729G/C polymorphism in Toll-like receptor 4 results in increased susceptibility to bladder cancer

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Genet. Mol. Res. 14 (4): 15482-15487 (2015)
Received June 20, 2015
Accepted September 20, 2015
Published November 30, 2015
DOI http://dx.doi.org/10.4238/2015.November.30.26

ABSTRACT. In this study, the association between the 729G/C polymorphism in Toll-like receptor 4 (TLR4) and the risk of bladder cancer was investigated. A total of 376 patients with bladder cancer and 380 healthy volunteers from the Third Xiangya Hospital of Central South University (China) were enrolled in this study between January 2008 and February 2014. The TLR4-729G/C polymorphism was detected by the polymerase chain reaction-restriction fragment length polymorphism assay. There was a significant difference in the distribution of the TLR4-729G/C genotype between bladder cancer patients and healthy controls ($P < 0.001$). Our analysis showed that the GC genotype (OR = 2.99; 95%CI = 1.01-4.81, P = 0.046) and CC genotype (OR = 3.67; 95%CI = 2.11-7.27, P = 0.017) were significantly associated with increased bladder cancer risk when the GG genotype served as a reference. Furthermore, carriers of the C allele had a significantly increased risk of developing bladder cancer (OR = 3.89; 95%CI = 2.88-8.53; P = 0.009). Our results suggest a correlation between the TLR4-729G/C polymorphism and the risk of developing bladder cancer in this Chinese population.

Key words: Bladder cancer; Toll-like receptor; Gene polymorphism; PCR-RFLP

INTRODUCTION

Bladder cancer is the most common malignancy of the urinary tract, ranking sixth in cancer incidence worldwide, and is more prominent in men than women (Shariat et al., 2010; Siegel et al., 2015). Bladder cancer has a complicated etiology and there are several major risk factors for bladder tumorigenesis, including chemical and environmental exposures and genetic factors (Wu et al., 2008).

Toll-like receptor 4 (TLR4) belongs to the toll-like receptor (TLR) family, which is an important class of pattern recognition receptors (PRRs) in humans that recognize pathogen-associated molecular patterns (Ishihara et al., 2004). The human TLR4 gene is located on chromosome 9 (9q32-q33) and consists of four exons and three introns with an overall length of approximately 19 Kb (Horie et al., 2009). TLR4 is critical in the recognition of viruses and bacteria, serving as a key immune system effector. Dysregulation of TLR4 signaling owing to single nucleotide polymorphisms (SNPs) may alter ligand binding and the balance between pro- and anti-inflammatory cytokines, thereby increasing the risk of chronic inflammation and cancer.

The potential association between TLR4 genetic polymorphisms and the risk of breast cancer, gastric cancer, prostate cancer, ovarian cancer, hepatocellular carcinoma (HCC), cervical cancer, and colorectal cancers has been investigated earlier (Zhu et al., 2013; Zhou et al., 2014; Jiang et al., 2014; Zidi et al., 2014a,b; Kopp et al., 2015). However, there has been little research on the association between the 729G/C polymorphism in the TLR4 gene and the risk of bladder cancer.

MATERIAL AND METHODS

Study subjects

Our study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University (China), and all research subjects provided informed consent. A total of 376 patients with bladder cancer and 380 healthy volunteers from the Third Xiangya Hospital of Central South University were enrolled in this study between January 2008 and February 2014. All cases of bladder cancer were histopathologically confirmed and staged according to the tumor-node-metastasis staging system of the Union for International Cancer Control. All cases and controls completed a face-to-face questionnaire to obtain information on demographic characteristics (age and gender), history of smoking, alcohol consumption, and family history of cancer.

Genotyping

Genomic DNA was extracted from venous blood samples using the QIAamp DNA blood mini kit (Qiagen, Germany) and then stored at -80°C until use. The TLR4 -729G/C polymorphism was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. In brief, PCR was performed in a 20 µL mixture containing 200 ng genomic DNA, 1.5 mM $MgCl_2$, 0.5 µM primer, 2 µL 10X PCR buffer, 0.2 mM dNTP, and 1.2 U Taq polymerase. After an initial denaturation at 95°C for 5 min, DNA was amplified by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final elongation step at 72°C for 5 min on the GeneAmp PCR System 9700. The digested PCR products were separated on 2% agarose Tris-borate-EDTA gels and stained with ethidium bromide.

