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Original Article

Association between *MLL3* genetic polymorphisms and development of laryngeal cancer in a Chinese population

Jiancheng Tan, Xinguo Liu, Huixian Zhao, Jinyan Xing

Department of ENT and HN Surgery, Zhumadian Central Hospital, Zhumadian, China

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Abstract: We firstly conducted a case-control study to investigate the role of two SNPs in *MLL3* gene (rs6943984 and rs4725443) in the development of laryngeal cancer in a Chinese population. Between May 2012 and October 2014, a total of 171 patients with laryngeal cancer and 251 control subjects were collected from the Zhumadian Central Hospital. Genotyping of *MLL3* rs6943984 and rs4725443 were analyzed using polymerase chain reaction coupled with restriction fragment length polymorphism. The chi-square test revealed that individuals with the laryngeal cancer were more likely to be males, have a habit of tobacco smoking and alcohol drinking, and have a habit of family history of cancer. By unconditional logistic regression analyses, we found that subjects carrying GG genotype of rs6943984 was associated with increased risk of laryngeal cancer compared to the AA genotype (Adjusted OR=3.58, 95% CI=1.54-8.81). In the dominant model, the AA+GG genotype of rs6943984 was marginal significant correlated with an increased risk of laryngeal cancer compared to the wide-type genotype (Adjusted OR=1.60, 95% CI=1.03-2.49). Moreover, the GG genotype of rs6943984 was associated with a heavy increased risk of laryngeal cancer compared to the AA+AG genotype (Adjusted OR=3.37, 95% CI=1.47-8.23). However, we did not find any significant association between the rs4725443 gene polymorphism and laryngeal cancer risk. In conclusion, our study suggests that the rs6943984 polymorphism was associated with increased risk of laryngeal cancer in co-dominant, dominant and recessive models.

Keywords: *MLL3*, polymorphism, laryngeal cancer

Introduction

Laryngeal cancer is the most common malignancy in the head and neck tumors, and it is estimated that this cancer accounts for 30%-40% of all malignant head and neck tumors [1, 2]. It is reported that laryngeal cancer is the thirteenth most common cancer in men (138,102 cases, 1.9% of the total) and the twenty fourth in women (18,775 cases, 0.3% of the total) worldwide [3]. The real causes of laryngeal cancer are still unclear. The development of laryngeal cancer is involved in a complex and multifactorial processes, and epidemiological studies have reported that many environmental factors play an important role in the development of laryngeal cancer, such as smoking, alcohol consumption, exposure to carcinogens in the work environment, nutrition, and viral infections with human papilloma virus (HPV) and Eostein-Barr virus (EBV) [4-6]. However, individuals who are exposure to the same risk factors of laryngeal cancer would not develop this cancer, which suggest that genetic factors would contribute to laryngeal cancer. Previous molecular studies have reported that many genes play an important role in the development of this cancer, such as GSTM1, XPG Asp1104His, XRCC3, CYP1B1, MTHFR, RECQL5 and TP53 [7-13].

Mixed-lineage leukemia 3 (*MLL3*) is a member of the TRX/MLL gene family, and it maps to chromosome 7q36.1. *MLL3* is encodes a predicted protein of 4911 amino acids comprised of two plant homeodomains (PHD), an ATPase alpha/beta signature, a high mobility group, a SET (suppressor of variegation, enhancer of zeste, Trithorax) and two phenylalanine tyrosine rich domains. Both PHD and SET domains are

Table 1. Characteristics of laryngeal cancer patients and control subjects

Variables	Patients N=171	%	Controls N=171	%	χ^2 test or t test	*P* value
Age, years	57.35±11.45		55.80±9.42		1.52	0.06
<60	98	57.31	161	64.14		
≥60	73	42.69	90	35.86	2.00	0.16
Gender						
Females	52	30.41	106	42.23		
Males	119	69.59	145	57.77	6.07	0.01
Tobacco smoking						
No	95	55.56	172	68.53		
Yes	76	44.44	79	31.47	7.36	0.01
Alcohol drinking						
No	78	45.61	166	66.14		
Yes	93	54.39	85	33.86	17.56	<0.001
Family history of cancer						
No	159	92.98	245	97.61		
Yes	12	7.02	6	2.39	5.33	0.02
TNM stage						
I-II	54	31.58				
III-IV	117	68.42				

