic regression analyses. All the statistical analyses were performed with the Statistical Analysis System software (v.9.1.3; SAS Institute, Cary, NC, USA). Two-sided tests were used for statistical analysis and $p<0.05$ represented that the difference was statistically significant.

Results

As shown in Table I, there were no significant differences in the distributions of age and gender between the cases and controls ($p=0.202$ and 0.637, respectively), suggesting a successful matching. However, the proportion of drinkers in the cases was higher than that in the controls (39.0% *vs.* 22.0%, $p=0.011$).

The minor allele frequency (MAF) and the genotyping rate of the three selected SNPs are presented in Table II. The observed genotype frequencies for these three SNPs were all in Hardy-Weinberg equilibrium in the controls ($p=0.183$, 0.224 and 0.336 for rs874945, rs4759314, and rs7958904, respectively). The genotype frequen-

Table I. Selected characteristics in HNSCC cases and controls.

Variables	Case N (%)	Control N (%)	p^a
All subjects	366 (100)	732(100)	
Age			0.202
• <60	148 (40.4)	267 (36.5)	
• ≥60	218 (59.6)	465 (63.5)	
Gender			0.637
• Females	159 (43.4)	329 (44.9)	
• Males	207 (56.6)	403 (55.1)	
Smoking			0.120
• Ever	144 (39.3)	253 (34.6)	
• Never	222 (60.7)	479 (65.4)	
Drinking			0.011
• Ever	106 (39.0)	161 (22.0)	
• Never	260 (71.0)	571 (78.0)	

[a]Two-sided chi-squared test.

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

SNP	Base change	MAF in our controls	HWE
rs874945	G>A	0.292	0.183
rs4759314	A>G	0.386	0.224
rs7958904	C>G	0.419	0.336

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

cies of the three SNPs in the cases and the controls are summarized in Table III. After the adjustment for age, sex, smoking, and alcohol status, multivariate logistic regression analysis revealed that rs4759314 was significantly associated with an increased risk of HNSCC (GG *vs.* AA: adjusted OR=1.23, 95%CI=1.01–1.50, additive model: OR=1.21, 95%CI=1.01–1.46). No significant associations of variant genotypes with the other two SNPs and HNSCC risk were observed.

The stratification analysis on the associations between rs4759314 and HNSCC risk from age, sex, smoking, and drinking were further conducted. As shown in Table IV, the significant association of rs4759314 with HNSCC risk was found among the older group (adjusted OR=1.32, 95%CI=1.01-1.72, p=0.040) and drinkers (adjusted OR=1.50, 95%CI=1.02-2.19, p=0.040).

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

SNP	Genotype	Case (%)	Control (%)	Adjusted OR[a] (95%CI)	p-value[a]
rs874945	GG	191 (52.2)	358(48.9)	1.00	
	AG	152 (41.5)	317(43.3)	0.91 (0.70-1.18)	0.471
	AA	22 (6.0)	55(7.5)	0.87(0.67-1.13)	0.304
	Additive model			0.88(0.72-1.09)	0.241
rs4759314	AA	113 (30.9)	268(36.6)	1.00	
	AG	188 (51.4)	362(49.5)	1.24(0.93-1.66)	0.148
	GG	65 (17.8)	101(13.8)	1.23(1.01-1.50)	0.042
	Additive model			1.21(1.01-1.46)	0.043
rs7958904	CC	140 (38.3)	241(32.9)	1.00	
	CG	167 (45.6)	369(50.4)	0.77(0.58-1.02)	0.063
	GG	59 (16.1)	122(16.7)	0.90(0.74-1.09)	0.273
	Additive model			0.87(0.72-1.04)	0.132

[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 rs4759314 and HNSCC risk.

Variables	rs4759314		Adjusted b OR(95%CI)	p^b
	Cases[a] AA/AG/GG	Controls[a] AA/AG/GG		
Age, yr				
• <60	46/65/37	98/102/66	1.11 (0.85-1.44)	0.460
• ≥60	67/123/28	170/260/35	1.32 (1.01-1.72)	0.040
Gender				
• Females	63/67/29	140/151/37	1.23 (0.93-1.62)	0.143
• Males	50/121/36	128/211/64	1.18 (0.92-1.53)	0.199
Smoking				
• Ever	34/80/30	79/130/44	1.27 (0.93-1.74)	0.134
• Never	79/108/35	189/232/57	1.18 (0.93-1.50)	0.165
Drinking				
• Ever	26/55/25	53/88/20	1.50 (1.02-2.19)	0.040
• Never	87/133/40	215/274/81	1.13 (0.91-1.41)	0.268

[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 study, the associations of 3 selected SNPs in HOTAIR with HNSCC risk were evaluated in a case-control study, which showed that rs4759314 was associated with HNSCC risk in this Chinese population. As far as it concerns, this is the first study to evaluate the associations between HOTAIR polymorphism and HNSCC risk.

As reported, HOTAIR can function as a molecular scaffold, targeting Polycomb Repressive Complex 2 (PRC2)[6] and Lysine Specific Demethylase 1 (LSD1) complexes[12] to specific genes on a genome-wide level and lead H3K27 to methylate and H3K4 to demethylate and silence the expression of related genes. Additionally, HOTAIR can influence the suppression of target miRNAs by acting as a miRNA sponge[13]. Recently, increasing evidence has revealed aberrant expression of HOTAIR in a wide range of cancers, and the expression level of HOTAIR may serve as a potential biomarker for diagnosis, metastasis, and prognosis, indicating HOTAIR is involved in the etiology of related cancers[6-8].