Statistical analysis

Allele and genotype frequencies were calculated by the gene counting method. ORs and 95%CIs were used to represent the risk of developing bladder cancer. ORs were calculated using multivariate logistic regression analyses after adjusting for age and gender. All statistical tests were two-sided probability tests and a P value of less than 0.05 was considered statistically significant. Statistical analyses were performed using the SPSS 18.0 software (SPSS, Chicago, IL, USA).

RESULTS

Characteristics of the study population

The frequency distribution of demographic characteristics in the 376 bladder cancer patients and 380 healthy controls is shown in Table 1. The patients and controls were shown to be adequately matched for age (P = 0.166) and sex (P = 0.481). The frequency of relatives with cancer was higher in the bladder cancer patients than in the controls (22.07 *vs* 11.84%; P < 0.001). No significant difference in tobacco (P = 0.283) or alcohol (P = 0.549) consumption was found between the bladder cancer patients and healthy controls.

Table 1. Frequency distribution of selected characteristics of the bladder cancer patients and healthy controls.

Characteristic	Bladder cancer patients (N = 376)		Healthy controls (N = 380)		P value
	N	%	N	%	
Age (years)					
≥60	189	50.27	211	55.53	0.166
<60	187	49.73	169	44.47	
Gender					
Male	254	67.55	266	70.00	0.481
Female	122	32.45	114	30.00	
Family history of cancer					
Yes	83	22.07	45	11.84	<0.001
No	293	77.93	335	88.16	
Tobacco consumption					
No	241	64.10	258	67.89	0.283
Yes	135	35.90	122	32.11	
Alcohol consumption					
No	139	36.97	149	39.21	0.549
Yes	237	63.03	231	60.79	

Allele and genotype frequency of the TLR4-729G/C polymorphism

Table 2 summarizes the genotype and allele distributions of the TLR4-729G/C polymorphism in bladder cancer patients and the healthy control group. The observed genotype frequencies of the TLR4-729G/C polymorphism were in agreement with the Hardy-Weinberg equilibrium in both the bladder cancer and control groups (both P > 0.05). Genotypes GG, GC, and CC were detected in 268 (71.28%), 84 (22.34%), and 24 (6.38%) of 376 bladder cancer patients and in 331 (87.11%), 47 (12.37%), and 2 (0.53%) of 380 healthy control samples. There was a significant difference in the distribution of the TLR4-729G/C genotype between bladder cancer patients and healthy controls (P < 0.001). The frequency of allele G was 620 (82.45%) and allele C was 132 (17.55%) in the bladder cancer cases, and 709 (93.29%) and 51 (6.71%) in the healthy controls, respectively.

The frequency of the C allele was significantly higher in the bladder cancer group as compared to the control group (P < 0.001).

Table 2. Allele and genotype frequencies of the TLR4-729G/C polymorphism in bladder cancer patients and healthy controls.

	Bladder cancer patients (N = 376)		Healthy controls (N = 380)		P value
Genotype	N	%	N	%	
GG	268	71.28	331	87.11	<0.001
GC	84	22.34	47	12.37	
CC	24	6.38	2	0.53	
Allele					
G	620	82.45	709	93.29	<0.001
C	132	17.55	51	6.71	

Association between the TLR4-729G/C polymorphism and bladder cancer susceptibility

Our analysis showed that the occurrence of the GC (OR = 2.99; 95%CI = 1.01-4.81, P = 0.046) and CC genotype (OR = 3.67; 95%CI = 2.11-7.27, P = 0.017) significantly correlated with increased bladder cancer risk when the GG genotype served as the reference. The GC+CC genotype was significantly associated with increased bladder cancer risk under the dominant model (OR = 3.18; 95%CI = 2.24-6.19, P = 0.016). Furthermore, carriers of the C allele were likely to have a significant increase in risk of bladder cancer (OR = 3.89; 95%CI = 2.88-8.53; P = 0.009). These results are summarized in Table 3.