reported to be chromatin regulators, and some of them are altered in cancers [14]. Previous experimental study has suggested that inactive MLL3 could cause epithelia tumor formation, and have a role of tumor suppression [15]. Several epidemiologic studies have reported that *MLL3* gene polymorphisms contribute to the development of different kinds of cancers, such as oesophageal squamous cell cancer, colorectal cancer and gastric cancer [16-18]. However, no study has reported the association between *MLL3* gene polymorphisms and development of laryngeal cancer. In this case-control study, we firstly investigated the role of two SNPs in *MLL3* gene (rs6943984 and rs4725443) in the development of laryngeal cancer in a Chinese population.

Materials and methods

Subjects

Between May 2012 and October 2014, a total of 183 patients with laryngeal cancer were collected from the Zhumadian Central Hospital. All the patients with laryngeal cancer were confirmed by pathological examination within one month before enrolling into our study. Patients who had a secondary or recurrent tumors and a history of other tumors as well as a serious kidney or liver disease were excluded from this study. Ultimately, 171 patients were collected into this study, and the participation rate was 93.44%.

A total of 266 controls free of cancers were collected from individuals who came to receive regular health check-ups at the Zhumadian Central Hospital between May 2012 and October 2014. Controls which had a history of malignant tumor or digestive system disease as well as laryngeal and pharyngeal diseases were excluded from this study. Ultimately, 251 controls were collected into our study, and the participation rate was 94.36%. Written informed consents were obtained all patients with laryngeal cancer and control subjects before enrollment into the study. The ethical approval of the study protocol was in line with the standards of the Declaration of Helsinki.

A self-designed questionnaire and medical records were used to collect the demographic and lifestyle as well as clinical information of laryngeal cancer patients and control subjects. The demographic and lifestyle characteristics included age, sex, tobacco smoking, alcohol drinking, and family history of cancer. The clinical characteristics included TNM stage.

Genotyping

Peripheral blood sample (5 mL) was drawn from all subjects, and was stored at -80°C until use. Genomic DNA was extracted from the collected blood sample using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China). Genotyping of *MLL3* rs6943984 and rs4725443 were analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primer sequences for *MLL3* rs6943984 and rs4725443 polymorphisms were designed using the Primer pre-

Table 2. Genotype frequencies of *MLL3* rs6943984 and rs4725443 gene polymorphisms between laryngeal cancer patients and controls

MLL3	Patients N=171	%	Controls N=251	%	χ^2 test	*P* value	*P for HWE*	Minor allele frequency	
							In controls	In database	In controls
rs6943984									
AA	108	63.16	184	73.31					
AG	42	24.56	57	22.71					
GG	21	12.28	10	3.98	11.19	0.004	0.06	0.1593	0.1534
rs4725443									
CC	134	78.36	207	82.47					
CT	27	15.79	36	14.34					
TT	10	5.85	9	3.59	1.51	0.47	<0.001	0.0962	0.1076

mier v5.0 software (PREMIER Biosoft. Ltd, Palo Alto, USA). PCR reactions were carried out in a Perkin-Elmer 9700 thermocycler with the following cycling protocol: initial denaturation step at 94°C for of 8 minutes, followed by 30 cycles at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute. All assays were conducted blindly by two researchers without the knowledge of case or control status. Additionally, about 10% of the samples were randomly selected and retested, and the results were 100% concordant.

Statistical analysis

The SPSS 17.0 for windows was used for the statistical analysis. Differences of lifestyle characteristics and genotype distributions between laryngeal cancer patients and control subjects were analyzed using chi-square (χ^2) test or student T test. Whether the genotype frequencies deviated from the Hardy-Weinberg equilibrium (HWE) was calculated using a χ^2-test with one degree of freedom. Multiple logistic regression analysis was used to assess the role of *MLL3* rs6943984 and rs4725443 polymorphisms in the development of laryngeal cancer, and the results was described using odds ratio (OR) and their related 95% confidence intervals (CIs). All tests were two-sided with a significant level of *P*-value <0.05.

