JBUON 2021; 26(2): 587-591
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Correlation between lncRNA H19 rs2839698 polymorphism and susceptibility to NK/T cell lymphoma in Chinese population

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Summary

Purpose: *To explore the influence of long non-coding ribonucleic acid (lncRNA) small nucleolar RNA host gene 1 (SNHG1) on the proliferation and apoptosis of gastric cancer cells.*

Methods: *NKTCL patients (n=573) and healthy participants (n=688) were recruited. Their blood samples were collected for detecting H19 rs2839698 polymorphism and its genotypes using PCR-RFLP. The correlations of H19 rs2839698 polymorphism with NKTCL susceptibility and pathological indexes were analyzed by logistic regression analysis.*

Results: *No significant differences in age, body mass index (BMI), smoking, drinking and family history of cancer were detected between NKTCL patients and healthy participants. Hypertension and diabetes were statistically significant between groups. H19 rs2839698 polymorphism and its genotypes were correlated with NKTCL susceptibility. Moreover, compared with NKTCL patients carrying GG allele, patients carrying AG, AA and AG+AA alleles had more advanced tumor stage and higher incidence of EBV(+) and original involvement of non-paranasal structure.*

Conclusions: *LncRNA H19 rs2839698 polymorphism and its genotypes are correlated with NKTCL susceptibility.*

Key words: *LncRNA H19 rs2839698, polymorphism, NKTCL, genetic susceptibility*

Introduction

NK/T cell lymphoma (NKTCL) is a relatively rare invasive lymphoma, mainly attacking NK cells and/or T cells. These cells are responsible for defending against viruses, bacteria and tumor cells [1,2]. NKTCL is named because most of malignant components are derived from mature NK cells, and a few are derived from NK-like T cells [3,4]. In fact, NK/T cells do not exist. Histological characteristics of NKTCL include vascular centered lesions and extensive infiltration of lymphoma cells, resulting in obvious ischemic necrosis and formation of neoplastic tissues [5]. NKTCL is relatively rare in European and North American population, accounting for less than 1% of all lymphomas. However, it is more common in East Asia and South America. In China and Japan, NKTCL covers 6-10% of lymphomas cases [6,7]. Adult males are more attacked by NKTCL, and the sex ratio is about 3-4:1. The mean age of NKTCL onset is 40-50 years. Children and adolescents are occasionally affected by NKTCL [7,8]. It is generally accepted that EBV infection, race and geographic location are related to the occurrence of NKTCL [9,10]. Great efforts are required on exploring its pathogenesis.

LncRNAs belong to noncoding RNA family exceeding 200 nucleotides long. They are extensively

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Received: 22/11/2020; Accepted: 15/01/2021

involved in biological activities [11,12]. Several lncRNAs have been identified to affect cell functions of NKTCL [13,14]. The correlation between lncRNA polymorphism and NKTCL susceptibility remains largely unclear. Previous studies have proven that H19 SNPs remarkably reduces the risk of bladder cancer in European and American population, and its protective effect is more obvious on superficial bladder cancer [15,16]. This study selected tag single nucleotide polymorphisms (SNPs) of H19 based on the data of the Thousands Genome Project. Differential distribution of H19 genotypes in NKTCL patients and healthy participants was compared, aiming to explore the correlation between H19 rs2839698 and the risk of NKTCL in Chinese population.

Methods

Study population

This was a case-control study. NKTCL patients (n=573) of our hospital were recruited January 2008 to December 2019. They did not have other malignancies, urinary system diseases or smoking-related diseases, and were not treated by chemotherapy or radiotherapy. During the same period, age- and sex-matched healthy participants (n=688) had physical examination and were recruited as controls. All participants were of Han nationality, and they did not have blood relations. Non-smokers were defined as less than one cigarette a day and a year. Drinkers were defined as drinking at least three times a week, for more than six months. Family history of cancer was defined as the presence of cancer in any first-degree relatives (parents, siblings or offsprings). This study was approved by the Ethics Committee of Jingzhou Central Hospital. Signed written informed consents were obtained from all participants before the study entry.

PCR-RFLP Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP)

Genomic DNA was isolated and purified from peripheral blood lymphocytes by proteinase K digestion and phenol-chloroform method. H19 rs2839698 and its alleles were detected using PCR-RFLP (Applied Biosystems, Foster City, CA, USA). SNP primers were amplified at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. PCR products were cleaved by BccI, loaded on 1% DNA agarose gel containing $C_{21}H_{20}BrN_5$, and analyzed.

