RESEARCH ARTICLE

Association Study of Single-Nucleotide Polymorphisms of STAT2/STAT3/IFN-γ Genes in Cervical Cancer in Southern Chinese Han Women

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Abstract

Objective: Interferon-γ (IFN-γ) and signal transducers and activators of transcription (STATs) each play an important role in carcinogenesis associated with viral infection. Cervical cancer is almost invariably associated with infection by human papillomavirus (HPV), and previous studies suggested that dysregulation of the signal pathway involved in IFN-γ and STATs is associated. Our objective was to evaluate the association of SNPs in STAT2, STAT3, and IFN-γ with cervical cancer susceptibility in Chinese Han women in Hunan province. Materials and Methods: Genomic DNA was extracted from peripheral blood samples of 234 cervical cancer patients and 216 healthy female controls. STAT2 and STAT3 genotyping was performed using polymerase chain reaction-restriction enzyme (PCR-RE) analysis. IFN-γ genotyping was detected by PCR-amplification of specific allele (PASA). Results: For STAT2 rs2066807 polymorphisms, there was no significant difference of genotype distribution (P=0.827) and allele frequencies (*P*=0.830, OR=1.09, 95% CI: 0.51-2.31) between cases and controls. For STAT3 rs957970 polymorphisms, there was no significant difference of genotype distribution (*P*=0.455) and allele frequencies (*P*=0.560, OR=0.92, 95% CI: 0.71-1.20) between cases and controls. For IFN-γ +874A/T polymorphisms, there was no significant difference of genotype distribution (*P*=0.652) and allele frequencies (*P*=0.527, OR=1.12, 95% CI: 0.79-1.59) between cases and controls. Conclusion: These results suggest that polymorphisms in STAT2, STAT3 and IFN-γ genes are not likely to be strong predictors of cervical cancer in Han women in southern China.

Keywords: STAT2 - STAT3 - IFN-γ - single-nucleotide polymorphisms - cervical cancer

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Introduction

Most cervical cancer is caused by human papillomavirus (HPV) infection. It is estimated that the prevalence of HPV infection in young adult females is as high as 40-80% in many developed countries. However, not all the infected females developed cancer. Furthermore, the average time from infection with a carcinogenic HPV to invasive cervical cancer is 25-30 years or more (Bogaert, 2013). Therefore, other factors are also implicated in the development of cervical cancer. The underlying endogenous and exogenous factors driving the process from persistent infection to carcinogenesis are still unclear.

Interferon gamma (IFN-γ), produced during viral infection, is important in controlling both HPV infection and HPV-associated neoplasms. A single nucleotide polymorphism (SNP) +874 T/A located in the first intron of the IFN-γ gene can influence the secretion of IFN-γ. In vitro study has shown the association of +874 alleles T to A with a low (AA), medium (AT) and high (TT) IFN-γ production (Pravica et al., 2000). Several studies have reported the association between IFN-γ gene polymorphisms and cancer risk (Ge et al., 2014). However, the results vary between ethnic groups. The meta-analysis study showed that IFN-γ (+874 T/A) polymorphism was likely to increase the risk of cervical cancer in the overall population (Sun et al., 2015). Gangwar et al indicated that low production of IFN-γ with +874 AA genotype was associated with high risk of cervical cancer in a north-Indian population (Gangwar et al., 2009). However, Govan et al indicated that there was no significant association between IFN-γ +874 polymorphisms and susceptibility or resistance to cervical cancer in South African women (Govan et al., 2003).

Signal transducers and activators of transcription (STATs) are transcription factors, including STAT1-4, 5a, 5b, and 6, which are responsible for stimulation of cytokines. STAT2 is an indicator of IFN signaling against

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viral infections (Zimmermann et al., 2005; George et al., 2012). Studies have shown that STAT2-deficient mice have decreased tumor incidence in response to carcinogens, suggesting that STAT2 plays a positive role in tumorigenesis (Gamero et al., 2010). Our previous study revealed an association between cervical cancer progression and augmented STAT2 expression (Liang et al., 2012).