Efforts were made to explore whether polymorphisms could influence the expression or the structure of HOTAIR, thus affecting individual susceptibility to cancers. The rs4759314 polymorphism is located in the first intron of HOTAIR. Du et al[14] found that rs4759314 contributes to an increased risk of gastric cancer. This study revealed that rs4759314 was associated to HNSCC risk. A recent meta-analysis[15] including 28,527 cases and 37,151 controls has concluded that rs920778 in high LD (r^2=1) with rs7958904 was related to HNC susceptibility. However, the association between rs7958904 and HNSCC risk was not found in this study. This difference may due to the relatively small sample size or the complex function of HOTAIR. Further studies on the interaction effect of rs7958904 C allele and rs920778 T allele on the biological behavior of CC cells are warranted. The rs874945 polymorphism is located in the 3' near the gene of HOTAIR. Wang et al[16] reported that there is no association between rs874945 and noise-induced hearing loss. Jin et al[17] found no association between rs874945 and cervical cancer risk. Another study[18] indicated that rs874945 makes no contribution to colorectal cancer susceptibility.

Conclusions

rs4759314 is in the first intron of HOTAIR, which is significantly associated with increased risk of HNSCC. Further functional studies on the effect of rs4759314 on biological behavior are needed to confirm and extend our findings.

Conflict of Interest:

The authors declare no conflict of interest.

References

1) Liu B, Ding JF, Luo J, Lu L, Yang F, Tan XD. Seven protective miRNA signatures for prognosis of cervical cancer. Oncotarget 2016; 7: 56690-56698.
2) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
3) Maier H, Dietz A, Gewelke U, Heller WD, Weidauer H. Tobacco and alcohol and the risk of head and neck cancer. Clin Investig 1992; 70: 320-327.
4) Zhang CG, Yin DD, Sun SY, Han L. The use of lncRNA analysis for stratification management of prognostic risk in patients with NSCLC. Eur Rev Med Pharmacol Sci 2017; 21: 115-119.
5) Gerstein MB, Kundaje A, Hariharan M, Landt SG, Yan KK, Cheng C, Mu XJ, Khurana E, Rozowsky J, Alexander R, Min R, Alves P, Abyzov A, Addleman N, Bhardwaj N, Boyle AP, Cayting P, Charos A, Chen DZ, Cheng Y, Clarke D, Eastman C, Euskirchen G, Frietze S, Fu Y, Gertz J, Grubert F, Harmanci A, Jain P, Kasowski M, Lacroute P, Leng JJ, Lian J, Monahan H, O'Geen H, Ouyang Z, Partridge EC, Patacsil D, Pauli F, Raha D, Ramirez L, Reddy TE, Reed B, Shi M, Slifer T, Wang J, Wu L, Yang X, Yip KY, Zilberman-Schapira G, Batzoglou S, Sidow A, Farnham PJ, Myers RM, Weissman SM, Snyder M. Architecture of the human regulatory network derived from ENCODE data. Nature 2012; 489: 91-100.
6) Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.
7) Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 2011; 71: 6320-6326.
8) Li T, Mo X, Fu L, Xiao B, Guo J. Molecular mechanisms of long noncoding RNAs on gastric cancer. Oncotarget 2016; 7: 8601-8612.
9) Troiano G, Caponio V, Boldrup L, Gu X, Muzio LL, Sgaramella N, Wang L, Nylander K. Expression of the long non-coding RNA HOTAIR as a prognostic factor in squamous cell carcinoma of the head and neck: a systematic review and meta-analysis. Oncotarget 2017; 8: 73029-73036.
10) Guo W, Dong Z, Bai Y, Guo Y, Shen S, Kuang G, Xu J. Associations between polymorphisms of

HOTAIR and risk of gastric cardia adenocarcinoma in a population of north China. Tumour Biol 2015; 36: 2845-2854.

11) Bayram S, Sumbul AT, Dadas E. A functional HOTAIR rs12826786 C>T polymorphism is associated with breast cancer susceptibility and poor clinicopathological characteristics in a Turkish population: a hospital-based case-control study. Tumour Biol 2016; 37: 5577-5584.

12) Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010; 329: 689-693.

13) Ma MZ, Li CX, Zhang Y, Weng MZ, Zhang MD, Qin YY, Gong W, Quan ZW. Long non-coding RNA HOTAIR, a c-Myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer. Mol Cancer 2014; 13: 156.

14) Du M, Wang W, Jin H, Wang Q, Ge Y, Lu J, Ma G, Chu H, Tong N, Zhu H, Wang M, Qiang F, Zhang Z. The association analysis of lncRNA HOTAIR genetic variants and gastric cancer risk in a Chinese population. Oncotarget 2015; 6: 31255-31262.

15) Pan W, Wu C, Su Z, Duan Z, Li L, Mi F, Li C. Genetic polymorphisms of non-coding RNAs associated with increased head and neck cancer susceptibility: A systematic review and meta-analysis. Oncotarget 2017; 8: 62508-62523.

16) Wang B, Ding E, Shen H, Wang J, Sun K, Chen S, Han L, Zhang H, Zhu B, Xu M. Association of TagSNP in lncRNA HOTAIR with susceptibility to noise-induced hearing loss in a Chinese population. Hear Res 2017; 347: 41-46.

17) Jin H, Lu X, Ni J, Sun J, Gu B, Ding B, Zhu H, Ma C, Cui M, Xu Y, Zhang Z, Lercher M, Chen J, Gao N, Wang S. HOTAIR rs7958904 polymorphism is associated with increased cervical cancer risk in a Chinese population. Sci Rep 2017; 7: 3144.

18) Xue Y, Gu D, Ma G, Zhu L, Hua Q, Chu H, Tong N, Chen J, Zhang Z, Wang M. Genetic variants in lncRNA HOTAIR are associated with risk of colorectal cancer. Mutagenesis 2015; 30: 303-310.