Table 3. Correlation between the TLR4-729G/C polymorphism and risk of bladder cancer.

	Bladder cancer patients	Healthy controls	OR (95%CI)	P value
General genotype				
GG	268	331	1.00 (Reference)	
GC	84	47	2.99 (1.01-4.81)	0.046
CC	24	2	3.67 (2.11-7.27)	0.017
Dominant genotype				
GG	268	331	1.00 (Reference)	
GC + CC	108	49	3.18 (2.24-6.19)	0.016
Recessive genotype				
GG + GC	352	378	1.00 (Reference)	
CC	24	2	2.35 (0.73-5.75)	0.298
Allele frequency				
G	620	709	1.00 (Reference)	
C	132	51	3.89 (2.88-8.53)	0.009

DISCUSSION

Cancer is a multifactorial disorder that stems from the combined effects of various genetic, environmental, and behavioral risk factors. These factors are unique to each individual and studies have shown that environmental factors such as diet and smoking can be more important risk factors than genetic susceptibility (Hahn and Weinberg, 2002). The genomic DNA sequences of two unrelated people are quite similar, differing in only 0.1% of total DNA content. This percentage is important because it includes the genetic variants that influence

the susceptibility of individuals to different diseases and response to drugs and environmental factors (Levy et al., 2007).

Chronic infection and inflammation are important factors contributing to tumorigenensis and tumor progression (Balkwill and Coussens, 2004). TLRs play a pivotal role in the immune response and are involved in the regulation of inflammatory reactions and activation of the adaptive immune response to eliminate infectious pathogens and cancer debris (Akira et al., 2001; Akira and Takeda, 2004; Krieg and Vollmer, 2007). TLR4 expression has been investigated in tumor cells or cell lines, including gastric carcinoma, extranodal marginal zone B-cell lymphomas, pituitary epithelial tumor cell lines, hepatocellular carcinoma cells, colon cancer cells, and human prostate epithelial PC3 cells. Although TLR4 is expressed in numerous non-immune and tumor cells, the functional association of TLR4 with tumor progression requires further elucidation.

The potential association between TLR4 genetic polymorphisms and the risk of breast, gastric, prostate, ovarian, HCC, cervical, and colorectal cancers has been investigated (Zhu et al., 2013; Zidi et al., 2014a,b; Jiang et al., 2014; Zhou et al., 2014; Kopp et al., 2015). Li et al. (2014) found that the TLR4-2081G/A polymorphism affected the risk of gastric carcinogenesis and may play a protective role against *Helicobacter pylori* infection. Zidi et al. (2014b) found that a specific gene variant in TLR4 (Asp299Gly) increased susceptibility to cervical cancer in Tunisian women. In the study by Wang et al. (2014), cases of ovarian cancer were analyzed with regard to the Asp299Gly and Thr399Ile mutations in the TLR4 gene. Their results indicated that these mutations occurred at a lower frequency in ovarian cancer patients than healthy controls. However, Priyadarshini et al. (2013) found that the same mutations were associated with a higher risk for developing prostate cancer. Theodoropoulos et al. (2012) found that the Asp299Gly mutation in the TLR4 gene might confer an increased susceptibility to breast cancer development. However, there has been little research on the association between the TLR4-729G/C polymorphism and the risk of bladder cancer.

In the present study, we found that there was a significant difference in the distribution of the TLR4-729G/C genotype between bladder cancer patients and healthy controls. The frequency of allele C was significantly higher in the bladder cancer group compared to the control group. Our analysis showed that the GC and CC genotypes were significantly associated with increased bladder cancer risk when the GG genotype served as a reference. The GC+CC genotype was significantly associated with increased bladder cancer risk under the dominant model. Furthermore, carriers of the C allele were likely to have an increased risk of developing bladder cancer. In summary, we report a statistically significant association between the TLR4-729G/C polymorphism and the risk of developing bladder cancer in a Chinese population.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the Hunan Provincial Natural Science Foundation of China (Grant #14JJ3044), and the Science Foundation of Health and Family Planning Commission of Hunan Province (Grant #B2012-032).

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