Statistics

SPSS 22.0 statistical software (IBM, Armonk, NY, USA) was utilized for statistical analyses. Enumeration data were expressed as frequency (%). The HWE of control genotype distribution, and comparison of enumeration data were evaluated using the x^2 test. Risk factors of NKTCL were assessed by logistic regression analysis, and results were expressed as odds ratio (OR) and 95% CI. P<0.05 indicated statistical significance.

Table 1. Distribution of selected variables between the NK cell lymphoma cases and the control subjects

Variables	*Cases (n=573)* *n (%)*	*Controls (n=688)* *n (%)*	*p value*
Age (mean ± SD), years	45.44±10.04	45.23±11.23	0.545
BMI (mean±SD), kg/m^2	24.34±2.65	24.15±3.08	0.765
Sex			
Male	358 (62.48)	383 (55.57)	0.687
Female	215 (37.52)	305 (44.33)	
Smoking status			
Never	484 (84.47)	519 (75.44)	0.515
Ever	89 (15.53)	169 (24.56)	
Drinking status			
Never	406 (70.86)	540 (78.49)	0.908
Ever	167 (29.14)	148 (21.51)	
Family			
No	408 (71.20)	578 (84.01)	0.826
Yes	165 (28.80)	110 (15.99)	
Hypertension			
No	415 (72.43)	454 (65.99)	<0.001
Yes	158 (27.57)	234 (34.01)	
Diabetes			
No	418 (72.95)	508 (73.84)	<0.001
Yes	155 (27.05)	180 (26.16)	

T-test for age distributions between the cases and controls; Two-sided x^2 test for others selected variables between the cases and controls.

Table 2. Summary of the clinicopathologic features of NK cell lymphoma studied

Variables	*Cases (n = 573)* *n (%)*
Clinical stage	
I	346 (0.60)
II	103 (0.18)
III	79 (0.14)
IV	45 (0.08)
EBV serology test	
Negative	248 (0.43)
Positive	325 (0.57)
Originally involved site	
Paranasal structure	480 (0.84)
Other sites	93 (0.16)

Results

Characteristics of NKTCL patients and healthy controls

NKTCL patients (n=573) and healthy participants (n=688) were recruited and their clinical data were recorded for analysis. No significant differences in age, BMI, smoking, drinking and family history of cancer were detected between NKTCL patients and healthy participants. However, the incidence of hypertension and diabetes were significantly different between groups (Table 1).

Further analysis on the clinical stage, Epstein B virus (EBV) serological test, and original involvement site of NTKTL patients was conducted. Classified by clinical stage, the number of stage I-IV NKTCL patients was 346, 103, 79 and 45, respectively. A total of 248/573 NKTCL patients had nega-

Table 3. The basic information of the genotyped polymorphisms in lncRNA H19 rs2839698 associated with NK cell lymphoma risk

Polymorphisms	*Cases (n=573)* *n (%)*	*Controls (n=688)* *n (%)*	*p**	*Adjusted OR (95% CI)*
rs2839698				
GG	192 (33.51)	351 (51.02)		1.00 (reference)
AG	244 (42.58)	248 (36.05)	0.001	1.20 (1.07-1.34)
AA	137 (23.91)	89 (12.93)	0.001	1.44 (1.15-1.79)
AG+AA	381 (66.49)	426 (48.98)	0.011	1.21 (1.05-1.40)
G allele	628 (54.80)	950 (69.04)		1.00 (reference)
A allele	518 (45.20)	426 (30.96)	0.032	1.22(1.01-1.91)

*Two-sided x^2 test for either genotype distributions or allele frequencies between the cases and controls. Adjusted for age, smoking status, drinking status and family history of cancer in logistic regression model; 95% CI: 95% confidence interval.

