RESEARCH ARTICLE

Lack of Associations between TNF-α Polymorphisms and Cervical Cancer in Thai women

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Abstract

The risk of developing cervical cancer in women infected with human papillomavirus (HPV) may be influenced by an individual's genetic susceptibility. Published data linking single nucleotide polymorphisms (SNPs) in the tumor necrosis factor-alpha (TNF-α) promoter region at positions -308G>A (rs1800629) and -238G>A (rs361525) to cervical cancer risk have been inconclusive. In this study, we examined 251 cervical specimens and classified them into two groups according to their cytological findings: 121 cancer cases and 130 controls (low-grade squamous intraepithelial lesion and normal cytology). All specimens were typed by PCR and sequencing for TNF-α promoter -308G>A (rs1800629) and -238G>A (rs361525). The genotype distribution of SNPs in either rs1800629 or rs361525 did not significantly demonstrate higher frequency in the cancer group (p=0.621 and p=0.68, respectively). Based on these results, neither the TNF-α promoter -308G>A (rs1800629) nor the -238G>A (rs361525) polymorphism presents a major risk factor for cervical cancer among Thai women. Larger studies are necessary to elucidate possible genetic mechanisms influencing cervical cancer development.

Keywords: Human papillomavirus - TNF-α - sequencing - single nucleotide polymorphism - cervical cancer risk

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Introduction

Cervical cancer is the fourth most common cancer in women worldwide and represents 7.5% of all female cancer deaths. In 2012, it is estimated that there were 528,000 new cases of cervical cancer and 266,000 deaths worldwide. In developing countries, limited access to screening, vaccination, and effective treatment contributes to relatively high morbidity and mortality. The age-standardized incidence rate of cervical cancer in Thailand is approximately 17.8/100000 person-year (Ferlay et al., 2015). Infection with the human papillomavirus (HPV) is attributed to the development of nearly all cervical cancer (Snijders et al., 1999; Bosch et al., 2002; Bosch and de Sanjose., 2003; Chin'ombe et al., 2014; Ermel et al., 2014). In addition, hormonal, nutritional and environmental factors as well as host genetic susceptibility such as single nucleotide polymorphisms (SNPs) further contribute to the risk of cancer development (Kohaar et al., 2007).

Genetic polymorphisms in several important genes have been examined for correlation between specific allele variants and cancer progression (Chansaenroj et al., 2013). These genes encode cytokines, which mediate the immune response and have been implicated in the development of cancer (Dranoff, 2004). Tumor necrosis factor-alpha (TNF-α) is a potential pro-inflammatory cytokine, which plays a critical role in a wide range of inflammatory, autoimmune, and malignant diseases (Bazzoni and Beutler., 1996; Beutler and Bazzoni., 1998). TNF mediates carcinogenesis by inducing proliferation, invasion, and metastasis of tumor cells (Shishodia et al., 2003). SNPs have been identified in the promoter region of TNF-α and potentially affect the regulation of TNF-α transcription. TNF-α gene lies in the major histocompatibility complex class III region on the short arm of chromosome 6 (6p21.3) (Hajeer and Hutchinson, 2000). One of the two most common polymorphisms in the promoter region of TNF-α, G to A substitution at position -308, resulted in higher level of TNF-α production than the -308 G allele (Kroeger et al., 1997). Additionally, there is a putative repressor site located in a 25-bp stretch including the position -238, resulting in G to A substitution at position -238 in the TNF-α promoter, which might also affect TNF-α expression (Fong et al., 1994).

Case-control studies have been performed to examine the association between TNF-α promoter region -308G>A and -238G>A polymorphisms with cancer susceptibility,

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including breast, lung, nasopharyngeal, and cervical cancer (Deshpande et al., 2005; Shih et al., 2006; Pooja et al., 2011; Sousa et al., 2011). The available data linking these SNPs and cervical cancer, however, have been inconclusive or contradictory. The aim of this study is to compare the polymorphisms of the TNF-α promoter region (-308 G>A and -238 G>A) in HPV-infected Thai women with normal or low-grade squamous intraepithelial lesion (LSIL) versus cancerous cytology in order to determine possible association between the risk of developing cervical cancer and these polymorphisms.

Materials and Methods

Study population

One hundred and twenty-one patients from the National Cancer Institute in Thailand with cancerous cervical cytology were included in this study. In addition, the control group comprised 130 patients with normal or LSIL cytology from Bangpakok 9 International Hospital and Samitivej Srinakarin Hospital in Bangkok (Table 1). Cervical cells were sampled and all patients had HPV infection. This study was approved by the Institutional Review Board (IRB number 071/56) of the Faculty of Medicine, Chulalongkorn University. Informed consent was obtained from all participants.

Polymorphism analysis

Germline DNA was extracted from cervical cells by the standard phenol/chloroform extraction method. The TNF-α -308G>A and -238G>A genotypes were determined by PCR and sequencing. Briefly, polymerase chain reaction (PCR) amplifications were performed with the master mix (5 PRIME Inc., Hamburg, Germany), DNA template (25-100 ng) and 0.2 μM solution of the specific primers including SNP_F8092: 5'-GAA GGA AAC AGA CCA CAG AC-3' and SNP_R8521: 5'-TTG CTT CTC TCC CTC TTA GC-3'. The final volume was made up to 25 μl with sterile water. The reaction conditions were 95ºC for 5 min, 40 cycles at 95ºC for 30 s, 55ºC for 30 sec and 72ºC for 45 s, followed by a final extension of 7 min at 72ºC. All PCR products were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide, and visualized with ultraviolet light. The PCR products were purified using the gel extract kit (GeneAll, Seoul, Korea) according to the manufacturer's instructions and were sequenced (First BASE Laboratories, Selangor, Malaysia).