STAT transcription factors are activated by Janus kinases (JAKs). The JAK/STAT signaling pathway is frequently dysregulated in primary tumors, causing increased angiogenesis, enhanced survival and immunosuppression (Xiong et al., 2014). The STAT3 gene, which is an oncogene, plays an important role in tumor progression, particularly in tumors associated with chronic inflammation (Rajbhandary et al., 2013; Pandurangan et al., 2014). HPV constitutively activates the STAT3 signaling pathway, thus generating a suppressor environment for T cells (Stone et al., 2014). It was reported that IFN-γ activates STAT3 strongly (Qing et al., 2004). Inhibition of STAT3 provides a rational strategy to block carcinogenesis at an early stage of cancer development. Recently, Zhang et al demonstrated that selective inhibition of STAT3 activation commits SiHa and HeLa cells to apoptosis (Zhang et al., 2014).

Given the critical role in IFN-γ, STAT2 and STAT3 in cervical carcinogenesis, using data from a population-based case-control study conducted in Chinese Han women, we investigated the association of SNPs in STAT2, STAT3, and IFN-γ with cervical cancer susceptibility.

Materials and Methods

Study Participants

216 patients with cervical cancer were recruited from Hunan Cancer Hospital between January 2012 and August 2012, and 234 cancer-free controls were recruited from the health administration center of China International Trust and Investment Corporation - Xiangya Hospital between October 2011 and August 2012. All participants were genetically unrelated Han Chinese. There were no age restrictions for the two groups (t=0.078, $P>0.05$). The eligible patients were histopathologically confirmed. This study was approved by the Ethics Committee of Central South University.

Materials

2×Taq Master Mix and DNA marker (20 bp DNA ladder and100 bp DNA ladder) were purchased from CWBio Co. (Beijing, China). Restriction enzymes Psy I and Xba I were purchased from Fermentas (Ottawa ON, Canada). Primer synthesis and sequencing of the PCR products were conducted by Beijing Genomics Institution (BGI). Agarose, ethidium bromide and protease K were purchased from Dingguo Biotech. Co. (Beijing, China).

Genetic Analysis

We selected SNPs of STAT2 rs2066807, STAT3 rs957970, IFN-γ rs2430561 using genotype data of the Chinese population of the HapMap database (www.hapmap.org). The gene sequences of three SNPs were acquired from NCBI (www.ncbi.nlm. nih.gov/SNP/). Primer 5.0 was used to design primers for these SNPs.

Genomic DNA was extracted from peripheral blood according to a standard salting-out protocol. STAT2 rs2066807 and STAT3 rs957970 genotypes were performed using polymerase chain reaction-restriction enzyme (PCR-RE) analysis. IFN-γ +874A/T (rs2430561) genotype was detected by PCR-allele specific (PCR-AS) method. 20% of the samples of each group were randomly selected for DNA sequencing. The concordance rate for each of the two methods was more than 99%.

Statistical analysis

The T test was used to examine the differences in age between cases and controls. The χ^2 test was used to assess the Hardy-Weinberg equilibrium and to examine the differences in the distributions of genotypes between cases and controls. The association between polymorphisms in each SNP variant and the risk of cervical cancer was estimated by computing odds ratios (OR) and 95% confidence intervals (95%CI), using an unconditional logistic regression analysis. All statistical tests were two-sided tests, and significance was set at $P<0.05$.

Results

We genotyped three SNPs of three genes, STAT2, STAT3 and IFN-γ. SNP frequencies are shown in Table1 and Table2. All markers were in Hardy-Weinberg equilibrium.

STAT2 and STAT3

PCR products of STAT2 rs2066807 showed specific positive signal in 470bp (Figure 1A), which were digested by Psy I. The enzyme-digested products were electrophoresed in 2% agarose. The result showed that three genotypes differed in the electrophoretogram (Figure 2A). Homozygote C/C showed a single electrophoresis strip in 470bp (this type of DNA fragments didn't appear in present studied groups); homozygote G/G showed two strips in 262bp and 207bp; and heterozygote C/G showed three strips in 470bp, 262bp and 207bp.

