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Association between H19 polymorphisms and osteosarcoma risk

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Abstract. – OBJECTIVE: The long non-coding RNA (lncRNA) H19, a maternally expressed imprinted gene, has involvement in cancer susceptibility and disease progression. However, the association between H19 polymorphisms and osteosarcoma susceptibility has remained elusive. We designed this case-control study to explore the association between H19 polymorphism and osteosarcoma risk.

PATIENTS AND METHODS: In this study, we genotyped 4 tagger SNPs of the H19 gene in a case-control study including 193 osteosarcoma cases and 393 cancer-free controls.

RESULTS: For the main effect analysis, rs217727 (G>A) was associated with osteosarcoma risk (GA/GG: adjusted OR = 1.51, 95% CI: 1.06-2.17, *p* = 0.024; AA/GG: adjusted OR = 1.89, 95% CI: 1.23-2.91, *p* = 0.004; additive model: adjusted OR = 1.35, 95% CI: 1.01-1.80, *p* = 0.043).

CONCLUSIONS: This finding indicates that rs217727 polymorphism may play a role in genetic susceptibility to the risk of osteosarcoma, which may improve our understanding of the potential contribution of H19 SNPs to cancer pathogenesis.

Key Words:
Osteosarcoma, H19, Polymorphism, Susceptibility, Association.

Introduction

Osteosarcoma is an aggressive bone malignancy originating from the mesenchyme cells predominantly intrudes the long tubular bones including the distal femur, proximal tibia, and humeral. Osteosarcoma occurs generally in youths between 10 and 25 years old, and represents the eighth most common cancer in children[1]. According to record, 4.4 out of 1 million were newly diagnosed as osteosarcoma each year among individuals less than 24 years[2]. The pathogenesis of osteosarcoma is not totally understood; however, findings showed that osteosarcoma is a polygenic disease, and heritage and environment are related to its development and progression[3-5]. Long non-coding RNAs (lncRNAs) are non-coding, single stranded RNAs of more than 200 nucleotides which are lacking of protein encoding capability[6,7]. LncRNAs are reported to regulate the expression of cancer related genes at transcription level or post-transcription level[8]. Several lncRNAs were shown to be aberrantly expressed in different cancers, and this suggests that lncRNAs contribute to carcinogenesis and deliver functions in regulating cell cycle progression, apoptosis, invasion, and migration[9]. LncRNA H19, an imprinted gene and located on human chromosome 11p15.5, encodes a 2.3kb long, capped, spliced, and poly-adenylated noncoding RNA, which plays important roles in embryonic development and growth control[10-13]. Increasing evidence suggests that H19 is abnormally expressed in lung, breast, liver and esophageal tumors[14-17]. H19 level was increasingly expressed in bladder cancer tissues, and elevated expression promoted bladder cancer cell proliferation and migration *in vitro*[18]. Additionally, the differentially methylated regions (DMRs), which located upstream of H19, were reported to play a role in the regulation of H19 gene expression[19, 20]. Gao et al[21] reported that the methylation status of H19 DMRs had a relation to the occurrence, development, and metastasis of esophageal squamous cell carcinoma (ESCC). H19 genetic variants were reported to play important roles in the development of cancers. CT+TT genotypes of rs217727 in h19 were significantly associated with decreased risk of breast cancer and increased risk of gastric cancer and bladder cancer[22-24]. Mutation

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Table I. Primers and probes for H19 tagSNPs genotyping.

TagSNPs	Primer (5′-3′)	Probe (5′-3′)
rs2735971	F: CACCTCCGATTCCACAACTACA	T allele: FAM-CCAATTCTGTGCCATC-MGB
	R: GAGGCTTCCCCTTCAGTCTCA	C allele: FAM-CAATTCCGTGCCATC-MGB
rs217727	F: CAAAGAGACAGAAGGATGAAAAAGAA	G allele: FAM-TCAACCGTCCGCCG-MGB
	R: CGGCGACTCCATCTTCATG	A allele: FAM-TCAACCGTCCACCGC-MGB
rs2839698	F: CATCGTCCCCAGCTGATGTC	G allele: FAM-CTGGGCGCCTACT-MGB
	R: GGAGTGATGACGGGTGGAG	A allele: FAM-CCTGGGCACCTAC-MGB
rs3024270	F: CTTCCTGGTGAGCGTGTCTG	C allele: FAM-TCACTGCCCCCACCT-MGB
	R: AAGTAAAGAAACAGACCCGCTTCTT	G allele: FAM-TCACTGCCCCGACCT-MGB