Results

The demographic, lifestyle and clinical characteristics of laryngeal cancer patients and control subjects are shown in **Table 1**. No significant difference was found between laryngeal cancer patients and control subjects in terms of age by chi-square test. The chi-square test revealed that individuals with the laryngeal cancer were more likely to be males (χ^2=6.07, *P*=0.01), have a habit of tobacco smoking (χ^2=7.36, *P*=0.01) and alcohol drinking (χ^2=17.56, *P*<0.001), and have a habit of family history of cancer (χ^2=5.33, *P*=0.02).

The genotype frequencies of *MLL3* rs6943984 and rs4725443 gene polymorphisms were shown in **Table 2**. By chi-square test, a significant difference in the frequencies of rs6943984 genotypes was established between the laryngeal cancer patients and controls (χ^2=11.19, *P*=0.004). In the control group, the distribution of rs6943984 was consistent with the Hardy-Weinberg equilibrium (*P*=0.06), while that of rs4725443 was not (*P*<0.001). The minor allele frequencies of *MLL3* rs6943984 and rs4725443 were similar with those in the SNP database of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp/).

The association between *MLL3* rs6943984 and rs4725443 gene polymorphisms and risk of laryngeal cancer was described in **Table 3**. By unconditional logistic regression analyses, we found that subjects carrying GG genotype of rs6943984 was associated with increased risk of laryngeal cancer compared to the AA genotype (Adjusted OR=3.58, 95% CI=1.54-8.81). In the dominant model, the AG+GG genotype of rs6943984 was marginal significant correlated with an increased risk of laryngeal cancer compared to the wide-type genotype (Adjusted OR=1.60, 95% CI=1.03-2.49). Moreover, the GG genotype of rs6943984 was associated with a heavy increased risk of laryn-

Table 3. Association between *MLL3* rs6943984 and rs4725443 gene polymorphisms and risk of laryngeal cancer

MLL3	Patients N=171	%	Controls N=251	%	OR (95% CI)[1]	*P* value
rs6943984						
Co-dominant						
AA	108	63.16	184	73.31	Ref.	
AG	42	24.56	57	22.71	1.26 (0.77-2.05)	0.34
GG	21	12.28	10	3.98	3.58 (1.54-8.81)	0.001
Dominant						
AA	108	63.16	184	73.31	Ref.	
AG+GG	63	36.84	67	26.69	1.60 (1.03-2.49)	0.03
Recessive						
AA+AG	150	87.72	241	96.02	Ref.	
GG	21	12.28	10	3.98	3.37 (1.47-8.23)	0.001
rs4725443						
Co-dominant						
CC	134	78.36	207	82.47	Ref.	
CT	27	15.79	36	14.34	1.16 (0.64-2.06)	0.6
TT	10	5.85	9	3.59	1.72 (0.61-4.90)	0.25
Dominant						
CC	134	78.36	207	82.47	Ref.	
CT+TT	37	21.64	45	17.93	1.27 (0.76-2.12)	0.33
Recessive						
CC+CT	161	94.15	243	96.81	Ref.	
TT	10	5.85	9	3.59	1.68 (0.60-4.77)	0.27

[1]Adjusted for sex, age, tobacco smoking, alcohol drinking and family history of cancer.

Table 4. Association between *MLL3* rs6943984 and risk of laryngeal cancer based on environmental factors

Variables	Patients		Controls		OR (95% CI)	*P* value
	AA	AG+GG	AA	AG+GG		
Tobacco smoking						
No	60	35	127	45	1.65 (0.93-2.91)	0.07
Yes	48	28	57	22	1.51 (0.73-3.15)	0.23
Alcohol drinking						
No	51	27	121	45	1.42 (0.76-2.63)	0.23
Yes	57	36	63	22	1.81 (0.91-3.62)	0.07
Family history of cancer						
No	104	55	180	65	1.46 (0.93-2.31)	0.08
Yes	5	7	4	2	2.80 (0.25-40.51)	0.32

geal cancer compared to the AA+AG genotype (Adjusted OR=3.37, 95% CI=1.47-8.23). However, we did not find any significant association between the rs4725443 gene polymorphism and laryngeal cancer risk.

