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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
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 χ² 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) Lane1 is 100bp DNA ladder, lane2-7 are PCR products of rs2066807 from six samples. The length of products is 470bp. B) Lane1 is 100bp DNA ladder, lane2-7 are PCR products of rs957970 from six samples. The length of products is 382bp.
Association Study of SNPs of STAT2/STAT3/IFN-γ Genes in Cervical Cancer in Southern Chinese Han Women

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).

IFN-γ

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 +874 A/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 no significant association between cervical cancer risk and IFN-γ +874 A/T polymorphism.

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

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Controls (n=234)</th>
<th>Cases (n=216)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT2 rs2066807</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>220 (94.0)</td>
<td>202 (93.5)</td>
<td>0.827</td>
</tr>
<tr>
<td>C/G</td>
<td>14 (6.0)</td>
<td>14 (6.5)</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>14 (3.0)</td>
<td>14 (3.2)</td>
<td>1.09 (0.51-2.31)</td>
</tr>
<tr>
<td>G</td>
<td>454 (97.0)</td>
<td>418 (96.8)</td>
<td></td>
</tr>
<tr>
<td>STAT3 rs957970</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>39 (16.7)</td>
<td>38 (17.6)</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>126 (29.5)</td>
<td>104 (34.3)</td>
<td>0.455</td>
</tr>
<tr>
<td>T/T</td>
<td>69 (53.8)</td>
<td>74 (48.1)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>204 (43.6)</td>
<td>180 (41.7)</td>
<td>0.92 (0.71-1.20)</td>
</tr>
<tr>
<td>A</td>
<td>387 (82.7)</td>
<td>364 (84.3)</td>
<td></td>
</tr>
</tbody>
</table>

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

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

between cases and controls (P=0.560, OR=0.92, 95% CI=0.71-1.20) (Table 1).
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.

Acknowledgements

This study was supported by grant 30771122 from the National Natural Science Foundation of China, and grant 12JJ2016 from the Key Project of Natural Science Foundation of Hunan Province.

References