Table II. Selected characteristics in osteosarcoma cases and control

Variables	Case N (%)	Control N (%)	*p*-value[a]
All subjects	193 (100)	383 (100)	
Age			0.206
<20 (median)	104 (53.9)	185 (48.3)	
≥20 (median	89 (46.1)	198 (51.7)	
Gender			0.729
Females	80 (41.5)	153 (439.9)	
Males	113 (58.5)	230 (60.1)	
Tumor location			
Extremities	156 (80.8)		
Other site	37 (19.2)		
Metastasis			
Yes	64 (33.2)		
No	129 (66.8)		
Enneking stages			
I- II	120 (62.2)		
III	73 (37.8)		

of rs2839698 in h19 had decreased risk of bladder cancer[25]. Nevertheless, no study has investigated the role of H19 polymorphisms in osteosarcoma. In this work, we hypothesized that single nucleotide polymorphisms (SNPs) in h19 may be associated with the risk of osteosarcoma. To test this hypothesis, we conducted a case-control study including 193 osteosarcoma cases and 383 controls to examine the effect of 4 selected SNPs in H19 on osteosarcoma risk in a Chinese population.

Patients and Methods

Patients

This study is a hospital-based case-control study. The study subjects were obtained from the First People's Hospital of Changzhou between January 2009 and May 2016. All newly osteosarcoma patients historically were confirmed by two pathologists and none of them had received adjuvant chemotherapy or radiotherapy before surgery. The controls had no self-reported history of malignancies, and they were matched to the cases in terms of age (±5 years) and sex. All participants were genetically unrelated, ethnic Han Chinese population. Each subject donated 5 ml of blood for genomic DNA extraction and they were surveyed by face-to-face interviews to collect their information on demographic data, such as age, sex, hypertension, diabetes mellitus, smoking history and residence.

Ethics Statement

This case-control study was approved by the institutional Review Board of the First People's Hospital of Changzhou. All participants were voluntary and would complete the informed consent in written before taking part in this research.

SNPs Selection and Genotyping

The region including lncRNA H19 and its promoter, the DMR, was targeted in chromosome 11, position 2016406-2021693 (data obtained from

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

SNP	Genotype	Case (%)	Control (%)	Adjusted OR a(95%CI)	*p*-value[a]
rs2735971	CC	88 (45.6)	169 (44.1)	1.00	
	CT	94 (48.7)	182 (47.5)	1.05 (0.72-1.52)	0.816
	TT	11 (5.7)	32 (8.4)	0.92 (0.61-1.38)	0.674
	Additive model			0.95 (0.70-1.29)	0.740
rs217727	GG	79 (40.9)	195 (50.9)	1.00	
	GA	102 (52.8)	165 (43.1)	1.51 (1.06-2.17)	0.024
	AA	12 (6.2)	23 (6.0)	1.89 (1.23-2.91)	0.004
	Additive model			1.35 (1.01-1.80)	0.043
rs2839698	GG	83 (43.0)	178 (46.5)	1.00	
	GA	98 (50.8)	175 (45.7)	1.35 (0.93-1.95)	0.112
	AA	12 (6.2)	30 (7.8)	1.03 (0.70-1.52)	0.892
	Additive model			1.23 (0.91-1.66)	0.175
rs3024270	GG	85 (44.0)	173 (45.2)	1.00	
	GC	91 (47.23)	179 (46.7)	1.03 (0.72-1.48)	0.882
	CC	17 (8.8)	31 (8.1)	1.14 (0.81-1.62)	0.457
	Additive model				
	1.08 (0.82-1.42)	0.607			

[a]Logistic regression with adjustment for age, sex.

the UCSC, Feb. 2009 assembly). Linkage disequilibrium value ($r^2 < 0.8$) and minor allele frequency (MAF ≥ 0.05) in the Chinese Han population (CHB) were set as thresholds for the SNPs selection. Finally, four tag SNPs were identified: rs2735971, rs217727, rs2839698 and rs3024270.

H19 SNPs Genotyping

Genomic DNA was isolated from leukocyte pellets of venous blood by proteinase K digestion and followed by phenol chloroform extraction. Nanodrop and DNA electrophoresis were used to check the quality and quantity of the DNA samples before genotyping. Genotyping was carried out using the TaqMan allelic discrimination assay on an ABI 7900 system (Applied Biosystems, Foster City, CA, USA). Briefly, PCR primers and Taqman minor groove binder probes (Table I) were designed and reactions were performed in 384-well micro-plates with ABI 9700 thermal cyclers (Applied Biosystems). The genotyping results were determined by System SDS software version 2.3 (Applied Biosystems, Foster City, CA, USA). 10% of the total samples were randomly selected for genotyping twice and accordance achieved 100%.

Statistical Analysis

Distributions of selected variables, age and sex, between the cases and controls were evaluated using the χ^2 test. Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies to expected frequencies among the control subjects. Logistic regression analyses were performed to compute odds ratios (ORs) and their 95% confidence intervals (CIs) for the associations between the genotypes and the risk of osteosarcoma adjusted by age and gender. All the statistical analyses were performed with Statistical Analysis System software (v.9.1 SAS Institute, Cary, NC, USA). The significance was considered at $p<0.05$ with two-sided tests.