We further conducted gene-environmental interaction analysis between *MLL3* rs6943984 polymorphism and environmental factors in the risk of laryngeal cancer (**Table 4**). We found that no significant interaction between rs6943984 polymorphism and tobacco smoking, alcohol drinking and family history had affect on the laryngeal cancer risk (P>0.05).

Discussion

Genetic susceptibility to disease has attracted increasing attention in recent years, and gene polymorphisms that are involved in various diseases are of particular interest. It is well known that individuals would not develop the same type of disease although they are exposing to the same environmental and lifestyle factors. Therefore, inherit factors may contribute to the development of diseases. Single nucleotide polymorphisms refer to the gene sequence of a single nucleotide bases inserting, missing or replacing to cause the polymorphism of the nucleic acid sequence [19]. Among millions of SNPs, the incidence of gene polymorphism is more than 1%, including transition, transversion, and insertion and deletion of single nucleotide. Gene polymorphisms could alter the gene expression, structure and quantity of the products, and thus influence the function of the gene [20, 21]. In our study, we firstly investigate whether *MLL3* rs6943984 and rs4725443 polymorphisms could influence the susceptibility to laryngeal cancer. We found that rs6943984 polymorphism was correlated with an increased risk of laryngeal cancer.

MLL3 is located at chromosome 7q36.1, which is frequently deleted in myeloid leukemia (Ruault et al., 2002; Tan and Chow, 2001). Previous studies have reported that *MLL3* plays an important role in the susceptibility to carcinogenesis, such as myeloid leukaemia, colorectal, glioblastoma, melanoma, pancreatic, breast and gastric carcinoma [22-30]. Ruault et al. conducted a study in a Korean population, and they reported that absence of *MLL3* mutations was found in colorectal carcinomas [22]. Vakoc et al. reported that low frequency of *MLL3* mutations was associated with susceptibility to colorectal carcinomas [25]. Another systematic sequence analysis of well-annotated human protein coding genes or consensus coding sequences in colorectal cancers and breast cancers indicated that mutations of *MLL3* were frequent in these cancers [26, 27]. Balakrishnan et al. reported that *MLL3* mutations were correlated with glioblastoma, melanoma and pancreatic carcinoma [28]. Je et al. indicated that *MLL3* mutations showed loss of MLL3 expression in all cancers, and frameshift mutations of *MLL* genes and loss of expression of MLL3 protein are common in colorectal cancer and gastric cancer [29]. Li et al. indicated that rs6943984 and rs4725443 gene variants of *MLL3* gene were associated with increased gastric cancer risk in a Chinese population [30]. Currently, no study reported the association between *MLL3* mutations and carcinogenesis of laryngeal cancer in a Chinese population, and our study firstly found that rs6943984 polymorphism contribute to the development of laryngeal cancer. Further studies with large sample size are greatly needed to confirm our results.

There were two limitations in the present study. First, there may have been selection bias due to the hospital-based design and deviation from HWE for rs4725443 in controls; therefore, this study may not represent the general population. Second, the statistical power of determining the association between polymorphisms in *MLL3* gene and risk of laryngeal cancer could be limited. Therefore, further studies with larger sample sizes must be performed to validate our findings.

In conclusion, our study suggests that the *MLL3* rs6943984 polymorphism was associated with increased risk of laryngeal cancer in co-dominant, dominant and recessive models. Future studies with larger sample sizes may help to further elucidate the impact of *MLL3* rs6943984 polymorphism in the risk of laryngeal cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jinyan Xing, Department of ENT and HN Surgery, Zhumadian Central Hospital, Zhumadian 463000, China. Tel: +86-396-2926217; Fax: +86-396-2926217; E-mail: jinyanxin2@sina.com

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