PCR products of STAT3 rs957970 showed a specific positive signal in 382bp (Figure 1B), which were digested by Xba I. The enzyme-digested products were electrophoresed in 2% agarose. The result showed that three genotypes differed in the electrophoretogram (Figure 2B). Homozygote C/C showed a single electrophoresis

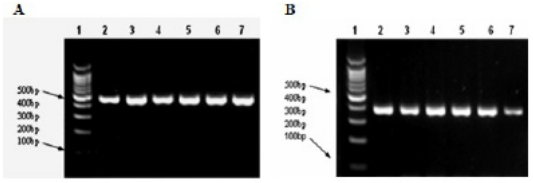

Figure 1. Electrophoretogram of PCR products of rs2066807 and rs957970. A) Lanel is 100bp DNA ladder, lane2-7 are PCR products of rs2066807 from six samples. The length of products is 470bp. B) Lanel is 100bp DNA ladder, lane2-7 are PCR products of rs957970 from six samples. The length of products is 382bp.

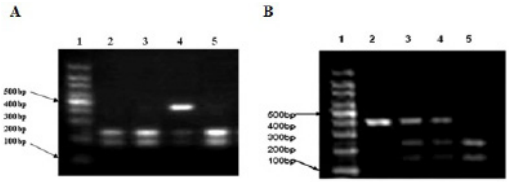

Figure 2. Electrophortogram of enzyme-digested PCR products of rs2066807 and rs9579709. A) Electrophoretogram of rs2066807 enzyme-digested PCR products: Lane 1 is 100bp DNA ladder; Lane2, 3 and 5 presented the gemotype G/G; Lane 4 presented the gentype C/G. B) Electrophoretogram of rs9579709 enzyme-digested PCR products: Lane 1 is 100bp DNA ladder; lane 2 presented the presented the genotype C/C; Lane 3 and 4 presented the genotype C/T; Lane 5 presented the genotype T/T

Table 1. Associations between STAT2, STAT3 Gene Polymorphisms and Risk of Cervical Cancer

SNPs	Controls (n=234) n (%)	Cases (n=216) n (%)	OR (95% CI)	P
STAT2 rs2066807				
G/G	220 (94.0)	202 (93.5)		0.827
C/G	14 (6.0)	14 (6.5)		
C/C	0 (0.0)	0 (0.0)		
C	14 (3.0)	14 (3.2)	1.09 (0.51-2.31)	0.83
G	454 (97.0)	418 (96.8)		
STAT3 rs957970				
C/C	39 (16.7)	38 (17.6)		
C/T	126 (29.5)	104 (34.3)		0.455
T/T	69 (53.8)	74 (48.1)		
C	204 (43.6)	180 (41.7)	0.92 (0.71-1.20)	0.56
T	264 (56.4)	252 (58.3)		

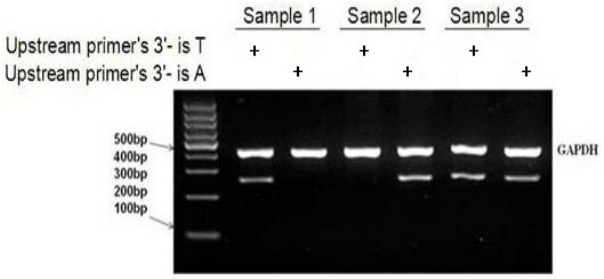

Figure 3. Electrophoretogram of IFN-y +874A/T PCR products. The left lane is 100bp DNA ladder; Sample l's genotype is A/A; Sample 2's genotype is T/T; Sample 3' genotype is T/A.

strip in 382bp; homozygote T/T showed two strips in 236bp and 146bp; and heterozygote C/T showed three strips in 382bp, 236bp and 146bp.