Table 4. The association of lncRNA H19 rs2839698 polymorphism and clinicopathologic characteristics of NK cell lymphoma patients

Variables	*Risk allele*		*p**	*Adjusted OR (95% CI)*
	GG *n (%)*	*AG+AA* *n (%)*		
Clinical stage	192	381		
I	107 (55.73)	176 (46.19)		1.00 (reference)
II	45 (23.44)	105 (27.56)	0.904	0.97 (0.60-1.58)
III	20 (10.42)	33 (8.66)	0.021	1.67 (1.47-1.94)
IV	20 (10.41)	67 (17.59)	0.015	1.50 (1.29-2.88)
EBV serology test				
Negative	102 (53.13)	164 (43.04)		1.00 (reference)
Positive	90 (46.87)	217 (56.96)	0.024	1.96 (1.41-3.29)
Originally involved site				
Paranasal structure	108 (56.25)	218 (57.22)		1.00 (reference)
Other sites	84 (43.75)	163 (42.78)	0.016	1.63 (1.29-2.36)

*Two-sided x^2 test for number of alleles in cases and controls; 95% CI: 95% confidence interval. Adjusted for age, BMI, gender, smoking status, drinking status and history of hypertension and diabetes in logistic regression model.

tive result of EBV serological test. In addition, 480 patients had an original involvement of paranasal structures, while 93 were originally involved by other sites (Table 2).

Association between NKTCL risk and genetic polymorphism of H19 rs2839698

H19 rs2839698 polymorphism and its genotypes were correlated with NKTCL susceptibility. Compared with NKTCL patients carrying GG allele, patients carrying AG, AA and AG+AA alleles had higher susceptibility to NKTCL [OR=1.20 (1.07-1.34), 1.44 (1.15-1.79) and OR=1.21 (1.05-1.40), respectively]. Moreover, higher risk of NKTCL was detected in people carrying A allele of H19 rs2839698 [OR=1.22 (1.01-1.91)] (Table 3).

Combined analysis between H19 rs2839698 polymorphisms and clinical data of NKTCL

Compared with NKTCL patients carrying GG allele, patients carrying AG, AA and AG+AA alleles were more possible to have stage III, stage IV, EBV (+) and original involvement of non-paranasal structures (OR=s1.67, 1.50, 1.96 and 1.63, respectively) (Table 4).

Discussion

Targeted drug therapy has become an inevitable trend in the clinical treatment of human cancers, including sequential and concurrent methods. Besides, hematopoietic stem cell transplantation (HSCT), especially allogeneic HSCT (Allo-HSCT), is recommended to recurrent or refractory cancer [1,5,9]. There is increasing evidence that gene variations not only lead to lymphoma genesis, but also regulate the growth of lymphoid tissues and the immune system [1,3]. It is reported that polymorphisms of some immune-related genes are involved in the pathogenesis of NKTCL and its subtypes, suggesting the great significance of gene polymorphisms in NKTCL [17].

LncRNAs are a hotspot in the field of life science research [11,12]. Although lncRNAs are not directly involved in encoding proteins, they exert vital functions in cell behaviors [12]. The biology of lncRNAs in human cancers has been emerged [18,19]. H19 locates on human chromosome 11p15.5, with a length of 2300 bp [15]. Except for adult skeletal muscles and the heart, H19 expression is induced in the embryonic stage and its level is downregulated after birth [15,20]. H19 acts as an oncogene in gastric cancer, bladder cancer and pancreatic cancer, while it is a tumor-suppressor gene involved in prostate cancer [20,21]. High activity of H19 rs2839698 polymorphism may affect cancer development. Recent studies obtained inconsistent findings on the correlation between H19 rs2839698 polymorphism and NKTCL susceptibility. In the present study, H19 rs2839698 polymorphism and its genotypes enhanced NKTCL susceptibility. Compared with NKTCL patients carrying GG allele, patients carrying AG, AA and AG+AA alleles had more advanced tumor stages, and higher incidences of EBV (+) and original involvement of non-paranasal structures.

Notably, selected participants were all of Han nationality from Liaoning Province. A small sample size and a single race may lead to potential biases. Besides, the analyzed H19 SNP was located in the intron regions. Whether SNPs will affect H19 expression should be further analyzed.

Conclusions

LncRNA H19 rs2839698 polymorphism and its genotypes are correlated with NKTCL susceptibility, and it is an important genetic susceptibility gene of NKTCL. Our findings should be further validated in larger sample size experiments.

Funding acknowledgements

Hubei Provincial Health Committee scientific research projects (No.WJ2019M081).

Conflict of interests

The authors declare no conflict of interests.

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