Table 1. Demographic Data of All Cervical Samples in this Study

	Control	Cancer
No.	130	121
Age		
Range	19-88	35-81
Average	39.9	51.7
SD	12.3	10.5
Cytology normal	36	
LSIL	94	
CA		121

Statistical analysis

Allele and genotype frequencies of the TNF-α -308G>A and -238G>A positions were analyzed manually based on the chromatogram of nucleotide bases using Chromas Lite (v.2.01) and compared to the reference sequence retrieved from GenBank database (http://www.ncbi.nlm.nih.gov/). Odds ratios (ORs) with 95% confidence intervals (95% CI) were used to evaluate the strength of the associations between the TNF-α -308G>A and -238G>A polymorphisms and two groups of cervical cytology. Statistical analyses were performed by Microsoft Excel using χ^2 contingency table analysis. A P-value <0.05 was considered statistically significant.

Results

All samples in this study were HPV-DNA positive by PCR and sequencing. Although there were roughly equal numbers of individuals in the control and the cancer group, the age distribution for the cancer group was narrower (35-81 years) (Table 1). Mean age of the cancer group and the control group are 51.7 and 39.9 years, respectively. Among the 130 cervical samples in the control group, there were twice as many samples with LSIL cytology than with normal cytology.

Genotyping of the rs1800629 showed that most individuals in both groups possessed the GG alleles (Table 2). The frequency of either heterozygous or homozygous AA was also similar between the two groups. These differences were not statistically significant (P=0.621). Similarly, the majority of the individuals in both groups possessed genotype GG at the locus rs361525 (P=0.68). There were no individuals with the AA alleles.

Discussion

Cervical cancer is a common gynecological cancer among women especially in developing countries.

Table 2. Genotype Frequencies of TNF-α Promoter Gene Polymorphism in Cancer and Control Groups

SNP	Genotype	Cancer (N=121)	Control (N=130)	OR (95% CI)	*P*-value
TNF-α -308	GG	108 (89.3%)	113 (86.9%)	1.19 (0.59-2.40)	0.621
(rs1800629)	GA	11 (9.1%)	15 (11.5%)		
	AA	2 (1.7%)	2 (1.5%)		
TNF-α -238	GG	114 (94.2%)	124 (95.4%)	0.80 (0.27-2.40)	0.68
(rs361525)	GA	7 (5.8%)	6 (4.6%)		
	AA	0	0		

Although HPV infection is responsible for the oncogenic transformation, disease development also depends in part on the infected individual's genetic factor. In recent years, the associations between genetic polymorphisms and cancer susceptibility have been extensively investigated, and there is evidence to suggest that certain alleles located in the proximity of key regulatory genes could contribute to individual differences in cancer susceptibility.

Inflammation enables precancerous and early cancerous lesions of the cervix, leading consequently to cervical cancer (Luthra et al., 1987; Bornstein et al., 1995). Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine secreted by activated macrophages (Kroeger et al.,1997; Dranoff, 2004) and plays a pivotal role in human immune response to a number of pathogens, such as HPV. Several studies revealed that TNF-α is a versatile cytokine with multiple functions, including defending cells against viral infections and at times serving as a cancer promoter. (Brinkman et al., 1995; Kroeger et al., 2000; Eksteen et al., 2001)

Previous studies have shown that TNF-α may be linked to the development of HPV-associated cervical cancer. Elevated levels of a number of cytokines including TNF-α were increased in the cervicovaginal washings of patients with cervical cancer (Tjiong et al., 2001). Levels of TGF-β and TNF-α mRNA in HPV-positive cells were increased compared to the nontumorigenic control cells (Bequet-Romero and Lo´pez-Ocejo, 2000). Chronic inflammation and the release of proinflammatory cytokines such as IL-1α and TNF-α might provide a selective growth advantage for abnormal cervical cells in vivo (Woodworth et al., 1995).

In our study, comparison of the genotype frequencies between the HPV-positive cancer and the control group did not reveal statistically significant association for either rs1800629 or rs361525. It is possible that this study was limited by the sample size whereby minor genetic effects may be masked. Age-matching between cancer and control groups may also be needed, but this presents a challenge because advanced cancerous progressions are usually found in the older individuals compared to those with normal or early stage abnormal cytology. HPV genotypes may also play a role in combination with the polymorphism. Although all samples were HPV-positive, specific HPV genotypes were not identified in these samples. It is known that high risk and low risk HPV genotypes gave the different in cervical cancer pathogenesis.

Previous study demonstrated that the less common TNα (-308A) allele (Wilson et al., 1992) is strongly associated with the MHC haplotype HLA-A1, B8, DR3 which is associated with high TNF production (Wilson et al., 1993). In our study, rs1800629 genotype AA was found in both groups of patients, albeit in very few individuals. The most common HLA types among Thais are HLA-A2, B46 and DR9. Moreover, association of these 2 SNPs with risk of cervical cancer using meta-analysis suggested that TNF-α -238A allele significantly decreased cervical cancer risk and the TNF-α -308G>A polymorphism is associated with the susceptibility to cervical cancer in Caucasian and African population (Pan et al., 2012). For common HLA alleles and Asian population, polymorphism in TNF-α promoter gene is not a risk factor for cervical cancer in the Thai population.

In conclusion, based on the results of the present study, neither the TNF-α -308 G>A nor the TNF-α -238 G>A polymorphism is a major risk factor among Thai women with cervical cancer. The further studies on the correlation between host and viral factors are necessary to reveal potential diagnostic and therapeutic benefits from such association.

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