It was found that the distribution of STAT2 rs2066807 genotype was 94.0% G/G, 6.0% C/G in the controls and 93.5% G/G, 6.5% C/G in the cases, respectively .There was no significant difference of genotype distribution (P=0.827) and allele frequencies between cases and controls (P=0.830, OR=1.09, 95% CI=0.51-2.31) (Table 1). The distribution of STAT3 rs957970 genotype was 16.7% C/C, 29.5% T/T, 53.8% C/T in the controls and 17.6% C/C, 34.3% T/T, 48.1% C/T in the cases, respectively. There was no significant difference of genotype distribution (P=0.455) and allele frequencies between cases and controls (P=0.560, OR=0.92, 95% CI=0.71-1.20) (Table 1).

Table 2. Associations between IFN-γ Gene Polymorphisms and Risk of Cervical Cancer

SNPs	Controls (n=234) n (%)	Cases (n=216) n (%)	OR (95% CI)	P
IFN-γ +874A/T (rs2430561)				
A/A	162 (69.2)	157 (72.7)		0.652
T/A	63 (26.9)	50 (23.1)		
T/T	9 (3.8)	9 (4.2)		
T	81 (17.3)	68 (15.7)	1.12 (0.79-1.59)	0.527
A	387 (82.7)	364 (84.3)		

IFN-γ

IFN-γ +874A/T (rs2430561) genotyping was performed by using PCR-AS analysis. The house-keeping gene GAPDH was used as the control. Therefore, all amplification products of rs2430561 showed a positive signal of GAPDH in 430bp. The result showed that three genotypes differed in the electrophoretogram (Figure 3). PCR products amplified by using the specific upstream primer, whose 3'- is T, showed a specific positive signal in 264bp presenting A/A. PCR products amplified by using the specific upstream primer, whose 3'- is A, showed a specific positive signal in 264bp presenting T/T. PCR products of upstream primers, whose 3'- is T and A, respectively, showed a specific positive signal in 264bp presenting T/A.

The result showed that the distribution of IFN-γ +874 A/T genotype was 69.2% A/A, 26.9% T/A, 3.8% T/T in the controls and 72.7% A/A, 23.1% T/A, 4.2% T/T in the cases, respectively. There was no significant difference of genotype distribution (P=0.652) and allele frequencies between cases and controls (P=0.527, OR=1.12, 95% CI=0.79-1.59) (Table 2).

Discussion

This study found no statistically significant association between cervical cancer risk and polymorphisms in the STAT2, STAT3 and IFN-γ genes in Han Chinese women in the Hunan region.

To our knowledge, we are the first to evaluate the relationship between STAT2 SNP polymorphisms and cervical cancer risk. STAT2 contains 24 exons, encoding 851 amino-acid residues. STAT2 rs2066807 is a non-synonymous coding SNP in the nineteenth exon region, encoding amino-acid mutated from Methionine (Met) to Isoleucine (Ile). We found that the physicochemical property and structure of Met and Ile are similar. Therefore, it is hypothesized that the effect of STAT2 rs2066807 SNP on HPV clearance and carcinogenesis may not be obvious.

Previous studies analyzing the effect of SNPs of IFN-γ or STAT3 genes on risk of cervical cancer offer conflicting results. Homozygous IFN-γ +874A/A polymorphisms may be associated with increased cervical cancer risk in certain Chinese populations (Wang et al., 2011). However, several meta-analyses from published case-control

studies showed that IFN-γ +874 T/A was not associated with increased cancer risk (Ge et al., 2014). One of the polymorphisms of the STAT3 gene, rs4769793, was associated with susceptibility and poor differentiation of cervical cancer in certain Chinese women (Wang et al., 2011). One possible explanation for the inconsistencies in findings of prior and current studies is that host susceptibility may vary in different ethnic populations. Another possibility is that only limited SNPs of each gene were investigated in our study.

In conclusion, our study suggests that genetic variations in IFN-γ, STAT2 and STAT3 genes were not associated with the risk of cervical cancer. Further studies with a larger sample size and larger number of polymorphisms of SNPs should be considered.

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