Results

Our study included 193 osteosarcoma cases and 383 healthy controls. Distributions of physiological characteristics in case and control groups are presented in Table II. There was no significant difference in the distributions of age and sex (p = 0.206, 0.729, respectively), suggesting a successful matching. Of the 193 cases, osteosarcomas occur in extremities among 156 cases and occur in other sites among other 37 cases. No significant deviations from Hardy-Weinberg equilibrium for the 4 polymorphisms was found among the 383 healthy controls ($p > 0.05$). Logistic regression analyses revealed rs217727 was associated with increased risk of osteosarcoma (GA *vs.* GG: adjusted OR = 1.51, 95% CI = 1.06-2.17; AA *vs.* GG: adjusted OR = 1.89, 95% CI = 1.23-2.91; additive model: adjusted OR = 1.35, 95% CI = 1.01-1.80). No significant association was founded between the other three SNPs and osteosarcoma risk (Table III). Further, we performed a stratifica-

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

	rs217727			
Variables	Cases a GG/AG/AA	Controls[a] AA/AG/GG	Adjusted[b] OR (95%CI)	p^b
Age, yr				
<20	8/47/49	14/72/99	1.02 (0.68-1.52)	0.932
≥20	4/55/30	9/94/95	1.60 (1.01-2.54)	0.047
Gender				
Females	7/45/28	9/56/88	1.63 (1.02-2.59)	0.042
Males	5/57/51	14/109/107	1.20 (0.81-1.78)	0.367
Tumor location				
Extremities	9/88/59	23/165/195	1.45 (1.06-1.98)	0.021
Other site	3/14/20	23/165/195	1.19 (0.66-2.13)	0.560
Metastasis				
Yes	8/76/45	23/165/195	1.32 (0.85-2.05)	0.213
No	4/26/34	23/165/195	1.43 (1.02-2.01)	0.039

[a]Rarer homozygote/heterozygote/major homozygote between cases and controls;
[b]Logistic regression with adjustment for age, sex.

tion analysis between rs217727 and osteosarcoma risk by age, gender, tumor location and metastasis or not. In this analysis, we assumed that the individuals with age < 20 can be considered as younger subjects, and the ones with age ≥ 20 can be defined as older subjects. We found effect of rs217727 on osteosarcoma risk was evident in females (adjusted OR = 1.63, 95% CI = 1.02-2.59, p = 0.042), older subjects (adjusted OR =1.60, 95% CI = 1.01-2.54, p = 0.047) and tumor in extremities (adjusted OR =1.45, 95% CI = 1.06-1.98, p = 0.021). However, heterogeneity test showed no significant heterogeneity exits in every two stratums (p>0.05) (Table IV).

Discussion

In this case-control study, we examined associations between tagSNPs in lncRNA H19 and osteosarcoma susceptibility. We identified rs217727 to be significantly associated with osteosarcoma risk in a Chinese population. To the best of our knowledge, this is the first research to examine the role of H19 polymorphism in osteosarcoma risk. Increasing studies are revealing the important role of lncRNAs play in cancer pathogenesis and metastasis[26,27]. H19 expressed with a high level in bladder cancer tissues when compared with paired normal controls and that could promote the proliferation of bladder cancer cell[18]. Further research showed that patients with a larger fraction of H19-positive cells in biopsies are potentially at higher risk cancer metastasis[28]. SNPs in the H19 gene or promoter region may affect the expression of H19 or the structure and function of the H19 RNA. As a consequence, H19 variants may play a role in osteosarcoma risk. Previous studies have found the association between H19 SNPs and cancer risk[22,23]. In our work, we detected that rs217727 was significantly with increased risk of osteosarcoma; however, no association was found between the other three selected SNPs. In the stratification analysis, we found effect of rs217727 on osteosarcoma risk was evident in females, older subjects, and cases of tumor in extremities. Heterogeneity test showed no significant heterogeneity existing. Hua et al[24] reported rs217727 polymorphism is significantly associated with increased risk of bladder cancer; however, Verhaegh et al[25] found that rs217727 decreased the susceptibility of breast cancer. This difference may due to the various pathogenesis of different cancers. The H19 gene is located in a cluster with the insulin-like growth factor 2 (IGF2) gene on chromosome11p15.5. Researches reported an association between the T allele of rs217727 and enhanced birth weight as well as increased IGF2 cord blood levels[29]. It has been shown that H19 RNA can interfere with IGF2 in transcription[30]. IGF2 have been founded with a high expression in cancer tissues[31]. It seems implausible that rs217727 polymorphism may increase osteosarcoma risk by increasing IGF2 levels.

Conclusions

Our work provides the evidence that H19 polymorphisms may be associated with osteosarcoma risk in Chinese Han population. However, an independent replication study is needed to confirm

our findings in diverse populations, and further functional analyses are also necessary to uncover the underlying mechanism.

Conflict of interest

The authors declare no conflicts of interest.

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